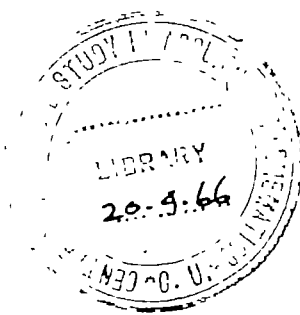


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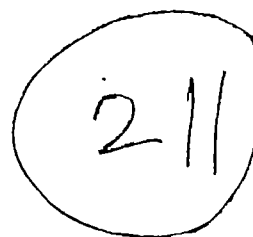
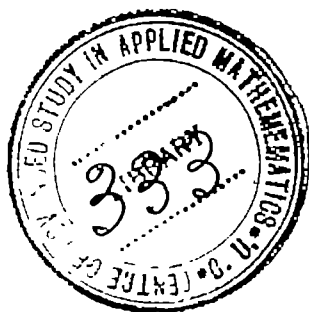
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## DISTRIBUTION OF SCIENTIFIC EFFORT

THE thirteenth annual report of the National Science Foundation\*, covering the year July 1, 1962–June 30, 1963, is the first annual report of the Foundation to be transmitted over the signature of the new director, Dr. L. J. Haworth. The activities described were carried out under Dr. A. T. Waterman's directorate and much of the interest of the report lies in Dr. Waterman's farewell statement as director. Essentially it follows the pattern of the twelfth annual report†, covering the year ended June 30, 1962, and earlier ones. Dr. Waterman's statement in the twelfth annual report is of particular interest, however, as it emphasizes the important new dimension acquired by science and one fraught with far-reaching social problems, namely, its potentiality for creating radical or large-scale changes in man's environment. Dr. Waterman's statement first directs attention to the major limitations imposed in the overall planning process by finance. In this connexion he observes that it is not sufficient merely to identify the immediate objectives, and proceed on the basis of studies of practicability, organization and procedure with a selected series of undertakings, basing the support of all scientific and technological activities on these specific objectives, in spite of the appeal of this approach from the point of view of management practice and efficiency. He emphasizes that our planning must encourage research in areas that are highly significant from the scientific point of view, and insists that we should not be limited by whether or not such research appears to have any immediate practical application. Basic research should contribute to the achievement of foreseeable objectives by providing assistance in all directions that science itself regards as significant and feasible.

Finance, however, is not the only limiting factor, and Dr. Waterman once again insists that the most important limitation is that imposed by manpower. He reminds us of the fact that there is a firm limit provided by the proportion of the population possessing ability to enter the professions of science and engineering—a factor to which insufficient attention is sometimes directed in planning the expansion of higher education in Britain. For a free society the problem, he suggests, is to identify and to stimulate the fraction of the population possessing such aptitudes and to provide for their education and training. Nor should it be overlooked that something like 40 per cent of students specializing in science and engineering should undertake academic careers if we are to be sure that sufficient research, and instruction of the high quality required, will be available to achieve our overall objectives.

This said, however, Dr. Waterman insists, as does the Commoner Report (*Nature*, 206, 1277; 1965), that it is

man's new capacity to effect major changes in his environment and in himself that should engage our most serious attention. Technical developments directly influence our environment on an unprecedented scale, and it may well be asked whether man is giving sufficient consideration to the possible consequences of experimenting on the present scale. Dr. Waterman points out that it is essential that we should fully recognize the dangers as well as the benefits that may attend modern research programmes and the application of new products on an ever-increasing scale. If every possible precaution is to be taken in experiments and pilot operations on a global scale, international co-operation is indispensable. He insists that it is essential that nations should come together in an effort to determine the extent of the hazards involved in such matters as nuclear explosions, water pollution and the manipulation of weather and climate, and to consider the types of controls that should be instituted. This is quite apart from the increasing extent to which the scale of the products involves resources far beyond those which a single nation could afford. Dr. Waterman concludes this statement by referring to some striking examples of successful international co-operation in this field, such as the International Geophysical Year and the programmes growing out of it, the International Year of the Quiet Sun and research on the upper mantle of the Earth's crust. The World Health Organization, the Food and Agriculture Organization, and the World Meteorological Organization of the United Nations provide other examples of successful international co-operation in science, while the United Nations Educational, Scientific and Cultural Organization has also played an important part, especially among the undeveloped nations. Necessity as the primary motive for discovery is fast disappearing, but we are now faced with innovation on a constantly growing scale, and with the question of whether we will continue to exploit Nature (including man himself) for immediate practical objectives with the minimum regard for possible long-range consequences. The greater the innovation and the larger the effort we devote to it, the heavier the responsibility for a thorough study of its purpose, motive and consequences. We should be seeking not merely knowledge but understanding and, to the extent that it is possible, wisdom. In this basic research the outstanding individual will always provide the most important and vital contribution.

Dr. Waterman's valedictory statement in the thirteenth annual report follows appropriately on his previous year's statement, being essentially a review and critical analysis of overall trends in research and development in scientific manpower, and their significance for national strength in science and technology. While the Federal Government played a leading part in providing funds and introducing and supporting large and critical programmes in the spectacular development of science and technology in the United States in recent years, Dr. Waterman points out

\* National Science Foundation. Thirteenth Annual Report for the Fiscal Year ended June 30, 1963. Pp. xxii+874. 1.25 dollars. (Washington, D.C.: Government Printing Office, 1964.)

† National Science Foundation. Twelfth Annual Report for the Fiscal Year ended June 30, 1962. Pp. xvii+868. 1.50 dollars. (Washington, D.C.: Government Printing Office, 1963.)

that the scale of the national effort demands careful consideration of certain questions. Among these questions the most important are: whether the grand total for research and development can be justified in the national interest in terms of money, manpower and other resources; whether the objectives of the undertaking represent a wise, prudent and adequate selection of national priorities; to what extent the component programmes in the effort are feasible and are intelligently designed to meet these objectives; whether the effort is conducted with proper efficiency and economy; and whether there exists, now and for the foreseeable future, the requisite scientific and engineering manpower. These are all points which call for consideration in framing scientific policy whether in the scientific field narrowly considered or on the wider national scale: they call for consideration by other Governments as much as by that of the United States, where the National Science Foundation was formed to facilitate the analysis of the facts and the review of the situation by competent and experienced persons.

Dr. Waterman returns fairly confident answers to the questions he asks. Since the National Science Foundation began to analyse the data in 1953, the sources of funds have changed very little, and while the Federal contribution increased from 53 per cent to 65 per cent, this increase occurred between 1953 and 1957 and has remained practically constant since. In basic research, however, the total funds are now nearly 1,500 million dollars, or about three times what they were in 1953, but as a percentage of the total they remained nearly constant at 8 per cent until two years ago, when they rose to 10 per cent, mainly through major new undertakings in such fields as oceanography, the atmospheric sciences, high-energy physics and space research. Somewhat less than 60 per cent of these basic research funds come from the Federal Government, while rather more than 25 per cent of the basic research is conducted in colleges and universities and about 16 per cent by Government. Statistically and fundamentally, the growth of science and technology among the three economic sectors over the past decades appears to have been balanced and consistent, and Dr. Waterman does not display the concern over space research which is manifested in the Commoner Report. Most of the research and development is being carried out directly in the national interest, and although the Federal Government is not acquiring a larger proportionate share in the investment in research and development, or encroaching increasingly on private and other sectors, these conditions may not always apply within particular programmes, projects or areas of science. If, therefore, any substantial economies are to be effected, they must be applied to the 90 per cent of the effort directed towards practical objectives and not in the basic research effort, unless the economy is to be disastrously affected.

Basic research, Dr. Waterman insists, provides the foundation for technological advance, as well as determining the potentialities of scientific progress and providing the essential advanced training for scientists and engineers. As to the capacity of the United States to support its present effort, he again believes that the limiting factor is the number of scientists and engineers available and the extent to which facilities for their education and training are available. Here it is the time scale that is immediately critical. Generally, he points out, the studies of the National Science Foundation suggest that the employment of scientists, engineers and technicians will be doubled by 1970, and the education and training of

this particular group can be achieved without depriving the country of the professional skill required in other fields. Although the scientific manpower problem is very urgent the underlying problem is the much broader one of providing thoroughly competent training in all fields, and Dr. Waterman suggests that the opportunities for radical improvement in general education in the United States are very great. He refers to evidence that careful attention to the improvement of elementary courses in standard subjects could be decisive in producing effective long-range results. His observations fully substantiate the argument for giving much greater attention in Britain, also, to the revision of courses in science subjects at all levels.

Dr. Waterman has some cautionary words about the support of basic research by the project system which should be noted carefully by those who are inclined to extend the contract system in Britain. He also notes the problem of continuity involved in the establishment of special research centres with only limited interests, whether by the Government or otherwise. In a similar context, he suggests that the sponsorship of special integrated programmes in such areas as oceanography, atmospheric sciences and high-energy physics should not be allowed unduly to curtail support for individuals in other fields of science. In his 'farewell review', however, the emphasis is placed on the critical importance of fundamental research and on ensuring that this is allowed to develop freely, uncontrolled by political or social considerations. For this he recognizes that there must be a fuller public understanding of science as well as of technology and that to clarify the understanding of the distinction between science and technology provides an important starting point. Like the Commoner Committee, he recognizes that some of the decisions cannot be taken by scientists or technologists themselves, because they involve social and political issues; but there must be some general public understanding if the decisions taken are to be intelligent or appropriate. As in his previous statement, he concludes with a reference to the importance of international co-operation in this field. The statements illustrate how much the National Science Foundation has owed during the years to Dr. Waterman's leadership and how well deserved are the tributes which his successor pays to him in his own statement in the fourteenth annual report of the Foundation\*, covering the year ended June 30, 1964.

Dr. Haworth devotes that statement to outlining his views concerning the role of science and technology in national affairs, the responsibilities of the Federal Government for promoting science, technology and education in the public interest and, particularly, the part played by the National Science Foundation. It is interesting to note the extent to which he places his chief emphasis on exactly the same points as does Dr. Waterman, and especially on those which have recently been stressed in the Commoner Report on the integrity of science. He insists, for example, that in spite of their interaction and overlap, research and development are separable and separate entities, and that expenditure on development should be justified on grounds relating to its final purpose and should not be competitive with expenditure on basic research, or even on broadly based applied research. Again, while all development, and much

\* National Science Foundation. Fourteenth Annual Report for the Fiscal Year ended June 30, 1964. Pp. xxix+128. 45 cents. (Washington, D.C.: Government Printing Office, 1965.)



applied research, is directed towards specific national goals, it is more important to ask, not how much we can afford for research and development but how necessary a particular undertaking is to the achievement of a national goal. Criticism and appraisal must be specific, and Dr. Haworth is as clear as Dr. Waterman as to the vital importance of public understanding: the real objective in basic research is to develop an understanding of Nature.

There is no misconception of the importance of all those areas of activity—basic research, applied research, and development—or their interrelationship. Dr. Haworth suggests that scientists might have done more to explain clearly the nature of basic research. One of the most important features of his statement is his survey of the role of the National Science Foundation, including some specific criticism of the project grant system as hitherto used. This is the more valuable in that it is placed in the context of the historical perspective rather than simply expressing a conviction that scientific and technological progress depends alike on strengthening education in science and on maintaining and augmenting a fund of scientific knowledge, derived mainly from basic research. In this historical perspective he notes particularly the limitations which manpower, facilities and finance always impose on policy, the necessity of guarding against fragmented research effort or dissipation of resources, the extent to which scientific activities transcend the responsibility or interest of a single agency, and the need for continuous reappraisal from fresh vantage points.

In the United States Dr. Haworth considers that the key individual in co-ordinating the national research and development effort is the Special Assistant to the President for Science and Technology, who is chairman of the President's Science Advisory Committee and of the Federal Council for Science and Technology as well as head of the Office of Science and Technology. The Federal Council for Science and Technology consists of the chief scientific officers of the nine Federal agencies most heavily involved in scientific activities and it leans heavily on the scientific advice of the Science Advisory Committee and National Academy of Sciences. It is under this system that the National Science Foundation operates and it has in the past relied heavily on project grants in supporting research. Dr. Haworth now asks whether the Foundation should attempt instead to devise new or modified programmes of support; how the Foundation can be sure that the relative amounts of support provided to the various fields of science are approximately correct; and whether the Foundation should change its policies and procedures in response to increasing concern over the geographical concentration of Federal funds for research and development.

Even in the United States these problems are not limited to the National Science Foundation, and in Britain they are implicit in the concern at present being expressed over recent changes in the organization of civil science. Dr. Haworth points to some specific defects in the project grant system. • It leads, for example, to decisions being made outside the institution on the nature and amount of support to be given to the parts within a particular institution, and this may adversely affect its balanced growth or administration. Again, scientists and administrators may alter the proposed balance of research in favour of efforts judged most likely to receive Federal support. Younger, unknown investigators tend to have difficulty in securing support, and it is also difficult for

an institution to establish new activities, particularly interdisciplinary units or programmes. Under this system, too, funds are not often available for flexible or co-operative use, and experienced research workers are sometimes required to submit fresh applications for continued support every year or two, when the nature of the work makes it evident that completion of the project requires much longer.

Dr. Haworth does not suggest that there is any simple way to overcome these difficulties, although they might become less pressing or acute if more generous funds were available. What is clear from his statement is that under his leadership the Foundation will continue to seek for new and improved methods of allocating its funds effectively, including the assessment of priorities as between different fields of science and their probable contribution to the solution of national problems. He is obviously animated by essentially the same convictions as his predecessor and, so far, he appears to have limited himself to some measures to encourage decentralization within the Foundation itself. However, on the evidence of his first statement the Foundation should continue to function on the sound lines established in the past, but with constant appraisal of its methods and procedures in the light of past experience, new needs and resources and changing national requirements. To scientists in Britain, the Foundation's report and the director's statement should provide a wealth of material for appraisal of the reorganization of scientific effort and the structure most appropriate for that effort and for the formulation of scientific policy.

## ORGANIZATION AND EVOLUTION IN PLANTS

### Organization and Evolution in Plants

By Prof. C. W. Wardlaw. Pp. xiii + 499 + 24 plates. (London: Longmans, Green and Co., Ltd., 1965.) 80s. net.

IN Prof. Wardlaw's latest book, a worthy successor to earlier and familiar volumes<sup>1-3</sup>, the author faces the problems which biological organization presents. He therefore deals with what many regard as one of the most outstanding scientific problems of our time. The book relates specifically to plants, and here one should frankly recognize that it is in their organization that higher plants and animals differ in essential respects. The breadth and scholarship with which Wardlaw develops his theme will be appreciated by all plant scientists, who accept the great challenge of interpreting growth, development, differentiation, and morphogenesis, for they are, and will increasingly be, preoccupied with organization in living things. Nevertheless, there are those to whom the topic of organization carries a suggestion of vitalism and even mysticism. But even they must see that, whereas comparative biochemistry and now molecular biology tell us much about what cells and organisms have in common, we still face the problems of their diversity. It obviously takes something more than a general knowledge of the separate parts of a complex system to understand its smoothly co-ordinated, integrated behaviour.

Prof. Wardlaw's book, which deals with problems of organization at various levels of complexity, is therefore a timely one, for biologists must now turn increasingly to the consideration of the implications of organization as they investigate biological functions, not only in isolation, but also in the full complexity of their interactions in living systems which can grow and develop. While it is a laudable chemical ambition—and even a necessary first

step—to achieve as much as possible in cell-free systems, knowledge is rarely complete until we interpret the behaviour *in vivo*. In this respect the present-day popular term 'molecular biology', if too literally interpreted, can become a *reductio ad absurdum*; for the problems of biology really begin when one considers levels of organization higher than the purely chemical interpretation of molecules—molecules *per se* are not alive. Indeed, without the examination and interpretation of viable systems, which have evolved and which can grow and develop, biology would be like 'Hamlet without the Prince of Denmark'. Prof. Wardlaw therefore summarizes the knowledge that has been gleaned from the various branches of natural science, and which is now needed to formulate concepts of the organization of plants. However, he also draws extensively on a wide knowledge of works which deal with the comparative morphology of plants and with the philosophy of organisms. Ever appreciative of the essential contributions which have been made and are still to be made by students of chemistry and physics, etc., he nevertheless stresses—and rightly so—the need to see and understand the organism as a whole.

This book is therefore to be welcomed. It will be welcomed by those botanists who are steeped in a descriptive tradition, because of the skill with which Prof. Wardlaw summarizes knowledge drawn from a scattered and extensive literature. It should be welcomed by experimentalists, who may turn to it for an appreciation of the systems with which they often do their work. It should be helpful to those in biochemistry, biophysics and molecular biology if they really seek to understand the full range of problems which the interpretation of plants as organisms presents. But all biologists will readily appreciate the twenty-four carefully chosen and well-executed plates in this attractive volume, and the large number of figures which illuminate the text and develop the author's theme around concrete and well-chosen examples. Thus, this book should be useful to students, teachers and research workers alike for the wealth of documented material it contains. The bibliography comprises into some 750 citations the selected references by which the author skilfully exposes his readers to a very extensive body of literature. The subject index also functions as an index by plant names.

Prof. Wardlaw views the biological scene with the perspective of a morphologist and he emphasizes a comparative approach; his essential and indisputable claim is that biological organizations have evolved and, to survive, they have adapted and must function effectively. But, with the scope and breadth of interest which distinguishes Prof. Wardlaw's writings, this new work ranges from current knowledge of genetically determined events as they occur in nature at the molecular level to the recognition of organization at successively higher levels. Thus organization is considered at the level of sub-cellular organelles, cells, tissues, organs, and organisms; and only at the level of populations, where problems of organization also arise, does Prof. Wardlaw content himself by reference to other authorities. But, throughout, the author is essentially true to that which he knows best, namely, the comparative morphological approach. Collectively, organisms are seen as the product of their history of evolutionary adaptation, and, individually, as the outcome of their ontogenetic development. Thus time is an essential parameter.

Prof. Wardlaw's book is particularly needed, as investigators now make use of more and more refined techniques but also tend to draw broader generalizations from a narrower base of observations. Not all investigators find themselves able to build on the philosophical writings of the past nor to formulate that broad appreciation of plants as successful functioning organisms which is the necessary base from which to approach the still unolved biological problems. Prof. Wardlaw's book seems very successfully to have contributed to this end; it

sketches in the biological background against which the emerging drama of the growth, differentiation and morphogenesis of plants may now be viewed.

It is neither possible nor profitable to make a digest, or a précis, of the sixteen separate chapters which comprise *Organisation and Evolution in Plants*. Were that possible, neither the book nor the subject to which it refers would have the fascination of a continuing, unfolding, unfinished story. The author would clearly have it so. He concludes, however, on a somewhat wistful note, designed to preserve the unity of the subject of botany.

Some (as Prof. Wardlaw says) seem now to regard botany as a "Cinderella-like science", only likely to attract general interest in so far as its problems are "tackled by those with competence in the physical sciences". It is even feared that botanical science will almost certainly suffer a setback "even cease to be botany" if "purely physico-chemical concepts of some of the processes in plants were permitted to displace and supervene upon the comprehensive biological concepts which are essential to any adequate understanding of plants as functional entities". Surely, however, Prof. Wardlaw need have no fears. Plants are not animals; neither are they mere assemblages of molecules any more than a chemical analysis alone describes the Five Sisters' window of York Minster or the beauty of the stained glass in Chartres Cathedral. So long as there are those who set down with skill and scholarship the facts as Prof. Wardlaw has presented them, and others take up the problems as he, and they, see them, there will be need for those who can take all the knowledge that chemistry and physics and molecular biology can furnish to develop concepts of what plants are and how they function through growth. One may even say that the students of plants as organisms are needed not only to pose the problems that the new disciplines may solve but to suggest the systems on which the attack may best be made. The ancient injunction to "consider the lilies of the fields how they grow" is still apt, to be heeded with wonder and humility by botanists and chemists alike.

To put the matter more bluntly, the dominant organisms of to-day are plants, for they collectively represent the bulk of living protoplasm now on the Earth, and without them there would be no animals or insects. This is not alone because of photosynthesis but also because it is plants that have solved the problem of incorporating inorganic forms of nitrogen into protein and which keep the nitrogen cycle going. One could have an extensive biology without animals, but not as we know it without plants. Higher plants also have distinctive ways by which they construct their organs out of cells and by which they correlate and regulate their behaviour; they have obviously achieved a very different way of life than have higher animals. Therefore, that branch of biological science, hitherto called botany, will and must persist. Botany is no "Cinderella science"—the stately theme which Prof. Wardlaw himself develops will not fade like a fairy-tale at midnight. It contains too much that is essential to human welfare and too much that still remains to challenge the combined abilities of biologists and of those who, following Wardlaw's advice, will add to the skills of the chemists and physicists that intimate knowledge of, and respect for, organization at all levels which is the distinguishing characteristic of the biologist.

The cell theory of 1838 defined the minimum viable morphological unit. Huxley's famous aphorism that "protoplasm is the physical basis of life" focused attention on a "stuff of life". Since then past progress has so often been made by, as it were, taking living organisms apart to achieve an ever more detailed knowledge of them at lower and lower orders of magnitude. But with each new technique that basis of life which used to be thought of as a substance becomes ever more obviously an arrangement, a design, composed of organelles and granular units in complex and intimate proximity. All this knowledge of fine structure is in a descriptive morphological tradition.

Meanwhile the molecular biologists, approaching the the problems from the other end of the scale, strive to interpret the living system in terms of the unit properties of its matter. But after all this essential learning of 'more and more about less and less', biologists must still attack the difficult task of synthesis. They must, in effect, put the pieces back together again; not merely to visualize the reconstructed wholes built out of their identifiable parts (granules, membranes, organelles, cells, tissues, organs) but also to interpret how organisms function—in short, how they grow and develop. (Having in fact understood the watch by taking it to pieces, we must not only visualize how it should be reconstructed but understand what makes it tick.) It is here that the seemingly more intangible concepts of organization apply, for they relate to features which—though they may distinguish the "quick from the dead"—are more evident in the subtleties of the assembly and interrelationships between component parts than in their identity alone. It is all this which makes for a smoothly operating integrated whole.

Prof. Wardlaw would have us understand plants, whether algae or angiosperms, in this way; and his book *Organization and Evolution in Plants* makes a real contribution to this end, if only because it directs attention to the problems that are to be encountered.

F. C. STEWARD

<sup>1</sup> Wardlaw, C. W., *Morphogenesis in Plants*, Methuen's Monographs in Biological Subjects, 176 (London: Methuen and Co., Ltd., 1962).

<sup>2</sup> Wardlaw, C. W., *Physiology and Morphogenesis*, 536 (London: Macmillan and Co., Ltd., 1963).

<sup>3</sup> Wardlaw, C. W., *Embryogenesis in Plants*, 381 (London: Methuen and Co., Ltd., 1965).

## INTEGRATED BIOLOGY

The Behaviour of Arthropods

By J. D. Carthy. Pp. vii+148. Paperback 12s. 6d.

The Biology of Hemichordata and Protochordata

By E. J. W. Barrington. Pp. vi+176. Paperback 15s.

The Physiology of Nematodes

By D. L. Lee. Pp. x+164. Paperback 12s. 6d.

The Metabolism of Insects

By Darcy Gilmour. Pp. xii+195. Paperback 15s.

Reproduction in the Insects

By K. G. Davey. Pp. x+96. Paperback 12s. 6d.

Cybernetics and Biology

By F. H. George. Pp. viii+138. Paperback 12s. 6d.  
(Edinburgh and London: Oliver and Boyd, 1965.)

THE past twenty-five years have seen the disappearance of the once well-defined boundaries separating classical biology from biochemistry, physics and chemistry. Recognising this fact, some universities have now begun to establish integrated courses in biological science in which biochemistry and the exact sciences carry equal weight with botany and zoology and in which the latter subjects are themselves biased toward experiment and analysis. But the biologist is still primarily concerned with the organism as an integrated whole; the ultimate explanations of physiology and morphology in terms of physics and chemistry must still account for the diversity of living things and the ways in which organisms exist in relation to their particular environments.

The six volumes included so far, in the series of *University Reviews on Biology*, separately and together form an admirable addition to the literature at present available to support this new approach to biology. The books combine classical zoological information with the results of modern experiment, and stress particularly the importance of biochemical processes in arriving at a deeper

understanding of biological function. The reviews should play an important part in any integrated course in biological science, although since the individual subjects are largely unrelated, the books may be bought separately according to particular interest.

*The Behaviour of Arthropods* is another example of Dr. Carthy's happy knack of communicating fairly complicated concepts in terms readily understandable to the non-specialist. The book eschews purely descriptive accounts of behaviour patterns and consequently deals largely with insects, the group in which most detailed analysis has been made; where equally rigorous research has been carried out in other arthropod groups, this has been included. The various characteristics of different kinds of behaviour are related, where possible, to the neurophysiological mechanisms involved. The excellent summaries of Mittelstaedt's analysis of the control pattern of prey localization in mantids, and Harker's work on cockroach diurnal rhythms, are typical of the quality of this book.

*The Biology of Hemichordata and Protochordata* discusses the morphology and interrelationships of the groups in a way which might be thought to follow the more classical accounts of animal phyla. But the anatomical descriptions provide a firm basis for a great deal of recent work on movement, feeding, reproduction, etc. And, as is to be expected in a book by Professor Barrington, there is an amount of stimulating speculation on the origins of the thyroid and pituitary glands and hormones. A special feature of this volume is the concise summary following each chapter.

*The Physiology of Nematodes* also includes sufficient anatomical detail, including ultrastructure, to make more meaningful the biochemical events underlying the physiology of the various kinds of free-living and parasitic nematodes. The metabolic processes of digestion and absorption, oxygen uptake and transport, osmoregulation and excretion, etc., in nematodes are continually compared with similar processes in other animals, to illustrate clearly the universality of these mechanisms and their specific variations related to the mode of life of particular forms.

*The Metabolism of Insects* is a biochemical text related particularly to insect biology. Where information about metabolic processes in insects is incomplete, Dr. Gilmour has indicated the probable course of events by drawing upon general biochemical knowledge. But the sections concerned with mechanisms more specific to insects, for example the metabolism of insecticides, tanning of the cuticle, or the intervention of hormones in the control of metabolism, are dealt with in remarkably lucid detail. Each chapter is introduced in general terms, describing principles in a way easily appreciated by the non-specialist, before moving to the more detailed biochemistry involved. This book will be invaluable both to the general entomologist and to the biochemist.

*Reproduction in the Insects* does not attempt a comprehensive review of insect reproductive mechanisms, but concentrates more upon some of the specific problems involved. Dr. Davey shows how modern research has brought a greater insight into the kinds of reproductive mechanisms evolved by the largest animal phylum as well as a clearer understanding of the ways in which different insects have developed a variety of processes to deal with similar problems. The chapters on spermatophore formation, sperm transfer and hormones are particularly good. The author's enthusiasm and clear exposition illuminate the whole of this little book.

*Cybernetics and Biology* is perhaps the least successful of this generally very successful series. Every field of intellectual activity tends to develop its own esoteric jargon; this becomes particularly confusing in psychology and cybernetics, where words and phrases lose their everyday usage and have specific and perhaps unusual meanings. Dr. George suggests that the Penguin Dictionary of Psychology be consulted for the definition of some of the terms

he uses—a revealing admission in a book about the science of control and communication! But, once this barrier is overcome, the book repays careful study; it can add considerably to an understanding of some of the problems discussed, for instance, by Dr. Carthy in his book. In fact, Dr. George stimulates thought about the various principles which may be involved in a great many biological processes that are usually taken for granted.

The authors of these volumes have evidently had few limitations imposed upon them by the publishers—as should be the situation when they are as expert in their subjects as these. The books are of various lengths, and exhibit a variety of treatment; but each one contains a longer or shorter list of references for broader or more detailed reading. In paperback or hard covers, the series is very well produced, with illustrations that are generally good although differing a little in quality from volume to volume. If subsequent volumes in the series maintain the standards set by the first six, then *University Reviews in Biology* will become a valuable, almost indispensable, part of the libraries of university students and teachers alike.

K. C. HIGHNAM

## CONTROL OF GLYCOGEN METABOLISM

### Control of Glycogen Metabolism

Edited by Dr. W. J. Whelan and Margaret P. Cameron. (A Ciba Foundation Symposium.) Pp. xiv + 434. (London: J. and A. Churchill, Ltd., 1964.) 60s.

THE latest addition to the invaluable series of Ciba Foundation symposia generously covers the field described in its title. The range of the numerous papers presented permits discussion of the synthesis and catabolism of glycogen, and its control by enzymatic and hormonal influences, together with an important section on the glycogen storage diseases. The editors, and in particular Prof. W. J. Whelan, are to be congratulated on the skilful way in which the complex assembly of papers and discussions on papers has been welded into the unified text of a book of great importance.

The book begins with a most stimulating article by French on the structure of glycogen evolving a concept of steric limitation in size of the Meyer model. This, together with the paper by Orrell, Bueding and Reissig, which offers a careful evaluation of the methods of preparing undegraded glycogen, serves to visualize the molecule and to restate the problems still unsolved in the structure of the particle.

A large section of the symposium is given up to papers on the present-day viewpoints of such enzymes as phosphorylases, branching and debranching enzymes. Here particularly the papers by E. G. Krebs *et al.* on the nature of the phenomenon leading to the appearance of catalytic activity in the phosphorylase molecule and its control are of great interest. This work receives interesting cross-illumination from the papers of Cori and Brown, who individually discuss the contribution of phosphorylase to the determination of the structure of glycogen and its role in the regulation of glycolysis in skeletal muscle. The further paper by Sutherland on the location of adenylyl cyclase and adenosine 3',5'-phosphate deserves close attention, as do the discussions which follow each of these accounts. Here perhaps is the most stimulating part of this book and clearly by the co-operative picture that emerges on present-day concepts of phosphorylase action more than justifies the assembling of so many talents together in a symposium.

Though so much work on one enzyme might suggest the neglect of others, the great variety of articles by the groups of Whelan, Lerner, Brown and Hers would firmly refute such an imputation. Part of the problem would appear to be that there are more enzymes known than

there are physiological functions for them to fulfil. However, much information is now available on the mechanism of debranching of glycogen structure. One wish that I would make is that some suitable enzyme nomenclature for these catabolic reactions should emerge in the near future. In addition, the papers by Lerner on the branching enzyme and Hers on the mechanism of action of amylo-glucosidase demonstrate the subtlety of experimental approach needed in elucidating the action pathways. Rosenfeld's paper reports a new mammalian enzyme— $\gamma$ -amylase—and reports on  $\gamma$ -dextrins. The paper by Prof. Whelan *et al.* lies at the heart of what may be called the Cori-Whelan controversy as to the structure of the branched chains in glycogen; there can be little doubt that Prof. Whelan is correct in his deductions.

The paper by Leloir on the role of uridine diphosphate glucose in the synthesis of glycogen is a model of lucid presentation and relevance, as anyone who heard his opening lecture in New York in 1964 will agree. One aspect which he mentioned there and which provoked much thought was his interpretation of the phosphorylase activation mechanism. Following Macfarlane's explanation of blood clotting as a multiplying system serving as a biochemical amplifier (*Nature*, 202, 498; 1964), Leloir postulated that a similar cascade of proenzyme-enzyme transformations might serve just such a purpose in the interconversions centred around the phosphorylase molecule.

Later articles in the symposium deal with the difficult territory of hormonal interaction on physiological systems, and the interpretation of these and other effects in terms of control over the enzyme sequences involved. Noteworthy in this class, of course, are the papers by Morgan and by Lerner. The former is a closely argued account of the allosteric properties of phosphorylase *b* studied in the perfused rat heart where glycogenolysis had been stimulated by glucagon and anoxia. Lerner's paper deals with the role of insulin in the control of glycogen synthetase. Together with these papers, the account of Bueding on the effects of adrenaline on intestinal smooth muscle, and the speculations of Randle on some indirect actions of adrenaline on glycogen metabolism, give this section of the symposium a pleasing sense of integrity.

The final section of the symposium deals with glycogen storage diseases. This is well presented; many of the previous contributors who dealt with the biochemical implications return to deal with the clinical quandaries of the subject. In particular Manners, whose excellent review of glycogen metabolism appeared in 1963, discusses type I glycogenosis, and is followed by other workers discussing the known diseases. Stimulating possibilities exist in the finding by Spencer-Peet of cases of glycogen synthetase deficiency.

The symposium closes with a discussion and a recapitulation of the main thoroughfares of glycogen metabolism.

C. F. PHILLIPS

## RECENT ADVANCES IN PSYCHOPHARMACOLOGY

### Animal Behaviour and Drug Action

Edited by Dr. Hannah Steinberg, A. V. S. de Reuck and Julie Knight. (Ciba Foundation Symposium jointly with the Co-ordinating Committee for Symposia on Drug Action.) Pp. xiv + 491. (London: J. and A. Churchill, Ltd., 1964.) 67s. 6d. net.

THE major part of *Animal Behaviour and Drug Action* is a compilation of papers and discussions presented at an international symposium, the aim of which was to examine the role of animal behaviour in investigations of drugs affecting the central nervous system. The book constitutes a welcome addition especially to the bookshelf

of the experimental psychologist, whose knowledge of psychoactive drugs could well be broadened, because the participants representing physiology, pharmacology, psychology and biochemistry laid emphasis, as could be expected, on the drug action rather than on the animal behaviour side of psychopharmacology.

A number of important issues were raised at the symposium. One which surely must merit future consideration is the role of the blood/brain barrier and blood/cerebro-spinal fluid barrier in psychopharmacological investigations. For example, RNA administered systemically may affect the rate of learning in an animal (Cook), yet there is no evidence that RNA penetrates the blood/brain barrier. It is also well known that if given intracerebrally rather than systemically, the biogenic amines can exert very different actions (Feldberg). In this connexion, one of the highlights of the symposium was the elegant work on the development of the blood/brain barrier in chicks, which showed how pharmacological, neurophysiological and behavioural techniques could be simultaneously applied to one problem (Dewhurst and Marley).

Another issue introduced was the importance of studying effects of drugs on social behaviour in animals (Chance and Silverman). Although this sort of approach appears promising, interpretation of the action of psychotropic drugs on social interactions among animals is difficult. It is questionable whether a drug which suppresses aggressive behaviour of rats possesses a corresponding effect on analogous behaviour in humans. However, in spite of methodological limitations, it is encouraging to learn that astute attempts are being made to translate the subtle psychogenic action of a drug from rodent to man.

The difficulties which may arise in the usage of behaviour analyses for drug evaluation were stressed (Irwin). For example, drugs can exert different effects if the animals tested differ in their "emotional and intellectual" states (Rushton and Steinberg), genetic backgrounds (Broadhurst), nutritional states (Watson), or even their familiarity with fellow test-subjects (Wilson and Mapes). With the use of the operant bar press as a research tool, a single drug may enhance a particular response topography with one schedule of reinforcement and depress it with another (Dews). Since reinforcement schedules can be varied *ad infinitum*, some interpretation is obviously desired in the meaning of the response change associated with changes in such schedules. One always wonders whether psychopharmacological studies utilizing an operant paradigm in animals with little or no cerebral cortex (birds) really bring us any closer to the understanding of psychoactive drugs, especially if these compounds are ultimately intended for clinical extension to humans.

Papers on the long-term effects of chronic administration of drugs were noticeably absent. Some coverage could have been given to those drugs which create profound social disturbances and on which experimental findings have emerged recently, namely ethyl alcohol, morphine, and other so-called 'addictive' compounds.

The discussion sessions which make up the final portion of the book were intended to be an organized exchange of ideas between the frequently divergent disciplines within the area of psychopharmacology. As a general impression it seemed that, in coming together, each discipline could be likened to an orchestral ensemble but each playing a different melody at the same time in the same concert hall. Because each contributor learned almost entirely on the literature within his own discipline, discussion often was disjointed, lacking in continuity, and sometimes switched abruptly from one topic to another with important points hanging in the air.

In spite of this criticism, the organizers of the meetings must be congratulated on the final endeavour. By virtue of discussion sessions of the quality recorded in this

volume, and as disciplines become better acquainted with one another's experimental techniques and points of view, future interdisciplinary meetings will become increasingly more fruitful.

R. D. MYERS

## PALAEOCLIMATE AND PALAEOMAGNETISM

### Problems In Palaeoclimatology

Edited by A. E. M. Nairn. (Proceedings of the NATO Palaeoclimates Conference held at the University of Newcastle upon Tyne, January 7-12, 1963.) Pp. xiii + 705. (London and New York: Interscience Publishers, a Division of John Wiley and Sons, Ltd., 1964.) 147s.

THE introductory article to *Problems in Palaeoclimatology*, by Prof. W. H. Bucher, is aptly entitled "The Third Confrontation". It draws analogies between the present impact on geological science of ideas on palaeogeography and palaeoclimates stemming from studies of rock magnetism, and the better-known geological-geophysical conflicts of the past concerning the age of the Earth and the origin of ocean basins. The reader of the book will doubtless find a fourth confrontation, between the apparent precision and repeatability of the palaeomagnetic observations (although their interpretation is still far from certain) and the failure of geologists to agree on the very nature of the phenomena which they seek to elucidate.

Thus in a series of successive geological articles on Precambrian glaciations, we find the conflicting conclusions: (1) "At present no Precambrian tillite has been sufficiently studied for us to accept glaciation without reservation"; (2) "The whole globe was glaciated in Eocambrian times"; and (3) "The evidence for late Precambrian glaciation is remarkably widespread". Without attempting to resolve these opposing opinions, it should perhaps be observed that recent dating work has shown many of the supposed late Precambrian 'tillites' to be more widely separated in age than is the Lower Cambrian from the Pleistocene; and that the presence of extensive phosphorites in the Sinian of China and Riphaean of Russia is scarcely compatible with the hypothesis of a lengthy and world-wide Precambrian glaciation, the end of which triggered off the rapid evolution of a Cambrian fauna.

A similar conflict is seen in the separate contributions of two authors on the late Precambrian conglomerate near Caen in Normandy, one of whom concludes that this formation is "as indicative as the Eocambrian fluvioglacial deposits of Norway or even the Quaternary fluvioglacial deposits of Poland", whereas the other asserts that "None of the data permits us to make inferences about climate. The climate could have been anything from tropical to glacial".

The work is, however, concerned with a great deal more than problems of Precambrian glaciation. It comprises no less than 54 contributions, arranged in chapters on the use of fossil plants in palaeoclimatic interpretation, the evidence of climate from coal and coal measures, the recognition of ancient glaciations, Precambrian glaciations, geophysical techniques and ancient climates, Devonian climate, theoretical considerations and Quaternary climate, the recognition of arid climate and wind direction studies, Permian climate, palaeontology and climate, and problems of red beds and soils. Dr. A. E. M. Nairn's editorial work is of outstanding quality, the diverse papers being bound together into these chapters by a unifying theme and by means of a well-summarized discussion at the end of each. Many of the contributions are entertainingly controversial and the book certainly deserves a place in every geological library for the use of mature readers, but it is to be regretted that the price is much higher than one expects to see for the report of a meeting which was financed from public funds.

C. F. DAVIDSON

### Massive Radiation Techniques

By Sidney Jefferson. Pp viii+324. (London: George Newnes Ltd., 1964.) 70s.

THE development of nuclear reactors during the past two decades has made possible the large-scale production of very strong radioactive sources. One result of this has been that some of the useful effects of ionizing radiation found in research laboratories can now be applied on the grand scale to a variety of technological problems. *Massive Radiation Techniques* surveys such applications in a number of industries with particular reference to their commercial viability. In some of them, for example the preservation of food and the sterilization of medical equipment, irradiation has to compete with well-established methods. In others, such as insect pest control by sterile male release, sterilization by gamma-irradiation may be the only feasible method. This is illustrated by the carefully planned operation of rearing, sterilizing and releasing 2.5 billion screw-worm flies in the south-eastern states of the United States which resulted in the virtual eradication of this livestock pest from the selected area in 1959.

The non-specialist will find that the lucid introductory section on the interaction of ionizing radiations with matter will make few demands on his previous knowledge while the final chapter on dosimetry will provide him with a short but adequate account of methods at present in use and the dose ranges over which they are best used.

The most widely used sources in large-scale irradiations are  $^{60}\text{Co}$  and electron accelerators and the engineering problems associated with their use, including automatic feed systems for packaging materials, are illustrated by examples of installations which have been operating for some years. Other topics covered at length include the biological effects of massive radiation doses in relation to agricultural problems and plant breeding and the use of radiation in the chemical industry.

The text is very free from errors and the numerous figures are with few exceptions clear and well prepared. The extensive bibliography which accompanies each chapter will be welcomed by the serious student.

This is a book which should stimulate ideas among industrialists and also provide a basis for planning the exploitation of useful radiation effects. R. J. MUNSON

### Automatic Methods in Volumetric Analysis

By D. C. M. Squirrell. Pp. x+201. (London: Hilger and Watts, Ltd., 1964.) 42s. net.

NINE-TENTHS of *Automatic Methods in Volumetric Analysis* is devoted to the description of automatic methods of end-point detection in volumetric analysis and as such is a bench working manual of considerable value. It is complementary in content to the work by J. F. Phillips, entitled *Automatic Titrators*.

The subject-matter is subdivided into four major parts: titrations to present end-points; full-scale recording potentiometric methods; full-scale titrations in non-aqueous media; and some other instrumental titration methods. Each part is headed by a section entitled "Principles and Instrumentation", which is followed by detailed description of a number of methods to illustrate the points elaborated. Many of these have been drawn directly from the author's own experience and are products of his fruitful association with Dr. Haslam. The methods described have been chosen to cover a wide field of general analytical interest and include determinations of total nitrogen in organic matter, water by Karl Fischer reagent, halides, sulphates and metals by ethylenediamine tetraacetic acid.

Much of the apparatus described has been assembled from a variety of component parts, some home-made, and many others are commercially available units. In some examples sufficiently detailed diagrams and circuitry are given to enable the reader to design and build similar equipment for a particular method.

Further stages in the development of completely automatic analytical systems are only briefly described, but an understanding of the basic principles elaborated in this book is a necessary prerequisite to such developments.

Minor editorial faults, which serve as irritants, exist in the text, for example, the calculation of titrant volumes from recorder chart inches, titration times or syringe plunger movement are stated and re-stated in full. A uniform presentation of reagents, used in the various methods is also required.

The book is, however, an invaluable collection of techniques and instrumental methods and as such is worth its place on any analytical laboratory bookshelf.

R. SAWYER

### Genetics In the Atomic Age

Second edition. By Charlotte Auerbach. Pp. vii+111. (Edinburgh and London: Oliver and Boyd, 1965.) 7s. 6d.

THIS is the sort of book which ought to be freely available in common-rooms, school libraries and reading rooms, wherever people, especially young people, with enquiring minds might be likely to pick it up and thumb through it. It is small, short and inexpensive: the sketches by Miss I. G. Auerbach invite perusal: the text is entirely readable, without technical terms or quantitative expressions: and a curious glance stands a good chance of being followed by a thorough reading. The result would be many more people with an interest in the genetic future of man, and the knowledge needed to form a rational view of the dangers of exposing his germ-plasm to ionizing radiations.

Since the first edition in 1956, Dr. Auerbach has revised and brought the book up to date. The first few chapters deal with what mutations are, how they affect the organism and the species, and how they are inherited. Then comes a discussion of the ways in which ionizing radiations act on genes and chromosomes. In the last chapter the application of this knowledge to man in relation to his genetic future is set forth with the clarity, reasonableness and force for which the author is already well known.

For those whose point of view is already formed and who are trying to reduce mankind's present genetic exposure to radiation, the book offers authoritative support and invaluable ammunition for argument.

ALMA HOWARD

### Analytical Chemistry of Molybdenum

By A. I. Busev. Translated from the Russian by J. Sohmorak. (Analytical Chemistry of Elements Series.) Pp. vi+247. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1964.) 96s.

A. I. BUSEV has set out to give a critical survey of the whole of the analytical chemistry of molybdenum. He has used his own experience as much as possible to decide the relative merits of the methods, and has, I think, succeeded very largely in 'separating the sheep from the goats'. After a comprehensive account of the chemical and analytical properties of molybdenum, chapters follow on the detection and isolation of the metal. Further chapters on gravimetric, titrimetric, photometric and other methods for the determination of molybdenum take up the second half of the book. There is a very extensive combined bibliography and author index. The omission of any reference to Granger's aqueous *n*-butanol dithiol method for the photometric determination of molybdenum (*Analyst*, 83, 609; 1958) is, therefore, surprising. Not unnaturally, the author appears to be strongest on the Russian literature and this does have its advantages.

One cannot detect that the book is a translation, so well has it been done, and the text is remarkably free from errors. It is very well produced, and should be of considerable value to anyone whose work involves molybdenum.

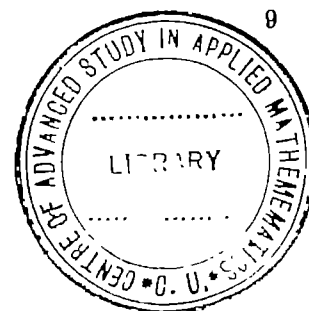
E. J. DIXON



## SIGNS OF LIFE

## CRITERION-SYSTEM OF EXO BIOLOGY

By PROF. JOSHUA LEDERBERG

Department of Genetics, Stanford University School of Medicine,  
Palo Alto, California

THE imminence of interplanetary traffic calls for systematic criticism of the theoretical basis and operational methods of 'exobiology', the initial search for and continual investigation of the life it might encounter. Very little science is totally irrelevant to it, and the policy-maker must face a riot of potential approaches to space flight experiments. By every standard, this is an epochal enterprise: a unique event in the history of the solar system and of the human species, and the focus of an enormous dedication of cost and effort. It requires a new perspective in experimental policy. The broader interfaces of *exo*-(Earth's own) biology, by contrast, permit its fruitful growth within the context of methodologies and instruments that can lag behind broadly established needs and imaginative possibilities. A system for orderly appraisal of the problem would rationalize the partition of labour, our only means of managing a complex problem.

Mars is our prior target. Our premised information is only: (1) terrestrial observation: *esobiochemistry*; (2) the implications of Mars being a 'terrestrial planet'; (3) a very small body of definite observational results. The choice of our first experiments must take account of a wide range of theoretical possibilities not yet narrowed by the experimental process. Over this broad reach, logical necessity rarely coincides with logical sufficiency. The most compelling inferences might stem from the least likely event. Our speculation will be narrowed and policy simplified by tangible information about any aspect of Mars, especially if it encompasses the variability of the planet's features in space and time.

## Evolutionary Stages and the Definition of 'Life'

Fundamental to all biological theory, *exo*- or *exo*-, is the evolutionary principle. As is now commonplace, we recognize the following stages in the Earth's history:

(A) *Chemogeny* (*organic chemistry*). The production of complex organic compounds by a variety of non-replicative mechanisms—the primitive cosmic aggregation, photochemistry of isolated atmospheres, thermal and spontaneous reactions of inorganically catalysed, previously formed reagents.

(B) *Biogeny* (*biology*). The replication of a specifically ordered polymer, DNA being the terrestrial example, which specifies the sequence of its own replicas, and of the working materials, like RNA and proteins, from which cells and organisms are fashioned. Random experiments of error in replication, and natural selection of their developmental consequences, result in the panoply of terrestrial life.

(C) *Cognogeny* (*history*). The evolution of the mechanisms of perception, computation, and symbolic expression and interpersonal communication, whereby tradition can accumulate, culture unfold.

Mars must be supposed to have had an initial history similar to Earth. To ask whether Mars has life is to ask how far has its *chemogeny* gone; how like and how unlike the Earth's; has its evolution passed through the biogenic (ordered macromolecular) stage? Then through the *cognogeny*?

In evaluating a complex set of possibilities it is helpful to find classifying parameters which can be scanned

systematically, if sometimes only implicitly, to generate a probability space. In this case, the evolutionary principle furnishes the parameter: chemical complexity.

The initial planetogeny and the consequent differences in physical and chemical environment determine the possible points of departure of the evolutionary processes. On these grounds, Jupiter must have special interest for comparative *cosmochemistry*; but it is still much less accessible to close investigation, and we have even less of a basis to predicate a homologous *chemogeny* there than we do for Mars. In so far as Mars does retain some environmental analogies to Earth we might at least predicate, for one branch of our analysis, that any Martian life is based on chemical linkages, predominantly  $-C-C-$ ,  $-O-O-$ ,  $-C-N-$  and  $-O-P-$ , which are barely stable in aqueous medium. We leave to hypothesis the extent to which the constructions from these and other radicals emulate terrestrial *biochemistry* at each level of complexity.

The cosmic abundance of these elements is relatively high, and there is every reason to believe that Mars is at least as richly endowed as Earth in them. If the initial budget of carbon has not, like that of the Earth's crust, been completely requisitioned by life, then what form will we find it in?

*Chemogeny* generates a vast mixture of products through the level of random macromolecules. Mars must have nurtured such chemistry, whether or not it had progressed to *biogeny*. A negative assay for organic materials would preclude biology, but could we believe such a result? It would properly be blamed on deficiencies in the particular sample. The positive assay, if it told something of the concentration and composition of organic molecules, would add to our understanding of Mars's development, and would contribute to our judgment of the life-detection problem. But it would not answer it. On the other hand, once life has appeared on a planet, it would dominate its organic chemistry—most carbon compounds would be witnesses of biogenic (or *cognogenic*) specificity. The cataloguing of organic molecules is a description of the consequences of evolution and must make up a large part of our effort.

## The Chemical Scan

To promise an actual complete scan of hypotheses of molecular complexity would be pretentious and witless, notwithstanding that a computer can now be programmed to visualize all the possibilities. However, the fantasy of such a scan is a constructive exercise in evaluation of evidence for life. For each chemical species the imagination of the specialist might be challenged to ask: (a) is there any information concerning the existence of this item relevant to scientific inference in exobiology; (b) what are my prior expectations on the distribution of this species, with and without life; (c) what other data could contribute; (d) how would the observation be interpreted from a terrestrial foray; (e) what special methods are available or could be devised to detect the species?

We might nurture a hope of turning up a special treasure, a rare example of a molecule which would reveal something about the evolution of the planet and help narrow our choices among the confusing array of possible



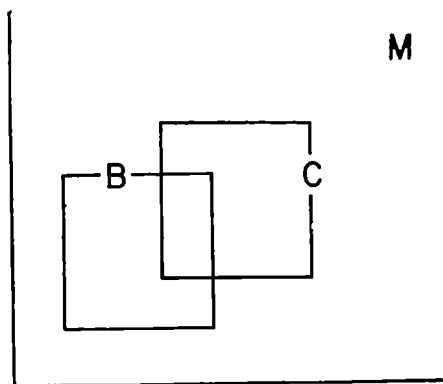


Fig. 1. Contingency space. The universe of possible observations  $I$  includes the overlapping domains  $B$  and  $C$ , the predictions of biogeny and chemogeny, respectively. The remainder is  $M$ , contradictions of these models, and possible consequences of cogency. Should this also be bounded?

targets. In practice this advantage does not materialize so easily, for the hope is false. Not that no chemical species is potentially informative; paradoxically, every one is.

Consider hydrogen. In terms of the simple Venn diagram (Fig. 1), most expected observations would fall in the region  $(B \cdot C)$ , that is, would be consistent with biogeny but not imply it. However, the sensible absence of hydrogen from Mars' surface would fall in region  $(-B \cdot C)$ , that is, virtually preclude life. But if we could produce no plausible physical model for the disappearance of hydrogen, we would have to reconsider the region  $(I - C)$ , that is, to ask whether the anomaly implies a biogenic or cognogenic sequestration of the element. On the other hand, certain microscopic distributions of H are hard to reconcile with any chemogenic model, and point to the region  $(B - C)$ , that is, an inference in favour of biogeny. This verges on morphology, but can still be formulated as molecular statistics.

On another tack, suppose a specimen consisted of pure protium,  $^1\text{H}$ , to the exclusion of deuterium,  $^2\text{H}$ . The price of pure protium on the terrestrial market hints at the obstacles to a chemogenic model. Apart from cognogenic activity, if a biogenic system were exquisitely sensitive to deuterium-toxicity it might evolve a discrimination against it.

The arguments have been laboured, but are quite typical of those that discovery of any other species would arouse.

### Entropy or Unlikelihood?

Given the evolutionary continuity of life and our understanding of the organism as a chemical machine, there can be no absolutely distinctive signature of life. Some conjunctions—like a planetary depot of protium—would be so unaccountable to our present model of chemical behaviour that we would feel obligated to postulate the operation of a goal-directed system (biogeny or cognogeny) rather than accept the improbability of such a conjunction by chance. This choice plainly depends on our freedom of choice of models. For example, our present knowledge of chemogeny permits a wide latitude of hypotheses as to the range of molecular species that atmospheric photochemistry might generate. Further developments in our knowledge of chemogeny or of the available chemical and physical resources of Mars might confer useful constraints on the data that might now be 'explained away' as chemogeny, and thus cannot yet make a crucial contribution to our search.

From terrestrial experience we judge that the occurrence of any of a number of compounds in high purity is a sign of life. Such deposits at a macroscopic level tend to signify cogency—a smelter, a chemical laboratory,

a communications cable, rather than biogeny—organic structure usually being built of microscopically defined components. Negentropy is a necessary, but not sufficient, sign of life. However, it can help filter out the most promising situations. Then only the details of experience or confident use of available theory can decide whether the eddy has a chemical-kinetic explanation or a bio- or cognogenic one. Lacking our experience, a Martian visitor might credit diamantane carbon to some mysterious biogenic function, inhibited by Y chromosomes; if he were cleverer, to the General Electric Co. He would need very special knowledge of the Earth to predict that diamonds would be found in the ground (and even more to understand why men dig them up, only so that women will wear them).

Kinetic instability in the context of local chemical and physical conditions is another clue. For example, cover of photosensitive pigments (witness terrestrial chlorophyll) requires special attention to the magnitude of plausible synthetic processes, atmospheric-chemical versus biogenic, by which their steady-state concentration could be maintained. Analogous reasoning would apply to compounds which are thermolabile in relation to the ambient temperature, or chemically unstable species which should reach equilibrium with coexistent oxidant. Do we see a forest fire? Then we must think of the efficient system of photosynthesis which will restore the steady-state vegetation. Top-heavy structures, which high-altitude reconnaissance could perceive even without resolving single trees, houses, or bipeds, likewise tell of kinematic instability, and in turn, some process to re-raise what must some time fall. But geophysics competes with biophysics, and we have to discriminate life from vulcanism and orogeny.

In sum, unlikelihood in terms of the chemogenic model gives weight to any finding as a datum for exobiology. It should be possible to quantitate chemogenic likelihood, essential if a datum is to be given a measured value in any decision-making programme. The resolution of the measurement need not be very high to make it still very useful in comparing disparate approaches.

In more general terms, biota have a high density of internal information: the root of our conceptual distinction between matter and life is the rich story that life can tell about itself, a plot the details of which we can scarcely deduce from our simple knowledge of the initial conditions. But there must be a plot, that is, the information must have some interesting pattern, or we would not distinguish a cell from the dislocations in a snowflake.

### Optical Activity

Many molecular species can contribute in an important way to our appreciation of life. *A priori*, we have a very limited basis to predict which species will be most cogent. We should, of course, give high, but not exclusive, priority to terrestrial prototypes like amino-acids and nucleotides. Fortunately, there is a generic classification of compounds which is relatively independent of detail of structure, yet should pervade a biogenic chemistry. This is optical activity.

The argument for logical necessity of net optical activity has nothing to do with optical rotation. It depends on the crucial role of the informational macromolecule in a definition of life. When tetravalent carbon is incorporated into macromolecular structure, each carbon stands a reasonable risk of being an asymmetric centre, of having a distinctive substituent on each of its four valences. Such an atom is subject to stereo-(optical) isomerism, and its orientation, D- or L-, must be specified if the macromolecule is to be fully ordered, more concretely, if it is to have a well-defined three-dimensional shape. Conversely, biogenic macromolecules, having ordered asymmetric centres, have the necessary information to discriminate among the isomers of monomeric substrates.

On Earth, where biogeny has dominated the statistics of organic molecules, we find that the ratio of D- to L-glucose residues is at least  $10^{14} : 1$ .

Logical sufficiency can also be argued. Chemical enantiomorphs should be generated in equal proportions except under the influence of a catalytic system which is already asymmetrically organized. The global organization of a planet into one catalytic system of particular orientation is a catastrophe of a magnitude unique to biogeny. Spontaneous resolution might occur locally. Hence this criterion has its greatest weight when applied to a species which flows through the planetary circulation. A paragon would be labile molecules condensed from the atmosphere: optically active smog.

Within the biogenic system, both enantiomorphs of a metabolite might be generated, but this would be in precisely equal amounts with no greater likelihood than any two species designated at random. Chemogeny and sterically ordered biogeny thus give sharply contrasted expectations on these statistics, and biogenic chemistry can hardly avoid becoming sterically ordered.

These lines of inference do not account for such parochialisms as the undeviating series of L-isomers of amino-acids in esobial proteins. For example, D-alanine would be at least as interesting as L-valine in expanding the homologues of glycine. But, for that matter, why is  $\alpha$ -amino butyric acid passed over? We may know better when the rules of polypeptide conformation are better known; more likely, from the details of evolution of amino-acid anabolism and problems of discrimination among analogues. It is precisely at this level that the local biological theory fails, and thereby points to the crucial issues of a cosmic biology.

In view of the theoretical generality and historical tradition of the Pasteurian principle, it is paradoxical that the direct measurement of optical activity is weak by comparison to other instrumental approaches. However, the basic criterion is not optical rotation but molecular statistics. Enantiomorphs can be assayed with optically active reagents to give resolvable diastereo-isomers, and exploit the most sensitive methods known to chemistry.

### Macromolecules

Informational macromolecules define the boundary of chemogeny and biogeny, of chemistry and life. Their description on another planet is the fundamental challenge of exobiology. Replication of macromolecules (genes) and the inevitability of random error (mutation) open the door to natural selection and the evolution of more and more complex forms of life. A random polynucleotide is not life; routes to its photochemical-synthesis from simple gases and inorganic phosphate are in sight. Can we deduce the replication of a polynucleotide by any means short of the most recent achievements of direct observation and *in vitro* enzymology? Historically, we could deduce the informationality of macromolecules just from compositional data. When the same sequence occurs in many molecules—as in a sample of crystalline haemoglobin—we have to invoke an informational process to programme and implement the synthesis of the protein. In fact, only recently and rarely could we gain complete specifications of an actual sequence. This is usually inferred from fragmentary analyses of a fraction found to be monodisperse on a few measures and then assumed to be sequentially homogeneous.

The sequence need not be the gene itself. Macromolecular sequencing is also manifest in gene products, RNA and proteins. It is important that the sequence imply ordering from a template which selects from an abundance of kinetically equivalent choices, not merely a pattern inherent in the chemistry of the monomer, as in crystallization.

Molecular esobiology faces the same methodological problems. This challenge gives us the groundwork for

exobiology and assures that any instrumental advances will have redoubled utility. But it is a chastening note that biochemistry has barely reached the point of affirmation that antibody  $\gamma$ -globulin has an informational sequence or is specified by a polynucleotide. That this abundant and medically important molecule can still be so controversial must evoke some humility in our postulations and experimental efforts concerning macromolecules on another planet.

### How We Detect Informational Macromolecules

(A) *Compositional analysis.* (a) Does the sample contain macromolecules? (b) What is their composition? (c) Any evidence of informational ordering?

Esobiology is firmly founded on the isolation of macromolecular species and their purification before attempts at analysis. Some of the most successful methods are empirical recipes of extraction and precipitation.

More rational techniques include diffusional properties of large molecules, free diffusion, sedimentation, dialysis, molecular sieves, and electrophoresis, in principle also vapour phase diffusion (to remove monomers)—molecular distillation, gas chromatography and mass spectrometry. Solution chromatographic methods may also rely on the coincidence of functional groups on one molecule, for example, a polyelectrolyte.

Similar principles underlie non-separative methods of detection which have not been extensively developed to date. Rotational relaxation times can be measured by flow or electric birefringence, or the analogous polarization of fluorescence. Polyfunctionality is tested by intermolecular interactions of adsorbed dyes (for example, optical shifts in acridine orange on DNA) or the monomeric units with one another in special cases (hypochromicity of DNA, diagnosable on heat-denaturation). More direct chemical tests for polyfunctionality also suggest themselves.

The previous methods, in so far as they lack perfect generality, may give only a clue as to the composition of the macromolecule, as well as its molecular size. At the other extreme, we would seek the complete primary structure to emulate the recent *tours de force* of chemical technique. Reasonable inferences might be drawn from less complete evidence of structural individuality, hard to evaluate in advance: homogeneity in molecular weight or end-group analysis, crystallinity, or sharp fractionation by any other procedure. A sharp X-ray diagram of a heteropolymer sample could imply its individuality long before it had yielded to full analysis.

Other partial measures of great utility include the isolation of the polymer by specific reagents, especially enzymes, to give a pattern of characteristic fragments (the polypeptide 'fingerprint').

The underlying generalization is 'molecular speciation'. Chemogenic synthesis of macromolecules should generate a continuum of nearly equiprobable forms. Biogeny chooses a few of these and generates a sharply discontinuous polydiscrete spectrum, that is, it speciates. Speciation can be discerned by many measures, for example, the distribution of molecular weight. Thus a sample under analysis by a sophisticated instrument might reveal a sac of haem-polypeptide, containing about a billion (a thousand million) atoms of iron. Virtually all the iron-polypeptide consists of a single species, that is, almost all the molecules have 2,936 carbon atoms, no more, no less. After removal of iron and porphyrin, equal numbers of sub-units containing just  $C_{211}$  and  $C_{711}$  are assayed. It would be difficult to escape an allusion to life after a single encounter with the red blood cell that has just been described.

(B) *Functional analysis.* The adaptive values, the uses that biology has discovered for some species of macromolecules, reveal short cuts to their singularity. These functions are all reducible to a structural specification:

Function	Complex with	Example
Auto-replication	Inert polymer (same species) and polymer-building monomer	DNA: DNA + deoxynucleoside triphosphates
Hetero-replication	Inert polymer (different species) and polymer-building monomer	DNA: RNA + nucleoside triphosphates
Morphogenesis: fibres, membranes, vesicles	Formed polymer, similar species	Collagen: collagen sub-units
Enzyme	Substrate—catalytic effect Cofactors—to form holo-enzyme Analogues—complexes Inactive, <i>pro</i> enzyme	
Neutralizing	Whatever	Antibody: antigen, that is, any chemical species foreign to the reacting organisms
Transport	Hormones, toxins, nutrients	Serum albumin: hormones, toxins Permeases: nutrients and metabolites for transport in and out of cells

the stereospecificity of the polymer in reacting with other molecules.

In this list, the enzymatic functions are particularly promising in the light of their specificity and amplifying capability. Many enzymes have turnover numbers of  $10^4$  substrate molecules/sec/enzyme molecule. If suitable precursors (nutrients) can be defined, integrated enzyme-sequences or metabolic systems, like respiration or photosynthesis, extend the versatility of this approach.

The simpler the level, the more likely are we to find a metabolic analogue on Mars, for example, for the assimilation of elementary nutrients, C, N, O, S or P, into organic molecules. The next more complex molecules,  $H_2O$ ,  $CO_2$ , and  $O_2$  and  $NH_3$ , are the most pervasive metabolites of terrestrial life, and the choice among them for searching for evidence of their conversion into other compounds will depend mainly on instrumental considerations. In general, the more complex the metabolite being tested, the less our prior expectation that it was part of an extraterrestrial biogenic system. However, the complete system offers the largest amplification—a single bacterium could grow and multiply into tonnage masses in a few days, but might make the most exacting demands of the environment.

### Morphology

Biogeny rapidly elaborates higher forms of organization: cells, tissues, organisms, populations, which might be recognizable according to their own forms and to their rectifications of the environment. However, what systematic rules distinguish biological forms in general? Some forms are recognizable, for example, a friend's face, and recognition then contains many bits of useful information. Compound vesicles, apparent cells, are most inescapable in morphogenesis; their absence would at least set an upper limit to the stage of biogeny. Their presence would be extremely provocative, but properly would raise many scepticisms of chemogenic artefact. Nevertheless, esobiology has so many roots in morphology that we could scarcely ignore the insights that our historic practice of it would offer. Any recognizable forms would provoke tangible and hence useful working hypotheses of the Martian system.

Some aspects of morphology can be systematized. As an example which might illustrate speciation, ultrastructural spacings in the range of 20–500 Å could be detected by powerful optical (electron microscope, X-ray diffraction) as well as separative techniques. Approaches so cogent to esobial ultrastructure must play an important part in exobiology. Unfortunately, we have little empirical basis to prejudge the morphological detail that might be exhibited by an infra-biogenic planet, since so much of the chemical diversity of Earth has been pre-empted by life.

As is well known, five-fold symmetries are anathematic in crystallography. Hence, regular pentagonal and

dodecahedral forms might occur as elementary units, for example, perhaps a ferrocene, but no simple law of crystal growth could account for their occurrence in diverse sizes. A glen of periwinkles has a deductively simple signature of life.

### Signals

So far I have tacitly assumed that whether or not Mars has achieved biogeny, it has not passed to cognogeny. Reaction to the once notorious Schiaparellian *canals* may account for a position which has no rigorous basis. True, we have had no scientifically admissible sign of intelligent activity on or communication from that planet. However, we can only fancy whether an exotic culture would have either the means or the motive to effect recognizable communication. We can generalize that the works of cognogeny would constitute the most startling unlikelyhoods, exceptions to biogeny and chemogeny alike.

It is no trivial exercise to speculate how we could most compactly summarize our scientific culture. For example, a description of DNA and our amino-acids could portray the convergence of physical and chemical ideas in biology, and some of the least predictable aspects of esobiogeny. If we could but do it, a detail of the inter-neuronal synapse and the cytoarchitecture of the cerebral cortex would go even farther. How much of our cognogeny would then be deducible from these facts and our awareness of them?

Purposeful emissions cost enough more than mere listening that we do not undertake them ourselves, but we have made casual efforts to hear them. Further, we might hope to eavesdrop on the internal communications of another planet, perhaps more likely far beyond the solar system. Among other difficulties, efficient information is, by definition, indistinguishable from noise to the unbrieffed eavesdropper.

While a rigorous answer to any notions of Martian intelligence is difficult, a realistic policy is not. Cognogeny would reveal itself in divers ways, and, at least for Mars, we have no better recourse than to keep eyes, ears and noses alert for any signs of it as we make progressively closer approaches to the planet.

### Instrumentation

The rational classification of existing instruments, or those proposed for analytical purposes, is a task as difficult as it is urgent. The real aim, a classification of possible instruments, requires a total knowledge of physics, and some system for classifying this information that will help us to understand the relationships among existing instruments and suggest new ones. A proposed scan parameter is the energy-level of the transition by which the molecule is recognized. Further parameters include whether photons are introduced or emitted, whether chemical reagents are employed, including auto-reactions, whether the displacement or state of the analyte and of the probe is diagnostic, and, for radiation probes, the role of power, polarization, phase, wave-length, or flux vector of the probe. The first step in a detailed rationalization is to determine whether any more dimensions are needed for our matrix of possible configurations.

Radiation probes are usually limited, either in selectivity—say, absorptiometry, or sensitivity—say, nuclear magnetic resonance—but they have special value in conjunction with chemical reagents. Absorption (power loss) measurements have dominated instrumental analysis. Conventional optical methods rarely stabilize or measure input power better than 1:1,000, with corresponding limitations to detectivity. For example, optical molar absorptivity rarely exceeds  $10^4$  so that  $10^{-6}$  molar solutions ( $6 \times 10^{13}$  molecules in a  $1\text{-cm}^2$  cell) would give the lowest useful signal under the most favourable conditions. By contrast, fluorometric measurement (which can

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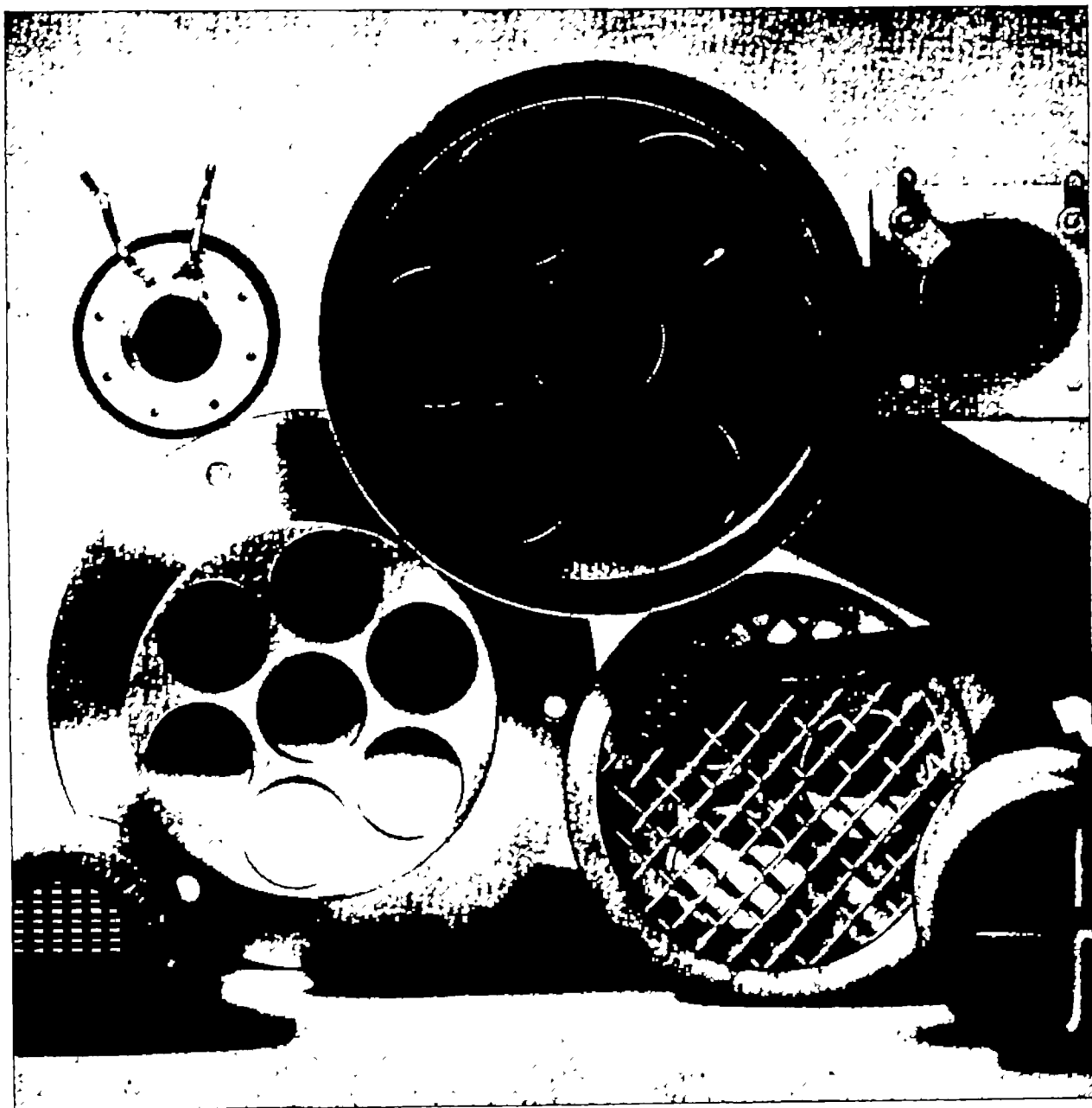
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exploit shifts in wave-length, flux vector, polarization and phase) can easily measure  $10^4$  molecules and can be extended at least to  $10^6$ . The delicacy of excitation methods (which could also include chemical, nucleonic and thermal excitation) stems from the measurement of the data signal merely against a detector noise background as compared with the much larger power fluctuations of practical probes.

Optical activity is also usually measured via loss of power (attenuation of polarized light by a crossed analyser): the molar rotations are relatively small, present detectivity being about  $10^{11}$  molecules. If some method of transforming optical rotation to an excited signal were developed, it would enormously enhance the power of this technique.

The most sensitive approaches to analysis are two-stage mechanisms: the selective displacement of the analyte, then a sensitive detection. In principle, such methods might detect a single molecule, as in mass spectrometry: selective  $m/e$  displacement followed by the sensitive detection of an ion that can be accelerated to arbitrary energy. The potential information content of a mass spectrum is especially high since the theoretically measurable mass of a single molecule is defined to a resolution far better than  $10^{-6}$ , independent of the variety of energetic states, which broaden other physical features. Existing instruments still lag behind theoretical limits of mass resolution; yet have already demonstrated their power in organic analysis. Further, the mass datum at high resolution for an intact molecular ion is deductively reducible to a molecular composition, unlike the inferential data given by most other spectroscopic techniques, and the statistics of the fragments also give detailed insight into the complete structure of the molecule. From these considerations the combination of a mass spectrometer with a simple, rugged, separative device, like the gas chromatograph, promises to be the most powerful component of analytical systems for biochemistry. However, a science of metrology, the orderly study of methods of measurement, remains to be developed. I can have little confidence that the last word has been said on this issue.

### Some Private Thoughts on Exobiological Strategy

The multitude of possible means and detailed ends in exobiology leaves little hope that a brilliant flash will

illuminate the whole picture as a happy substitute for the diverse paths of exobiology. Nor should there be any discouragement of the variety of talents and insights that would be needed in any event for the full development of the subject. The overriding problem in planning is, of course, how little we actually know about surface detail and atmospheric composition of Mars. We are also bedevilled by the uncertain hazards, but immense stakes, to either planet of an intemperate rupture of the inter-planetary barrier. Earth-based telescopes can, to be sure, add significantly to our present appreciation of Mars, and hence of the hazards of landing. But the next significant step would be a Mars-orbiting observatory, keeping the planet under a constant synoptic scrutiny from a safe distance, close enough to measure significant surface detail, and large enough to maintain the most sophisticated instrumentation, telemetry to Earth, and perhaps even some Earth-based regulation of its surveillance schedule and precautions against accidental intact landing.

Such an approach to Mars would also open the way to political agreements to unify terrestrial strategy and can allow constructive co-operations like the International Geophysical Year of recent history, for example, to facilitate the relaying of synoptic data. While it is essential to mount vigorous instrument development efforts to assure that a landing can ever be implemented, the detailed specifications of experiments should take full advantage of the most up-to-date planetological information. That is to say, the final decision to implement a landing on Mars should be suspended until we can have digested the data from a Mars orbiter. This criterion lends further weight to the strategy of designing a general-purpose laboratory for planetary investigation, in which many investigators can participate, and which has the flexibility to be readily reprogrammed in the light of new data. At present, for a biologist to participate actively in space research requires a commitment to engineering efforts which few are willing to undertake.

The deliberate staging of the exploration of Mars, perhaps with international agreement to proceed first with reconnaissance while preparations are made for comprehensive landed missions, would allow for the widest participation of interested scientists, both in the design of experiments in exobiology and in the prudent determination of global policy for the solar system.

## DEFINITION AND USE OF ZEBU, BRAHMAN OR *Bos indicus*\* CATTLE

By PROF. JOHN FRANCIS

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THERE has been a steadily increasing interest in Brahman cattle and their hybrids in Queensland during the past ten years. This has been due to their resistance to ticks and general ability to thrive better than British breeds under the various stresses of a tropical environment<sup>1-3</sup>. These facts, and anatomical investigations of British and Brahman cattle<sup>4</sup>, stimulated our interest in zebu cattle in various parts of the world.

In trying to determine the boundary between zebu and other cattle it was surprising to find that no real definition or concise description of the characteristics of 'a zebu', or of the apparently precise binomial *Bos indicus* (literally Indian ox), could be found. Linnaeus<sup>5</sup> described it as "a bovine whose horns are shorter than its ears, that has a

humped back and no mane"—the latter probably distinguishes it from *bibos*. Grasse<sup>6</sup> is scarcely more precise. Zeuner<sup>7</sup> has given much the most satisfying account of the origin of cattle, although his description of the zebu is not in complete accord with the definition proposed here. He says: "That the primigenius breeds came from temperate or western Asia is evident from the distribution of their wild ancestor in these parts". He discusses interesting breeding experiments in Europe during which these early animals were "reconstituted", and reports that they not only had the physical characteristics of the aurochs but also their agility and wildness. He describes the zebu as "characterized externally by a prominent hump on the shoulder, a long face, usually steeply upright horns, drooping ears, small brow-ridges which give it a peculiar expression, a dewlap and slender legs. Its colour varies, but is commonly of the grey type varying to white or black. Many crosses have occurred between

\* It is considered that these three terms are practically synonymous, but the name Brahman tends to be reserved for a particular type of zebu developed in the U.S.A. In some of the old Australian and other reports the name Brahman is used which doubtless originated from the sacred Brahmin bulls of India<sup>11</sup>.

zebu and *primigenius* breeds, especially in Africa, and these should be left out of consideration . . . the zebu with its long face, steep horns and hump is so distinct an animal that its origin has been discussed frequently".

Zeuner<sup>7</sup> discounts the possibility that the zebu could have derived from a cross between *primigenius* and *bibos* species and relates that the first archaeological record of the zebu is about 4500 B.C. Both *primigenius* cattle and zebu appear on Indian seals of 2500 B.C. and, allowing for some artistic licence, the zebras on these seals have the main characteristics of the modern Brahman. He<sup>7</sup> accepts the very early separate origin of the zebu, probably from the wild *Bos namadicus* of India or some region to the west of India, and says that this would make it possible to understand why there is a specifically tropical breed of cattle present from the earliest times onwards, and now distributed from China to Africa, with India as the focus. The sculptures and hieroglyphics of Egypt show three different types of *primigenius* cattle, and from about 1570 B.C. onwards zebras are also frequently shown. The earliest record in Palestine is in the ninth century B.C., and zebras then became well known all over the eastern shores of the Mediterranean. "They never succeeded, however, in ousting *primigenius* and longifrons breeds in the rest of the Mediterranean littoral, presumably because humped cattle were climatically unsuited to non-tropical countries".

Zeuner and Mourant<sup>8</sup> propose a nomenclature for the Bovinae. The first species listed is *Bos (Bos) primigenius* Bojanus (wild European aurochs, extinct since 1627). *Bos taurus* is described as *Bos (Bos) primigenius* f.d.\* *taurus* Linnaeus (domestic humpless cattle of Europe, type locality Sweden), and *Bos indicus* as *Bos (Bos) primigenius* f.d. *indicus* Linnaeus (domestic zebu, type locality China). The foregoing name for *Bos indicus* can scarcely be regarded as suitable because it is generally accepted that this species or sub-species arose from the wild cattle of India, *Bos namadicus*, and the zebu is clearly not the "forma domestica" of the European *Bos primigenius*. Why China is given as the type locality is also difficult to understand, as all the evidence indicates that India is the origin and focus of zebu cattle. Zeuner<sup>7</sup> says that cattle reached China from the west and south-west, and he clearly uses the term "primigenius breeds" to exclude *Bos indicus*.

The name, *Bos (Bos) namadicus* f.d. *indicus* (domestic zebu, type locality India), is suggested as the most suitable name as it is felt that it accords well with Zeuner's statement that "there is a specifically tropical breed of cattle present from the earliest time onwards, and now distributed from China to Africa, with India as the focus".

The descriptions by various authors<sup>9-13</sup>, and my own observations in Australia and Africa, lead to the following tentative definition of a 'typical zebu'†, or *Bos indicus*: A bovine with a well-developed musculo-fatty thoracic hump and dependent dewlap. In the male the prepuce is pendulous and in the female the umbilical fold well developed. It has a coat consisting largely of straight medullated hair fibres and the sweat glands are larger and more numerous than in *Bos taurus*. The thoracic spines are bifurcated and the caudal vertebrae do not extend into the distal portion of the tail covered by the switch. (The fact that the thoracic spines are a variable characteristic for intermediate type cattle does not reduce the value of the differentiation from *Bos taurus*.)

The shape and size of the horns are considered to be unimportant as they may vary enormously, and typical zebras may be polled. Some authors have placed considerable emphasis on the long narrow skull as being characteristic of the zebu, but again this can scarcely be accepted, as the gir, which must be regarded as a zebu, has a broad, domed forehead<sup>11</sup>. This breed and the Kankrej, so well illustrated by Olver<sup>14</sup>, are probably

\* *Forma domestica*.

† Henceforth zebu, Brahman or *Bos indicus* refers to a 'typical zebu'.

responsible for the head characteristics of the American Brahman<sup>11</sup>. The ears, although generally large and dependent, also vary much in shape and size.

It should be added that the term 'thoracic hump' is ill-defined. As used by Kelley<sup>11</sup> and Mason and Maule<sup>12</sup> the definition appears to depend on external appearance and applies to a hump which is more or less squarely above the forelegs. On the other hand, Epstein<sup>10</sup> defines a thoracic hump as one developed from the thoracic, as distinct from the cervical, portion of the m. rhomboideus. On the former definition the American Brahman cattle have a thoracic hump as well as many other zebu characteristics. However, limited dissection by Butterfield (personal communication) shows that their humps are developed from the cervical portion of the m. rhomboideus. Nevertheless, they extend caudally as far as the fifth thoracic dorsal spine, a limit not exceeded in photographs of bisected Boran cattle kindly supplied by H. P. Ledger of Kikuyu, Kenya. The fatty surface of the bisected hump has led many people to conclude that the hump consists almost entirely of fat; but dissection in Queensland, and inspection of photographs from transverse sections of humps supplied by H. P. Ledger, show that there is a considerable amount of muscular tissue, particularly in the anterior portion. In this article it is the external appearance of the hump which is used as a guide in distinguishing thoracic from cervical humps, although further work may permit a more satisfactory definition. Some authors have suggested that there are

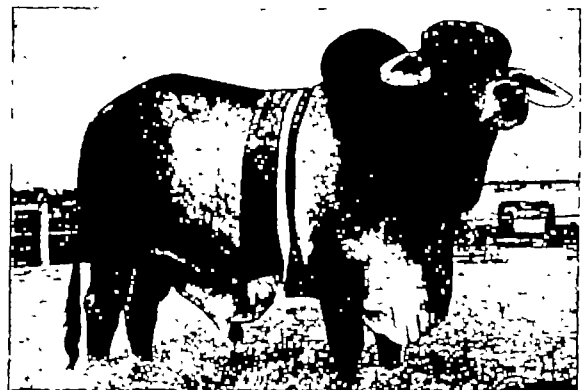


Fig. 1. Young Brahman bull of American descent from Cherokee stud, Queensland. This bull shows the broad forehead and good beef qualities typical of the breed.



Fig. 2. Champion Boran bull, Nairobi, Kenya. This bull is from the fine herd developed by Mr. W. D. Hinde, of Nanyuki. In addition, with the aid of artificial insemination, he has developed an outstanding herd of hybrid cattle which are about five-eighths Boran and three-eighths Red Poll. The cows are milked in corrals as well as raising a calf.



zebu cattle with no hump, but they do not specify the breeds or other characteristics of these animals, and the expression 'humpless zebu' would seem to be a contradiction of terms.

The American Brahman (Fig. 1), the Boran (Fig. 2) and the Kankrej<sup>14</sup> may be cited as examples of cattle with typical thoracic humps, and Africander and numerous hybrid cattle as animals with cervico-thoracic or cervical humps. The hump is, of course, best seen in the uncastrated male, and Jersey or Angus bulls may have as much crest or cervical hump as some African or Indian cattle.

The zebu has a characteristic voice and temperament well described by Kelley<sup>11</sup>. The temperament *vis-à-vis* the British breeds of cattle may be loosely compared with the difference of temperament between the alert or fiery thoroughbred and the heavy 'cart horse'. When properly handled by their attendants, zebu cattle may be quieter and more co-operative than British cattle; but this does not alter their characteristic lively temperament under different circumstances and, in my experience, temperament is broadly similar in all zebu cattle including the Sahiwal and American Brahman, and I regard these cattle, and probably all those included in group 2 of Mason and Maule<sup>13</sup>, as coming within the definition of zebu.

As might be expected, Youatt<sup>15</sup>, with his great powers of observation and descriptive ability, excellently portrayed the general characteristics of the zebu, and Fig. 3, reproduced from his book, shows a zebu which was the 'Nagore' bull exhibited at the Christmas Cattle Show in London in 1832, together with a cow of the same breed. (The prize carcass at Smithfield in 1848 was from a zebu × Ayrahire.) "They were beautiful animals and attracted much attention. . . . They are not buffaloes, but of the highest breed of Indian cattle. They are used in India by the higher orders, to draw their state carriages, and are much valued for their size, speed and endurance and sell at very high prices. . . . They will travel, with a soldier on their backs, 15 or 16 hours in the day, at the rate of six miles an hour. Their action is particularly fine—nothing like the English cattle, with the side-way, circular motion of their hind legs—the Nagore cattle bring their hind legs under them in as straight a line as the horse. They are very active, and can clear a five-barred gate with the greatest ease. Mr. Perkins has a calf which has leaped over an iron fence in order to get at the water, and when he has drunk his fill, leaps back again. . . . The bull is employed in a light cart, in various jobs about the farm: sometimes he goes fore-horse in the waggon-team, to deliver corn; he also drags the bush-harrow, and draws the light roller over the ploughed land. He is very docile and tractable, when one man drives him, and attends upon him, but he has now and then shown symptoms of

dialike to others. . . . He is very fond of being noticed; and often, when he is lying down, if any one to whom he is accustomed goes and sits down on him and strokes him over the face, he will turn round and put his head on their lap, and lie there contentedly as long as they please." The latter part of this quotation perfectly portrays the temperament of the Brahman.

The descriptions of African and Asian cattle show that there are innumerable gradations and variations between the zebu and non-humped cattle. Some of these have been designated as types or breeds, and there is much discussion concerning their origin. This is doubtless justified; but the varied and extensive crossing with the British breeds, following the introduction of 24 Brahman bulls and 25 females into Queensland between 1933 and 1954 has produced almost all the types of conformation (excluding horns and the very dwarfed breeds) illustrated in the articles and books mentioned here.

It is therefore possible that all the various intermediate types have derived from crosses between the zebu, which originated in India or some adjoining part of Asia, and non-humped cattle; and Curson and Thornton<sup>8</sup> (p. 621) do state that the Sanga cattle, so widely distributed in Africa, originated by the intermixture, probably in Egypt, of the Hamitic longhorn and lateral-horned zebu types.

Mason and Maule<sup>13</sup> divided the cattle of Africa into: Group 1, Sanga; Group 2, zebu; and Group 3, intermediate; but both groups 1 and 3 could be regarded as intermediate between *Bos indicus* and *Bos taurus*. Distribution of blood groups or haemoglobin types<sup>16</sup> may throw further light on the origin and classification of various types of cattle, although the presence of certain blood groups is not necessarily more significant than anatomical or other general characters.

Kelley<sup>11</sup> has dealt with the 'average characteristics' of the various hybrids, and some cattlemen in Queensland consider they can assess quite accurately the amount of 'Brahman blood' in an individual by its conformation and other characteristics, and that this is broadly related to hardihood and disease resistance. The Africander cattle in Queensland are considered to have anatomical and other characters similar to those of an animal with three-quarters Brahman 'blood' and one-quarter from a British beef breed. Differences of one-quarter Brahman 'blood' can be easily recognized on inspection, and experienced cattlemen distinguish one-eighth amounts of Brahman 'blood' in hybrid cattle, but this can scarcely be very accurate.

Discussing the results of dissecting various British breeds of cattle and Brahman crosses, we<sup>17</sup> concluded that, contrary to general belief, there was no difference between the various breeds, in the proportional distribution of the different 'muscle groups' based either on weight or on economic value. In the recent past there has been a trend to produce quickly-maturing cattle and to consider that the larger, later-maturing cattle lacked 'quality'. But it is evident from the foregoing results that the latter types of cattle, whatever their breed, can be used with confidence that there will be no deterioration of muscle-weight distribution.

The beef industry of tropical areas must be based on cattle well adapted to the local environment, and from the point of view of muscle-weight distribution there can be no reason for rejecting adapted cattle, no matter what their conformation, from areas where they are established. There has been much difference of opinion concerning breeding policies in tropical countries, but zebu cattle are considerably more resistant to most diseases than are cattle of European breeds<sup>18</sup> and, after a careful survey, Faulkner and Brown<sup>19</sup> expressed considerable doubt concerning the use of European breeds for improvement in the tropics.

Leaving aside dairy cattle, which can be kept under special conditions more suitable to the high-yielding



Fig. 3. A Brahman bull in England about 1830. "He is very docile and tractable when one man drives him." (From ref. 15)

European breeds, the only modification to the foregoing opinions would be that a judicious infusion of European 'blood' may introduce desirable characteristics to some local breeds. This has become much easier with the development of artificial insemination, and it is now realized<sup>1,2</sup> that the stabilization of suitable hybrid cattle is far easier than had been supposed. Thus, over much of Queensland, which stretches from latitude 29° to 10° S., the best adapted hybrids will probably vary from three-eighths to five-eighths Brahman 'blood' and it is very unlikely that there will ever be large commercial herds of pure Brahmans. In the same way, suitable experiments and observations could lead to decisions concerning the correct admixture in other tropical areas, and this could move more towards the European breeds with improvements in disease control and agricultural practice.

I thank Dr. R. M. Butterfield for his advice, and the Australian Cattle and Beef Research Committee for financial support.

<sup>1</sup> Francis, J., in *Cattle Country*, 201 (F. H. Johnston Publishing Co., Sydney, 1960).

<sup>2</sup> Francis, J., *Aust. Vet. J.*, 40, 114 (1964).

<sup>3</sup> Francis, J., and Little, D. A., *Aust. Vet. J.*, 40, 247 (1964).

<sup>4</sup> Butterfield, R. M., and May, N. D. S., *Muscles of the Ox* (Univ. Queensland Press, 1965).

<sup>5</sup> Linnaeus, C., *Systema Naturae*, 1, tenth ed., 72 (1758).

<sup>6</sup> Grassie, P. P., *Traité de Zoologie*, 17, 625 (1959).

<sup>7</sup> Zeuner, F. H., *A History of Domesticated Animals* (Hutchinson and Co., Ltd., London, 1963).

<sup>8</sup> Zeuner, F. H., and Mourant, A. H., *Roy. Anthropol. Inst., Occas. Paper* No. 18, 168 (1963).

<sup>9</sup> Ouseley, H. H., and Thornton, R. W., *Onderstepoort J. Vet. Sci.*, 7, 613 (1938).

<sup>10</sup> Epstein, H., *East Afr. Agric. J.*, 31, 83 (1956).

<sup>11</sup> Kelley, R. B., *Natives and Adapted Cattle* (Angus and Robertson, Sydney, 1969).

<sup>12</sup> Mason, I. L., and Manle, J. P., *The Indigenous Livestock of Eastern and South Africa* (Commonwealth Agricultural Bureau, Farnham Royal, Bucks., 1960).

<sup>13</sup> Inago, E., *Science*, 137, 195 (1962).

<sup>14</sup> Oliver, A., *Misc. Bull. Imp. Council Agric. Res.* No. 17 (New Delhi Government India Press, 1938).

<sup>15</sup> Youatt, W., *Cattle: Their Breeds, Management and Diseases* (note: the material in this paper was taken from a reprint published by Sumpkin, Marshall and Co., London (1870), 1834).

<sup>16</sup> Hall, J. G., and Bangham, A. D., in *Roy. Anthropol. Inst., Occas. Paper* No. 18 (1963).

<sup>17</sup> Butterfield, R. M., and Francis, J., *Proc. Seventeenth World Intern. Vet. Congress, Hannover*, 1, 321 (1963).

<sup>18</sup> Francis, J., *Brit. Vet. J.* (in the press).

<sup>19</sup> Faulkner, D. H., and Brown, J. D., *The Improvement of Cattle in British Colonial Territories in Africa* (Col. Adv. Council Agric. Animal Health and Forestry, No. 8, H.M.S.O., London, 1953).

## SEEING IN DEPTH

By R. L. GREGORY

The Psychology Laboratory, University of Cambridge

EYES are biological early warning systems. By giving information of events distant in space they serve to probe the immediate future, allowing brains to transcend simple reflexes and control strategic behaviour. Without information of distant objects there can be no anticipation of danger, no organized attack, no knowledge of the world. Indeed, the development of brains must have depended on seeing in depth.

The brain has a most difficult task interpreting retinal information from distant objects. The retinal image has lost a dimension: somehow the brain must construct depth from the projection of three dimensions reduced to two. For near objects the different views of the two eyes are used to compute depth, but the base line between the eyes is too small for distances beyond 50 ft. or so, when we are effectively one-eyed. With a single eye we generally see the world more or less accurately in three dimensions. For distant objects we use many 'clues' to depth, with a subtlety in the best traditions of the sleuth. With increasing distance, outlines look more blurred and fine detail is lost, objects look blue from increasing atmospheric haze, and more distant objects are in part hidden by those nearer the observer. These are some of the available clues to depth.

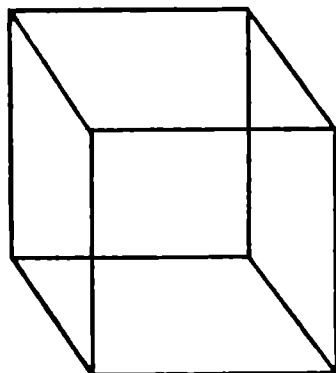


Fig. 1. The Necker cube. This spontaneously reverses in depth. There is no information available for deciding which is the nearer and which the further face. The perceptual system entertains first one hypothesis, then the other. The brain never makes up its mind!

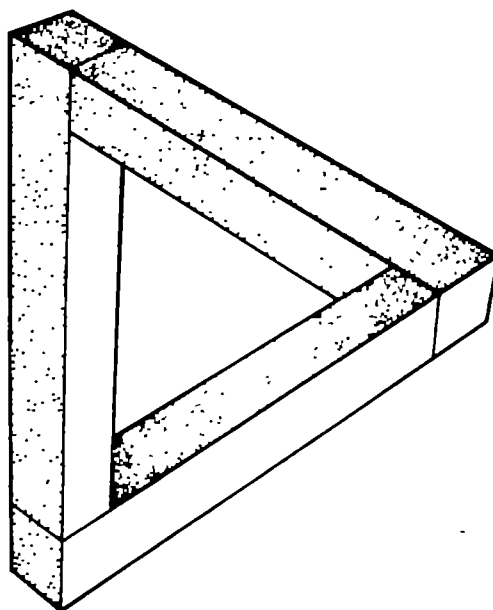


Fig. 2. An impossible object. This cannot represent any possible physical object, for it has conflicting depth 'clues' (after L. S. and R. Penrose, *Brit. J. Psychol.*, 49, 31 (1958)).

One can see why perception of depth is so difficult by thinking about pictures. Although a picture is itself two-dimensional, it represents objects lying in three dimensions. But this is strictly impossible, and so pictures are essentially ambiguous in depth. Consider a drawing of a simple ellipse: Is the object represented distant and large, or small and near? Is it an elliptical object, or a circle tilted at an angle? The two-dimensional drawing could represent any of an infinite set of objects. Add shading, perspective—or an indication that it is a wheel—then we see one specific object. Visual ambiguity in depth is seen dramatically in figures which could equally well lie in more than one orientation. For example, Fig. 1: a flat drawing of a skeleton cube. A given face is seen first as the front, then the back. The alternative 'hypotheses'

are entertained perceptually in turn, and we never do see the figure as a unique unchanging object.

Pictures are not only ambiguous; they are also paradoxical. A picture is seen to be lying flat on its paper or canvas, and yet is also seen in three dimensions as indicated by its perspective and other depth cues. It is paradoxical in being seen as both flat and in depth at the same time. Conflicting cues to depth can produce 'impossible objects'. Fig. 2 cannot be an object lying in space. Fig. 3 cannot even be seen. In both cases, the trouble is over the third dimension.

Why should the eye accept a picture as representing objects lying in a space different from its own? It does so because a picture is essentially like a retinal image—both are flat projections of three-dimensional space. Pictures give simplified images, and very likely distorted in various ways, but the brain is so familiar with the problem of adding the third dimension from information given by the flat retinal image that we might expect it to cope with pictures. But there is an important difference between pictures and retinal images. Both are ambiguous, but retinal images do not lie perceptually in both two and three dimensions. We do not 'see' the flatness of the retina, or its texture: they are not signalled to the brain. Thus the brain has a more difficult task dealing with a picture than with normal objects. The textured background imposes a highly artificial problem to the visual system which it cannot completely solve. It is indeed unfortunate that experiments in perception have largely used figures drawn on paper. It is only when the double reality of the picture and what it represents is being explicitly investigated that pictures should be used in visual experiments.

It is, however, possible to produce pictures which, like retinal images, have no information of their flatness. This we may do by avoiding all background texture, and viewing with a single eye. When Fig. 1 is shown in this way—luminous, glowing in the dark—it appears as a truly three-dimensional cube. It still reverses in depth—it is still ambiguous—but it is no longer paradoxical in depth. The luminous figure looks different in another way—the apparently further face always looks larger than the apparently nearer face, whichever this may be. We see this distortion most dramatically in a truly three-dimensional skeleton cube, made of wire and coated with luminous paint to make it glow in the dark. The true cube also reverses in depth, and when it reverses it changes shape—the apparent front appearing too small. It becomes a truncated pyramid. Also, it rotates in the most odd way when the observer moves round it, but that is another story<sup>1,2</sup>.

Why should the luminous cube change shape when reversed in visual depth? This is answered by asking a silly-sounding question—why does a cube normally look like a cube? This needs some explanation, for since the

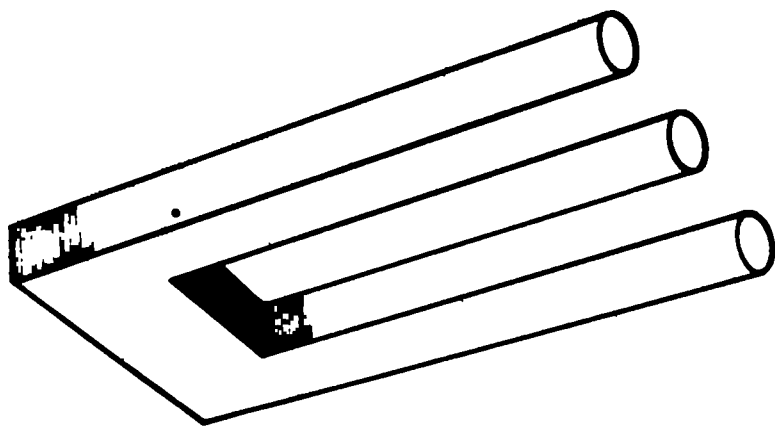


Fig. 3. This figure cannot even be seen. Again the difficulty is over the third dimension.

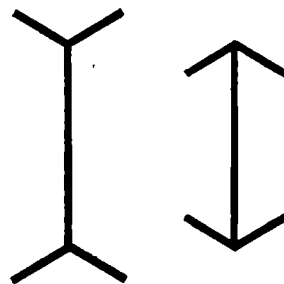


Fig. 4. The Müller-Lyer arrow illusion. Outgoing fins expand the shaft joining them, the ingoing shrink it. The 'fins' can be regarded as perspective lines of corners. When shown with no textured background (luminous) they look like true corners. The perspective depth appears to produce the illusion by triggering primary constancy scaling.

back face is further away, it must give a smaller image to the retina. But it does not look smaller—it looks the same size as the front. Although all objects give smaller retinal images as they recede from the eye, this geometrical shrinking is generally compensated by the brain, to give 'size constancy'. Size constancy was known to Descartes<sup>3</sup> in the seventeenth century, and has been investigated intensively since, notably by R. H. Thouless<sup>4</sup> in the 'thirties. It serves to give immediate recognition that a distant bottle is pint or half-pint, or whether it is a cat or a tiger about to spring. There are various theories about constancy<sup>5,6</sup>, but I believe it to be produced by an active scaling process in the brain, either set according to the apparent distance of viewed objects or set directly by various depth cues. We may call the underlying processes 'constancy scaling'.

When we see the skeleton wire cube distorted, when reversed in depth, we see our constancy scaling at work. But it is working backwards. For it is working according to the apparent and not the true depth of the object. Although constancy scaling normally corrects for the shrinking of retinal images with distance, reversal of depth makes the normally useful compensation distort visual space. The cube looks more distorted than it would if there were no constancy scaling correcting for the shrinking of the image with distance. These distortions occur whenever depth reverses in non-paradoxical figures. It could happen in real conditions, such as landing aircraft, or in space flight, and the consequences might be serious<sup>7</sup>.

### The 'Geometrical Illusions'

We know, then, that visual space is distorted when depth is seen wrongly. Can this somehow explain the distortions of the well-known 'illusion figures'? Fig. 4 shows the most familiar example: the outgoing arrow heads expand the line (or the space) between them, while the ingoing heads shrink it. Now these 'arrow heads' can be thought of as perspective drawings of corners lying in depth. They are the same shape as the retinal images of real corners<sup>8</sup>. With the outgoing arrow heads the vertical line would be distant, the heads representing, for example, the lines of the ceiling and walls of the inside corner of a room. The ingoing heads are perspective drawings of an outside corner, say, of a building or box, where the joining line would be near.

If the perspective features of retinal images do indeed serve to set constancy scaling, then when these features are present in flat pictures we must expect them to produce distortions of visual space. Constancy scaling corrects for shrinking of the retinal image with increasing distance, but pictures present perspective depth features with no

change in distance to compensate, since they are physically flat, and so the scaling must be inappropriate. We must expect objects indicated as further away to be systematically expanded<sup>9</sup>. This is just what happens, apparently for all the illusion figures, with people familiar with corners and parallel lines.

It has been known for sixty years that people who live in environments largely free of right angular corners and parallel lines—such as the Zulus, who live in a 'circular culture' of round huts—do not suffer these distortion-illusions<sup>10</sup>. Miss Jean Wallace and I found that a man of middle-age, who recovered his sight by corneal graft

after being blind since early infancy, was not subject to the illusions<sup>11</sup>. His perception of depth was also most odd. It seems that early experience of perspective features is important: apparently we learn to use perspective for setting constancy scaling.

Can we demonstrate experimentally a close connexion between depth and distortion illusions? There are several hints in the literature of such an origin of the illusions<sup>9,12</sup>, but there is a difficulty. It is always assumed that size constancy works simply according to apparent distance<sup>3</sup> (which is indeed true for the luminous cubes), but if this were always the case it could not produce distortions in figures seen as flat. But the illusion figures are generally seen as lying flat on their paper backgrounds, so how can we invoke constancy to account for these distortions?

### Measuring Depth In Pictures

The apparatus is shown in Fig. 5. The illusion figure is presented as a back-illuminated transparency. Light from it is polarized, and cross-polarized at one eye. Both eyes, however, view a small dim movable light which is optically introduced into the figure with a part-reflecting mirror. Now this light may be adjusted in distance, until it matches the apparent distance of any selected part of the figure seen with a single eye. The figure's depth is given by its perspective features, but the light's distance is given by convergence of the two eyes. Positions of the light are recorded on the graph paper at the top of the apparatus, and so we plot visual space in three dimensions, using the two eyes as a range-finder to measure the effect of perspective on a single eye. Fig. 6 shows how the arrow illusion is related to its apparent depth, as measured with this technique. The similarity of the distortion and depth functions, for various angles of the fins, demonstrates the close relationship we should expect—if indeed perspective features can set constancy scaling—to produce illusions when the perspective is inappropriate to true distance.

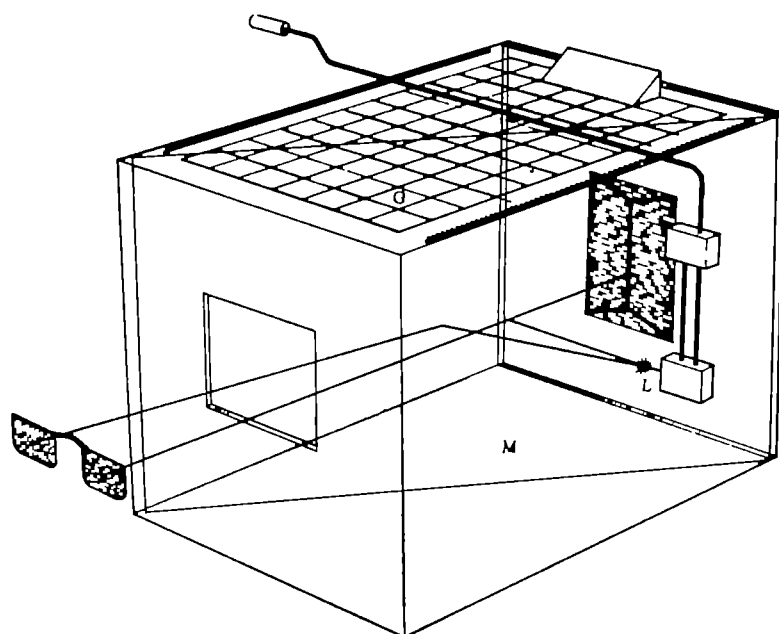


Fig. 5. Apparatus for measuring visual depth in pictures. The picture (for example, an illusion figure) is back-illuminated, to avoid background texture. It is seen monocularly, the light being polarized, and cross-polarized at one eye. The reference lamp (L) is seen by both eyes, by reflection from the part-reflecting mirror (M), and is seen as lying in the picture. Its distance is adjusted to equal the apparent distance of any selected feature of the picture. Positions are marked on the graph paper (G), giving a plot of visual space in three dimensions (Gregory and Townes).

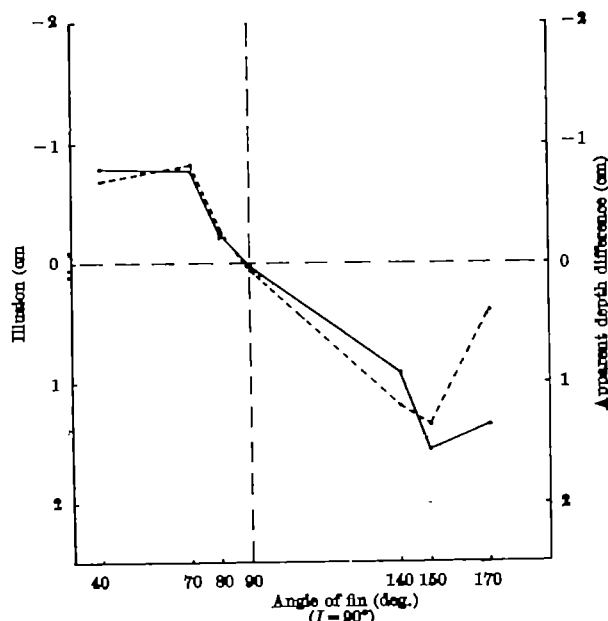


Fig. 6. Full line shows extent of Muller-Lyer arrow illusion (measured by adjusting a 'neutral' line to apparent equality) for a range of fin angles. Figures presented without background texture, to a single eye. The depth (broken line) is the difference in apparent distance between the shaft and ends of the fins.

### Depth Optics

Can we improve instruments, or devise new instruments, for extending the eye's ability to see in depth?

The microscope is a direct extension of the eye, extending its ability to see the very small by effectively making it see objects extremely near. But when used at high magnification, its depth of focus is so small that structures lying only a few microns further or nearer the plane of sharpest focus are degenerated to be unrecognizable. It cannot provide the separated views to the two eyes to give stereoscopic depth. This limitation, however, can be overcome<sup>13,14</sup> by vibrating the objective lens of the microscope so that the plane of sharp focus scans rapidly up and down through the specimen, extracting the depth information with each scan. But if this were presented on a plane we would see confusion, for we would be compressing three into two dimensions. Somehow the information must be reconstituted into three-dimensional visual space. This can be done by projecting the image on to a screen kept vibrating in phase with the scan through the specimen. The image on the vibrating screen then changes systematically as it moves to and from the observer, and builds in the volume swept by the screen a 'solid image', magnified in depth. We can see, for example, brain cells magnified a thousand times in three

dimensions. In practice the vibrating screen introduces difficulties, but it can be replaced by a rotating helical screen, or a screen avoided altogether, by sweeping the pair of images in opposition across the eyes, in a way the observer's brain accepts as signalling depth.

Could we devise a way of drawing pictures in three dimensions? Do artists have to be for ever limited to flat planes on paper? The problem is to produce a pair of lines, one for each eye, produced under the control of the artist, so that correct stereoscopic depth is given by the horizontal separation of the lines. We have recently built just such a device. The depth artist holds a stylus, bearing a small bright light which is imaged on a pair of Thorn electroluminescent image-retaining panels. As he draws with the light, in three dimensions, glowing lines are presented to each eye and fused by the brain into a single picture in depth. He sees and creates in a

three-dimensional world, where artist and scientist meet.

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- <sup>12</sup> Teuber, H.-L., in *Handbook of Physiology*, Sect. 1, *Neuropsychology*, edit. by Field et al. (Amer. Physiol. Soc., Washington, 1960).
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## NEWS and VIEWS

### The Royal Society: S. G. Brown Award and Medal

THE Royal Society's S. G. Brown Award and Medal has been won this year by Mr. F. T. Bacon, consultant to Energy Conversion, Ltd., for his work in the development of fuel cells, on which he has been continuously engaged for the past twenty years. The award is made annually by the Council of the Royal Society, on the nomination in turn of the Institutions of Civil, Mechanical and Electrical Engineers, for an outstanding contribution to the promotion and development of mechanical inventions. The nomination is based on work carried out during the year of the Award and the preceding five years. The 1965 nomination was made by the Institution of Mechanical Engineers, of which Mr. Bacon is an Associate Member.

### Prof. A. C. Haddow, F.R.S.

THE cross of Chevalier de la Légion d'Honneur has been awarded to Prof. Alexander Haddow, director of the Chester Beatty Research Institute of the Institute of Cancer Research, London. This award is an indication of the esteem in which Prof. Haddow is held in scientific circles in France and is at the same time an expression of appreciation of much friendly collaboration over many years. Recently, Prof. Haddow has been closely associated with discussions about the international support of cancer research, in particular with the proposals put forward two years ago by the French Government.

### Space Research in the Ministry of Aviation:

#### Mr. J. G. Lewis

MR. J. G. LEWIS has been appointed director (space) at the Ministry of Aviation Headquarters in succession to Mr. C. J. Stephens, who is now attending the Imperial Defence College. Mr. Lewis was born in Skipton, Yorkshire, in 1921 and educated at Skipton Grammar School and later at Christ's College, Cambridge, where he specialized in mathematics and physics. In 1942 he entered the Air Defence and Research Establishment at Malvern (now the Royal Radar Establishment), where he worked on searchlights control and surveillance radars. In 1956 he attended the 17th course at the Joint Services Staff College. He was promoted to senior principal scientific officer in 1957 when, as superintendent of the Special Projects Branch at the Royal Armament Research and Development Establishment in Kent, he became more directly involved in the development of guided weapons. For the past three years he has been an assistant director in the Defence Research Staff in Washington, D.C., concerned with U.S. guided weapons and space activities and Anglo-American co-operative programmes.

### Psychology in the University College of Swansea:

#### Prof. C. E. M. Hansel

MR. C. E. M. HANSEL, who has been on the staff of the Department of Psychology in the University of Manchester since 1949, has been appointed to the newly established chair of psychology in the University College of Swansea. Mr. Hansel was born in 1917 and educated at Bedford School. From 1938 until 1946 he served in the Royal Air Force and reached the rank of squadron leader. After demobilization he proceeded to Fitzwilliam House, Cambridge, where he read psychology in Part II of the Moral Sciences Tripos. His principal research interests have been in the field of visual perception and, in particular, he has been developing a theory intended to integrate the physical and psychological phenomena of colour vision. In this sphere he is an authority. He has recently designed a new type of teaching machine. He has also excelled as the leading critic of the experimental basis of claims for extra-sensory perception. His thorough-going examination of this field of enquiry is to be published in the spring of 1966 (by Scribners, of New York) and will, no doubt, create considerable consternation among para-psychologists and their adherents. The topic will now be placed in its correct scientific perspective. While at Manchester, Mr. Hansel collaborated with Prof. John Cohen in researches into subjective probability, temporal phenomena, the spread of ideas, and other topics. This resulted in a joint book, *Risk and Gambling* (1956), and numerous papers on decision-making, the measurement of linguistic usage, and the kappa effect. Mr. Hansel is a talented musician, and for many years played first violin in the Alderley Edge Orchestra.

### Second Chair of Electrical Engineering in the College of Advanced Technology, Birmingham:

#### Prof. J. E. Flood

DR. J. E. FLOOD has been appointed to the second chair of electrical engineering at the College of Advanced Technology, Birmingham. He gained his initial education at the City of London School and then went on to take an engineering course at Queen Mary College. After war-time service at the Admiralty Signal Establishment, he joined the Research Laboratories of the British Post Office and for the next five years was occupied with the application of electronics to automatic telephone switching. Deciding to get nearer the product, he joined the then Siemens Brothers of Woolwich, now the Telecommunications Division of Associated Electrical Industries, Ltd. Here he took an active part in furthering electronic applications to the telephone and, for a number of years, was chief engineer of the Advanced Development Laboratories

engaged on this work. During this time he played a leading part, together with other manufacturers and the British Post Office, first in the establishment of the first electronic automatic exchange at Highgate operating on the time-division multiplex principle, and latterly in the establishment of the first large electronic exchange in Britain in which reed relay speech circuits are controlled by electronic logic circuitry. In recent years he has also been engaged in electronic applications to other forms of telephone switching, in data transmission studies, and methods of achieving economy of speech-band paths over linked transmission circuits. He is now extending his activities to the wider basis of the academic life, where his experiences, especially those of industrial research laboratories, should prove of great value in university research activities with which he will now become associated. He was recently granted a D.Sc. (Eng.) by the University of London for his publications in the fields of electronics and telecommunications, and his new career at the Birmingham College of Advanced Technology will be watched with interest by all who were associated with him in his early activities.

### Nuclear Power Stations

In a statement in the House of Commons on May 25, the Minister of Power, Mr. F. Lee, said that the Central Electricity Generating Board, in inviting tenders for the second nuclear power station to be built at Dungeness, had stated that besides tenders for an advanced gas-cooled reactor station of the kind developed by the Atomic Energy Authority, it was also ready to consider tenders from British industry for water-moderated reactor systems of proved designs such as those developed in the United States. The tenders, which included proposals for both these types, had now been assessed by the Generating Board in conjunction with the Atomic Energy Authority; the advanced gas-cooled reactor showed clear economic and technical advantages over the alternative systems, and had a good potential for further development. It would also generate base-load power more cheaply than a contemporary coal-fired station. Mr. Lee said he had accepted the joint recommendation that an advanced gas-cooled reactor should be adopted at Dungeness, and later added that the advantage would be at least 10 per cent. A corresponding statement was made the same afternoon in the House of Lords, when the advanced gas-cooled reactor was described as a remarkable technical break-through, and Lord Numburnholme asked if some scheme for converting sea-water into fresh water could be incorporated in the contract.

### Expenditure on Overseas Development

In reply to a question in the House of Commons on June 1 as to the aid given by the Ministry of Overseas Development in agriculture, education and technology, Mr. A. E. Oram, Parliamentary Under-Secretary to the Ministry, said that in agriculture estimated expenditure on aid in 1964 was £1·885 million, excluding the cost of the Anti-Locust Research Centre (£140,000) and Desert Locust Control (£12,000), besides new commitments for grants (£3·8 million) and loans (£4·5 million). In addition, expenditure of £12·6 million was incurred in respect of earlier commitments. There were some 180 unfilled vacancies for technical assistance, but 1,342 Overseas Service Aid Scheme posts were filled in 1964 and 61 technical assistance posts. The demand was concentrated largely in East and Central Africa. In addition, under the National Agricultural Advisory Service and the Department of Agriculture for Scotland, 30 posts were created to enable experienced officers to be seconded overseas, and under a studentship scheme 20 British graduates were being trained for overseas service in agriculture. Britain's direct expenditure on education and training overseas in 1964-65, including Common-

wealth Education Co-operation, was more than £16·5 million, about £12·5 million being in the Commonwealth; there were also substantial contributions to international agencies such as the United Nations Educational, Scientific and Cultural Organization, the Expanded Programme of Technical Assistance, and the Special Fund. There were more than 42,000 Commonwealth students in Britain, and expenditure on training in the United Kingdom of students for developing Commonwealth countries, other than under Commonwealth Educational Co-operation arrangements, was estimated at £1·7 million. For 1964-65 estimated expenditure on British teachers overseas under the Overseas Services Aid Scheme was estimated at £1·6 million. Estimated expenditure on aid to technology in 1964 was £4·05 million, including expenditure by the British Council. New commitments for grants totalled £451,000 and for loans £6·86 million, while further expenditure of £18 million was incurred in respect of earlier commitments. It is expected that the whole of the new commitments in all the fields will require some years to disburse.

### Reports of the Estimates Committee

Two recent Special Reports from the Estimates Committee have attracted little attention although they bear closely on the efficiency of Parliament, particularly on its ability to criticize the Executive effectively. Of these, the Fifth Special Report for the Session 1964-65 (Pp. 4. London: H.M.S.O., 1965. 6d.) deals with temporary technical or scientific assistance for sub-committees of the Estimates Committee, which works through such sub-committees. For its evidence, it depends largely on senior officers of Government departments who are experts in the subject under examination, although informed and responsible persons from outside the Civil Service are also consulted. The Estimates Committee has reviewed the present system under which it examines the Estimates with the view of deciding whether its existing powers are sufficient. It considers that on occasion it might be valuable for it to engage the services of someone with scientific or technical knowledge on an *ad hoc* basis for the purpose of a particular enquiry, either to supply information which was not readily available or to elucidate matters of complexity within the Committee's order of reference. Such a person would attend meetings of the Sub-Committee conducting the enquiry when invited to do so, but without power to vote or to examine witnesses. There are two precedents for this procedure. When, in 1921, a Select Sub-Committee was set up to enquire into the organization and administration of the telephone service, the Committee was empowered to appoint from outside its own body such persons as it thought fit to obtain special expert or scientific information, or to advise on the subject-matter of its enquiry. Likewise in 1944, the Select Committee on House of Commons (Rebuilding) was empowered to invite any specially qualified persons it might select to attend any of its meetings in an advisory capacity. The Estimates Committee now recommends that as an experiment the House should pass an order, granting it a similar power limited to the two purposes indicated and on the understanding that anyone appointed will function in the way described. The sixth Special Report for the session (Pp. 4. London: H.M.S.O., 1965. 6d.) deals with sittings of sub-committees overseas. The Estimates Committee and its sub-committees are at present limited to sittings within the United Kingdom. When in the last two sessions members of one of the Sub-Committees were investigating military expenditure overseas they were only able to travel abroad to do so by courtesy of the Minister of Defence, who issued invitations to them and bore the expense on his Vote. Even so, they were not able to sit as a sub-committee to take evidence. The Estimates Committee does not think that it is satisfactory

that it should be dependent on invitations from departments which it may wish to investigate, and it accordingly recommends that its sub-committees should be able, with the agreement of the Committee and by leave of the House, to hold sittings in any overseas territory with the consent of that territory.

### The Australian Academy of Science

THE report of the Council of the Australian Academy of Science for the year ended March 31, 1965 (Pp. 14. Canberra: Australian Academy of Science, 1965), reiterates its concern at the absence of Government replies to the various proposals submitted to it since 1962, covering the establishment of a research museum of Australian biology and compilation of a Flora of Australia, the provision of rockets to enable Australian space research to be developed, and the establishment of a National Science Fund for supporting, on an individual basis, high-quality projects for scientific research. Such apparent discourtesy must be far more frustrating and disheartening to Australian scientists than the reasoned rejection of proposals. During the year the senior fellowship scheme was established on a continuing basis, and the Council accepted responsibility for preparing a new secondary-school text-book on biology for use in Australian schools. Publication of the *Australian Journal of Applied Science* was stopped, but the number of papers published in Australian journals of scientific research increased by 67 during 1964.

### The Smithsonian Institution

THE report of the Smithsonian Institution for the year ended June 1964 is a massive publication of nearly 300 pages (Pp. xiii + 293 + 14 plates. Smithsonian Publication 4595. Washington, D.C.: Government Printing Office, 1965). It expresses the hope that in its wider usefulness the Institution may be able to broaden the traditional co-operation with museums throughout the world. Museums and their related laboratories are entering a new era and their resources are being drawn on as never before for general education, since more than 90 per cent are involved, ranging from simple school-extension programmes to postgraduate fellowships. For these reasons, it is felt that the Smithsonian, with its superb resources, has a great opportunity to serve the museum world in the role of leadership and co-operation. Research on wild populations and undisturbed conditions in Nature is now a matter of urgency. A related objective is to strengthen the position, within science as a whole, of those fields of biology which have the entire organism as their object: ecology, genetics, systematics, botany, zoology, oceanography, microbiology and palaeontology as well as the sciences of man which have for so long been central concerns of the Smithsonian. The Institution has also been active in the international field, and has made a determined effort to carry its activities beyond traditional expeditions and research to co-operation with other Government agencies and private institutions in the development of exchange of persons and exhibition programmes.

### The Zoological Society of London

THE annual report of the Zoological Society of London for 1964 reviews all the activities of the Society during the year. There were 1,816,000 visitors to Regent's Park, a figure only marginally below the seven-year average, and 38,000 more than in 1963. The total number of visitors to Whipsnade Park was 675,000, which, though an improvement on 1963 (616,000), was still well behind the 757,000 visitors in 1961. 39,000 cars were brought into the Park by visitors, compared with some 33,600 in 1963. A particularly important acquisition by the Society during the year was a pair of European bison, presented to the British Forestry Commission by the Polish Forestry Commission and deposited in the Col-

lection. Since February 1963 there has been only a single male of this species in the London Zoo, but it is now hoped to be able to breed this extremely rare animal. Among the more interesting additions to the mammal collection have been a binturong, a Siberian weasel, two hog badgers, two giant pangolins and two spectacled bears. The outstanding exhibit in the aquarium still continues to be the *Arapaima* (*Arapaima gigas*) from South America, which is now almost 5 ft. long—more than three times its length on arrival in June 1962. A new nocturnal section housing pottos, douroucoulis, lemurs, flying squirrels and bushbabies was opened in the Children's Zoo at Regent's Park in the early summer, and has proved a most successful exhibit.

### Desert Locust Control Organization for Eastern Africa

THE first annual report of the Desert Locust Control Organization for Eastern Africa, which covers the period October, 1962–June 30, 1963, is of particular interest (Pp. 32 + map. Nairobi: Government Printer, 1964. 2s.). It records cogently and concisely the stages by which the former Desert Locust Survey developed from an organization financed entirely by Kenya, Tanganyika, Uganda and the British Government, but operating almost exclusively in Ethiopia and Somalia, into a fully constituted international organization of which Ethiopia and Somalia are members. This marks the culmination of the efforts that have been made for a number of years to foster international co-operation in locust control in Eastern Africa. During the period of the report desert locust activity was at a minimum throughout almost its entire area, and the Organization itself was not required to undertake control measures. Some control was carried out by national locust organizations in Somalia and Ethiopia assisted by the United States Regional Insect Control Project. The control potential of the Organization itself was used to conduct surveys which, with the co-operation of the countries concerned, were extended to the Sudan and Arabian Peninsula. The report contains a useful record of such locust activity as occurred, and an account of the research which the Organization is conducting or planning. This research includes locust population dynamics, use of radar in quantitative survey, morphometric studies, aircraft spray equipment and operational methods. The outcome of these projects will be of importance to all concerned with locust control, and future reports by the Desert Locust Control Organization for Eastern Africa will be awaited with interest.

### The London School of Hygiene and Tropical Medicine

THE report on the work of the London School of Hygiene and Tropical Medicine for the year 1963–64 (Pp. 127. London: London School of Hygiene and Tropical Medicine, 1965) outlines the progress made with the modernization and expansion of the School, following the Wolfson Foundation benefaction of £800,000. It is supplemented by reports from the Departments of Bacteriology and Immunology, Biochemistry, Clinical Tropical Medicine, Entomology, Human Nutrition, Medical Statistics and Epidemiology, Occupational Health and Applied Physiology, Parasitology and Public Health, and from the Ross Institute of Tropical Hygiene and the Medical Research Council Environmental Physiology Research Unit. A list of publications during the year is appended.

### The National Institute of Child Health and Human Development

THE Public Information Office of this Institute, which is part of the U.S. Public Health Service, plans to distribute on request every 2½–3 months a news service to workers in this field, which will give information about the Institute's activities and progress. The *News Letter* dated February 1965 contains information about the higher values of the



3 enzymes acid phosphatase, galactose-1-phosphate uridyl transferase and glucose-6-phosphate dehydrogenase found in mongoloid individuals, and also about Public Health Service research grants, and work on the diagnosis and study of retardation done by a new unit in the Institute. There is also a report of a discussion on saving life before birth and items on 'fetology', the name given to the study and treatment of the human foetus *in utero*, and on grants for the study of adult development and ageing.

#### 'Miche' Stains and Reagents for Biology

BUILDING extensions to the premises of Edward Gurr, Ltd., at East Sheen, London, S.W.14, have now been completed at a cost of £25,000 with the view of streamlining operations, more particularly in connexion with the firm's exports expansion programme. This is the second extension since Edward Gurr acquired the original premises in November 1950. The first was built in 1952 (*Nature*, 169, 483, 1952), when the Mayor of Barnes laid the foundation stone of a new laboratory block. The ceremony was performed in the presence of officials of the Board of Trade (Export Promotion Department), Central Office of Information, the British Broadcasting Corporation and the Press. The Company was founded shortly after the Second World War by Edward Gurr, an organic chemist and biologist, primarily to supply the export markets which had formerly drawn on German manufacturers for their needs of biological stains. Another object of the founder was to gain freedom and facilities for research and writing in his own style on the topics closest to him, namely the chemistry and use of biological stains and the simplification and rationalization of histological staining. He has published several books and has contributed a number of research papers to various journals, all with this object in view.

#### Introductory Courses in Genetics

THE fourth edition of Dr. Charlotte Auerbach's *Notes for Introductory Courses in Genetics* (Pp. 42. London: Oliver and Boyd, Ltd., 1965. 8s. 6d.) again emphasizes that the booklet is not intended to compete with any textbook, however elementary. Even less is it meant to be a popular introduction to genetics, although it may prove useful to scientists in other fields who require a concise exposition of the basic concepts and laws of genetics. The object of the book is to help students who attend courses in elementary genetics, especially the short courses given to medical students and first-year agricultural students, and for their special requirements two appendixes have been added. The *Notes* should relieve the students of the necessity of taking their own notes on a subject which requires great accuracy and clarity of terminology. The book has, in fact, grown out of dictations to students, and has successfully replaced these dictations in Dr. Auerbach's own introductory courses.

#### Translation and Interpretation

THE chief features in *The Incorporated Linguist*, the journal of the Institute of Linguists for April 1965, are Mr. S. Soubbotnik's article on "Interpretation in the United Nations and its Specialized Agencies"; Mr. R. Hartman's comparative review of the bilingual dictionaries of English and German; and Dr. van Abeé's discussion of overcoming initial difficulties in teaching a modern language.

#### Psychological Investigation of Card Punching

BEHIND each brief summary of multifarious fact provided by data-processing lie not only the achievements of electronic engineering but hours of humdrum card punching by young women, some of whose supervisors report that for this work the level of intellectual attainment represented by General Certificate of Education (Ordinary Level) passes is a positive drawback. Yet

their work demands manipulative speed and accuracy, with considerable mental alertness in ordering and coding the raw data, and they need 3-6 months training. The National Institute of Industrial Psychology has made a preliminary survey of the problems of selection, training and methods of work that this entails (National Institute of Industrial Psychology. Report No. 18: *Card Punching: a Study of the Work and the Factors that Affect It*. By Leonora Johnston. Pp. 40. London: National Institute of Industrial Psychology, 1965. 8s. 6d.). That problems really exist is indicated by the high leaving-rate among operators, and the standard view that it results mainly from marriage and pregnancy is shown to be untenable. Any programme of selection and training in this field has to face unexpected difficulty in assessing performance, largely because of wide variations in the nature and layout of the information to be punched. The Institute's investigator met the familiar industrial situation of rather elaborate record keeping which everyone concerned knows to be misleading, and although she tactfully accepts the view that it serves well enough for practical purposes it will obviously not meet scientific needs. The report, however, begins to bring the problem within range of systematic investigation, and it is to be hoped that further work can be undertaken in an area where widely differing practices prevail, each at present supported with more conviction than evidence.

#### North Staffordshire Field Club

THE North Staffordshire Field Club celebrated its centenary on April 18. Founded for the purpose of studying the natural history of the district, its scope was soon extended to include local history and archaeology, and early members contributed notably to the knowledge of the North Staffordshire Coalfield. In recent years a close liaison has developed between the Club and the University of Keele; in 1960 the *Transactions of the North Staffordshire Field Club* were incorporated in the *North Staffordshire Journal of Field Studies*, which is edited in the University. The president of the Club for the centenary year is Mr. H. V. Thompson, formerly head of the Department of Chemistry in the North Staffordshire College of Technology.

#### Fossil Tapirs

IN contrast to the voluminous writings on other fossil perissodactyls, tapiroids have been long neglected. Prof. Radinsky's recent monograph, entitled *Early Tertiary Tapiroidea of Asia*, does much to redeem this—a work all the more remarkable when one realizes that the material which is described has lain in the American Museum for more than forty years (*Bulletin of the American Museum of Natural History*. 129, Article 2. By Leonard B. Radinsky. Pp. 181-264+plates 1-4. New York: American Museum of Natural History, 1965. 3 dollars). Radinsky's earlier works have clarified the location of Mongolian Tertiary sites and their stratigraphy. This work sheds new light on the earliest ceratomorphs, a taxum which bristles with taxonomic difficulties. Radinsky describes three new tapiroid genera and rearranges the classification of early tapirs in the light of all known material, mostly Russian and Chinese. His re-grouping of genera within families still leaves an uncomfortably large number of *incertae sedis* specimens. In spite of this, his descriptions of dentitions and limb bones, adequately illustrated, make a welcome contribution. Prof. Radinsky comments on the interesting contrast between the diverse tapiroid fauna of the Asiatic Eocene with the relative paucity of artiodactyls, and the reverse position in contemporary North America and Europe where artiodactyls predominated; he suggests the influence of ecological factors. The 'grande coupure' still remains between early and late Tertiary tapiroids, as it does for numerous other mammalian taxa.

### Pyrenean Prehistory

THE late M. and Mme. St. Just Péquart were well known before the Second World War as amateur prehistorians. They were at the village of Mas d'Aixil during the German occupation and worked on the new gallery found in 1937, completing their investigations four years later. Mas d'Aixil is in the Pyrenees; a river plunges down a valley and turns into the mountain-side, emerging just above the village. The tunnel is large enough to accommodate both the river and a road. Half-way through, to one side, there are extensive caves—so extensive indeed that it is said that during the Albigensian Wars an army took refuge in them and was not discovered. Prehistoric man used some of the galleries, and Stone Age industries have been unearthed from them. M. and Mme. St. Just Péquart had the luck to find a new gallery full of implements and fauna, some of it Aurignacian, but mostly Magdalenian in date, and including some remarkable examples of prehistoric art. The exact location of the gallery is described in the *Annales de Paléontologie* (48-49, now published in one volume), and an account is given of the section obtained; these are followed by a description of the finds—both stone and bone. The implements are very numerous and well fashioned. A number of small engraved rondels occur, as well as pieces of bone sculptured into the form of animals' heads. A few pieces of modelled clay were also found as well as a small, somewhat crude sculpture in bone of a screech owl. Many of the flat pieces of bone carry engravings of animals. But the really remarkable object is a spear thrower, the end of which is carved with a figure of a fawn looking back over its shoulder towards its tail where the head of a bird is incorporated. Very few prehistoric drawings or sculptures show an animal turning its head over its shoulder. The engraved reindeer from Langerie Basée is an example, but the new find from Mas d'Aixil is especially well executed. Many other finds of interest are described, but enough has been said to make it obvious that the *Annales* in which the account of the new finds appears should not be missed by prehistorians.

### Geological Maps of Sweden

THE first of a new series of geological maps of Sweden, combining solid and drift geology on the scale 1:50,000, covers the north-eastern environs of Stockholm (*Geologiska Kartblad*, Serie Ae No. 1, Stockholm NO; with explanatory memoir. Pp. 148. Stockholm: Sveriges Geologiska Undersökning, 1964. Map, 20 kr.; memoir, 5 kr.). In view of criticisms in several countries that the publications of the national geological surveys are becoming increasingly unintelligible to the intelligent layman, this map and pocket-size memoir merit recognition not only as an outstanding piece of geological cartography, but also as a praiseworthy attempt to present a description of the geology in a comprehensive and yet readily comprehensible form. The memoir, which carries a coloured petrological map on the scale 1:100,000, is in Swedish, save for a seven-page English summary; both maps and all illustrations have bilingual legends. In addition to the geological features, the location of ancient monuments is shown on the map and listed in the text. Stockholm now joins the relatively few capital cities—Oslo, Edinburgh and Paris are among the others—where the geological tourist is well catered for.

### The Macaulay Institute for Soil Research

THE latest bound collection of scientific papers from the Macaulay Institute for Soil Research, published during the period 1961-63, has been issued (7. Pp. 24+papers 1-74. Edited by Alexa M. B. Geddes and Rosaleen Noble. Craigiebukler, Aberdeen: The Macaulay Institute for Soil Research, 1964). The titles of 74 papers are listed in chronological order and there is an author index. The work, from seven major departments, naturally

covers a wide range of topics embracing both fundamental research and the application of scientific discoveries to agricultural practice, but an idea of the scope of the publications may be gathered from the following extracts from the titles. Soil formation, the principles of the classification of soils, the genetic soil groups of Scotland; sensitivity in differential thermal analysis, various aspects of clay minerals; microbiology of rocks and weathered stones; the rate of growth of peat and its use in horticulture; the fungal metabolism of compounds related to lignin, the release of ions from minerals by fungal activity; the fractionation of organic phosphate; soil fertility, evaluation of phosphate status, effects of fertilizers on plant production; placement of radioisotopes in the study of root systems; changes in the composition of leaves during growth, aconitase activity in leaves; trace elements in soil, plant and animal.

### Announcements

THE Secretary of State for Education and Science has appointed the Right Hon. Viscount Amory a member of the Medical Research Council as from October 1, in succession to the Right Hon. Lord Shawcross, whose term of membership expires in September. Lord Amory has agreed to be chairman of the Council. He was chairman of the Council during December 1960-July 1961, when he resigned on taking up his appointment as United Kingdom High Commissioner in Canada.

A SYMPOSIUM on "Physiology and Biochemistry of Muscle as a Food" will be held in the University of Wisconsin during July 12-14. Further information can be obtained from E. J. Briskey, Department of Meat Science, University of Wisconsin, Madison.

AN international seminar on "Ekistics and the Future of Human Settlements", organized by the Graduate School of Ekistics, Athens, will be held in Athens during July 20-24. Further information can be obtained from Dr. D. Iatridis, Athens Center of Ekistics, 24 Strat. Syntekmou Street, Athens 136.

AN international symposium on "Swine in Biomedical Research", sponsored by the Atomic Energy Commission and the Pacific North-west Laboratories of the Battelle Memorial Institute, will be held in Richland, Washington, during July 19-21. Further information can be obtained from Dr. L. K. Bustad, Biology Department, Battelle-Northwest, P.O. Box 999, Richland, Washington.

AN international conference on "The Education of Professional Physicists", arranged by the Institute of Physics and the Physical Society under the auspices of the Education Commission of the International Union of Pure and Applied Physics, will be held at the Imperial College of Science and Technology during July 16-21. Further information can be obtained from Miss P. N. Boston, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

A SYMPOSIUM on "Thermodynamics, with Emphasis on Nuclear Materials and Atomic Transport in Solids", sponsored by the International Atomic Energy Agency and the Commission on Thermodynamics and Thermochemistry of the International Union of Pure and Applied Chemistry, will be held in Vienna during July 22-27. Further information can be obtained from Dr. O. E. Holley, jun., Division of Research and Laboratories, International Atomic Energy Agency, Kärntnering 11, Vienna I.

ERRATUM. In the communication entitled "Mannosamine in the Cell Wall of *Clostridium welchii* (Type A)", by Dr. B. T. Pickering, which appeared on p. 400 of the April 24, 1965, issue of *Nature*: 1st para., line 10, the words "glutamic acid" should be inserted between "alanine" and "glycine". 2nd para., line 15, the first word should be "galactosamine".

## REORGANIZATION OF THE FORESTRY COMMISSION

IN a written answer in the House of Commons on May 10, the Minister of Land and Natural Resources, Mr. F. Willey, stated that he and the Secretaries of State for Scotland and Wales had comprehensively reviewed the organization of the Forestry Commission, taking account of the recommendations made by the Estimates Committee in its seventh report for the Session 1963-64, with which they found themselves in wide agreement. Among other changes, they had decided that the Commission should be reconstituted with four full-time executive members and only five part-time Commissioners, chosen for their knowledge and experience of commerce, the timber trade, trade union matters, and forestry and the countryside. There will be a part-time chairman as at present, and the staff will be reorganized functionally under the full-time members responsible for forest management, harvesting and marketing, and administration and finance, respectively, and there will be greater delegation to the Conservancies, with headquarters staff in Scotland and Wales. Mr. Willey added that the chairman of the Commission, Lord Waldegrave, was in full agreement with their proposals, and to facilitate the reorganization he and the other Commissioners had placed their offices at the Government's disposal.

These proposals and the other Government observations on the Committee's proposals are set forth in the eighth Special Report from the Estimates Committee\*. Responsibility for forestry has been transferred to the Minister for Land and Natural Resources and the Secretary of State for Wales, who will seek advice from their senior officers, as well as from the Forestry Commission on the broader aspects of forestry. The Forestry Ministers have discussed with representatives of the private woodland owners, and of the timber trade, arrangements to make themselves accessible to a direct formal approach. It is confirmed that the Commissions should continue to be responsible for promoting the interests of private as well as of State forestry, and the production and supply of timber. However, it is suggested that development of the position of the Home Grown Timber Advisory Committee would ease the dilemma of the private woodland owners and the timber trade. They accept that the administrative staff in the Commission's headquarters, and particularly the Commissioner in charge of administration and finance, should play a full part in determining

policy, and it is recognized that the higher posts should not be limited to any one class or profession. It is believed that the new functional organization will provide improved prospects of promotion for the executive class. A system of valuation for the Commission's forests, which is under discussion with the Treasury and the Comptroller and Auditor-General, will be introduced experimentally for the next two or three years. The recommendations that a brief explanation of the most significant items of income and expenditure should accompany the summary of the financial results in future annual reports, and that the Commission should establish a new Costings Branch, are also accepted.

On the recommended study of the present procedures for acquiring land for forestry purposes, the Ministers express the opinion that close consultation with the agricultural departments is essential concerning the change of use from farming to forestry, which is inherent in the forestry programme. They are satisfied that adequate machinery exists for resolving speedily, if necessary at Ministerial level, any conflict that may arise. In England the Forestry Commission is already undertaking a pilot survey of agricultural and forestry land use with the Ministry of Agriculture, Fisheries and Food in Northumberland and Cumberland, and possibilities of similar surveys in Wales are being explored. The Ministers also agree that Parliament and the public should be informed both of the planting programme to which the Government is working and of the considerations on which it has been formulated. However, they decline to commit themselves as to the frequency with which it would be appropriate to review the programme, or the means to be used to furnish information to Parliament. The position of marketing within the Commission will be strengthened by the appointment of a Commissioner as full-time head of harvesting and marketing, and a new branch has already been added to the Marketing Section. The Commission is also discussing with the Civil Service Union a revised grading structure and scales of pay for the forester class which, it is hoped, will significantly reduce the wastage of trained foresters. In the reorganized Commission it is proposed to amalgamate the Education and Training Branches, vesting responsibility for them in the Commissioner for Administration and Finance. In consultation with other Departments, the Forestry Commission is reviewing the procedure for making Tree Preservation Orders, and the Forestry Committee of Great Britain and other interests concerned will be consulted at the appropriate time.

\* Eighth Special Report from the Estimates Committee, Session 1964-65 *The Forestry Commission*—(Departmental Observations on the Seventh Report from the Estimates Committee in Session 1963-64). Pp 8 (London H.M.S.O., 1965) 9d. net.

## THE AUSTRALIAN DEFENCE STANDARDS LABORATORIES

THE Australian Defence Standards Laboratories with their establishments at Maribyrnong (Victoria), Alexandria (New South Wales) and Finsbury (South Australia) provide a scientific service for all branches of the Defence Service, Civil Defence and the Department of Supply. The Laboratories deal with problems which arise in the provision and use of defence material, within the fields of chemistry, physics, metallurgy and engineering.

On July 18, 1963, 'Operation Blowdown' was successfully carried out at Iron Range in North Queensland, and a reproduction in colour of the fireball produced by the trinitrotoluene explosion forms the striking cover illustration of the annual report of the Laboratories for the year ended June 30, 1964\*. As predicted, the blast of the

explosion produced extensive damage to the forest. Trees were uprooted and broken and there was a substantial pile-up of debris far from the seat of the explosion. A high order of spherical detonation was achieved, but the Mach stem of the explosion was severely distorted by the forest, an effect previously unknown. Army personnel stationed within the area of the operation assessed the tactical implications of the debris and the displacement hazards to troops were determined by articulated dummies placed at appropriate positions. Other scientific data of value were collected by instruments, some of novel design, placed on trees within the forest. The combined results provide a sound basis for the assessment of nuclear weapons effects in a tropical forest area.

In the Chemistry Division work has continued on the application of mass spectrometry in the structural analysis of organic compounds and a grating infra-red spectrophotometer has been used to obtain high-resolution

\* Commonwealth of Australia. Department of Supply. Annual Report of the Defence Standards Laboratories, 1963-64. Pp. 50. (Ascot Vale, Victoria: Defence Standards Laboratories, 1964.)

vibration-rotation spectra of small molecules containing phosphorus. A rapid and accurate photometric method, based on the reduction of dichromate, was developed for the determination of the excess of zinc in zinc oxide. Zinc oxide is of interest as a photoconductor in electro-photographic processes. Measurements were made with a low-frequency torsion pendulum of the dynamic modulus and internal friction of irradiated samples of polytetrafluoroethylene at temperatures between  $-180^{\circ}\text{C}$  and  $150^{\circ}\text{C}$  in order to obtain new information about the effects of  $\gamma$ -radiation. The results indicate that irradiation under high-vacuum conditions produces some random chain scission, but cross-linking also occurs and together with an unusual increase in crystallinity the polymer becomes more rigid and brittle.

The Metallurgy Division was mainly concerned with the investigation of chromium alloys with the object of developing strong high-temperature alloys. Metallographic investigations of the surface-damaged layer on abraded surfaces of germanium previously reported on have been extended to other semiconductors, in particular silicon and indium antimonide. It has been established that an extensive arrangement of cleavage cracks, each associated with an array of dislocations, is a common feature. In indium antimonide, the damaged layer also contained glide dislocations and twins. The general mechanism for abrasion seems to be that a system of cracks develops about the indentation made by the contacting abrasive particles, a fragment of the surface being removed whenever a number of the cracks intersect appropriately. Tests made on single crystals of germanium, polished and then etched with *OP-4*, showed that Griffith cracks did not appear until after the test pieces were stressed. Once the cracks formed they become preferred sites for the nucleation of dislocations and when

the cracks reached the critical Griffith size brittle fracture of the material occurred.

A new group was set up in the Physics Division to carry out research, under the supervision of Dr. P. W. A. Bowe, on the physics of lasers. The spatial and spectral coherence of a small ruby laser is being investigated. Pumping efficiency using a high-power Q-switch device is also being investigated and a Pockel-cell light switch with a rise time of less than 10 ns has been developed. A long-term programme of research on laser materials with initial emphasis on organic complexes has been initiated. In metrology, a screw-pitch measuring interferometer has been used to measure pitch over a 2-in. length to an accuracy of  $\pm 6 \times 10^{-4}$  in.; new techniques for flatness measurement and for the measurement of large jigs and components have been developed; and the accuracy of the frequency standard has been improved by the installation of a quartz crystal oscillator of the Warner type and a phase tracking receiver, for comparison with standard very low frequency transmissions.

Various other investigations and devices described in the annual report include a probe to detect the passage of a pressure front; photoelectric fuse-timing equipment; a rapid action dilatometer, based on the type used at the British Welding Research Association, to obtain continuous cooling transformation diagrams of low-alloy steels using heating and cooling rates appropriate to welded joints; the welding of thin-wall titanium tube; the tearing of textiles; the varnish-holding properties of timbers; and the detection and determination of toxic gases. The report concludes with a list of the various personnel and their status, the publications of, and lectures given by, members of the staff of the Laboratories and the names of the Governmental and other committees on which the establishment is represented. S. WEINTROUB

## TEXAS INSTRUMENTS, INC.

AT the annual meeting of Texas Instruments, Inc., on April 22, in Dallas, the president, Mr. P. E. Haggerty, reported on the activities of the company during 1964 and explained the principles and system of management. He mentioned that key personnel were deliberately and consistently moved from one job category to another and upward from one level of management to the next. During the nineteen years since the Second World War the company had grown to more than 150 times its original size, but it had not been necessary to go outside the company in order to fill any principal managerial position. The demand for highly trained personnel with advanced degrees continues, and if the growth rate of degree personnel—both first degree and higher degree—of 1960-64 is maintained, Texas Instruments will require some 12,000 college-trained personnel by 1974, including about 750 of Ph.D. status.

New record levels in both sales and products were achieved during 1964. Net sales, which totalled some 327.5 million dollars, were up by 18 per cent, and approximately one-fifth of the sales were from operations conducted outside the United States. The organization for the distribution of industrial supplies expanded its activities into international markets by the establishment of branches in Stuttgart, Germany, and in London. Branches in the United States are in Dallas, Houston, Kansas City, Tulsa and Boston.

Most of the company's products stem from investigations of materials at the fundamental structure-of-matter level, and a great variety of metals and non-metallic elements, including semiconductor materials, are refined and processed before fabrication into electrical or electronic components, or assembly into electronic systems. Zinc-clad aluminium for rotary-press printing plates, and copper-clad materials with performance characteristics

equal to or superior to those of solid copper, were two new developments during 1964. Semiconductor-grade silicon, and silicon-carbide clad parts, were in increased demand, and the company continued to be the main supplier to the U.S. Navy of fabricated nuclear fuel.

More than 200 new semiconductor and electronic component products were introduced during the year. Transistors for television receivers, and plastic-packaged silicon transistors for low-cost applications, were in growing demand for the industrial and domestic market. New components for the space and defence industries included an advanced series of silicon planar-epitaxial power transistors, a unique glass silicon rectifier with very high-voltage capability, and super-sensitive silicon-controlled rectifiers of reduced size and weight.

The semiconductor integrated circuit was first introduced in the Texas Instruments Laboratories during 1958, and now 86 standard circuit types are manufactured. A recent order for integrated circuits by North American Avionics Autonetics Division amounts to 11 million dollars. The circuits are to be used for the guidance and control system of the U.S. Air Force *Minuteman II* intercontinental ballistic missile. Texas Instruments semiconductor plant at Bedford, England, began the production of monolithic circuits during 1964 and the integrated circuits have been designed for the European six-nation space exploration effort called ELDO-ESRO\*, for a new hearing aid, and for several industrial programmes.

During 1962, Texas Instruments introduced, on a limited scale, a completely new approach to seismic exploration. It consists of the application of statistical communications theory to seismic data processing, and

\* European Launcher Development Organisation—European Space Research Organisation.

utilizes specially developed field techniques and signal-intensifying processes. The method has since been fully demonstrated and evaluated, and in many of the more difficult exploration areas of the United States and Canada it has proved to be an advanced tool for petroleum exploration. Advanced digital seismic data-processing centres have been established in New Orleans, Louisiana, and Midland, Texas, to augment the Dallas centre and to meet increased demands. A fourth centre began operation during January 1965 in Calgary, Alberta, and a fifth is to be opened shortly in London, England. A seismic detection system for perimeter defence in jungle warfare has been

delivered to the U.S. Marine Corps, and a scheme for geophysical experiments to be carried out on the surface of the Moon under the *Apollo* programme has been prepared. Mr. E. McDermott, who was co-developer of the seismic reflexion technology which has been applied universally in exploration for petroleum and other mineral deposits, and who was co-founder in 1930 of Texas Instruments' predecessor company, Geophysical Service, retired as chairman of the executive committee of Texas Instruments, Inc., on December 31, 1964, and Mr. Haggerty, president and chief executive officer, has assumed also the office vacated by Mr. McDermott.

## THIN FILM RESEARCH

THE twelfth meeting of the Thin Films Group (formerly the Dielectrics Evaporation Group) was held at Harlow during March 24-25. The Research Laboratories of Associated Electrical Industries and Standard Telecommunication Laboratories, Ltd., acted as joint hosts, and visits were made to both of these. The following papers were read: "Ellipsometry", by Mr. B. H. Clausen (Standard Telecommunication Laboratories, Ltd., Harlow); "Defects in Evaporated Silicon Films", by Mr. D. J. Thomas (Standard Telecommunication Laboratories, Ltd., Harlow); "Nucleation Studies on Freshly Cleaved Crystalline Surfaces", by Mr. D. Stirland (Alan Clark Research Centre, Caswell); "Nucleation and Charge on Glass Substrates", by Dr. R. Hill (Electrical Research Association, Leatherhead); "Lorentz Electron Microscopy: Magnetic Imaging in the Electron Microscope", by Mr. A. Green (International Computers and Tabulators, Stevenage); "Oxidation in a Glow Discharge to Prepare Dielectric Films", by Mr. D. White (Associated Electrical Industries, Research Laboratories, Harlow); "Conduction Processes in Dielectric Films", by Mr. J. Simmons (Standard Telecommunication Laboratories, Ltd., Harlow); "Some Readily Evaporated Semiconducting Compounds", by Dr. J. Zemel (Imperial College of Science and Technology—read by Mr. Juhasz, also of Imperial College); "Initial Stresses in Evaporated Films", by Mr. P. Carpenter (Alan Clark Research Centre, Caswell) and Mr. J. D. Wilcock (Imperial College of Science and Technology).

The theme of this meeting was the study of surfaces with special reference to the early stages of growth in thin films. Perhaps the most interesting new results presented were those of Stirland and Hill, whose papers were to a large extent complementary. In the hope of finding a reproducible surface, which is the dream of every electron microscopist, Stirland has cleaved single rock salt crystals *in vacuo* and evaporated gold from the same source on to the two faces produced. Topographically, one should be a mirror image of the other apart from damage produced in cleaving. He has used a combination of optical microscopy and numbered specimen grids to locate the equivalent areas on the two faces for electron microscopy. Results showed excellent correspondence of cleavage step and dislocation decoration, but no exact correlation of nucleation site position along the cleavage steps or on the flat areas in between. Exposure of one of the crystal faces to moist air before deposition showed considerable effects on the decoration pattern produced.

Hill has examined the effects on film structure both of electrons incident from the source and of the application of a d.c. field across the growing film. His micrographs show that both processes can affect the type of nucleation and growth and the number of nucleation sites available. His work was done with gold on glass substrates at pressures of  $10^{-4}$  mm mercury or better. The use of Hill's technique to prevent stray electrons from the source striking the substrate might perhaps affect some of

Stirland's results on nucleation site correspondence. In order to detect the presence of surface charges on the substrate prior to deposition, a potential probe was applied. This gave, in fact, a very sensitive indication of the beginning of nucleation.

The paper on ellipsometry described a technique which is somewhat unfamiliar to many. Using a collimated beam of elliptically polarized light reflected from the specimen surface, the growth of a thin film can be followed *in situ* by observing changes in the optical constants. The method can be applied to films immersed in liquids and hence is well suited to investigations of anodic film growth and of corrosion or adsorption processes. Owing to the number of variables involved it is necessary to use computer techniques to obtain calculated curves to which the experimental results are fitted as well as possible.

Lorentz electron microscopy is a highly specialized method for examining the magnetic structure of very thin films. A stream of electrons passing near a region of magnetic inhomogeneity is deflected in such a way as to cause an intensity distribution on the screen which corresponds to the variation in the magnetic structure of the film. This method can reveal not only domain walls but also the fine magnetic structure within the domains, and such applications were described in detail by Green.

Electron microscopy has been applied to the study of stacking faults in 'epitaxially' grown silicon deposited by evaporation and by vapour deposition. The incidence of such faults is greatly increased by the presence of silicon carbide formed from pump oils. It is interesting to see that, according to the workers at the Standard Telecommunication Laboratories, Ltd., the use of a gold silicon alloy to promote epitaxy (see Nielson, S., *Nature*, 205, 755; 1965) increases the liability to form faults in the deposit due to thermal strain.

The difficulty of depositing silicon throws into high relief the ease with which single crystal films of lead sulphide, telluride and selenide (and perhaps of related compounds) can be deposited by evaporation. Measurements on the electrical and optical properties of these materials by Zemel, and, in particular, of the electronic mobilities, show that the values obtained approach those of bulk single crystals. (Thus, lead telluride shows a bulk value of 30,000 and a value of 23,000 has been obtained for the film at 77° K.) Juhasz has examined these films by electron diffraction and was able to show an almost perfect Laue diffraction pattern. Since epitaxy was observed even with lattice-misfit greater than 50 per cent it seems that, for these materials at least, substrate surface state is by far more important than lattice correspondence. This was confirmed by Pashley during the ensuing discussion. Juhasz concluded with a graceful compliment to the pioneering work of Wilman, who demonstrated the epitaxy of these compounds as early as 1938.

The relation between details of films structure as revealed by electron microscopy and the mechanical and

electrical properties of the films is an essential link in any comprehensive investigation of film growth. Carpenter and Wilcock (both of whom are working under the direction of Mr. D. Campbell, one in a university, the other in a commercial laboratory) are using very sensitive measuring equipment for the study of initial stresses in thin films of a type on which structural data are available. They are at present seeking to relate the onset of visible nucleation with the first appearance of stress.

White has investigated the preparation of thin insulating films by 'field-assisted anodization' or 'biased sputtering'. Results so far do not fully confirm claims made by some workers in the United States that the process is comparable with aqueous anodization. It appears to differ not only in its low-current efficiency but also in the properties of the films prepared, and the independence of their thickness of applied bias voltage.

J. R. BALMER  
J. H. BRUM

## MECHANISM OF CELLULASE ACTION

A ONE-DAY symposium on the mechanism of cellulase action, under the auspices of the Molecular Enzymology Group of the Biochemical Society, was organized by the Shirley Institute on May 28, 1965. Dr. J. Honeyman, who presided, commented that Mr. Selby took this opportunity to arrange a symposium because both Dr. Elwyn T. Reese, from Natick, Massachusetts, and Dr. Bruce A. Stone, from the University of Melbourne, were at present working in the United Kingdom. The Shirley Institute had for several years been active in cellulase research, particularly in work designed to elucidate the mechanism of the biodeterioration of cotton.

K. Selby (Shirley Institute) gave the first paper on the enzymatic degradation of cotton. He stressed that since many 'cellulases' classified by Reese as  $C_6$  can attack soluble cellulose derivatives or swollen cellulose but not native cellulose, the structure of the solid cellulose must govern its susceptibility to attack; if we knew more about the structure we would understand the enzymes better, and vice versa. Although opinion is now tending towards the belief that cotton is entirely composed of crystalline microfibrils without amorphous regions, there exist inhomogeneities in the structure, ranging in size from interfibrillar holes to the layer-structure of the cotton hair, and these may provide sites for enzymatic attack. If cotton could be chemically modified in these accessible regions it might be protected against attack, and this has been done with some success by Mr. Colbran at the Institute by reaction with phenyl isocyanate under non-swelling conditions. Work on the cellulase system of *Myrothecium verrucaria* was reported; exclusion chromatography separated a  $C_6$  from two other components both capable of weakening cotton, and both, like the activity of the crude filtrate, 'exhausted' in the process. The cellulase of *Trichoderma viride* does not suffer from this disability and seems to be a better agent for examining cellulose structure.

In subsequent discussion, J. O. Warwick (Shirley Institute) made it clear that it is still possible to explain the physical and chemical properties of cotton which had previously been attributed to the amorphous part of its structure.

B. A. Stone (University of Melbourne) then gave an account of his work on the purification of the exo- $\beta$ -1,4-glucanase (cellulase) of *Aspergillus niger* and its action on glucans with mixed 1,4- and 1,3-linkages. Elution chromatography removed cellobiase (probably an exo- $\beta$ -1,4-glucanase) and endo- $\beta$ -1,3-glucanase (laminarinase) and the resulting cellulase digested carboxymethylcellulose with a fall in viscosity that demonstrated its chain-cleaving action. Barley glucan was broken down by this cellulase to give some glucose, cellobiose and mixed-link tri- and tetra-saccharides both with 1,4-linkages at the reducing ends. This result confirms that of Perlman and Reese with *Streptomyces* cellulase and should be interpreted in the same way. However, Stone found that the cellulase still contained exo- $\beta$ -1,3-glucanase and that after removing this by adsorption on insoluble laminarin the products of digestion of barley glucan were significantly changed. Glucose was practically absent, but there were present, as stable products, about 20 per cent of penta-

saccharides and higher sugars which, so far as their structures had been determined, all showed the cellobiose grouping at the reducing end. This purified cellulase should be a powerful tool in the investigation of mixed-link glucans.

E. T. Reese (Quartermaster Research and Engineering Center, Natick, Massachusetts) reviewed work on  $\beta$ -glucanases, leaving a vivid impression of the multitude and variety of these enzymes and the complexity of their specificity. In particular, the work of Perlman showed that several endo-glucanases are not specific to the bond being broken but to the nature of the reducing end-unit being liberated. A surprising example of this is that, acting on a glucan with alternate 1,3- and 1,4-linkages, cellulase, producing cellobiose, would attack the 1,3-linkages, and laminarinase, producing laminaribiose, would attack the 1,4-linkages. How far this is true of all endo-glucanases is not clear. The growing class of known exo-glucanases act from the non-reducing end of the glucan; many of them liberate disaccharide molecules, and some have been shown to attack more than one kind of bond. Some exo-glucanases are blocked in their endwise action by substituent groups and branches in the glucan, but a case was given of an exo-1,3-glucanase, from a basidiomycete, which is not stopped by 1,6-branchpoints in a 1,3-glucan.

C. C. Maitland (Shirley Institute) described present work on the cellulase of *Trichoderma viride* and its attack on cotton, which it is able to solubilize entirely. Exclusion chromatography on 'Sephadex' G-75 separates the system into three major components. One component, of low molecular weight, accounts for the greater part of the activity against carboxymethylcellulose but plays no recognizable part in the solubilization of cotton. The two other components are probably identifiable with Reese's  $C_6$  and  $C_1$ , the  $C_6$  having activity against carboxymethylcellulose and cellobiose, while  $C_1$  has little or no such activity but acts synergistically with  $C_6$  in solubilizing cotton. The nature of this synergism, first found by Reese and co-workers but now shown more markedly than before, was discussed. The implied division of labour could be based on the structure of the cotton hair, but this seems unlikely because acid degradation and ballmilling, although destroying the large-scale structure of cotton, do not entirely remove the synergistic effect. It is generally assumed that  $C_1$  initiates the attack on cotton and  $C_6$  follows; this is very likely, but there is no direct evidence for it. However, the small solubilizing power of  $C_1$  acting alone is enhanced when the incubation with cotton takes place on a dialysis membrane; this indicates that an inhibitory product of  $C_1$  action, which  $C_6$  would presumably have removed, is now able to diffuse away.

G. Halliwell (University of Strathclyde) then spoke of his work on cellulases at the Rowett Institute. Rumen bacteria, although capable of extensive breakdown of solid cellulose, do not yield powerfully cellulolytic filtrates, but *Trichoderma* species do both, and the breakdown of fibrous cellulose (cotton) was investigated by using their filtrates. Of particular interest was the observation that an early stage of attack, noticeable by the breakdown of the cotton into insoluble fragments, of which the smallest were capable of passing through a



No. 3 porosity glass sinter, was distinct from the subsequent conversion into reducing sugars. This initial attack was most rapid at a markedly lower pH and might perhaps be caused by a different enzyme.

N. J. King (Forest Products Research Laboratory) described the present state of his work on the cellulase of *Coniophora cerebella*. This is classified as a brown-rot fungus, capable of digesting the polysaccharide of wood and leaving a skeleton structure of lignin. Electron-micrographs showed how this attack was widespread and not confined to the immediate neighbourhood of the fungal hyphae. The widespread nature of the attack was also illustrated by the production, in the early stages of fungal invasion, of more soluble sugars than the fungus can utilize. *Coniophora* has been found to grow easily on cellulose in submerged culture, but cellulase active against compact native cellulose has not yet been obtained. Filtrates contain a cellulase active against swollen cellulose, carboxymethylcellulose, and against cellodextrins,

particularly cellotetraose and higher analogues. This cellulase has been successfully fractionated by elution chromatography on DEAE-cellulose giving three components active against carboxymethylcellulose, which were shown to be distinct by electrophoresis on polyacrylamide gel.

In a general discussion, C. van Bochove (T.N.O., Delft, Netherlands) mentioned his experience of an interesting case of protection of cotton from microbial decay by substitution reaction at the right sites. Methylol-chloroacetamide reacts with cotton and protects it against decay at a degree of substitution of 0.06. If the chlorine is removed by hydrolysis, protection remains; yet the resulting substituent group, applied directly as methylol-glycolamide, gives no protection, presumably because the substitution is not in the right place. J. Dlugosz (Shirley Institute) spoke of the interesting surface features shown by electron-microscopy of cotton attacked by the cellulase of *T. viride*. C. C. MATTLAND

## BINDING OF XENON TO SPERM WHALE MYOGLOBIN

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IN 1946 Lawrence *et al.*<sup>1</sup> and Tobias *et al.*<sup>2</sup> suggested that xenon might be expected to have anaesthetic effects in consequence of its relatively large inducible dipole. In 1951 Cullen and Gross<sup>3</sup> confirmed that xenon was indeed an anaesthetic agent in man<sup>4</sup>. The electron cloud of xenon is, of course, spherically symmetrical, so its narcotic activity cannot depend on any specific structural grouping as had often been supposed<sup>5</sup>, and therefore novel theories of anaesthesia were proposed to explain the action of anaesthetics in general and of xenon in particular<sup>6-8</sup>.

During further investigations of the anaesthetic action and distribution of xenon it became apparent that more xenon is transported by the blood than would be expected from its solubility in the plasma. Solubility experiments then proved that xenon reversibly binds to haemoglobin and myoglobin<sup>9-11</sup>. Since the structures of sperm whale met- and reduced myoglobin have been determined, and nearly all the 1,260 non-hydrogen atoms have been located (J. C. Kendrew and H. C. Watson, to be published), it seemed possible to determine the mode of attachment of xenon to myoglobin without difficulties by the difference Fourier method. Such an X-ray diffraction analysis of crystalline sperm whale myoglobin in equilibrium with xenon has now been carried out. The results prove xenon to be bound to one specific site which is buried in the interior of the molecule and is nearly equidistant from one of the pyrrole rings in the haem group and the ring of the haem-linked histidine. The experimental details and results of the investigation follow.

Sperm whale metmyoglobin, prepared by the method of Parrish and Kendrew<sup>12</sup>, was crystallized from an 80 per cent ammonium sulphate solution at pH 6.8. Crystals were mounted in a special cassette and equilibrated with xenon at 2.5 atm. for 12 h before and during their X-ray exposure. The intensities of the *hk0*, *h0l* and *0kl* reflexions were collected to 2.8 Å resolution on multiple film precession photographs with copper *Kα* radiation from a rotating anode X-ray tube. The exposure times were much longer than usual owing to the strong absorption of the X-rays by the xenon gas in the cassette. The intensities were measured with a semi-automatic microdensitometer; corrected for Lorentz, polarization and temperature factors and then scaled to the native metmyoglobin data.

Difference Fourier projections were calculated using appropriate versions of the general equation:

$$\Delta\rho_{(x,y,z)} = \frac{1}{V} \sum_h \sum_k \sum_l \{ |F_{(x,y,z)}| - |F_g| \} \exp \{ -2\pi i (hx + ky + lz) + i\alpha \}$$

$|F_g|$  and  $|F_{(x,y,z)}|$  represent the moduli of the structure amplitudes of the native myoglobin and its xenon derivative respectively,  $\Delta\rho_{(x,y,z)}$  the difference in electron density between the two compounds. The phase angles used were those for native sperm whale myoglobin, as determined by multiple isomorphous replacement<sup>13</sup>.

The use of a set of only approximate phases in a difference Fourier synthesis results in reduced peak heights for non-centrosymmetric projections. Luzzati<sup>14</sup> showed that the peak height of an atom not included in the phase determination depends on the ratio of the excluded electrons to the included ones. In this case, where the excluded electron density is small, the theoretical expected reduction of peak heights in non-centrosymmetric sections is of the order of 40 per cent and agrees well with the observed results.

Each of the difference electron density maps of the *hk0*, *h0l* and *0kl* projections (Fig. 1) shows only one, nearly circular, peak corresponding to a spherical atom with co-ordinates  $x = 0.177$ ,  $y = 0.864$ ,  $z = 0.168$ . The remaining areas of the maps are relatively featureless, indicating that the degree of isomorphism is high and that xenon atoms are not present at subsidiary sites to any appreciable degree.

The xenon atom is nearly equidistant from the haem-linked histidine and a pyrrole ring of the haem group; it is in contact with all the atoms in both rings. The neighbourhood of this binding site is shown in Fig. 2a and b, and the relevant approach distances are tabulated in Table 1.

The xenon atom in the myoglobin interior lies between a non-polar area and an area which is partially polar, indeed charged. This suggests that this complex is stabilized by charge-induced dipole moments, by dipole-induced dipole moments and by London interactions. In addition, there is an entropy gain due to the transfer of the xenon from the aqueous surface into the interior of the protein. Unfortunately the charge distribution on the



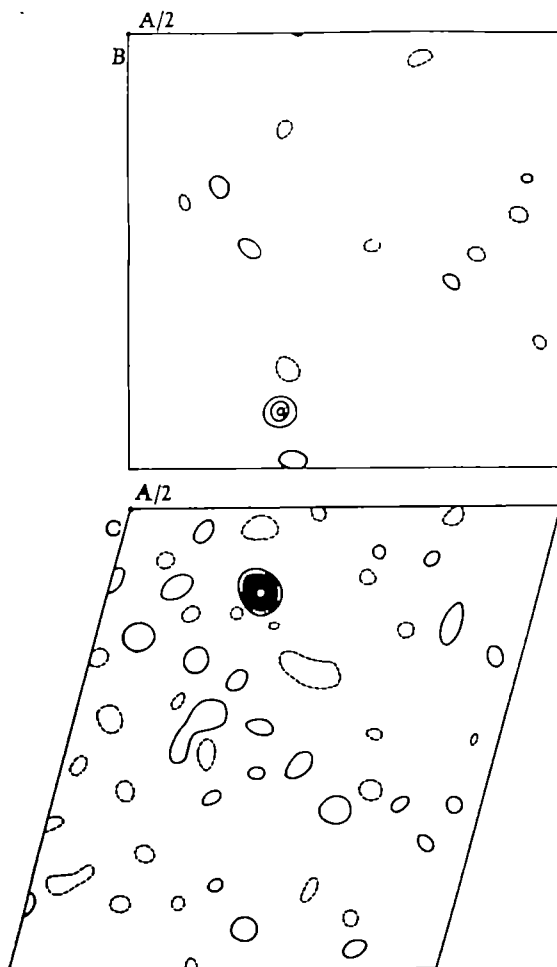


Fig. 1. a, 400 difference electron density map; b, 400 difference electron density map

haem group and the sizes of the various dipoles are still unknown; it is therefore not possible to calculate the degree of polarization and hence the charge-induced dipole and the dipole-induced dipole binding energy of the xenon atom. The entropy gain, which arises from the xenon transfer, could be estimated if the temperature-dependent solubilities of the gas in ammonium sulphate and in a non-polar solvent were known<sup>18</sup>.

Table 1		
Group	Atom	Distance < 5.5 Å
Leucine F4	O (carbonyl)	4.7
	O (carbonyl)	4.6
	Ca	4.9
	Ca	4.4
Alanine F5	N	5.1
	O	4.3
Histidine F8	Oy	3.7
	O	4.1
	N	4.0
	O	3.5
Leucine G5	N	3.3
	Oy	4.6
	Ca1	4.3
	Oy2	5.1
Phenylalanine H14	Ca1	4.7
	O	3.9
	Ca2	4.5
Isoleucine H18	Oy1	5.0
	Oy2	4.6
	O	3.3
Haem (Fig. 3)	Fe	5.3
	NVB	4.4
	OVVB	4.1
	OD	4.6
	O4VL	5.4
	OR	5.1
	O4VR	4.4
	O3VR	4.8
	O2VR	3.9
	O4VR	4.3
	OVVR	4.7
	OVVR	5.4

The size of the London interaction energy can, however, be calculated from the electronic dispersion interaction between molecules<sup>19</sup>. This interaction is given by

$$W = \sum_j \frac{3}{2} \frac{P_{ss} P_j E_{ss} E_j}{r_{ss,j}^6 (E_{ss} + E_j)}$$

$P$  = polarizabilities,  $E$  = electronic excitation,  $r_{ss,j}$  = distance between xenon and  $j$ th atom. Taking the excitation value for xenon to be 12 eV, the average excitation value for its neighbours to be 13 eV, and the polarizabilities to be 4 Å<sup>3</sup> and 2 Å<sup>3</sup> respectively, we obtain an interaction

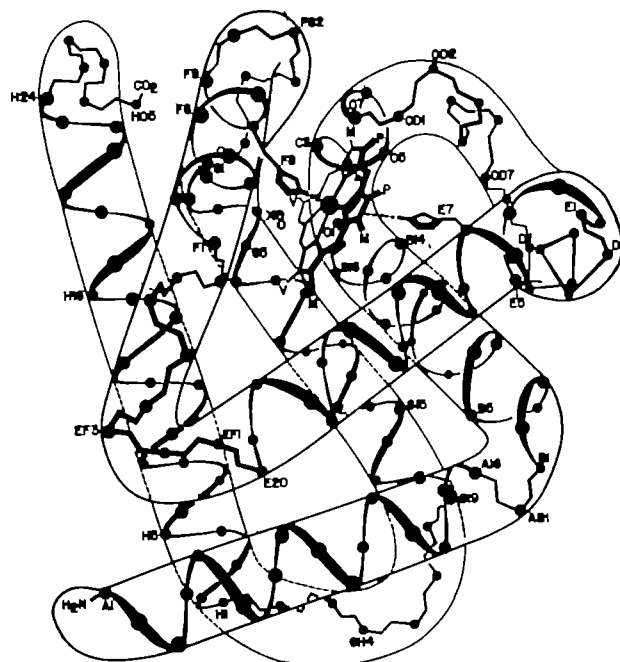


Fig. 2a. Location of xenon site in myoglobin

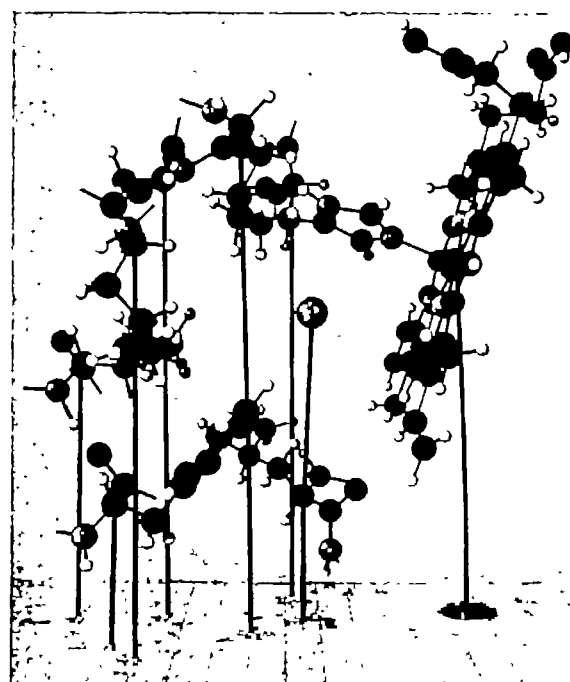


Fig. 2b. Model of structure in the region of the xenon atom. The haem group is to the right almost edge on, and the haem-linked histidine can be seen almost above the xenon atom. The other atoms shown are parts of the main chain of helices F (top left) and H (below)

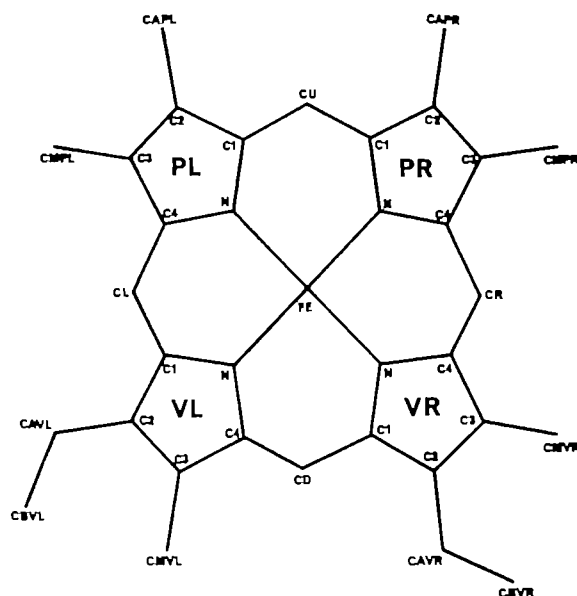


Fig. 3. Nomenclature of atoms in haem group (Table 1)

energy of about 10.2 kcal. This figure for the total energy is a minimum: in practice it will be increased by the other energy terms which have been mentioned here.

It should be pointed out that this is not a hydrophobic stabilization (hydrophobic stabilization occurs when two or more non-polar groups associate to decrease the interaction with water); there is no indication in the 1.4 Å three-dimensional Fourier synthesis of the native protein, under normal conditions, that a water molecule occupies the xenon site.

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## EVIDENCE FOR THE ROLE OF BOUND WATER AND PARTIAL DESICCATION IN CARCINOGENESIS

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RELATIVE humidity (*RH*), temperature and chemical additives have all been shown to determine the response of a microbial cell to drying in air<sup>1-3</sup>. Experimentation has pointed to structural changes in essential macromolecules resulting from loss of bound water as being responsible for cell death. This bound water can be replaced by certain chemicals during drying which are more stable than water and maintain the structural and functional integrity of the macromolecules<sup>4,5</sup>. Inositol (hexahydroxy cyclohexane) is the best stabilizing agent of many chemicals tested for protective ability against desiccation damage. This is assumed to be due to its structural similarities with water, its symmetry, its non-volatility and its lack of toxicity.

In metabolic experiments many enzyme changes were seen in desiccated cells whether protected with inositol or not. However, inositol did preserve the protein synthesizing ability of dried cells<sup>6,7</sup>, thus suggesting its interaction with ribonucleic acid (RNA). Further evidence for interaction with RNA was obtained when it was shown that Rous sarcoma virus (RSV) could be protected against desiccation death by inositol<sup>7</sup>.

Recently, studies on the effects of ultra-violet light, visible light and X-rays on survival of bacteria and viruses have produced added evidence for the important role of bound water in biological integrity<sup>8-11</sup>. It has become apparent that changes in molecular bound water are involved in radiation damage and can be prevented by inositol in carefully controlled experiments.

Finally, two other phenomena associated with the desiccation of bacteria were deemed important and stimulated further work with animal cells. One was the observation that RNA was released in greater amounts from cells protected with inositol than from unprotected cells. The other was that mutant cells appeared more frequently after desiccation with or without radiation treatment at certain *RH* levels<sup>12,13,14</sup>. When the work was extended to animal cells it became obvious that controlled desiccation in aerosols as employed with bacteria and viruses was not possible with the equipment available, due to mechanical destruction of the cells. However, a variety of cells held in suspension for varying lengths of time were protected against death by inositol<sup>14</sup>. Virus-infected cells released increased amounts of virus in inositol, and drying on Petri dishes in the presence of inositol resulted in increased virus release compared with cells dried from balanced salt solutions (BSS)<sup>15,16</sup>.

Quite independently of the work described here, Falk *et al.*<sup>17</sup> have published data showing that removal of bound water from deoxyribonucleic acid (DNA) results in the loss of structural integrity. This bound water begins to be removed as the *RH* is lowered below 70 per cent and about 5 molecules per nucleotide are removed when the *RH* reaches about 55 per cent. Still further water is removed below this level. A similar situation may exist for RNA and protein molecules, and Klotz<sup>18</sup> has discussed the importance of the hydration sheath as an ice-like lattice in fixing the structure and properties of protein

and nucleoprotein macromolecules. Warner<sup>17</sup>, using the stereochemical properties of inositol, has shown that these compounds can interact with protein layers and probably other biological macromolecules in the same way as water, but afford a much more stable system than water because of the rigidity of the hydroxyl positions in inositols. Thus the idea that bound water is intimately concerned with maintaining the structural, and thereby the functional, integrity of biological macromolecules is becoming increasingly recognized.

To discover whether tumour viruses other than RSV could be released in infectious form by drying the tumour cells an experiment was performed with Ehrlich ascites cells. This tumour is known to carry various murine leukaemia viruses<sup>18-20</sup>. Ehrlich cells were obtained from routinely transplanted Swiss mice, washed once in 5 per cent inositol solution and re-suspended in 5 per cent inositol to give about  $10^7$  cells/ml. 1-ml. aliquots were pipetted on to 60-mm Petri dishes and the cells were allowed to settle for 1 h at 37° C. After decanting the supernatant the cells were dried in a 'Blue M Vapor Temp' humidifier cabinet for 30 min at RH 40 per cent at room temperature. After drying, the cells were gently scraped off the glass into equivalent amounts of 5 per cent inositol solution, and centrifuged. The supernatant was filtered through a 'Millipore' 450-mμ filter to remove all cells and the filtrate was inoculated into 8-week-old female Swiss mice (0.2 ml. intraperitoneally). The cells were washed in 5 per cent inositol, re-suspended and inoculated into a similar group.

Some of those given the filtrate died with acute leukaemia beginning about 5 months after inoculation. At 12 months, when all survivors were killed, the incidence of leukaemia was as shown in Table 1. None of the animals developed ascites tumours, and since only 10 Ehrlich cells are required to produce tumours in 50 per cent of our Swiss mice this must indicate almost complete cell destruction.

However, all but one of the mice given the dried cell filtrate developed leukaemia and about half of those given cellular material did so. Most of the leukaemias were lymphoblastic in type with large thymus tumours as well as lymph node, liver, kidney and spleen involvement. Some of those developing later and discovered at autopsy appeared to be mixed lymphoblastic-lymphocytic neoplasms with cells characteristic of both types. Three of the leukaemias have so far been transplanted by both cells and cell-free filtrate.

The above experiment, along with the results already reported for RSV<sup>13</sup>, shows that some tumour viruses are released in highly infectious form by drying the host cells. The presence of inositol, at least in the case of RSV, results in increased amounts of infectivity either by protection of virus against desiccation death or by some other mechanism.

As already discussed, evidence has been obtained for a mutational effect of desiccation on bacteria<sup>10,13,16,21</sup>. In conjunction with the demonstrations of increased virus release and survival it was felt that experiments with dried normal cells should be undertaken. Initially, pooled spleen cells were obtained from 1-year-old Swiss mice by gentle homogenization and straining through 4 layers of sterile gauze. After centrifuging, half the cells were re-suspended in BSS and the other half in 5 per cent inositol solution. Approximately  $2 \times 10^7$  cells were pipetted on to 60-mm Petri dishes and allowed to settle

Table 2. EFFECT OF INOCULATING DRIED SPLEEN CELLS INTO SWISS MICE (EXPERIMENT 1) AND SUBSEQUENT BLIND TRANSFER OF TUMOURS AT THE HEIGHT OF 'PLASMA CELL REACTION' WITHOUT DRYING (EXPERIMENT 2)

Experiment	No. of animals	Percentage animals with tumour	Total No. tumours
1. Controls	36	11	5 (3L, 2MO)*
Non-dried cells	24	8	4 (2L, 2MO)
Dried cells	24	43	14 (7L, 7MO)
2. First passage	24	36	18 (9L, 9MO)
Second passage	12	42	6 (5L, 1MO)
Third passage	18	56	10 (10L)

\* Figures in brackets indicate number of leukaemias (L) and mammary carcinomas (MO).

for 2-3 h at 37° C. The supernatant fluid was removed and the cells were dried in the humidifier cabinet for 30 min at RH 70 per cent. The dried cells were re-suspended in BSS or 5 per cent inositol for inoculation and samples of non-dried cells were saved for inoculating control groups. Groups of 8-week-old female Swiss mice were inoculated intraperitoneally with about  $5 \times 10^5$  cells, and in addition to the non-dried cell controls other groups were given BSS or 5 per cent inositol solution. The experiment was allowed to run for 12 months after which time all the survivors were killed and autopsied. Sections were prepared from all animals not autolysed. Since little or no difference in tumour incidence appeared between the groups treated with cells dried in BSS and those treated with cells dried in inositol, the groups were combined for simplicity of presentation in Table 2 (Exp. 1). Two animals died during the experiment, one with lymphoblastic leukaemia and one with mammary carcinoma. Both of these were found at autopsy and confirmed histologically. It is apparent that the inoculation of dried spleen cells resulted in an increased incidence of tumours. The final column shows the total number of tumours scored. Some animals developed both leukaemia and mammary carcinoma and both conditions appeared in greater numbers in the group given dried cells.

All the leukaemias appearing in these animals were diagnosed either as lymphoblastic, involving the thymus, lymph nodes, liver, kidney and, usually the spleen, or as mixed lymphoblastic-lymphocytic in which similar but less extensive organ involvement was observed. It has already been mentioned that most of these tumours were found at autopsy 12 months after inoculation of the spleen cells. Attempts to reduce the latent period of leukaemia by transplantation of homogenates of affected organs have not so far been successful. However, it was noted in many instances that the mice receiving the dried spleen cell preparations developed a strong plasma cell reaction with abdominal ascites formation within 4 weeks after inoculation. This occurred in both adult and new-born mice whether the cells were given intraperitoneally or subcutaneously, and was usually accompanied by splenic enlargement. The reaction subsided over the following 2-3 weeks, but marked plasma cell infiltration of lymph nodes, liver, kidney and spleen persisted until the animals were killed at the end of the experiment. Close inspection of the plasma cells in the ascites fluid of many such animals failed to reveal signs of malignancy, but pooled fluid from 2 mice at the height of the reaction was nevertheless transferred to another group of 8-week-old mice along with homogenized spleen and liver from the same animals to make a 1:10 cell:fluid suspension. All the recipient animals developed marked ascites and splenomegaly within 3 weeks and, again, fluid plus spleen and liver cells was transferred to a third group. All animals again developed marked reactions similar to those seen in the previous passages. No evidence of bacterial contamination and transfer was found. The survivors were left for 12 months to see whether the incidence of tumours was altered by the serial transplantation of these cells. The results are presented as Exp. II in Table 2. It can be seen that the incidence of tumours increased at each passage. Again the final column indicates that some animals developed both leukaemia and mammary car-

Table 1. INCIDENCE OF LEUKAEMIAS IN SWISS MICE INOCULATED WITH DRIED EHRlich CELLS OR 450-mμ FILTRATE. CELLS DRIED AT RH 40 PER CENT FOR 30 MIN FROM 5 PER CENT INOSITOL

Group	No. animals	Percentage with leukaemia
Controls		8-17*
Washed cells	16	44
Filtrate	14	93

\* Range of leukaemia incidence among similarly sized groups of untreated Swiss mice.

Table 3. INCIDENCE OF TUMOURS IN SWISS MICE INOCULATED WITH NEWBORN WITH DRIED OR NON-DRIED THYMUS CELLS

Group	No. animals	Percentage animals with tumours	Total No. tumours
Non-dried	15	26	4 (2L, 2MO)*
Dried RH 60 per cent	17	24	5 (4L, 1MO)
Dried RH 40 per cent	18	60	9 (6L, 2MO, 1OO)
Dried RH 30 per cent	9	0	0

\* Figures in brackets indicate number of leukaemias (L), mammary carcinomas (MO) or ovarian carcinomas (OO).

cinoma. It is interesting to note that a high incidence of mammary tumours was only observed in the group inoculated with dried cells and that most of the increased tumour incidence in subsequent passages was due to leukaemia. It should also be noted that the previous experiment also showed high mammary tumour incidence in the group given dried cells. As before, most of the tumours were found at autopsy and did not kill the animals within the 12-month time limit of the experiment. Only 2 mice died with tumours during the experiment, one from the second passage at 19 weeks and one from the third passage at 33 weeks. Both of these were diagnosed as lymphoblastic leukaemias.

Since most of the leukaemias developing in these mice were of the lymphoblastic type an experiment was undertaken in which thymus cells from apparently normal 2-month-old Swiss mice were dried before inoculation into new-born mice. In view of the accumulated data from bacterial studies, which suggested that maximum biological changes when drying were found at low RH levels, 3 groups of new-born mice were inoculated with pooled thymus cells dried for 30 min at RH 60, 40 and 30 per cent, respectively, in the absence of inositol. The cells were prepared as before by gentle homogenization and straining through 4 layers of sterile gauze. Drying was carried out in Petri dishes with approximately  $10^7$  cells in the chemical drying chamber, and controls were inoculated with untreated cells from the same pool. Again the survivors were killed after 12 months and the final results are presented in Table 3.

It is apparent from the final column of Table 3 that the incidence of leukaemia in the groups given cells dried at RH 40 and 60 per cent was higher than in the controls. None of the animals given cells dried at RH 30 per cent developed tumours, which suggests that all biological activity had been destroyed. This is not surprising in view of previous results with bacteria and viruses. That activity survived drying to RH 40 per cent might appear surprising, but may be explained by the lack of precise humidity control that can be more easily achieved with bacterial aerosols. Another important feature of these results is that 4 of the 7 animals which developed tumours with 40 per cent dried cells did so before the 12-month

time limit. The earliest (19 weeks) developed an ovarian carcinoma as well as lymphoblastic leukaemia, the former being an extremely rare tumour in our colony. It is also interesting to note that the inoculation of thymus material did not result in a high incidence of mammary carcinoma.

Two biological phenomena associated with desiccation, namely, changes in function (mutation) and release of infectious viruses, led to the idea that carcinogenesis might be the occasional result of such changes in animal cells. There seems no doubt that in the preliminary experiments so far undertaken, the removal of molecular bound water from normal cells can result in the production of carcinogenic potentialities. Thus desiccation may be classed with carcinogenic chemicals, radiation and other tumorigenic agents, and the concept furthermore provides an attractive basis for a more general hypothesis of the mechanism of carcinogenesis. The removal of structurally important water could be accomplished by chemical means or by radiation. Local dehydration could also result from the implantation of plastics and other chemically inert materials. Tissue desiccation in old age could conceivably result in bonded water exchange with the environment, thus favouring the conditions necessary for a critical water loss. The macromolecular structural changes occurring in cells under these conditions result in marked changes in biological function, including mutation, virus release and death. Carcinogenesis could occur as a result of the production of viable mutated cells or, more probably in view of the apparent destruction of Ehrlich ascites cells, as a result of virus release or the appearance of altered, genetically competent macromolecules.

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## ACTION OF ACETYLCHOLINE ON COCHLEAR RESPONSES

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RECENT work, using the electron microscope, has shown that cochlear hair cells are synaptically connected with nerve endings, of which two different types have been demonstrated by Smith and Sjöstrand<sup>1</sup> and by Engström and Wersäll<sup>2</sup>. In 1959, Schuknecht *et al.*<sup>3</sup> investigated the localization of acetylcholinesterase in the cochlea of the cat and suggested that efferent nerve fibres in the olivo-cochlear bundle had high acetylcholinesterase activity. Hildings and Wersäll<sup>4</sup> reached a similar conclusion. Galambos<sup>5</sup> has shown that electrical stimulation of crossed efferent fibres in the olivo-cochlear

bundle diminishes the action potentials of the whole cochlear nerve, and Fex<sup>6</sup> has reported that the cochlear microphonics increase in amplitude. In a pharmacological investigation, however, Deamett and Monaco<sup>7</sup> have obtained results showing that strychnine and brucine can suppress these centrifugal effects of electrical stimulation in the olivo-cochlear bundle; but anticholinesterase cannot suppress them. This suggests that a kind of inhibitory transmitter substance, which is ineffective in the presence of strychnine, plays an important role in a feedback mechanism in the cochlea. Thus, there are some complexities in considering neurophysiological results on the basis of histochemical findings on the organ of Corti.

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In order to clarify such divergent experimental results, pharmacological agents were applied ionophoretically to the vicinity of cochlear hair cells. Eighty-nine guinea-pigs were used, and responses in the primary auditory neurones and the electrical stimulation of efferent nerve fibres were obtained from 22 cats.

The cochlear microphonics (CM) and the neural component  $N_1$  were recorded by means of vestibulo-tympanic leads from the basal turn of the cochlea. In recording single-fibre responses in cat, the flocculus of the cerebellum was retracted dorso-medially, and a capillary ultramicro-electrode was inserted into the cochlear nerve at its entrance into the internal auditory meatus.

Simultaneously with the recording of cochlear responses to sound, a capillary electrode with a tip diameter of  $2\mu$  was inserted into the hair cell region through the round window. The negative resting potential in the organ of Corti was considered as indicating penetration of the basilar membrane. Ionized chemical agents contained in the capillary were administered ionophoretically in the vicinity of the hair cells by means of a  $10^{-6}$  amp electric current in the form of a square wave, 500 msec in duration, applied at a frequency of once per sec for 8–10 min. The efferent nerve fibres were stimulated by electrodes placed in the floor of the fourth ventricle after removing the cerebellar vermis by suction.

The application of inorganic ions such as sodium, potassium and chlorine had no effect on the CM and  $N_1$  responses. Fig. 1 indicates that the CM and  $N_1$  were not influenced by potassium ions in  $8 \times 10^{-6}$  amp current. In contrast with the results for inorganic ions, acetylcholine (ACh) or prostigmine elicited gross changes in the cochlear responses. The CM decreased abruptly in amplitude during or shortly after ACh administration using the same amount of anodic current used for ionophoresis of the potassium ions, as shown in Fig. 1. However, separate administration of ACh or prostigmine diminished the amplitude of the CM to less than 50 per cent, usually within 20 min after the beginning of iono-

phoresis. Other drugs have been similarly tested but with negative results. Application of  $\gamma$ -aminobutyric acid (GABA), which is known to inhibit excitatory synapses in the cat brain, had no effect on this preparation; normal CM and  $N_1$  responses were maintained for as long as 120 min after applying the ionic current. Administration of strychnine produced no consistent change in the CM and  $N_1$  responses, apart from its effect in the olivo-cochlear inhibition as shown by Desmedt and Monaco. Local applications of adrenaline and atropine had no effect on the cochlear responses.

Since change in the  $N_1$  response due to ACh was not as obvious as that in the CM response, it was necessary to observe unitary response of the primary auditory neurones. The firing rate of fibres with characteristic frequencies above 5,000 c/s began to decrease first during the administration of ACh. This effect tended to extend gradually toward the low-tone frequency range, probably due to the diffusion of the ACh over the basilar membrane.

Other studies demonstrate that the action of ACh is blocked by curarization. As shown in Fig. 2, the applica-

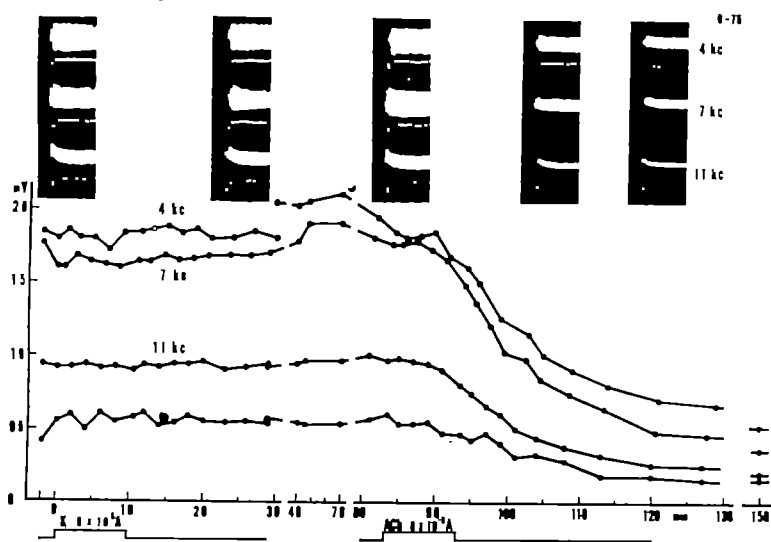


Fig. 1. Effect of ionophoretic application of acetylcholine on cochlear responses (administrations of potassium and acetylcholine have been combined). Photographs illustrate the cochlear microphonics and neural component  $N_1$  responses recorded from the basal turn of guinea-pig. Potassium ions were applied with electric current,  $8 \times 10^{-6}$  amp from 0 to 10 min, and acetylcholine, 85 to 95 min. Abscissa, time in min. Ordinate, amplitude of the cochlear microphonics response (open circles—4, 7 and 11 kc) and the  $N_1$  response (filled circles) in millivolts.

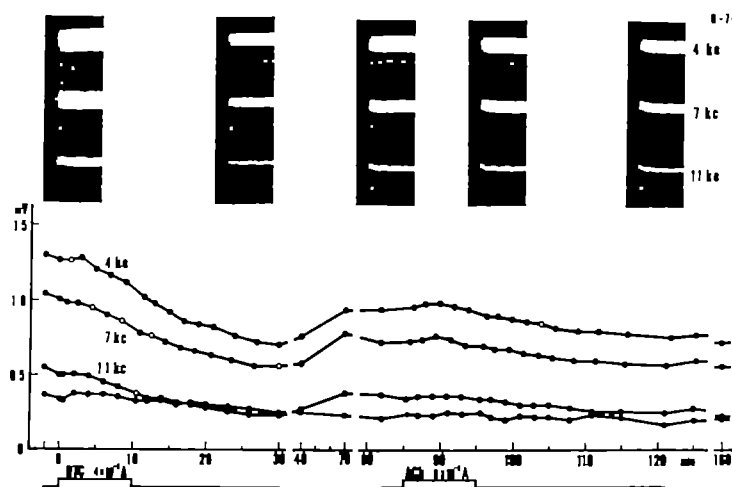


Fig. 2. Effect of acetylcholine application to curarized 'organ of Corti'.  $d$ -Tubocurarine was applied with current  $4 \times 10^{-6}$  amp from 0 to 10 min, and acetylcholine with  $8 \times 10^{-6}$  amp, 85 to 95 min. Cochlear microphonics to 4, 7 and 11 kc pure tone stimuli (open circles),  $N_1$  (filled circles).

tion of  $d$ -tubocurarine in  $4 \times 10^{-6}$  amp of current causes a gradual reversible decrease of the CM and  $N_1$  responses. When ACh was applied during this recovery the cochlear responses were only very slightly depressed even with twice the amount of ionic current that was used with  $d$ -tubocurarine. It is very significant, as compared with change of the CM and  $N_1$  responses to the ACh phoresis in Fig. 1, that ACh fails to diminish cochlear responses after administration of  $d$ -tubocurarine. Although Eccles *et al.*<sup>6</sup> have reported that intravenous injection of dihydro- $\beta$ -erythroidine (DHE) reduces the sensitivity of the Renshaw cell to ACh, 1–5 mg/kg of the DHE, enough to relax the respiratory muscles, did not influence the ACh effect on the cochlear responses.

Electrical stimulation of the crossed olivo-cochlear bundle inhibits neural response in the cochlea and augments the CM. This inhibitory effect is suppressed by intravenous injection of strychnine, in accordance with the results described here. When  $d$ -tubocurarine was applied to the hair cell region the amplitude of the  $N_1$  response was still reduced by as much as 15 dB attenuation of the

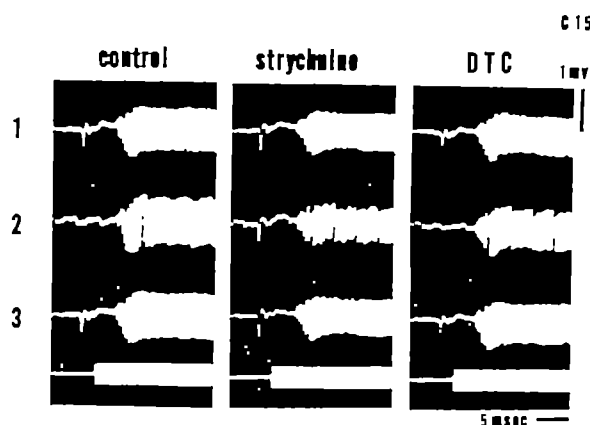


Fig. 3. Comparison of effects of strychnine and *d*-tubocurarine on olivo-cochlear inhibition. These photographs illustrate the  $N_1$  responses to sound (click, 40 dB) and the cochlear microphonic responses (3.5 to pure tone, 40 dB) recorded from the left round window in cat. 2. Electrical stimulation on the olivo-cochlear bundle at the floor of fourth ventricle, the bundle was stimulated with square wave 0.1 msec at 200/sec. Strychnine was applied intravenously and *d*-tubocurarine, ionophoretically in  $8 \times 10^{-4}$  amp current. 1, Before, and 2, 2 sec after the electrical stimulation.

tone stimuli by electrical shocks to the efferent fibres, as shown in Fig. 3.

It has not previously been recognized that the administration of ACh in the cochlea produces any effect there. In this experiment various ionized chemical agents were applied ionophoretically to the hair cell region; only ACh and anticholinesterase were effective. This is considered in relation to histochemical findings showing that the organ of Corti has intense cholinesterase activity, as reported by Schuknecht and Wersäll.

The effect of ACh on the nerve ending is generally known to be excitatory, in other words ACh depolarizes the membrane of the postsynaptic ending. But there has so far been no sign of excitatory effect there in the case of recording the responses of the single primary neurone to sound stimulation. It is therefore supposed that a part of the hair cell membrane is sensitive to ACh, since anticholinesterase acts like ACh to decrease the CM, and the ACh effect is prevented by curarization. Intravenous injection of DHE did not block the ACh effect. If cochlear potentials are cholinergically controlled, it would be expected that the ACh effect on the cochlear responses would be changed somewhat by systematic injection of DHE, as occurs in the central nervous system. It is imagined that the cochlea has a different blood barrier to DHE, but there is no evidence of it in this experiment. The ionophoretic application of *d*-tubocurarine did not influence the inhibition of the  $N_1$  response elicited by electrical stimulation of the crossed olivo-cochlear bundle. Recent experimental results show that strychnine suppresses the inhibitory action of both crossed and uncrossed efferent nerve bundles.

Consequently the results permit the conclusion that the efferent nerve fibres to the cochlea are not cholinergic, though acetylcholine depresses the activity of hair cells to a remarkable extent.

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## ELECTROPHYSIOLOGICAL EVIDENCE FOR OLFACTORY FUNCTION IN BIRDS

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IN describing the well-developed olfactory organs of some birds, Bang<sup>1</sup> criticizes the tendency to perform olfactory learning investigations on "feebly equipped" or "poorly endowed" birds such as pigeons, and justifiably concludes that these prove nothing about birds with "superior receptors". Concerning the turkey vulture, oilbird and albatross, she writes, "It is apparent that no definitive controlled experiments on the function or sensitivity of the chemoreceptors have been done".

It is now possible to record electrically the impulse traffic in the axons which comprise the primary olfactory nerve. The dorsal space between the eyes of most adult birds is occupied by spongy bone which is easily removed to leave a cavity bounded by orbital walls of thin bone, through which one can see the olfactory nerves. In the nighthawk (a goat-sucker) the posterior half of each nerve traverses the cavity, sheathed in a thin tube of transparent bone. After closing off connexions to the air spaces with bone wax and exposing a nerve, Ringer's solution is introduced to facilitate the neural twig dissection. The method is similar to that described for the gopher tortoise (*Gopherus polyphemus*)<sup>2</sup>. Although it is not really difficult to free small, live twigs of the bird's olfactory nerve, at first I found it difficult to perform the dissection without more stretching than is tolerable for some other animals. I discovered that useful results may be obtained by lifting the whole nerve up on the recording electrodes. Thus, it seemed sensible to search for a bird so feebly

equipped that its nerve bundle would fall in the desired diameter range of 10–100 $\mu$ . However, I have seen no olfactory nerves nearly so small in birds, but the nerve twigs seem to be unusually resistant to the effects of stretching, unlike rabbit olfactory nerve, for example. There is often a curious delay of several minutes before a preparation exhibits responsiveness, but once it develops the preparation is usually stable for many hours. This type of stability is distinct from the occasional appearance of a phenomenon which appears to reflect the reflex changing of accessibility to the olfactory organ. Table 1 lists species of birds in which successful olfactory receptor preparations were made.

We have recorded responses to odorants in various species of mammals, amphibians and reptiles as well as birds. The lack of obvious species differences has impressed us, since in the examination of taste with similar methods, species differences are readily apparent<sup>3,4</sup>. In these methods the neural electrical activity is continuously averaged with an integrator circuit of relatively short time constant. Thus, the magnitude of response is recorded directly, in deflexion units proportional to the number of nerve impulses per unit time. The olfactory responses occur in bursts synchronized with inspirations, but normally there is never a complete absence of neural activity, even with the cleanest air that we have been able to prepare. Such response records are shown in Fig. 1 for the sparrow hawk, goose, black vulture, chicken and, for

Table 1. BIRDS FROM WHICH OLFACTORY RECORDS WERE OBTAINED

Common name	Specific name	Body-wt.
Blue jay	<i>Cyanocitta cristata</i>	53-70 g
Common nighthawk	<i>Chordeiles minor</i>	80
Yellowthroat (warbler)	<i>Geothlypis trichas</i>	8-8
House sparrow	<i>Passer domesticus</i>	22
Ring-billed gull	<i>Larus delawarensis</i>	440
Chicken (white leghorn)	<i>Gallus gallus</i>	330-930
Sparrow hawk	<i>Falco sparsarius</i>	100
Homing pigeon	<i>Columba Hous</i>	360
Bob-white quail	<i>Coturnix virginianus</i>	170
Emden goose	<i>Anser anser</i>	4,100
Black vulture	<i>Coragyps atratus</i>	1,900-2,200
Turkey vulture	<i>Cathartes aura</i>	1,900
Muscovy duck	<i>Cairina moschata</i>	1,700-2,900
Common crow	<i>Corvus brachyrhynchos</i>	870-900

Olfactory records were obtained from these birds, listed chronologically, by the first specimen where a group is indicated by a range in weight.

comparison, rat. The chicken and goose might have been inspiring quickly enough to draw some room air into the breathing chamber, so the high background activity might be artefactual. Because the olfactory response is a function of the inspiratory flow rate, as well as of the kind of odorant and its concentration, the records reflect differences in inspiration and in the time of the respiratory cycle when the stimulus was exchanged for the clean air flowing through the breathing chamber. A few movement artefacts are evident in the first and last traces. Apart from the effect of differences in respiratory rhythm, the results were quantitatively similar for the two higher log units of concentration not shown in the figure. The olfactory receptors adapt considerably and rapidly at the highest concentration of amyl acetate.

The evidence indicates that the olfactory response as recorded from a small population of the receptors is quantitatively similar for diverse birds and other air-breathing animals. Birds were tested with various odorants without control of the stimulus concentration, as was done for amyl acetate, and in no instance was the nasal flow rate controlled, but the results were remarkably similar among the species. We appreciate the anatomical descriptions by Bang and recognize that the foregoing quotations, which imply superiority of the receptors in some species, might have been meant to apply collectively, for the organ. But the undoubted differences in olfactory performance of animals seem likely to be correlated with the extent of development of the olfactory apparatus, as Bang emphasized. However, the 8-g warbler might be the

olfactory equal of the 4,000-g goose (Table 1); thus a relative measure such as the ratio of bulb to cerebral hemisphere diameters used by Cobb<sup>8</sup> is probably pertinent.

Why the possession of a greater number of olfactory receptors should confer on an organism a heightened olfactory faculty, as is often implied, is not at all clear, since a relatively small number should be sufficient, in theory. Neuhaus<sup>9</sup> considered the sensitivity aspect of this question *vis-à-vis* the possibility of major differences in the sensitivity of receptors of related species. His discussion, however, rests heavily on the proposition that dogs have a much lower concentration threshold for butyric acid than do rats or humans, and not many more receptors. The influence of size and of three-dimensional folding and pocketing of the receptor sheet on quality discrimination and sensitivity was discussed by Adrian<sup>7</sup>: "We can see therefore why the dog should have a much larger and more elaborate organ than the rabbit. There is no reason why a large organ should be more sensitive to smells than a small one, but it will certainly provide a screen on which the pattern of excitation can be mapped in greater detail". His 'sensitivity' of the organ is logically not the same as the behavioural 'sensitivity' of Neuhaus, although it is the basis of it. Later, Adrian<sup>8</sup> emphasized the importance of differential receptor sensitivity for olfactory quality discrimination, but still maintained the likelihood that an animal obtains useful information from differences in the areal and temporal distribution of excitation of the organ by different odours. However, we are led to the idea that the olfactory stimulus is attenuated as it penetrates to the more inaccessible locations of the organ. Suppose that the electrophysiologist leads signals from a sample of receptors that includes some of a group most responsive to the odorant being used and that he controls the stimulus conditions enough to deduce the effective stimulus at those receptor sites<sup>8</sup>; the receptor sensitivity function determined in this way should constitute a limit for behavioural performance. Although the data shown in Fig. 1 do not yield this information, it is interesting that the differences are relatively so small among the species. The one that is obviously different correlates with the greater nasal complexity of the rat, though the relative stimulus attenuation in this instance is only about one half log unit, or a factor of three.

Neuhaus's<sup>9</sup> training experiments with various birds were fruitless. However, he succeeded in demonstrating changes in respiration of the goose on the presentation of odour. The possibility of trigeminal activation was considered. Although we have not yet studied trigeminal responses in birds, the threshold concentration of amyl acetate for the trigeminal receptors of the gopher tortoise and rabbit is near the relatively high value of 10 per cent saturation at 20° C. As Adrian<sup>8</sup> noted, rabbits under urethane anaesthesia seldom change their respiration with odours little above olfactory threshold values. In contrast to the mammals, birds and reptiles often exhibit reflex changes on breathing amyl acetate at concentrations lower than the trigeminal threshold cited here. The records in Fig. 1 show respiratory acceleration of the sparrow hawk and chicken at 1 per cent or less of saturation. It is notable that these reflexes persist in the anaesthetic state. Thus, we can conclude with Neuhaus that there probably exist innate reflexes mediated by the olfactory apparatus.

Reports vary as to the olfactory learning ability of birds<sup>10-12</sup>. I am convinced that the olfactory systems of birds are functional and, further, I emphasize how difficult it is to imagine that such functionality might

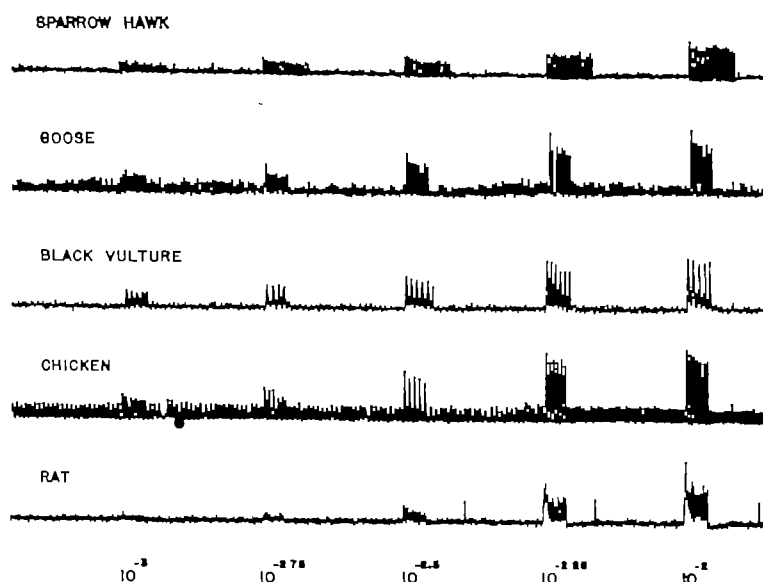


Fig. 1. Primary olfactory nerve responses to amyl acetate breathed in air at concentrations expressed as exponential fractions of vapour saturation at 20° C. Odour was presented every 3 min for durations of 1 min in the first trace and 0.5 min in the others. Records were processed with a Sanborn model '350-1,400' amplifier on the AO LINEAR mode of operation, for which the output indication is 'full wave average'.



persist without biological significance. That significance may be unlike our own subjective sensation of smells, because of the vast differences in mammalian and avian nervous systems, as Cobb and Neuhaus stressed. Some of the avian experimental preparations seem especially well-suited to the experimental needs of the electrophysiologists (see also Stager's<sup>14</sup> dissertation on the role of olfaction in food location by the turkey vulture).

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## AN UNEXPECTED ARTEFACT IN THE HUMAN ELECTROENCEPHALOGRAM CONCERNING THE ALPHA RHYTHM AND THE ORIENTATION OF THE EYES

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THE alpha component of the human electroencephalogram is one of the most commonly measured rhythms and its relationship to such factors as 'attention', visual input, and cognitive activity has been the subject of considerable investigation<sup>1</sup>. Summarizing a complicated and often somewhat confused field, the consensus of scientific opinion at present is that the optimum conditions for observing alpha occur when the individual is relaxed, with eyes closed, and neither drowsy nor alert. Under these conditions, present theory maintains that the characteristic alpha rhythm reflects synchronous fluctuations of dendritic potentials associated with a very large sample of cortical cells. The assumption is now general that by observing changes in alpha occurrence, inferences can be made as to the subject's degree of alertness, or to such matters as whether or not he is processing visual information. The results reported here, however, suggest that such assumptions may no longer be held with confidence, and that a variable as fundamental as the orientation of the eyes may drastically alter the probability of alpha occurrence.

The results were obtained from two male subjects, both of whom exhibited abundant 'resting' alpha. The electroencephalogram was recorded unilaterally using bipolar parietal-occipital scalp electrodes. The experiment was suggested as the result of chance observations made during a separate study by one of us (T. M.), who noticed that there appeared to be a marked increase in the occurrence of alpha when the eyes were rolled upwards. At first it seemed probable that this effect was due to the sharp reduction in visual input which occurs when the eyes are in this elevated position, despite the obvious increase in effort and 'attention' that maintaining this position involves. When it was noted, however, that the effect was maintained in dim illumination, it was decided to introduce appropriate experimental controls.

In the first phase of the experiment, alpha was recorded in normal room illumination and a comparison was made between the classic 'eyes open-eyes closed' test, and the

'eyes ahead-eyes up' phenomenon. Subjects were given ten 30-sec trials in each condition, the experimenter giving the command 'eyes open', followed in 30 sec by the command 'eyes closed', etc., or by the commands 'eyes ahead' followed by 'eyes up'.

The electroencephalogram was evaluated according to the presence or absence of alpha, this being defined in the resting record as a signal having a frequency of 8-13 c/s amplitude greater than 30  $\mu$ V. In the experimental conditions a rhythm was identified as 'alpha' when it was greater than 40 per cent of the average resting alpha amplitude. Despite the crudeness of the measures used, the magnitude of the effect was sufficiently great to render any more precise a criterion unnecessary.

Fig. 1 illustrates the changes in the EEG which occur when the eyes are moved from a straight ahead position to the elevated position. Table 1 illustrates the mean percentage of time for which alpha was present in the various conditions.

In the second phase of the experiment the previous conditions were repeated with the exception that the eyes were always covered to prevent patterned visual input. Table 2 summarizes the results.

It is evident from Tables 1 and 2 that there is an increase in alpha in the 'eyes up' condition, though this appears to be less in the case of subject R. R. than subject T. M. However, at this stage these results might yet be interpreted as showing that the effect of elevating the eyes is to

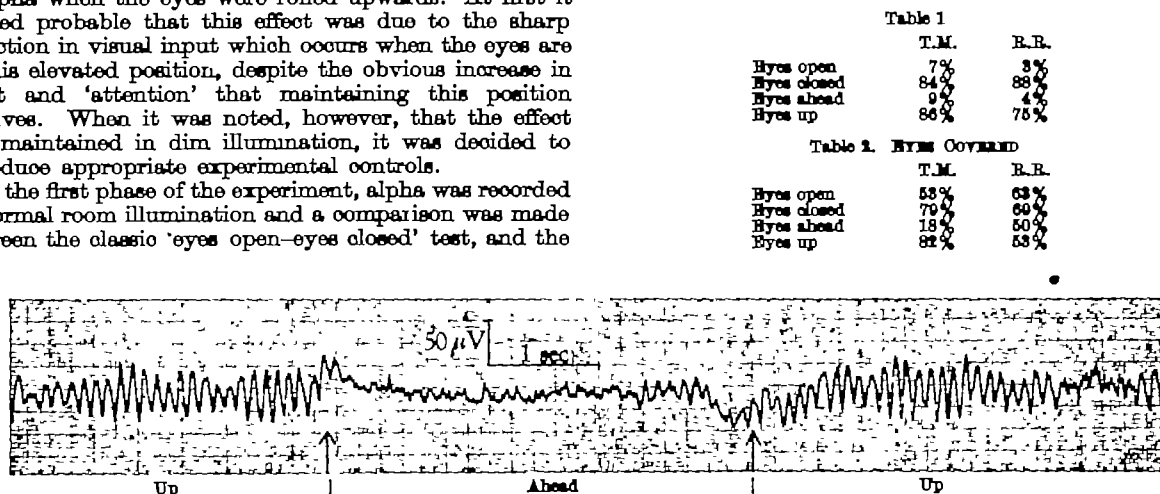


Fig. 1. Characteristic parietal-occipital EEG recorded from subject T.M. when eyes were elevated ('up'), or in normal straight-ahead position ('ahead'). Arrows indicate approximate time of shift of eye position.

Table 1

	T.M.	R.R.
Eyes open	7%	3%
Eyes closed	84%	88%
Eyes ahead	9%	4%
Eyes up	86%	75%

Table 2. EYES COVERED

	T.M.	R.R.
Eyes open	53%	63%
Eyes closed	79%	69%
Eyes ahead	18%	50%
Eyes up	81%	53%

reduce either: (a) overall illumination, or (b) overall patterned visual stimulation, and that it is not eye position *per se* that is the important variable. In the third and fourth phases of this experiment, an attempt was made to clarify these points.

(a) *Overall illumination.* If the increased alpha is a function of a decrease in effective overall illumination, then a simple control would be to introduce diffuse but intense illumination whenever alpha occurs, whether in the 'eyes ahead' or 'eyes up' condition. Such a closed-loop between stimulus and EEG has been described elsewhere<sup>2</sup> and such a system was used here. In effect, the occurrence of alpha turned on a brilliant light, flooding the visual field; this light was automatically extinguished when alpha disappeared. Table 3 illustrates that despite the coupling of a bright light to alpha, its occurrence in the 'eyes up' condition was very high and the remarkable differential between the two conditions was maintained.

	T.M.	R.R.
Eyes ahead	8%	41%
Eyes up	90%	48%

(b) *Patterned stimulation.* If, on the other hand, the increased alpha is a function of a decrease in overall 'patterned' stimulation, then further examination is necessary to clarify this point. The complications of ensuring that the subject receives patterned stimulation when his eyes are rolled up are simply overcome by the use of a patterned after-image. The basic method has been previously used in EEG investigations and is described in full elsewhere<sup>3</sup>. Simply put, subjects were given a brilliant after-image of a pattern (a circle with an inscribed cross), subtending approximately five degrees and foveally fixated. This could be readily perceived as a pattern by subjects for a full minute after the flash whether the eyes were in the 'ahead' or the 'up' position. Because of the relative impermanence of the after-image it was not possible to conduct 5-min trials as in the previous experiments. Shorter trials each of two 30-sec runs were therefore performed controlling for sequence. Eyes were closed and covered immediately following the flash, and subjects were then given the appropriate commands. Table 4 illustrates the mean percentage time for which alpha was present in the two conditions.

	T.M.	R.R.
Eyes ahead	18%	3%
Eyes up	28%	28%

It is clear that the patterned input has reduced the total amount of alpha present, but the differential between the 'eyes up' and 'eyes ahead' condition is maintained.

The relevance of these results to experiments involving the alpha component of the human EEG is obvious. It is clear that eye position is a significant variable which furthermore has been demonstrated to be sufficiently powerful to override such established variables as increments of homogeneous and patterned stimulation, normally quoted as markedly inhibiting alpha occurrence. In addition, as any individual may test for himself, keeping the eyes up not only involves continuous attention and muscular effort, but also becomes increasingly uncomfortable. Despite this combination of normally alpha-inhibiting conditions, more alpha occurs in this situation than in the comfortable, effortless 'eyes ahead' state.

These facts seem to suggest that a re-appraisal of the methods and design of human EEG experiments cannot long be delayed. This argument is rendered more stringent when one recalls that there is a tendency in the relaxed conditions prevailing when eyes are closed for the eyes to rotate upwards. Thus the classic 'eyes open'—'eyes closed' test for alpha (alpha normally appearing when eyes are closed) may not in fact reflect reduced visual input as has been almost universally supposed, but rather some such prosaic variable as the tendency for eyes to turn upwards under such conditions. Whether this is sufficient to account for all such differences seems unlikely, but it is now evident that: (a) much previous experimental work will need to be re-appraised with these facts in mind; (b) no experiments attempting to relate alpha presence or absence to such variables as 'attention' will be meaningful unless eye position is monitored in both the 'eyes open' and 'eyes closed' conditions.

In conclusion we will note that an extended series of experiments using a greater number of subjects is at present in progress. It is already clear that the effect already described may be found in marked fashion in 50 per cent of all subjects tested—though in some individuals its occurrence is less reliable than in others. A number of variables, such as subjects' general ability to hold their eyes continuously in any given position, no doubt contribute, and it is hoped subsequently to identify these variables. However, at the moment it seems unlikely that any 'screening' of individuals prior to their participation in an EEG experiment would be of much use, for the effect may suddenly appear in subjects who do not, on the first test, exhibit it.

Since performing the original experiments we have heard from Dr. M. D. Dewan, of the U.S. Office of Aerospace Research, who has independently observed that alpha occurrence is increased when his subjects were 'looking upwards' with eyes closed.

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## DIRECT X-RAY INACTIVATION OF THE VIRUSES OF TESCHEN AND TALTAN DISEASES, FOOT-AND-MOUTH DISEASE AND VESICULAR STOMATITIS

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THE inactivation of viruses by the direct effect of X- and γ-radiations has been shown by many workers to result in exponential survival curves from which it is possible to calculate the radiation-sensitive volumes of the virus particles<sup>1-4</sup>. Unfortunately, this calculation

requires a value for the density of the radiation-sensitive part of the virus and this is, in general, not known. However, using approximate values for this density, the sensitive volumes of several viruses have been determined, and those of the smaller viruses have proved to be numerically very close to the virus particle volumes as determined by other methods<sup>5,6</sup>. As a preliminary to the

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investigation of the degradation processes involved in this form of inactivation, a comparison has been made between the direct X-ray inactivation rates of the following viruses: foot-and-mouth disease virus (FMDV) of two immunologically different types, strain *M.11*, type 0, and strain *BV.11*, type *SAT* 1 (ref. 7); vesicular stomatitis virus of the Indiana immunological type and two viruses of the Teachen disease group, the original Teachen (Konratice) strain which produces a polioencephalomyelitis in pigs in Europe and the Talfan strain which affects young pigs in Britain<sup>8</sup>. Cross-neutralization tests have indicated that the two strains may be regarded as different serotypes of the same virus<sup>9</sup>. The results which are described here show that it is not possible to differentiate between these two strains by observation of their rates of direct inactivation by X-radiation.

The virus materials used in these experiments were obtained from a variety of sources as follows: FMDV strain *M.11* (type 0) from virus propagated on cell monolayers grown from pig kidneys<sup>10</sup> or from the *BHK* 21/13 cell line derived from baby hamster kidneys<sup>11</sup>. The pig kidney cell monolayers were grown in Roux flasks and the baby hamster kidney cell monolayers as continuous layers in cylindrical 3-l. bottles rotating with their axes horizontal at one revolution in 4 min. FMDV strain *BV.11* (type *SAT* 1) was attenuated for cattle by serial passage first in unweaned mice and later in older mice<sup>12</sup>. The viruses of Teachen and Talfan diseases were propagated on pig kidney cell monolayers and vesicular stomatitis virus (VSV) was grown on the chorioallantoic membrane of 7-day-old developing chick embryos. The allantoic and amniotic fluids were collected and pooled 24 h after inoculation of batches of about 30 eggs.

A Raymax-150 industrial X-ray unit manufactured by Associated Electrical Industries, Ltd., was used as the source of X-rays. All irradiations were performed with lightly filtered 150 kVp radiation having a first half-value-layer thickness of 0.5 mm copper. One set of FMDV samples were irradiated in the frozen state at a temperature of approximately  $-80^{\circ}\text{C}$ . The virus in these samples was suspended in 0.1 M ammonium bicarbonate at pH 7.6 so as to minimize inactivation due to pH variations on change of phase. All other samples were irradiated in a 20 per cent w/v solution of peptone in phosphate-buffered saline at pH 7.6. This was a sufficiently high peptone concentration to prevent significant inactivation of the viruses by the indirect effect of free radicals and other radiation products in solution<sup>13</sup>. During irradiations these samples were maintained at temperatures between  $0^{\circ}$  and  $5^{\circ}\text{C}$ .

The frozen and some of the liquid samples were irradiated in 'Lustroid' tubes set in a copper cooling bar fitted with a 'Perspex' cap which incorporated the beam filter. This bar was thermally insulated from the X-ray tube housing to which it was rigidly fixed during irradiations. The samples in this particular container were irradiated at a constant dose-rate of 3,000 r.p.m. for various times. Other samples were irradiated in a 'Perspex' jig of helical form so that the dose-rates to the eight samples, held at various distances from the X-ray tube anode, varied from 130 to 1,600 r.p.m. The slopes of the survival curves were found to be independent of dose-rate, which confirmed that the indirect effect had been eliminated.

Irradiation doses were measured by the ferrous sulphate dosimeter modified by the addition of sodium chloride<sup>14</sup>.

After irradiation the infectivities of the virus samples were estimated by inoculation of series of ten-fold dilutions into groups of 5-10 unweaned mice<sup>15</sup>, or by plaque counts of ten-fold dilutions on pig kidney cell monolayers or on baby hamster kidney cell monolayers<sup>16</sup>. Further details are given in Table 1.

Table 1 and Fig. 1 summarize the results of these inactivation experiments. The survival curves of Teachen and Talfan viruses have equal slopes which are 30 per cent greater than that for the two FMDV types. It

Virus	Source of infective material	Method of infectivity titration	Size from electron microscopy (m $\mu$ )	Diameter of equivalent radiation sensitive sphere (m $\mu$ )
FMD type 0, strain <i>M.11</i>	Pig kidney (PK) and baby hamster kidney (BHK) cell monolayers	Plaque assay on PK and BHK cell monolayers	25, sphere	25
FMD type <i>SAT</i> 1, strain <i>BV.11</i>	Unweaned mice	Intraperitoneal inoculation of unweaned mice	25, sphere	25
Teachen and Talfan diseases	PK monolayers	Plaque assay on PK monolayers	35, sphere	35
Vesicular stomatitis type Indiana C	Pooled egg fluids	Plaque assay on BHK cell monolayers and intracerebral inoculation of unweaned mice	Bullet-shaped length 156-176 and diameter 60-70	39

is also apparent that the radiation sensitivity of the cattle attenuated FMDV (strain *BV.11*) has not been changed by passage in mice.

The size of the virus particle of FMD has been estimated by several observers<sup>17,18</sup> from results obtained by ultracentrifugation sedimentation analysis and by electron microscopy. The diameter of the infective component of this virus is in the region of 25 m $\mu$ . If we assume that the density of the radiation sensitive part of the virus particle is 1.35 g/ml. (ref. 3), the slope of the inactivation curve gives a value of 25 m $\mu$  for the diameter of the radiation-sensitive volume. As for other small spherical viruses, there is numerical agreement between these two sizes although this is probably a chance agreement depending on the finite range of interaction between a rather smaller sensitive volume and the radiation-induced ionizations in surrounding non-sensitive virus material. Nevertheless, it seems reasonable to suppose that a similar agreement will prevail in the case of the viruses of Teachen and Talfan diseases, for which a diameter in the region of

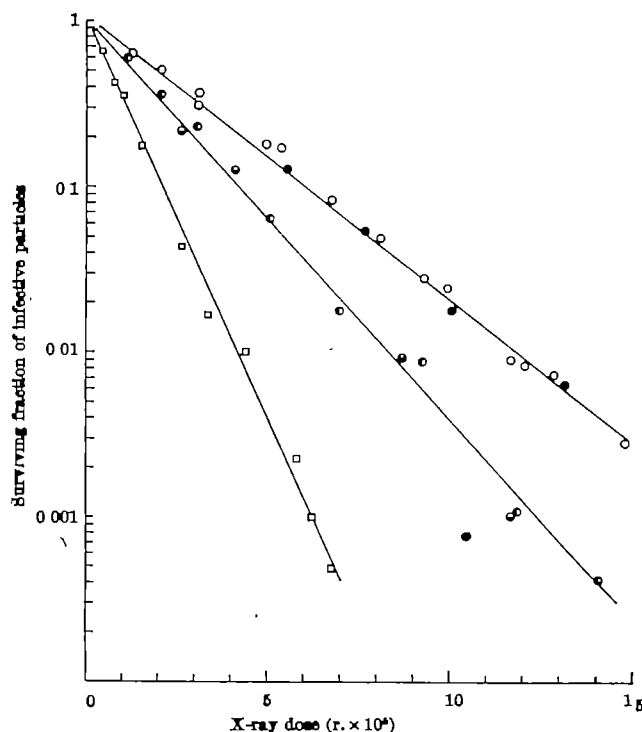


Fig. 1. Loss of infectivity of virus samples irradiated with lightly filtered 150 kVp X-rays:  $\circ$ , FMDV type 0, strain *M.11*;  $\bullet$ , FMDV type *SAT* 1, strain *BV.11*;  $\square$ , Talfan disease virus;  $\blacksquare$ , Teachen disease virus;  $\square$ , vesicular stomatitis virus.

28 m $\mu$  is indicated. This estimate of size depends on the assumption that the density of the radiation-sensitive volume is, in each case, the same as that of FMDV. Electron micrographs indicate that the diameters of the characteristic spherical particles of Teschen and Talfan diseases are both about 35 m $\mu$  (ref. 19).

The characteristic particle of vesicular stomatitis virus, as seen in electron micrographs, is bullet-shaped with a diameter of 60–70 m $\mu$  and a length of 155–175 m $\mu$  (refs. 20 and 21). The sensitive volume derived from the inactivation curve in Fig. 1 is that of an equivalent sphere having a diameter of 38 m $\mu$ . This is in agreement with a previous determination by Franklin<sup>22</sup>.

McCrea<sup>4</sup> has surveyed the irradiation data for a number of animal viruses, and he has shown that the size of the radiation-sensitive unit, which is close to its physical size for small spherical viruses, tends to a limit in the range 40–50 m $\mu$  for viruses larger than approximately 45 m $\mu$  diameter. The viruses investigated in the work reported here fit into this general scheme. Epstein<sup>23</sup> has demonstrated a close correspondence between the radiation sensitive volume and the nucleic acid content for a number of plant viruses and for phage T7. A similar correspondence may exist for these animal viruses.

Some preliminary results using the spray droplet counting technique with a polystyrene latex marker have indicated that the infectivity of FMDV is destroyed by the direct effect of X-rays without any immediate decrease in the concentration of characteristic particles. Virus samples which had lost up to 90 per cent of their original infectivity in mice or on cell monolayers, as a result of X-irradiation when frozen at  $-60^{\circ}\text{C}$ , showed no significant loss in numbers of characteristic particles counted in electron micrographs.

It was also observed that FMD virus samples, irradiated in aqueous solutions which contained lower protein concentrations than that required to protect fully from the indirect effects produced by inactivating agents generated by ionizations in solution, showed a loss of characteristic particles in proportion to their loss of infectivity.

The different sensitivities of the virus to changes in morphology, in relation to loss of infectivity, are not

unexpected on account of the dissimilar nature of the two inactivation processes, that is, the direct and indirect effects. The latter is the result of interactions between the virus and radiation products in solution. It is thought that there are many such interactions for every one which causes an inactivation<sup>24</sup>, and it is quite possible for the virus to be degraded more quickly under these circumstances than when suffering only direct ionizations which individually, although capable of destroying the virus infectivity, are apparently not sufficient to break up the particle itself.

I thank Dr. C. J. Bradish for his advice, Miss H. E. N. Ferrier for supplying vesicular stomatitis virus, Mr. B. J. Kirkham for the electron microscopy, and Mr. M. Ryat for his assistance.

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## LOW-FREQUENCY TARGET STRENGTHS OF PILCHARD SHOALS AND THE HYPOTHESIS OF SWIMBLADDER RESONANCE

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ECHOES from pelagic fish shoals off the Cornish coast were detected in June 1964 using a 'Boomer' as a sound source<sup>1</sup> and a line hydrophone receiving array towed by R.V. *Olione*. The peak energy of the 'Boomer' sound source occurred between 1,100 c/s and 1,800 c/s. By comparing the shoal echo with the bottom echo and assuming extreme values for the bottom backscattering coefficient, an approximate range of values for the target strength or acoustic sectional area ( $\sigma_s$ ) of the shoal around 1,400 c/s was calculated. The results for three shoals are given in Table 1.<sup>2</sup> Fish caught at the time using an Engel midwater trawl were almost exclusively pilchards 21–25 cm long. These fish have gas-filled swimbladders equivalent in volume to approximately 2-cm diameter spheres.

The acoustic cross-sectional area ( $\sigma_f$ ) of a single fish is strongly dependent upon wave-length (or frequency); for wave-lengths much shorter than the fish dimensions (the geometrical scattering region) it is well known<sup>3</sup> that the echo strength is very variable and strongly aspect-dependent; at very long wave-lengths no measurements are available, but it is expected that Rayleigh scattering

applies and so the acoustic cross-section is proportional to  $\lambda^{-4}$ . For fish without swimbladders the Rayleigh scattering region and the geometrical regions merge at wave-lengths comparable with the fish dimensions. It is reasonable to suppose that a swimbladder might exhibit volume resonance in the same way as a free gas bubble in water<sup>4</sup>, and thus have a magnified and omnidirectional acoustic target strength at a frequency well below the geometrical scattering region. At the depth observed the pilchard's swimbladder would resonate between 500 c/s and 600 c/s, but at these frequencies there was insufficient signal-to-noise ratio for a reliable measurement. The measurements were at more than an octave above the theoretical resonance, but still more than a decade below the geometrical region.

When the fish are sparsely distributed, the target spectrum of the complete shoal will probably have the same form as that of a single fish and an intensity in proportion to the number of fish in the shoal. This number has been calculated for: (a) Rayleigh scattering by each fish assuming no resonant effect from the swimbladder;

Table 1

Shoal	Apparent depth* of shoal (m)	Depth of bottom (m)	Ratio of energy in bottom echo to energy in shoal echo over the band 1100 c/s to 1800 c/s	Acoustic cross-section of shoal $\sigma_s$ (m <sup>2</sup> )		Number of fish in shoal, $N_f = \sigma_s/\sigma$ assuming:			
				(I)	(II)	Rayleigh scattering $\sigma_f = 10^{-8}$ (m <sup>2</sup> )		Resonant scattering $\sigma_f = 1.25 \times 10^{-8}$ (m <sup>2</sup> )	
						(I)	(II)	(I)	(II)
1	24	51	90	0.84	2.5	$8.4 \times 10^8$	$2.5 \times 10^8$	$6.7 \times 10^8$	$2.0 \times 10^8$
2	24	41	12	12	87	$1.2 \times 10^{10}$	$2.7 \times 10^{10}$	$1.0 \times 10^8$	$3.0 \times 10^8$
3	28	49	11	15	44	$1.5 \times 10^{10}$	$4.4 \times 10^{10}$	$1.2 \times 10^8$	$2.5 \times 10^8$

Columns (I), assuming bottom back-scattering coefficient at normal incidence = 0.3; columns (II), assuming bottom back-scattering coefficient at normal incidence = 0.9.

\*Due to the wide lateral beamwidth of the line array, the apparent depth may not be the true depth.

(b) scattering on the assumption that each fish swim-bladder resonates at 500 c/s. Only case (b) gives a credible number of fish in each shoal.

When the fish are densely packed the target area of the shoal will also depend on the concentration. The transition from sparse to dense packing occurs when the acoustic cross-sectional area of one fish begins to overlap that of a neighbour and interaction may occur. Both for Rayleigh scattering and for scattering at an octave above resonance, packing densities required to cause interaction become unrealistic, but at the resonant frequency itself this may not be so.

The pilchard shoals were detected by a higher frequency (30 kc/s) echo-sounder, together with other shoals (probably mackerel) at shallower depths than the pilchards and also more disperse pilchard larvae over a large depth range. It is significant that the mackerel, which do not have swimbladders, and the larvae, whose small swimbladders resonate about 30 kc/s, were not observed on the low-frequency Boomer records.

Though it is acknowledged that the values are only approximate, the measurements are believed to be unique since they were taken at a frequency more than a decade below the geometrical scattering region. The results are important since resonance, if observed directly on a wide-band echo-sounder or fishing sonar, may be used to provide classification of the shoals into size, number and possible species of fish. It is intended to repeat the observation this year using a Boomer modified to cover the expected resonance region. Controlled experiments to determine the echo spectral response of single fish at and just above resonance present severe instrumental problems which have not yet been satisfactorily solved.

We thank the staff of the Fisheries Laboratory, Lowestoft, for their co-operation, and the master and crew of R.V. *Olimos* for their assistance.

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## EFFECT OF RIBONUCLEASE ON ANTI-TUMOUR ACTIVITY OF RIBONUCLEIC ACID FROM NORMAL TISSUES

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SEVERAL authors have published the results of the anti-tumour effects of ribonucleic acids from normal tissues<sup>1-3</sup>. We have obtained similar results. The experiments were performed on the transplantable strain of liver mucous cancer obtained by Malugina. About 900 tumours were examined<sup>4</sup>.

Following on this work we found out that RNA preparations from normal tissues homologous to the tumours under investigation inhibited growth only in some of the tumour strains. But the results from single tests and experimental series even for these strains were found to differ greatly<sup>5</sup>. That is why we had to relinquish tumours of the Malugina strain on which the work recorded here had been started.

The difficulties which we met seem to be characteristic of many investigations aimed at revealing biological effects of RNA penetrating somatic cells from the outside. Thus when the question concerns the synthesis of the protein unusual for the cell it should be stated that this is induced by alien RNA (though not in all cases), or the level of it appears to be different<sup>6,7</sup>. It seems natural to suggest that these differences are due to changes in the competence of cell recipients. Unfortunately, it is difficult to say anything definite about this suggestion since the cause which conditions the state of competence was not known until now even in bacterial transformation, despite our knowledge of this process and homogeneity of the agent used (DNA)<sup>8-10</sup>.

There is another suggestion (it should not be considered an alternative) that the variability of the results is somehow related to the properties of the preparations used. In particular one cannot exclude the idea that the active component of the preparation is not RNA, but some unknown admixture extracted together with RNA from the tissue. The amount of these admixtures may be different in different tissues and also vary significantly in the preparations from one and the same normal tissue, thus determining the variability of the obtained effect. This article describes experiments performed to check this hypothesis.

Cells of the transplantable strain of rhabdomyosarcoma O 100-3/1 were used as recipients. The suitability of this strain for experiments of this kind has already been determined<sup>4</sup>. The strain O 100-3/1 is a progeny of the *in vitro* malignant cells (explanted cells of muscle tissues of new-born Wistar rats<sup>11</sup>). The strain was maintained in Wistar rats.

RNA was extracted from the bodies of rats 1-2 days old after removal of their paws, skin and viscera. The technique of removal has already been described<sup>12</sup>. One and the same preparation was used in 5 replications of the experiment (Table 1, II-VI); RNA was isolated from 3 groups of new-born rats. Combined sediment was obtained by ethanol reprecipitation. The preparation contained no DNA (tested by the method of Dische). The ratio of nitrogen/phosphorus was 1.63. The preparation contained glycoogen.

Table 1. EFFECTS OF INCUBATION FOR 10 H OF RAT RHABDOMYOSARCOMA C 100-3/1 CELLS WITH RNA PREPARATIONS EXTRACTED FROM MUSCLE TISSUE OF NEW-BORN RATS, ON THE WEIGHT OF TUMOURS 20 DAYS AFTER IMPLANTATION

Replication	Concentration (mg/ml.)	Control (incubation in Hanks's solution)		RNA			Additionally deproteinized RNA			RNase treated and deproteinized RNA		
		No. of animals	Average weight of tumours (g)	No. of animals	Average weight of tumours		No. of animals	Average weight of tumours		No. of animals	Average weight of tumours	
					In (g)	% of the control		(g)	% of the control		(g)	% of the control
I	2.5	18	4.60	11	2.25	48.9	9	2.43	52.8	12	3.85	83.9
II	2.0	8	7.35	9	0.45	6.2	6	2.13	28.9	7	3.18	43.2
III	2.0	9	2.89	10	0.02	0.7	10	0.24	11.1	10	0.01	0.3
IV	2.0	9	9.80	10	3.23	33.4	9	1.04	10.6	11	4.31	44.0
V	1.5	10	3.36	7	0.00	0.0	8	3.50	104.2	7	2.64	70.86
VI	2.0	14	5.89	14	2.18	36.9	19	2.89	49.1	9	3.24	139.9
Total			5.65		1.35	23.9		2.5	36.3		3.70	65.5

Probabilities of differences, control—RNA, > 0.99; control—additionally deproteinized RNA, > 0.99; control—RNase treated and deproteinized RNA, < 0.05; RNA—additionally deproteinized RNA, < 0.05; RNA—RNase treated and deproteinized RNA, < 0.95. Additionally deproteinized RNA—RNase treated and additionally deproteinized RNA, > 0.95.

Before implantation the minced tumours C 100-3/1 were incubated with RNA in Hanks's solution with antibiotics at room temperature for 10 h following a previously applied technique<sup>4,12</sup>. Cells were injected subcutaneously; the weight of tumours was determined on the twentieth day. Each replication of the experiment had four variants. (1) The first variant (the control), incubation in Hanks's solution. (2) The second variant, incubation with RNA. (3) The third variant, incubation with RNA, treated with RNase at 37° for 1–2 h. The content of the enzyme was 15 µg/ml. RNase was removed by treatment with phenol four times. Phenol was extracted by ether. Nitrogen was blown through the solution to remove traces of ether. The solution was tested for RNase activity with freshly extracted liver RNA as a substrate. The increase in soluble nucleotides was measured by spectrophotometry. Such testing always yields negative results. (4) The fourth variant, incubation with RNA which was not treated with RNase but underwent, as in the third variant, four times additional deproteinization.

Activity of preparations was checked for six months.

Before implantation the injected material was tested for sterility. In the first replication when only penicillin (2,000 units/ml.) was applied, the plating on meat peptone agar revealed the presence of about 30,000 bacterial cells per ml. of incubated tumour homogenate. In subsequent replications, besides penicillin, streptomycin was used (500 units/ml.) and complete sterility obtained.

Weights of tumours in all the replications and variants of the experiment are given in Table 1. Inhibition of growth of tumours due to RNA was noted in all the replications. Variability of tumour weight in the control (Table 1) shows that the differences between the variants and the differences between the replications are probably due to a considerable extent to the fact that the condition of animals and/or of tumours used in one replication of the experiment differs from that used in another.

RNA and RNA additionally deproteinized cause a statistically significant decrease in the weight of the tumours as compared with the control. RNA depolymerization abolishes this effect. There is no reliable difference between the effects of RNA and RNA additionally depolymerized, whereas this difference exists between the data on RNA and RNA treated with RNase.

This evidence taken as a whole shows that RNA inhibits the growth of tumours under investigation and that antitumour activity of preparations is eliminated as a result of RNA enzymatic hydrolysis. The absence of statistically reliable differences between the effects of additionally deproteinized RNA and the RNA treated with RNase probably is due to the high variability of the results obtained. In this connexion attention should be paid to sharp differences of the data in some repetitions of the experiment. For example, while in the sixth replication the weight of tumours grown from the cells incubated with RNA hydrolysates even exceeded the weight of control tumours, in the third replication of this

variant a sharp repression of tumour growth was noted (Table 1).

Nevertheless, the total result is clear: RNA is an active component of preparations. Correspondingly their antitumour activity is not retained after RNase treatment with a subsequent additional deproteinization whereas the deproteinization itself does not inactivate preparations.

For some years certain antitumour agents have been found in the organs of normal animals<sup>13–16</sup>. If the tumour cells are incubated with them before implantation, the growth of tumour is inhibited. The chemical nature of these agents has yet to be investigated. The foregoing results show that the depolymerization of RNA inactivates the preparations used in the work recorded here. Hence, when before implantation the tumour cells were incubated with our preparations, the active component causing the growth inhibition of tumours was RNA, but not other substances extracted from normal tissues together with RNA (or contaminating the latter during isolation).

Our results do not support the suggestion that variations of the results obtained in the experiments on the effect of RNA depend solely on changes in the competence of recipient cells. The properties of the preparations used can serve as another source of these differences. At any rate until the preparative extraction of RNA fraction responsible for the registered effect (that is, for decrease in weight of the tumour as compared with the untreated control) is performed, this suggestion remains plausible. For example, the finding of the anti-tumour effect in all the replications of the experiment might depend on the fact that during the whole investigation with the one exception (replication 1) only the same RNA preparation was used.

There are some suggestions that the biological effect of RNA can be explained by its toxicity<sup>17,18</sup>. The experiments we performed on a series of tumour strains did not confirm this suggestion<sup>14,15</sup>. Moreover, special experiments have shown that the eleventh incubation of monolayer cultures of rat rhabdomyosarcoma with RNA from muscle tissues of the rat (RNA concentration 3.5–5 mg/ml.) does not decrease the proliferating activity of cells. Data on organospecificity of anti-tumour effect of RNA extracted from various tissues also contradict the suggestion concerning the toxicity of RNA preparations. Tumours are sensitive to the action of RNA isolated from homologous tissues and not susceptible or less susceptible to RNA separated from other tissues<sup>4,6</sup>.

It is probable that under the influence of RNA from the normal tissue homologous to the tumour the ability of autonomous growth of the latter is decreased<sup>4</sup>. If this is so, it would be of interest to examine the effect of RNA on the development of primary tumours: it is possible that at relatively earlier stages of autonomization the tumour cells would be rather sensitive to this agent.

The experiments which we performed with C<sub>3</sub>H mice in order to check this suggestion gave negative results. The development of hepatomas induced by orthoamino-

azotholuene was not retarded due to the action of the liver RNA repeatedly injected subcutaneously into the mice. This work is being continued.

To explain the anti-tumour effect of RNA it is not necessary to suggest that changes it induces are preserved for a long time. It is not improbable that a decrease over a short period in the ability of implanted cells to autonomous growth similar to short-time changes induced in bacteria by specific RNA from the outside<sup>18-21</sup> is sufficient for protective mechanisms of the animal to perform their function.

Another possibility is the participation of stable informational RNA in the process. The existence of such stable RNA in nuclear cells has been proved<sup>22</sup>. It is possible that they play the principal part in the vital activity of somatic cells of higher animals<sup>23</sup>.

Finally, we refer to the hypothesis according to which RNA from normal tissues causes in tumour cells epigenetic changes reducing their malignancy. Had such a result been obtained it could be compared with cell differentiation under the influence of specific RNA (the question concerning the possibility of such an influence at different stages of ontogenesis cannot be considered as finally settled<sup>24-26</sup>). It should be noted that we have not found redifferentiation phenomena in tumours grown from the cells incubated with RNA<sup>4</sup>. However, the morphological criterion is evidently not enough to determine the character of anti-tumour effect of RNA. In this connexion we should mention the results of two investigations of the action of specific RNAs<sup>6,27</sup> which show that the ability to synthesize proteins not normally produced by recipient cells may be retained over many cell generations.

The possibility of the appearance of epigenetic changes under the influence of RNA from the outside should be taken into consideration when comparing the biological effect of RNA and the transformation phenomenon conditioned by DNA. The great frequency of changes caused by specific RNA is the main difference available for the analysis at the present stage of knowledge<sup>20,21</sup>.

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## ORGANOCHLORINE INSECTICIDE RESIDUES IN THE EGGS OF SEA BIRDS

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IN 1963, organochlorine insecticide residues were found in the eggs of terns and other species feeding in British waters<sup>1,2</sup>. In 1964, the survey was extended to include specimens from 13 species collected at four sites. A preliminary examination of residues in the food of the birds at one site was also made. In all, 90 eggs were collected from Scolt Head, Norfolk; the Farne Islands, Northumberland; St. Abbs Head, Berwickshire (all on the North Sea coast); and from Great Saltee Island, County Wexford, which lies at the junction of St. George's Channel with the Atlantic. The eggs were from different clutches and were taken at random except at Scolt Head National Nature Reserve where only deserted eggs were taken. Thus, while strict comparisons cannot be made between the results of Scolt Head and the other sites, as the organochlorine pesticide residues in the Scolt Head eggs were found to be of the same order as those from the other sites they are probably not unrepresentative of the site. In all cases the residues were low and so were unlikely to have affected hatching success. Ten fish (1 sand eel *Ammodytes tobianus*, 1 pipe fish *Syngnathus*

sp., 2 plaice *Pleuronectes platessa*, and 6 gobies *Gobius minutus*) and 8 molluscs (2 mussels *Mytilus edulis* and 6 cockles *Cerastium edule*) were also collected from the feeding area of the sea bird colony at Scolt Head.

The specimens were analysed in the Laboratory of the Government Chemist using the extraction and clean-up method of de Faubert Maunder *et al.*<sup>3</sup> followed by gas-liquid chromatographic estimation of the pesticides using both silicone and 'Apiezon' columns in all cases. Confirmation of some of the results was possible by a paper chromatographic method.

The results from the sea bird eggs are summarized in Table 1. Dieldrin and *pp'*-DDE were detected in all the eggs. In addition, 70 specimens contained residues of *pp'*-DDT and/or its metabolite *pp'*-TDE; 47 contained traces of benzene hexachloride (BHC), usually the  $\beta$ -isomer and in no case exceeding 0.2 p.p.m.; 48 contained small amounts of heptachlor epoxide, none exceeding 0.2 p.p.m. These residues are included in the total residue figures given in Table 1. All of the molluscs and fish except for one cockle contained insecticide residues but in no case



Table 1. ORGANOCHLORINE INSECTICIDE RESIDUES IN SEA BIRD EGGS FROM THE BRITISH ISLES  
(All results expressed in p.p.m. by weight with respect to total egg contents)

Species	Egg	Site	No. analysed 1963/ 1964	Range of pp'-DDH		Range of dieldrin		Range of total residue	
				1963	1964	1963	1964	1963	1964
Little tern ( <i>Sterna albfrons</i> )	Inv. fish	Scot Head	2/8	0.2-1.0	0.2-0.6	0.1-0.3	0.2	1.6-2.6	0.5-0.8
Sheildunk ( <i>Tadorna tadorna</i> )	Mol.	Scot Head	1/2	0.5	0.2-0.6	0.6	0.1	2.2	0.5-1.0
Oyster catcher ( <i>Haematopus ostralegus</i> )	Mol.	Scot Head	2/10	0.2-1.2	0.2-1.1	0.1-0.4	0.1-0.4	0.4-2.3	0.4-2.1
Common tern ( <i>Sterna hirundo</i> )	Pha.	Scot Head	0/8		0.2-0.7		0.1-0.3		0.5-2.3
Sandwich tern ( <i>Sterna sandwichensis</i> )	Pha.	Scot Head	3/8	1.0-1.5	0.3-1.1	0.2-0.5	T-1.6	2.6-3.2	0.5-3.4
[Black headed gull ( <i>Larus ridibundus</i> )	Om.	Scot Head	0/9		0.5-2.8		0.1-0.5		0.7-3.4]
Kittiwake ( <i>Rissa tridactyla</i> )	Mar. Inv.	St. Abbs Head	5/0	T-0.2		T		T-0.7	
[Herring gull ( <i>Larus argentatus</i> )	Om.	St. Abbs Head	6/0	0.2-0.9		0.1-0.4		0.4-1.6]	
Puffin ( <i>Fratercula arctica</i> )	Mar. Inv.	St. Abbs Head	1/0	1.0		0.4		1.9	
Guillemot ( <i>Uria aalge</i> )	Mar. Inv.	St. Abbs Head	4/0	1.5-4.0		0.1-2.0		2.2-6.8	
Razorbill ( <i>Alca torquata</i> )	Mar. Inv.	St. Abbs Head	1/0	2.9		1.6		4.5	
Shag ( <i>Phalacrocorax aristotelis</i> )	Large fish	St. Abbs Head	2/0	3.1-4.3		2.5-3.3		6.7-8.9	
Kittiwake ( <i>Rissa tridactyla</i> )	Plat.	Great Saltee Island	0/6		0.2-0.5		T-0.1		0.2-0.7
Shag ( <i>Phalacrocorax aristotelis</i> )	Large fish	Great Saltee Island	0/5		0.3-0.7		0.1-0.5		0.4-1.2
Guillemot ( <i>Uria aalge</i> )	Mar. Inv.	Great Saltee Island	0/6		1.3-2.1		T-0.2		1.6-2.2
[Cormorant ( <i>Phalacrocorax carbo</i> )	Large fish	Ferne Islands	0/3		1.5-1.8		0.2-0.8		1.8-2.6]
Shag ( <i>Phalacrocorax aristotelis</i> )	Large fish	Ferne Islands	0/3		1.5-3.2		1.3-2.6		3.4-6.7

T, trace (<0.1 p.p.m.). Inv., marine invertebrates (mainly Annelida, Crustacea) Mol., marine molluscs. Om., omnivorous. Species which feed partly on land or in fresh water are in square brackets.

did this exceed 0.1 p.p.m. Traces of pp'-DDT or its metabolites were present in 4 molluscs and all the fish and traces of dieldrin in 3 molluscs and 8 fish.

The following conclusions are drawn from the results: (a) Small amounts of organochlorine insecticides detected in marine life suggest that such residues may be widely distributed in British waters. (b) The total organochlorine insecticide residues found in the eggs of different sea birds are nearly all of the same order, most of them lying within the range of 0.4-3.5 p.p.m. (c) There are some indications that the eggs of birds which feed on large fish contain higher residues than those of species which feed on small fish or on invertebrates. (d) There is no evidence of an increase in contamination from 1963 to 1964, but some suggestion of a slight decrease. (e) Since there is little or no spraying of coastal waters with insecticides in Britain, the residues detected must derive either from the contamination of rivers or from aerial drift.

Analyses of freshwater molluscs, arthropods and fish in the fen rivers which drain into the Wash not far from Scot Head have indicated that traces of insecticides may be present in the molluscs and arthropods, with residues varying in the fish from a trace to a total of 19.5 p.p.m. in the case of the fat of a pike *Esox lucius*.

The existence and undesirability of environmental contamination by pesticides have been recognized by the Advisory Committee on Pesticides and Other Toxic Chemicals\*. Many uses of the more toxic organochlorine insecticides have been curtailed voluntarily in order to reduce this contamination; but in order to determine the effectiveness of these actions a measure of annual changes in residue levels in indicator organisms is required. The narrow limits within which the majority of the egg residues falls appear to be characteristic of marine species; residues for terrestrial species tend to vary within much wider limits<sup>1</sup>. This suggests that the eggs of sea birds may be useful indicator organisms for detecting general changes in environmental contamination by persistent pesticides. Since most sea birds range widely they would not be suitable indicators of local conditions.

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## CHROMOSOME CHANGES IN HUMAN DIPLOID-CELL CULTURES INFECTED WITH *Mycoplasma*

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CONTAMINATION of mammalian cell cultures by *Mycoplasma* was reported for the first time by Robinson *et al.*<sup>1</sup>. Subsequent publications have shown that these agents occur frequently as contaminants in many animal cell cultures and that contaminated cells sometimes show morphological changes and alteration in their rate of growth<sup>2-4</sup>.

During routine chromosomal checks on a human diploid cell strain WI-38 which normally retains a diploid complement of chromosomes for at least forty passages under optimal conditions<sup>5</sup>, certain chromosomal aberrations were observed by us; polyploid counts and break frequencies were high, and occasional cells with fragmented chromosomes were seen. At about the time that these chromosomal abnormalities were detected, a decrease in the rate of cell growth occurred and the cultures were found to contain *Mycoplasma*. Since chromosomal aberrations associated with *Mycoplasma* infection

in normal cells have not previously been reported, it was decided to infect a strain of human diploid cells and to observe any changes which occurred. These findings are reported here.

Medium for cell growth consisted of Eagle's basal medium<sup>6</sup> (in Hanks's balanced salt solution) containing 8 per cent calf serum and 0.05 per cent sodium bicarbonate. No antibiotics were included in the medium.

The human diploid cell strain WI-38 developed from normal embryo lung by Hayflick at the Wistar Institute was serially passaged from cells frozen in liquid nitrogen. Cells were dispersed and propagated in 4-oz. bottles using the methods described by Hayflick and Moorhead<sup>5</sup>.

The *Mycoplasma orale* used was isolated from a continuous line of *Cercopithecus aethiops* (vervet) monkey-kidney cells (V34). The diploid cells which had been propagated for four consecutive passages in antibiotic-free medium were infected with a heavy inoculum immediately

after trypsinization while the cells were still in suspension. Both infected and control cultures were then trypsinized every three or four days and samples from both infected and control cultures were pooled and tested each week for the presence of *Mycoplasma* and microbial contaminants. The infected cultures and control cultures were handled in separate rooms.

Bottles containing many cells in mitosis were selected at intervals for chromosomal investigation, either 24 or 48 h after trypsinization. The cells were pretreated for 3 h with colcemid, and fixed in acetic alcohol. They were then spread on slides, lightly flamed, hydrolysed and stained in Giemsa (a modification of the method described by Moorhead and Nowell<sup>7</sup>). All intact metaphase cells were examined for heteroploidy, although it was not possible to count the chromosomes accurately in all cases; endore-duplicating cells were scored as polyploids. Well-spread metaphases were examined more closely and in all cases in which there was no ambiguity their chromosomes were counted and breaks scored. The term 'breaks' applies to a distinct break of the chromosome or chromatid but does not include secondary constrictions or unstained gaps. The figures in column 5 of Table 1 show the number of cells with one break or more and include fragmented cells (Fig. 1) which contain many fragments, chromatid exchanges and structural abnormalities.

Table 1. COMPARISON OF CHROMOSOME ABNORMALITIES OCCURRING IN NORMAL AND INFECTED CELLS

Passage	Days after infection	Polyploid cells		Cells with breaks		Fragmented cells	Structurally abnormal chromosomes
		+ <i>Mycoplasma</i>	Control	+ <i>Mycoplasma</i>	Control		
22	12	(31/323) 9.6%	(12/374) 3.2%	(18/151)* 11.9%	(5/107) 4.6%	1	3
28	38	(38/161)* 23.6%	(12/232) 5.2%	(20/78)* 25.6%	(1/147) 0.6%	12	1
31	42	(6/60) 10.0%	(10/185) 5.4%	(6/85) 7.0%	(3/76) 3.9%	2	1
32	47	(51/343)* 14.8%	(44/728) 6.0%	(13/122) 10.6%	(7/148) 4.7%	7	2

\* Indicates that the difference between infected and control cultures is significant at 5 per cent level.

Compared with *Mycoplasma*-free control cultures, cells experimentally infected with *Mycoplasma* showed noticeable cultural and cytopathic changes beginning by the second passage following infection. Progressive reduction in the rate of cell growth and a decrease in final cell population occurred together with cell elongation and increased cytoplasmic granularity. Tests to detect the presence of microbial agents were repeatedly negative, except for the consistent growth of *Mycoplasma* obtained from cell samples removed from infected cultures.



Fig. 1. Chromosomes from normal and infected WI-38 cells. Left, normal cell; right, infected cell (arrows indicate chromatid changes)

Table 2

Passage	Days after infection	Polyploid cells		Cells with breaks	
		+ <i>Mycoplasma</i>	Control	+ <i>Mycoplasma</i>	Control
28	5	(28/295)* 9.5%	(12/452) 2.7%	(27/185)* 14.6%	(10/149) 6.7%
	5	(22/332) 6.6%	(8/199) 4.0%	(41/277)* 14.8%	(9/130) 6.9%
29	6	(27/270)* 10.0%	(4/350) 1.1%	(16/79)* 20.3%	(4/85) 4.7%

\* Indicates that the difference between infected and control cultures is significant at 5 per cent level.

Chromosome examinations of the cells taken at four passage-levels are shown in Table 1. It is clear that the percentage of breaks and polyploidy was higher in the *Mycoplasma*-infected cells than in the controls. This difference tended to increase with time after infection, whereas the frequency of breaks and polyploid cells in the controls remained at a low level. The majority of infected cells had 46 chromosomes and, when analysed, the complement was of normal karyotype. Some of the infected cells were suspected of containing structurally abnormal chromosomes, but only unequivocal dicentric and translocation configurations are included in Table 1. With two exceptions fragmentation always occurred in heteroploid cells, the damage observed being so extreme that it was not possible to identify any of the remaining intact chromosomes many of which appeared structurally abnormal but were not included in column 8 of Table 1. Fig. 1 shows chromosomes in a fragmented cell, and a typical control cell.

Although Table 1 gives the results obtained during a long period of infection, we have also examined the chromosomes of WI-38 cells within six days following infection with *Mycoplasma* (Table 2). Three days after infection some of the cultures were growing more slowly than the controls and analysis of chromosome preparations made 5-6 days after infection suggested that the frequency of polyploidy and the number of breaks was greater than in the controls. Statistical analysis of the polyploid counts shows this difference to be significant in two cases out of three.

It is now recognized that *Mycoplasma* are common contaminants of cell cultures and may induce one or all of the following changes in contaminated cells: (1) reduction in growth rate, usually associated with a morphological change of the cells<sup>3,4</sup>; (2) reduction of arginine and glutamine in the growth medium<sup>3,5</sup>; (3) alteration of sensitivity of the cell cultures to viral infection<sup>6</sup>.

This communication shows that *Mycoplasma* may also change cell chromosomes, and therefore emphasizes the need for caution in interpreting experimental results

without adequately testing for the presence of *Mycoplasma*. Chromosome damage similar to that described here has been induced by viruses in cell cultures and in blood cultures<sup>8,10</sup>. Chromosomal abnormalities have also been reported in blood cultures and bone marrow from leukaemic patients<sup>11</sup>.

Hayflick identified Eaton agent as *Mycoplasma pneumoniae*, which causes primary atypical pneumonia and may be associated with other respiratory diseases in man<sup>12</sup>. *Mycoplasma* species have also been isolated from the synovial fluid of patients with rheumatoid arthritis<sup>13</sup> and from both human tumours and bone marrow from subjects with leukaemia<sup>14,15</sup>.

In most of these reports the evidence that *Mycoplasma* causes human disease is inconclusive; but the accumulated data strongly suggest that an association exists. Our evidence that chromosomal aberrations occur in normal human cells after infection with *Mycoplasma* is of particular interest, but further investigations are needed to

establish the full significance of our observations. These preliminary findings, however, add support to the opinion that *Mycoplasma* may be involved in the aetiology of human disease.

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## CONTRALATERAL EFFECTS OF SODIUM AND POTASSIUM ON THE ELECTRICAL POTENTIAL IN FROG SKIN AND TOAD BLADDER

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THE effects of  $\text{Na}^+$  and  $\text{K}^+$  bathing the outside and inside surface of frog skin and the corresponding mucosal and serosal surface of toad bladder (hereafter referred to as outside and inside) on the transmembrane electrical potential are well recognized. Under conditions in which the anion permeability is negligible, that is, by using  $\text{SO}_4^{2-}$  as the predominant anion, Koefoed-Johnsen and Ussing<sup>1</sup> have stated that the total transmembrane potential is composed of two parts and described by the relation:

$$\psi_i - \psi_o = \Delta\psi = \frac{RT}{F} \left[ \ln \frac{[\text{Na}^+]_o}{[\text{Na}^+]_i} + \ln \frac{[\text{K}^+]_i}{[\text{K}^+]_o} \right] \quad (1)$$

where the subscripts, *o* and *i*, refer to the outside (mucosal) surface and inside (serosal) surface, respectively, and the subscript, *c*, refers to the cell cytoplasm. *R*, *T* and *F* are the gas constant, the absolute temperature and Faraday respectively, and the bracketed chemical symbols denote the concentration of the respective ions. Other investigators have confirmed this to varying degrees, although disagreement has arisen in two regards. In the case of frog skin derived from a variety of species, as well as toad bladders<sup>2</sup>, the magnitude of the slopes,  $\partial\Delta\psi/\partial \log [\text{Na}^+]_o$  (log refers to base 10) and  $\partial\Delta\psi/\partial \log [\text{K}^+]_i$ , is, more often than not, less than the value of  $2.3 RT/F$  as given in equation (1), that is, 59 mV per log unit at 25° C. Furthermore, these slopes, more often than not, are not constant over the range of experimental observation. Both of these kinds of findings have been 'explained' in terms of passive shunting, that is, leakiness of the bounding membrane to other ions. For example, in a rather extensive study, Lindley and Hoshiko<sup>3</sup> have interpreted their results, with some success, by applying the constant field equation of Goldman<sup>4</sup>.

No reports have appeared on the contralateral transmembrane effects of one of these ionic concentrations,  $[\text{Na}^+]_o$  or  $[\text{K}^+]_i$ , on the functional dependence of the potential on the other, that is,  $[\text{K}^+]_i$  or  $[\text{Na}^+]_o$ . For example, the question may be asked: does  $[\text{Na}^+]_o$  have any effect on the electrical potential as a function of  $[\text{K}^+]_i$ ,  $\Delta\psi([\text{K}^+]_i)$ , or does  $[\text{K}^+]_i$  have an influence on the function,  $\Delta\psi([\text{Na}^+]_o)$ ? We have carried out a brief but systematic investigation of such contralateral effects in both isolated frog skin (*Rana pipiens*) and toad bladder (*Bufo marinus*). The results would appear to have important implications on any interpretation of the origin of the transmembrane potential and reflect on the validity of the interpretation ascribed by the Koefoed-Johnsen and Ussing model<sup>1</sup>.

Either the abdominal skin of frog or the urinary bladder of toad was isolated in the usual manner. Within 1-4 h after isolation, it was mounted in a two-chamber cell

providing 3.8 cm<sup>2</sup> of area and so designed that the solutions bathing the surfaces could be conveniently changed, thereby to alter the ionic compositions. All experiments were carried out during the period April-November and were performed at room temperature of about  $25 \pm 1^\circ \text{C}$ . Both the open-circuit total transmembrane potential and the short-circuit current were measured<sup>5</sup>. The ionic compositions in the bathing solution were varied in a systematic manner. At a given  $\text{Na}^+$  concentration in the 'sulphate' Ringers' solution bathing the external surface,  $[\text{Na}^+]_o$ , the  $\text{K}^+$  concentration in the solution bathing the internal surface,  $[\text{K}^+]_i$ , was incrementally changed substituting  $\text{Na}^+$  with  $\text{K}^+$ , approximately doubling  $[\text{K}^+]_i$  for each successive change to cover the range 2.5-117.5 mM. (The sodium 'sulphate' Ringer's solution used was composed as follows:  $\text{Na}_2\text{SO}_4 = 57.5 \text{ mM}$ ,  $\text{CaSO}_4 = 1.5 \text{ mM}$ ,  $\text{K}^+ (\text{KH}_2\text{PO}_4 - \text{K}_2\text{HPO}_4) = 2.5 \text{ mM}$ , sucrose 60 mM for frog Ringer's solution, glucose 20 mM for toad Ringer's solution,  $\text{pH} = 7.4 \pm 0.1$ .) After each change, sufficient time was allowed for the potential and short-circuit current to become relatively stable. Following the highest  $[\text{K}^+]_i$  examined, sodium sulphate Ringer's solution containing 2.5 mM  $\text{K}^+$  was again applied to the surface and  $[\text{Na}^+]_o$  was then altered by substituting choline or  $\text{K}^+$  for  $\text{Na}^+$ . (Choline sulphate was prepared by neutralizing choline bicarbonate (Matheson 40 per cent aqueous solution) with sulphuric acid.) The changes in  $[\text{K}^+]_i$  were then repeated. In this manner, the total transmembrane potential was examined as a function of both  $[\text{Na}^+]_o$  and  $[\text{K}^+]_i$ . In some experiments, the procedure was to alter  $[\text{Na}^+]_o$  sequentially at given values of  $[\text{K}^+]_i$  to obviate bias in any irreversible effects of drifts in preparation behaviour. The results show clearly in both frog skin and toad bladder that as the  $\text{Na}^+$  concentration bathing the outside surface,  $[\text{Na}^+]_o$ , is decreased, the magnitude of the slope,  $\partial\Delta\psi/\partial \log [\text{K}^+]_i$ , decreases. Whereas this slope may be as much as -50 mV per log unit at 115 mM  $[\text{Na}^+]_o$ , it is often not more than -15 mV per log unit at 1 mM  $[\text{Na}^+]_o$ . In a like manner, the results show that as  $[\text{K}^+]_i$  is increased, the slope,  $\partial\Delta\psi/\partial \log [\text{Na}^+]_o$ , decreases. Results from a representative experiment from a total of ten are plotted in Fig. 1.

From the short-circuit current measurements, patterns analogous to those found for the open-circuit transmembrane potential emerge. Data from the same experiment illustrated in Fig. 1 are plotted in Fig. 2.

The electrical resistance, taken as  $\Delta\psi/I$ , where *I* is the short-circuit current, decreases with both increasing  $[\text{Na}^+]_o$  and increasing  $[\text{K}^+]_i$ .

According to the Koefoed-Johnsen and Ussing<sup>1</sup> model, the only means whereby these contralateral transmembrane effects could be realized would be through changes

in  $[Na^+]_o$  and  $[K^+]_o$  (equation 1) accompanying the changes made in the bathing solution. However, as MacRobbie and Ussing<sup>4</sup> have shown, such changes are not expected with the use of  $SO_4^{2-}$  Ringer's solutions since anion permeability is so small. Indeed, using  $^{35}SO_4^{2-}$ , we were unable to detect measurable transmembrane flux in 30-min periods<sup>5</sup>. It is not inconceivable that there may be cytoplasmic changes in composition, changes which in effect amount to exchanges of  $Na^+$  for  $K^+$  and vice versa, presumably with some preservation of the total osmotic concentration. These changes, it may be argued, would lead to slopes less than that given by equation (1), that is, 59 mV per log unit, but the reduction in magnitude would be expected to be greatest at high  $[Na^+]_o$  and low  $[K^+]_o$ , the reverse of that observed. Essig and Leaf<sup>7</sup> have interpreted the effect of bathing the serosal surface with  $K^+$  free Ringer's solutions, an effect characterized by a marked reduction in active sodium transport, as one attributed to an increased resistance of the mucosal bounding membrane. Presumably this increase in  $[K^+]_o$  should decrease the resistance of this bounding membrane and lead to an increase in active sodium transport, an effect again opposite to that observed (Fig. 2).

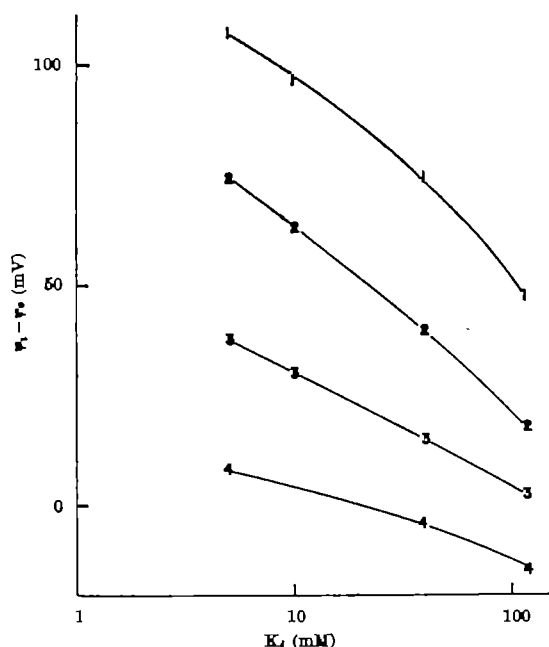


Fig. 1. Data from a representative experiment showing the effect of varying  $[K^+]_o$  on the potential,  $\Delta\psi$ , across frog skin at different values of  $[Na^+]_o$ . The numbers refer to the sequence of  $[Na^+]_o$  changes: 1,  $[Na^+]_o = 115$  mM; 2,  $[Na^+]_o = 30$  mM; 3,  $[Na^+]_o = 6$  mM; 4,  $[Na^+]_o < 1$  mM.

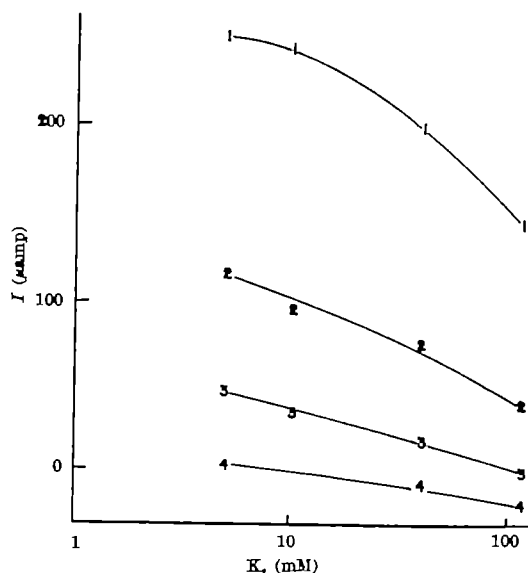


Fig. 2. Data from the same experiment as shown in Fig. 1 showing the effect of varying  $[K^+]_o$  on the short-circuit current,  $I$ , at different values of  $[Na^+]_o$ . The area of the skin was about 3.8 cm<sup>2</sup>.

In view of our recent findings from microelectrode investigations, showing that the slopes  $\partial\Delta\psi_m/\partial\log[Na^+]_o$  and  $\partial\Delta\psi_m/\partial\log[K^+]_o$  ( $\psi_m$  being the potential measured by the microelectrode) are continuous and are linear functions of distance in that tissue region manifesting the total electrical potential<sup>8</sup>, we are more inclined to attribute the contralateral effects of  $Na^+$  and  $K^+$  on the potential as mediated by their primary effects on the sodium transport system. In this respect, therefore, one must conclude that at concentrations in the range 0–2.5 mM,  $K^+$  bathing the inside (serosal) surface acts as an activator of the sodium ion transport system, but as the concentration is increased above this level,  $K^+$  serves to inhibit the system in a manner reminiscent of non-competitive inhibition of enzymes. Mechanisms at this point must remain a matter of conjecture, but it is not inconceivable that the  $Na^+$  and  $K^+$  sensitive ATPase<sup>9</sup> may be involved.

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<sup>6</sup> MacRobbie, R. A. O., and Ussing, H. H., *Acta Physiol. Scand.*, **53**, 348 (1961).

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<sup>9</sup> Skou, J. O., *Membrane Transport and Metabolism*, edit. by Kleinscheller, A. and Kotyk, A. (Academic Press, New York, 1961).

## CONTROL OF GLYCOLYSIS IN WASHED SUSPENSIONS OF *Streptococcus faecalis*

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IT has been proposed that control of the rate of anaerobic glycolysis is mediated by intracellular orthophosphate or feedback mechanisms involving adenosine triphosphate (ATP)<sup>1</sup> and adenosine diphosphate (ADP)<sup>2</sup>. It is postulated that, if an organism increases its energy requirements, the rate of hydrolysis of ATP increases; this causes the feedback system to operate to oppose any change in the level of ATP by increasing the rate of glycolysis so that the rate of production of ATP is correspondingly increased to balance the changed conditions. This argument would require that an organism should exhibit a fairly constant pool of ATP or ADP; but measurements

of the sizes of these pools have been rather inconclusive<sup>3</sup>, and Commoner<sup>4</sup> proposes that direct feedback control is inoperative in non-growing organisms.

It has apparently not been suggested that adenosine monophosphate (AMP) is involved in regulation of glycolysis; however, the recent suggestion that this compound is responsible for feedback control of gluconeogenesis<sup>5</sup>, makes it necessary to consider AMP also as a possible intermediate in control of glycolysis.

Measurements of rates of glycolysis and the level of the ATP pool in non-growing suspensions of cells of the facultative anaerobe, *Streptococcus faecalis*, suggest that there is

no feedback control of glycolysis in a washed suspension and that control is exerted indirectly through the endogenous metabolism of the organisms. ATP is involved in regulation, but the ATP pool concerned is not that directly participating in glycolysis.

In a washed suspension of cells suspended in a phosphate buffer the intracellular level of inorganic phosphate will be high and constant<sup>1</sup>. In addition, the cells can at most maintain the *status quo* since no extracellular materials for synthesis are present; accordingly the total concentrations of the adenine nucleotides will be constant, and the time-scale in the experiments to be described is long enough to permit the assumption that equilibrium between the concentrations of the nucleotides will be maintained. Making similar assumptions to these, Krebs<sup>2</sup> has calculated that under physiological conditions a small change in the concentration of ATP reflects larger changes in the other nucleotides. The relative changes are ATP1:ADP2:AMP15, so that examination of the ATP pool alone should indicate whether regulatory mechanisms exist in non-growing suspensions.

In experiments to examine the level of the ATP pool, *Streptococcus faecalis* (A.T.C.C. 4083) was grown on complex medium containing 2 per cent each of sodium citrate, dried yeast extract, peptone and glucose. Washed suspensions were prepared from cells 17 h after inoculation by washing twice in de-aerated 0.2 M phosphate buffer, pH 6.2, and suspending in the same buffer under a gas phase of nitrogen at a cell density of about 1 mg/ml. Pool-levels were measured by a modification<sup>3</sup> of the method of Strange *et al.*<sup>4</sup>, using 0.3 M sulphuric acid to extract ATP from cells and a recording photometer to measure the bioluminescence produced in the luciferin-luciferase assay method.

In a washed suspension of cells carrying on glycolysis, the kinetics of glycolysis are accurately of zero order and a constant rate of glycolysis has been observed for several hours when large quantities of glucose are added to a suspension<sup>5</sup>. If control were exerted through the nucleopools, a constant level should be observed in the ATP pool; but in fact the level of the ATP pool during glycolysis may increase to ten times the level found in a cell suspension in the absence of energy source (Fig. 1). The process operating is the combination of a constant rate of increase in pool level concomitant with a constant rate of generation of ATP from glycolysis, opposed by an exponential decay. These processes eventually balance, so that the pool-level asymptotes to an upper limit. When all the added glucose has been metabolized the decay process alone operates. Calculation of the rate of generation of ATP from glycolysis, compared with the measured level of ATP in the pool, indicates that the turnover time of the

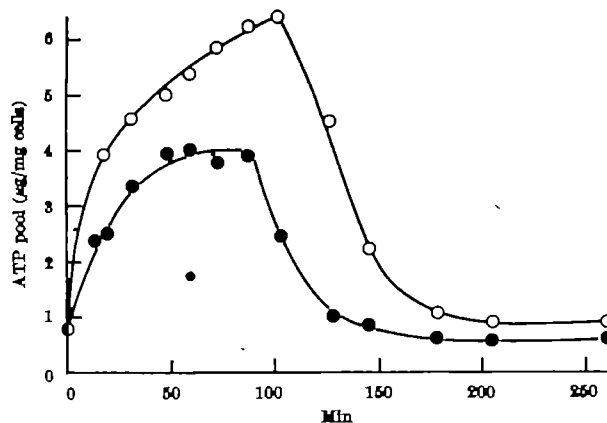


Fig. 1. The ATP pool in *S. faecalis* during glycolysis. Cells grown on 2 per cent complex medium, washed and suspended in 0.2 M phosphate buffer, pH 6.2, under nitrogen. Cell density 1.48 mg/ml. Cells aged 2.5 h before substrate added. ○, Substrate glucose 3 mg/ml; ●, substrate glucose 3 mg/ml plus casamino acids (Difco) 0.5 mg/ml.

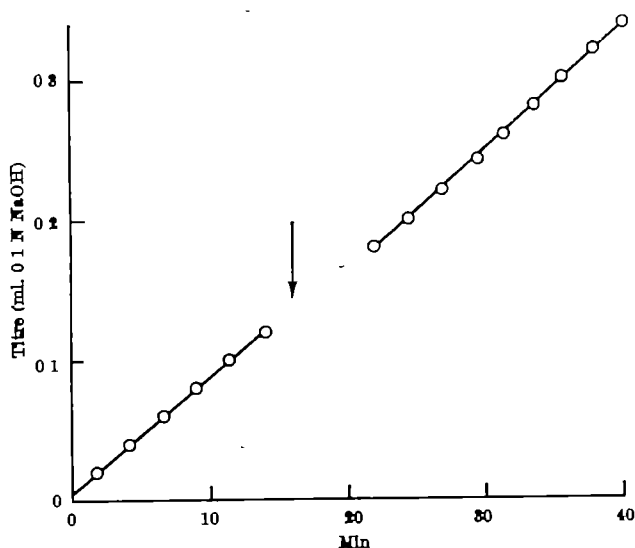


Fig. 2. The rate of glycolysis of cells with and without added amino-acids. Cells aged as in the experiment of Fig. 1, then resuspended in 0.01 M phosphate buffer, and rate of hydrogen ion production measured by titration with 0.1 N NaOH. 6.4 mg cells. Substrate glucose 4 mg/ml. At arrow 0.5 mg/ml casamino acids were added.

ATP in the pool is about 5 sec; this is short compared with the half-time of the decay process.

Cells of *S. faecalis* lacking reserve materials for endogenous metabolism can incorporate amino-acids into cellular material at a rapid rate<sup>6</sup>. In the experiment shown in Fig. 1 the cell suspension was aged for 150 min before the addition of substrate so that endogenous reserves<sup>10</sup> were depleted but not exhausted, then glucose alone was added to one aliquot of cells and glucose plus amino-acids from casein hydrolysate to the other. The synthetic reactions occurring during incorporation of amino-acids deplete the ATP pool so that the rise in the level of the pool is much less and the asymptotic limiting value is only about half that found with glucose alone. However, the rate of glycolysis is not significantly affected by the addition of casamino-acids to the suspension (Fig. 2).

These findings are in direct contradiction to the hypothesis of feedback control. A constant rate of glycolysis is accompanied by a very large change in the level of the ATP pool and presumably also the ADP and AMP levels, yet an increased demand for energy to perform synthetic reactions, though it lowers the ATP pool level drastically, does not affect the rate of glycolysis. The ATP pool appears to behave as a dependent variable rather than one exercising control.

When *S. faecalis* is grown on complex media with excess energy source, as in these experiments, reserve materials are accumulated; starved washed suspensions of such cells in phosphate buffer exhibit a large endogenous metabolism which continues for several hours<sup>10</sup>, and the glycolytic activity, determined by withdrawing a sample of cells from the suspension, adding glucose to this sample and titrating the hydrogen ion produced at pH 6.0, is constant over the whole of this period. The glycolytic activity found in these measurements has a characteristic value of 0.07  $\mu$ moles of glucose per min per mg dry-weight of cells at 37° C; it is unaffected by the environmental factors which influence endogenous metabolism<sup>11,12</sup>.

Fig. 3 shows that the level of the ATP pool in a starved suspension is constant for the period during which endogenous metabolism and constant glycolytic activity are observed. The pool-level is unaffected by the addition of thioglycollate, which markedly affects the rate of endogenous metabolism<sup>13</sup>. Similarly, it has been found that change in the concentration of cells in the suspension, which also strongly affects endogenous metabolism, has no significant effect on the pool level.

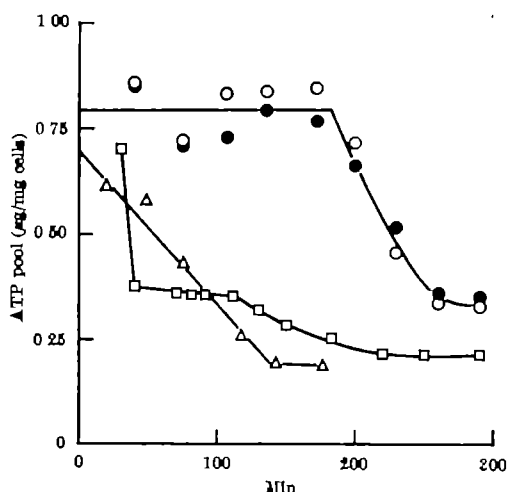


Fig. 2. The ATP pool in starved, washed suspensions. Cells with endogenous reserves, grown and suspended in phosphate buffer as in the experiment of Fig. 1. Cell density 0.69 mg/ml. ○, No thioglycollate; ●, 0.05 per cent sodium thioglycollate added; △, cells without endogenous reserves, grown on complex medium containing 0.1 per cent glucose, washed and suspended as in previous experiments. Cell density 0.73 mg/ml; □, cells with endogenous reserves, suspended in 0.2 M citrate buffer pH 6.2 under nitrogen. Cell density 1.23 mg/ml.

In contrast to this behaviour, suspensions in phosphate buffer prepared from cells grown on complex medium with the concentration of glucose reduced to 0.1 per cent so that the growth was limited by source of energy, have no detectable endogenous metabolism. The ATP pool has no period with a constant level. The glycolytic activity of the suspension is not maintained at the constant level characteristic of cells carrying on endogenous metabolism, but is initially higher than this and falls rapidly, losing about 10 per cent of the initial activity per hour.

It is found that phosphate buffer concentrations between 0.01 M and 0.2 M have no effect on glycolytic activity. However, incubation of washed suspensions in citrate buffer of cells grown with excess energy source abolishes the period of constant glycolytic activity<sup>8</sup> and the ATP pool stabilizes at a low level because of the depletion of intracellular phosphate<sup>1</sup>, even though, in this case, endogenous reserve materials are present.

The experiments suggest that glycolysis in washed suspensions of *S. faecalis* is not controlled by direct feedback. During the period of endogenous metabolism the glycolytic activity remains constant regardless of whether the suspension is incubated with or without glucose, so

that no feedback loop depending on glucose can operate. It seems that the cells have a fixed capacity for glycolysis which is not modified by their environment (provided phosphate is present) or their energy requirements. This behaviour can be explained, without postulating feedback, by normal kinetic mechanisms. We have not observed a glycolytic activity which is stable with time, lower than that found in such a washed suspension; cells which have exhausted their endogenous reserves and lost glycolytic activity after prolonged incubation have a glycolytic activity which continually decreases with time<sup>10</sup> in the absence of glucose, but when energy source is added they tend to restore their rate of glycolysis<sup>9</sup> towards the level characteristic of cells possessing endogenous metabolism.

For a fixed rate of glycolysis to be operative, it is necessary that a sufficient quantity of active enzymes should be present. Energy appears necessary to maintain these, and the supply of this energy seems to be one function of the endogenous metabolism. If there is no endogenous metabolism, or a sufficient ATP pool to couple the energy from this process to maintenance reactions is not available, no period of constant glycolytic activity is found, so that ATP seems to be involved in regulation of glycolysis in washed suspensions only in this limited sense.

The rate of endogenous metabolism and presumably of generation of ATP increases under conditions of environmental stress<sup>10</sup>. However, the ATP pool is unaffected by the change in endogenous metabolism, suggesting that this metabolism is regulated to the energy requirements of the organisms. The rate of generation of ATP by endogenous reactions gives rise to a pool-level small compared with the pool-level measured during glycolysis, yet it appears sufficient to allow the organisms to maintain themselves; glycolysis in washed suspensions may therefore generate ATP greatly in excess of the requirements of the cells, and the control systems of the organisms apparently cannot 'shut down' sufficiently to prevent this happening.

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## CONTINENTAL DRIFT

### Continental Drift and the Age of Angiosperm Genera

THE decades of dispute and indecision over the hypothesis of continental drift seem to be drawing to a close. Modern techniques such as rock magnetism measurement, investigations of ancient climates and palaeowinds, and also the computer investigations of continental outlines reported by Sir Edward Bullard<sup>1</sup>, all point to the fact that continental drift has been and probably still is occurring. A further major factor in the discussion may be the time at which drift began, and when the present continental areas were delimited. This question is of great significance to plant geography and evolution, where the problems of plant distribution in disjunct areas frequently suggest a correlation with continental drift or former intercontinental land connexions.

Such disjunctions in areas of distribution over the globe are perhaps most marked in the southern hemisphere, where the distances between continents are very great.

Several angiosperm genera can be cited, a classic example being the cotton genus *Gossypium*. Here, taxonomic sections or groups of sections have been shown to possess different genomes, most of which are confined to different continental masses. Thus the *D* genome (Sections *Ericoxyla*, *Klotzschiana* and *Thurberana*) is confined to the American continent and the *O* genome (*Sturtiana*) to Australia. Even though the *B* and *H* genomes are found in Africa it is possible that one of them might have migrated into that continent from India as suggested by Hutchinson, Silow and Stephens<sup>2</sup>. These authors have postulated Cretaceous fragmentation of a former super-continent in order to explain present-day distribution of the genus.

In the grass genus *Bromus*, distinct sections based on morphological, cytological, genetic, serological and biochemical criteria are found in the various southern continents. The very distinct section *Ceratochloa* occurs in South America, as does the peculiar *Neobromus* group. *Ceratochloa* in North America probably developed by

hybridization of South American species<sup>8</sup> when they migrated north over the Central American link in the Pliocene or later. The Section *Bromopsis*, although mainly North American and Eurasian, occurs also in Africa. *Bromus* distribution therefore suggests that the early primitive forms were widespread over a large land mass, but produced the present well-marked sections owing to geographical isolation caused by continental fragmentation and drift.

Although the genus *Solanum* is pantropical, of the six sub-genera recognized by Seithe<sup>4</sup>, four are mono-continental. Thus the sub-genera *Lycopersicon* and *Bassovia* are confined to South America, *Archaeosolanum* to Australia and New Zealand, and *Lyciosolanum* to Africa. The two other sub-genera, *Solanum* and *Stellatipilum*, are widespread. These latter have been divided by Seithe into 42 sections and sub-sections, and 32 of these (some 75 per cent) are also mono-continental. Many of the others may well owe their wide distribution to the 'woody tendencies' exhibited by certain species, which have enabled them to spread rapidly as adventives in more recent times.

To quite a large extent, therefore, the taxonomic groupings within the genus *Solanum* accord with the geographical separation of the three southern continents, South America, Africa and Australia.

Many other examples might be quoted, for example, leafy Hepaticae<sup>9,10</sup>, and marine Copepoda<sup>7</sup>, where the facts of distribution are most convincingly explained by continental drift.

Continental drift seems now to be a more acceptable theory than the alternative hypothesis of land bridges, since Heezen<sup>8</sup> states that the petrography of ocean floors is markedly different from that of continental areas. This clearly reduces the likelihood of the sea-bed ever having been a land area, except in a very few cases, for example, near the coast of South Africa, where submergence of continental blocks could have taken place. Another possible explanation of disjunct distributions of organisms is by chance long-distance dispersal. If this phenomenon were at all important we should expect the vegetation cover of the world to be much more uniform than it is. Furthermore the very high endemism in isolated oceanic islands seems to show that long-distance dispersal is a very inefficient method of spread.

The major objection to continental drift as an explanation of modern plant distribution has been that it occurred too early, before modern genera were in existence. However, although the break-up of the ancient continent of Gondwanaland has often been placed at the end of the Triassic, Lester King<sup>9</sup> quotes evidence that it started in the mid-Jurassic (140 million years ago), and not until mid-Cretaceous (about 90 million years ago) were the present outlines of the continents defined. Thus King regards drift as a primarily Jurassic to mid-Cretaceous occurrence. His basic evidence for the existence of the present continents in past times is the presence of continuous and uninterrupted sequences of marine deposits on modern continental margins dating from mid-Jurassic at the earliest. This dating of continental origin and drift is in the period when the angiosperms are thought to have been actively evolving, and it is likely that the angiosperms are older than is at present realized<sup>10</sup>.

On the basis of palaeomagnetic data, Creer<sup>11,12</sup> thinks that the beginning of the split occurred in Permo-Triassic times, some 200 million years ago. The polar wandering curves for South America, Africa and Australia begin to diverge at the Permo-Triassic point, although the indications are that Antarctica did not separate from South Africa until possibly Jurassic times. Apart from this, the separation of the various parts of Gondwanaland is thought to have been complete by the end of the Triassic, some 180 million years ago. Nevertheless, it is thought that drift continued up to the Tertiary. Considering these same data, again in the form of polar wandering curves, Khan, on the other hand (private communication), believes that

drift could have begun in the mid-Mesozoic and that the significant splitting and drifting of parts of Gondwanaland took place during the Cretaceous.

From the above-mentioned geological data we must conclude that genera such as *Gossypium*, *Bromus* and *Solanum* were already in existence by at least early Cretaceous (about 100–120 million years ago), since by that period, according to King<sup>9</sup> and many other geologists, the present southern continents had drifted apart. On Creer's rock magnetism data the formation of these genera must be considered to be even more ancient, and must date from Permo-Triassic times, at least 200 million years ago. If we take the more conservative estimate of King and others this still means that at least some of the angiosperm genera were in existence about 100 million years ago, and we are therefore forced to the conclusion that the families to which they belong must be older still. We naturally assume that the 'genus' when first formed would not have been recognizable as such but might rather have been a widespread—and perhaps highly variable—series of forms to which, if we had been able to see it then, we might have given the status of species.

One other point of interest to biologists emerges from these recent geological investigations on continental drift. This concerns the very rapid advance of the flowering plants in Cretaceous times, a fact which considerably excited the attention of Charles Darwin. To quote Darwin himself<sup>13</sup>: "The rapid advance, as far as we can judge, of all the higher plants within recent geological times is an abominable mystery". With what seems to us nowadays to be prophetic judgment, Darwin also states<sup>14</sup>: "I have been so astonished at the apparently sudden coming in of the higher phanerogams, that I have sometimes fancied that development might have slowly gone on for an immense period in some isolated continent or large island, perhaps near the South Pole". This thought has also occurred to various botanists since Darwin's time. For example, Camp<sup>15</sup> voiced the opinion that "the divergencies of the basic generalized familial groups had been accomplished on this southern land mass (i.e. Gondwanaland) certainly by the mid-Mesozoic". The same view was held by Seward<sup>16</sup> and by Hemsley Thomas<sup>17</sup>.

On the other hand, Stebbins<sup>18</sup> thinks that the apparently rapid evolution of the angiosperms could be accounted for by assuming that their origin was not much earlier, geologically speaking, than their first appearance in the fossil record, and that during this period they were undergoing a burst of particularly rapid evolution. Takhtajan<sup>19</sup> considered that the geographical changes associated with the break-up of Gondwanaland brought about a rapid spread of the angiosperms at the expense of other plant groups because of their "high evolutionary plasticity expressed by their extraordinary diversity" (English translation, p. 13).

While this view of the rapid extension of angiosperms in mid-Cretaceous times sounds very plausible, another possible explanation for their sudden appearance should be considered. The distributional data presented here indicate that angiosperm families must have been in existence, possibly as small populations only, at least by Jurassic times, and that the group as a whole may have originated even further back, in Carboniferous or Permian, in the southern land mass of Gondwanaland. Their virtual absence in pre-Cretaceous times from the better-studied rocks of the northern hemisphere may then be accounted for by assuming that the evolution of the earlier families and genera took place in Gondwanaland. According to Takhtajan<sup>19</sup> no undoubted angiosperm fossils other than spores are to be seen in the Jurassic; impressions of angiosperm leaves appear in the Lower Cretaceous (Neocomian) (though there is some doubt as to their authenticity); undoubted dicotyledonous leaves are seen in the second half of the Lower Cretaceous (Aptian and Albian), but it is not until the Upper Cretaceous (some 90 million years ago) that they become really abundant. So far as we can see,



all this information is obtained from northern hemisphere rocks.

These data could readily be explained by postulating an exclusively Gondwanaland origin for angiosperms, which were unable to colonize the northern land masses until the fragments of the southern super-continent had drifted like rafts, with the flowering plants growing on them, into the vicinity of Laurasia. According to King<sup>11</sup> (and private communication), so far as north Africa and south Europe are concerned, this collision "would seem to have taken place about the mid-Cretaceous (pre-Gosau) movements of the Alpine belt. For India the same Tethyan geosyncline was filled up by the late Cretaceous, by which time India would appear to be united with Asia and great mountains had been formed". King goes on to state, however, that "These dates are provisional only".

This palaeogeographical evidence of a mid-to-late-Cretaceous union of some of the southern land-masses with Europe and Asia accords very well with the generally accepted period in which the really abundant angiosperm fossils appeared in these latter regions. Before this, there is only scanty evidence of leaf impressions (lower Cretaceous) while even earlier (Jurassic) only spores have been observed. This would fit well with the assumption that in Jurassic times the light, wind-borne spores floated northwards from Gondwanaland over a gap that the heavier seeds themselves could not bridge. As the gap narrowed, a few genera, perhaps with lighter seeds or more efficient methods of dispersal, colonized the northern land mass, forming a vanguard to the main colonizers which followed in enormous quantities, once the land connexion had been firmly established in mid-Cretaceous. Axelrod<sup>12</sup> quotes evidence that the advancing angiosperms spread northwards from tropical regions once part of Gondwanaland, based on the fact that fossil angiosperm material occurs in lower strata at lower latitudes. His evidence for southward migration is very scanty.

This hypothesis of the advance of the angiosperms presupposes that the group was quite numerous in Gondwanaland, or at least must have been widely dispersed there in Mesozoic times. Angiosperms were certainly not numerous in the Jurassic of Yorkshire in one of the best known sites in the northern hemisphere, according to Harris<sup>13</sup>, who states: "I would say that the Jurassic flora of Yorkshire which has been studied scientifically for about 140 years must have had about as much time spent on it, in relation to its size, as any other flora on earth... and no one has found a real angiosperm in it". Although no undoubted angiosperm fossils have yet been found in the Palaeozoic to Mesozoic floras of the southern hemisphere, it could hardly be claimed that as much work had been done on them as on the Jurassic flora of Yorkshire. Plumstead<sup>14</sup> has contributed a great deal of knowledge to the palaeobotany of the southern hemisphere and especially of the *Glossopteris* flora. Although she cites and discusses the work of other investigators in the field, it becomes clear that the hoped-for abundance of angiosperms still cannot be verified, unless, as King (private communication) suggests, the *Glossopteris* flora could itself be considered as the earliest angiosperms. This seems rather unlikely, at the present state of our knowledge.

The apparent absence of fossil angiosperms in the southern hemisphere might be explained partly by assuming that the flowering plants occurred as small, rather isolated populations and that although some fossil material may exist it has not yet been discovered because of its relative scarcity. It could also be explained by assuming that the ecological distribution of the early angiosperms was such that they largely escaped preservation. Axelrod<sup>12</sup> and Heslop-Harrison (private communication) have discussed the possibility that the Gondwanaland flowering plants might well have exploited drier, non-fossilizing habitats (possibly montane) situated well away from the competition of the humid *Glossopteris* flora.

The facts of distribution of the genera quoted at the beginning of this paper, *Bromus*, *Gossypium* and *Solanum*, cannot readily be explained unless we assume that they were already present in Gondwanaland at least by early Cretaceous times, almost certainly during the Jurassic, and possibly even earlier. We therefore suggest that a re-examination of existing material and a renewed search in other southern continental areas should be made, especially in deposits that are known to have been separated from the delta and swamp conditions inhabited by the Glossopterids, either along the margins of these regions or in what are believed to have been upland, montane or semi-desert areas. Harris<sup>13</sup> states: "We want early angiosperms urgently, almost as a thirsty man needs water". We agree, and therefore suggest that renewed efforts should be made to examine Mesozoic rocks of the former land mass of Gondwanaland for such fossils, since it is there that the distributional evidence of present-day plants indicates that they should be found.

We may summarize our conclusions as follows:

(1) Distributional evidence of living plants indicates that angiosperms were almost certainly present on the super-continent of Gondwanaland in Mesozoic times, if not before.

(2) Representatives of some modern genera of flowering plants were in existence by at least early Cretaceous times (100-120 million years ago).

(3) From the present lack of evidence of abundant flowering plant remains in the southern continents one must assume that they were rare in those areas until mid-Cretaceous or were exploiting drier and therefore largely non-fossilizing habitats (possibly montane).

(4) A possible explanation for the apparently sudden appearance of flowering plants in the northern hemisphere is that they evolved in Gondwanaland and were able to migrate from it only after one or more of its fragments had drifted northward and come into contact with the northern land masses of Europe, Asia and North America.

(5) A more intensive search for fossil angiosperms should be made among Mesozoic deposits from the southern hemisphere (including parts of former Gondwanaland now in the northern hemisphere) and especially in deposits, if any can be detected, that lie marginally to, or completely away from, the main lowland fossil beds.

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## Palaeomagnetism and the Time of the Onset of Continental Drift

My conclusion that Gondwanaland started to break up in the Permo-Triassic<sup>1</sup> should be regarded as an early limit. It depends partly on palaeomagnetic work on the vast Mesozoic basalt and diabase formations of the Gondwanic continents<sup>2-4</sup>. The procedure was to compute mean palaeomagnetic directions for each continent, namely, South America, Africa, Australia, Antarctica, and India. These directions correspond to the mean age of each formation, and, in the absence of evidence to the contrary, these ages were taken to be the same. On this basis it was concluded that the palaeolatitudes and palaeomeridian directions were inconsistent both with the present arrangement of the continents and also with their arrangement as adjacent parts of Gondwanaland<sup>5</sup>: the palaeomagnetic data were consistent with an intermediate arrangement of continents. The assumption that the mean age of igneous activity was the same in all the Gondwanic continents was a reasonable one to make initially. However, it is not adequate for more precise work.

Potassium argon ages have now been determined for the Serra Geral formation (South America) and are grouped round 140 m.y. and 120 m.y.<sup>6,7</sup>. Stormberg lavas (South Africa) have been dated at 190 m.y. and 184 m.y.<sup>8</sup>. Tasmanian dolerites have been dated at 170 m.y.<sup>11</sup> and Ferrar dolerites (Antarctica) at 163 and 147 m.y.<sup>12</sup>. Hence the ages of the rocks sampled for palaeomagnetic

investigations in the Gondwanic continents could possibly differ by some tens of millions of years. If continental drift or polar wandering were rapid in those times, the conclusions drawn from the original surveys may have to be modified.

It is not possible to divide palaeomagnetic data at present available into radiogenically dated sub-groups. However, more complete surveys are now being carried out in South America and in Africa, and every lava flow and dyke used will have to be dated radiogenically.

In due course it should be possible to answer with more assurance the question of whether the onset of drift was associated with the first outpourings of lava 190 m.y. ago, whether it started rather earlier as I had concluded, or later (preceding communication).

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## DEPOSITION OF STRONTIUM AND CALCIUM IN SNAIL SHELL

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THE chemical similarity between strontium and calcium has led to widespread use of <sup>87</sup>Sr-Ca ratios in interpreting the movement of <sup>87</sup>Sr in the environment and in metabolic processes<sup>1-3</sup>. While the ratios are useful in assessing the hazard to large sections of the population, they add little to our understanding of the fundamental relationship between strontium and calcium in organisms. Mollusc shell is considered to be a biogeochemical sink since incorporated materials are physiologically, as well as physically, isolated and unavailable for the animal's metabolism, which is not the case in the bone of rats or other mammals<sup>4</sup>. The amounts of newly deposited strontium and calcium in the shells of snails should therefore be related to the concentrations of these elements in experimental environments. The object of this research was to demonstrate precisely the relative effect of strontium and calcium concentrations in the environment on their uptake and incorporation into snail shell. This experiment with the freshwater snail, *Physa heterostropha* Say, differs significantly from other work on strontium and calcium uptake, in that the strontium and calcium concentrations of the experimental media were rigidly controlled by assay of the strontium and calcium content of all chemicals used. While there is a constant relationship between radioactivity and mass for a given radioisotope, a more useful comparison of the deposition of calcium and strontium may be obtained by using atom concentrations. In this experiment <sup>45</sup>Ca and <sup>87</sup>Sr were used as tracers and specific activities (radioactive atoms/total atoms of the same element) were used to determine deposition.

As the functional relationship between uptake of strontium and calcium in snail shell and the concentration

of these elements in the environment is unknown, one can do no better than to approximate it by a general form. One such form is a truncated Taylor series expansion:

$$Y = \beta_0 + \sum_{i=1}^n \beta_i X_i + \sum_{i,j=1}^n \beta_{ij} X_i X_j + \epsilon$$

in which  $Y$  is a response (either strontium uptake or calcium uptake),  $X_i$  and  $X_j$  are concentrations of strontium and calcium in the medium,  $\beta_0$ ,  $\beta_i$  and  $\beta_{ij}$  are unknown coefficients, and  $\epsilon$  is the deviation of the truncated Taylor series from the unknown functional form. From experimental evidence it is possible to estimate the unknown coefficients and to assess the significance of  $\epsilon$ . An efficient way to accomplish this is to perform an experiment based on an experimental design of the factorial type. The second order rotatable designs of Box and Hunter<sup>5</sup> are especially suited to this purpose.

If the scales of strontium concentration and calcium concentration in the medium are transformed to equal scales,  $U_1$  and  $U_2$ , a suitable design is obtained by selecting six combinations of  $U_1$  and  $U_2$  which are equally spaced on a circle in the  $U_1$ ,  $U_2$  plane together with that combination of  $U_1$  and  $U_2$  which is in the centre of the circle. If a number of snails are treated in each of the solution media corresponding to these points the several  $\beta$ 's of the Taylor series may be estimated and the significance of the  $\epsilon$  term assessed. In addition, the predicted response (that response calculated at a point in the  $X_1$ ,  $X_2$  plane using the estimates of the  $\beta$ 's) will have an uncertainty which is dependent only on the distance of the point from the centre of the design and not on its direction. Furthermore, this uncertainty will be relatively uniform throughout the experimental region if the centre point of the design is performed twice.

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This seven-point design with the centre point duplicated was laid out in the  $U_1, U_2$  plane. Then, to achieve a random orientation, the design was rotated through a randomly determined angle ( $41^\circ$ ). The resulting co-ordinates were transformed back into units of strontium and calcium concentrations ( $X_1$  and  $X_2$ ) with the results as shown in the second and third columns of Table 1. These concentrations are essentially within the extreme range of strontium and calcium concentrations in natural waters.

Culture media were prepared from compounds of high purity, the strontium and calcium contaminants of which were accurately known. Reagent grade  $\text{CaCO}_3$  was further purified in the Analytical Chemistry Division of Oak Ridge National Laboratory and the compound used contained about  $1.8 \mu\text{g Sr/g CaCO}_3$ , the minimum level of detection. The basic medium was composed of  $0.5 \text{ g KNO}_3$ ,  $0.1 \text{ g NH}_4\text{H}_2\text{PO}_4$ ,  $0.25 \text{ g MgSO}_4$ ,  $0.1 \text{ g NaCl}$  and a trace of  $\text{FeCl}_3$  per litre. The solubility product constants were not exceeded for calcium or strontium salts in this medium. Calcium and strontium amendments in the form of chlorides were added to the basic medium according to the statistical design (Table 1). The pH of the media was adjusted to neutrality by the addition of small amounts of  $\text{NaOH}$ , and 200 ml. of solution were placed in 400-ml. 'Pyrex' beakers. Carrier-free  $^{88}\text{Sr}$  and  $^{45}\text{Ca}$  with negligible carrier ( $1.2 \times 10^{13}$  atoms/ml.) were added as tracers to all beakers in constant amounts.

Groups of ten snails, members of a graded series ranging in size from 9 mm to 13 mm long, were assigned randomly to each experimental concentration. The snails were maintained separately in beakers in a water bath at  $18^\circ\text{C}$ . Their food consisted of small amounts of the green, leafy portions of head lettuce. Minor amounts of calcium and strontium were included in the lettuce, which contained  $0.180$  and  $0.00062 \text{ mg/g}$ , respectively.

At the conclusion of the 21-day experimental period, snails were killed by freezing and the soft tissues were removed from the shell. Shells were then dried and weighed. Strontium-85 deposited in the shell was counted in a single-channel  $\gamma$ -scintillation spectrometer and  $^{45}\text{Ca}$ , as nitrate, in a  $\beta$ -liquid scintillation counter. Calcium-45 did not affect the  $^{88}\text{Sr}$  counting rate, but it was necessary to correct the  $^{45}\text{Ca}$  counts for a slight contribution from the  $^{88}\text{Sr}$  activity. Non-biological adsorption of both  $^{45}\text{Ca}$  and  $^{88}\text{Sr}$  was assessed by immersing fresh, 'Plasticine'-filled shells in the test solutions. However, adsorption of both elements was slight in comparison with biological deposition in the shells.

The average uptake of strontium and calcium in the snail shells is shown in the fourth and fifth columns of Table 1. The number of snails analysed at each experimental point is given in the last column. Losses occurred in the sample preparation, from mortality, and from snails crawling out of containers.

A rough idea of the variability in the data may be obtained by comparing the results of point 4 with those of point 8 which are the duplicated centre points. The standard deviations measured among snails treated alike are  $1.14 \times 10^{13}$  atoms/g for strontium and  $1.18 \times 10^{13}$  atoms/g for calcium with 39 degrees of freedom. However, the variability from one point of the design to another differed so much that the standard deviations at each point had to be considered.

The coefficients of the Taylor series were estimated by the method of least squares, weighting each observation by the reciprocal of the estimated variance at the point of observation.

Table 1

Experimental point	Design variables		Responses		No. snails
	Concentration in medium (atoms/ml.)		Uptake in shell (atoms/g)		
	Sr $\times 10^{13}$	Ca $\times 10^{13}$	Sr $\times 10^{13}$	Ca $\times 10^{13}$	
1	5.555	274.7	1.247	2.244	5
2	0.0111	162.5	0.00519	2.784	6
3	5.069	14.64	8.793	1.182	6
4	3.545	150.6	1.387	2.235	7
5	6.430	160.3	3.670	3.760	2
6	1.904	49.31	1.771	2.029	6
7	2.304	281.6	0.7676	3.145	8
8	3.545	150.6	1.326	2.011	7

Table 2. ANALYSES OF VARIANCE

Source of variation	Strontium		Calcium	
	D.F.	Mean square	D.F.	Mean square
Total	47		47	
Linear terms	3	105.89	3	73.04
Quadratic terms	3	13.20		
Residual	41	0.97	44	0.94

If the weights used were exact weights rather than estimated weights, probability tests would declare the residual mean squares non-significant and the other mean squares significant. Exact tests in the case of estimated weights are not known.

Table 3. REGRESSION COEFFICIENTS

Regression term	Sr uptake		Ca uptake	
	Coefficient $\times 10^{13}$	S.E. $\times 10^{13}$	Coefficient $\times 10^{13}$	S.E. $\times 10^{13}$
Intercept	96.78	63.2	19,800	4,970
Ca input	-2.12	0.50	54.14	15.94
Sr input	97.74	15.2	1,713	968
(Ca input) <sup>2</sup>	0.009	0.002		
(Sr input) <sup>2</sup>	11.88	3.37		
(Ca input) (Sr input)	-0.650	0.134		

For calcium uptake the data were adequately explained by the linear terms in the Taylor series. For strontium uptake, however, it was necessary to include all six terms of the truncated expansion. Reference to the analyses of variance in Table 2 will demonstrate the adequacy of the regression analysis on the two responses. The estimates of the coefficients in the regression equations are given in Table 3. For strontium these coefficients explain 90 per cent of the total variation and for calcium they explain 84 per cent.

The deposition of calcium and strontium by snails is illustrated graphically in Figs. 1 and 2, respectively. These are response surfaces calculated from a least squares fit of the data. Calcium uptake is represented best by a plane surface which is expressed mathematically as follows:

$\text{Ca uptake} = 19,800 + 54.14 \text{ Ca input} - 1,713 \text{ Sr input}$   
with uptake in atoms  $\times 10^{13}/\text{g shell}/21 \text{ days}$  and input in atoms  $\times 10^{13}/\text{ml}$ .

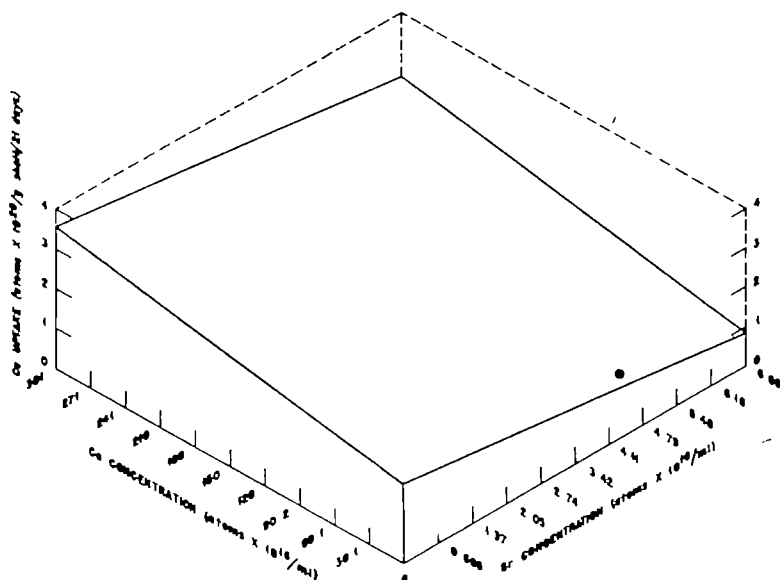


Fig. 1. Calcium deposition in snail shell as a function of environmental Ca and Sr concentrations ( $30.1 \times 10^{13}$  atoms Ca/ml. = 20 p.p.m. Ca;  $0.686 \times 10^{13}$  atoms Sr/ml. = 1 p.p.m. Sr).

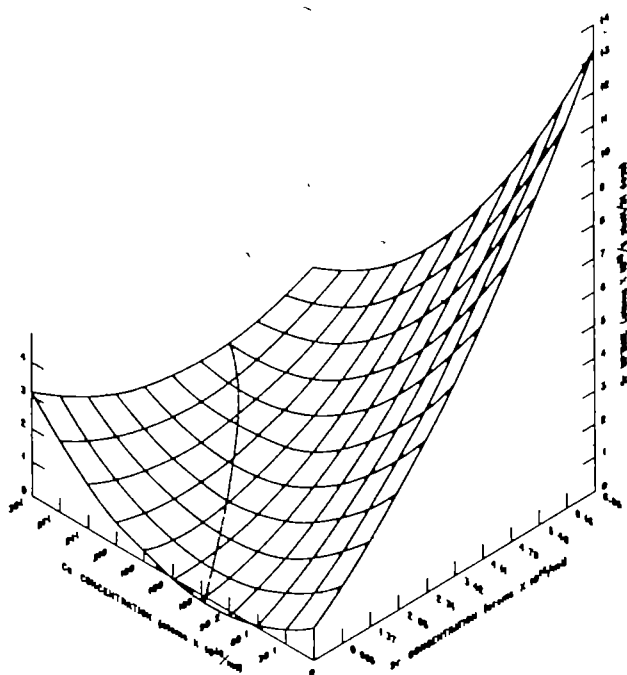


Fig. 2. Strontium deposition in snail shell as a function of environmental Ca and Sr concentrations ( $30.1 \times 10^{18}$  atoms Ca/ml. = 20 p.p.m. Ca,  $0.685 \times 10^{18}$  atoms Sr/ml. = 1 p.p.m. Sr)

Calcium carbonate is the basic structural component of mollusc shells, and a slight increase in growth is indicated by an increasing uptake of calcium with increasing calcium concentrations. However, growth differences in the seven experimental environments, as measured by calcium deposition, were only 0.17 times as much as the differences in the environmental calcium concentrations. While growth increased directly with the calcium concentration, the increases were relatively small when compared with the differences in environmental calcium concentrations.

The uptake of calcium is conservative when compared with that of strontium, which is best represented by a quadratic expression:

$$\text{Sr}_{\text{uptake}} = 98.78 - 2.12 \text{Ca}_{\text{input}} + 97.74 \text{Sr}_{\text{input}} + 0.009 (\text{Ca}_{\text{input}})^2 + 11.88 (\text{Sr}_{\text{input}})^2 - 0.695 (\text{Ca}_{\text{input}}) (\text{Sr}_{\text{input}})$$

with uptake in atoms  $\times 10^{16}$ /g shell/21 days and input in atoms  $\times 10^{18}$ /ml.

The range of strontium deposition in the snail shells was 2.9 times the range of environmental concentrations.

The surfaces depicting the deposition of calcium and strontium in Figs. 1 and 2 are based on least squares fits to the data in Table 1. Inspection of the data and Fig. 1 and Fig. 2 viewed grossly as plane surfaces indicates that deposition of both calcium and strontium in the shell of *P. heterostrophia* depends primarily on the respective concentrations of these elements in the immediate environment. Failure of the planes to intersect the zero axis is a geometric artefact arising from the extrapolation of the equations to zero; obviously there can be no incorporation of calcium or strontium when none is present. A slight negative interaction, that is, of environmental strontium on calcium deposition, and vice versa, is also evident from the skewed appearance of the incorporation planes. Biologically this interaction is probably best explained in the conventional terms of competition for incorporation sites.

The dotted line in Fig. 2 represents the minimum strontium deposition for the various combinations of strontium and calcium in the environmental media. To the right of the dotted line there is essentially a linear increase in strontium uptake with increasing environmental strontium concentrations. At lower calcium concentra-

tions (in this portion of the surface) there is a relatively greater uptake of strontium. This suggests a simple substitution of strontium uptake for that of calcium by snail shell when environmental calcium is deficient; it is in accord with the observations and conclusions of Odum<sup>8</sup> and Likens *et al.*<sup>9</sup> regarding strontium uptake by snails under conditions of high environmental strontium-calcium ratios (at low calcium concentrations). At all points to the right of the dotted line strontium uptake increases with increasing environmental strontium concentrations and decreases with increasing environmental calcium concentrations. To the left of the dotted line the opposite behaviour is noted, that is, increased environmental strontium depresses strontium uptake and increased environmental calcium enhances strontium uptake.

This phenomenon, the reversal of the relation of strontium deposition rate to strontium and calcium concentrations, is unexpected. Two possible explanations may be offered: (1) it is brought about by different reactions, purely chemical in nature, based largely on concentration differences; and (2) it is primarily biological and probably associated with a threshold or compensatory effect. There is no evidence, either specific or general, for accepting the first explanation. The effect would seem to be related to some physiological property of the mantle cells, possibly to membrane phenomena or secretory activity, and warrants further investigation.

Because of the interacting effects of different environmental strontium and calcium concentrations on strontium and calcium uptake, the presence of adventitious strontium in calcium compounds is an important factor in interpreting strontium uptake data. Recent analyses<sup>10</sup> of various reagent grade compounds show adventitious strontium ranges from 140 to 1,615  $\mu\text{g}$  strontium/g calcium. Hence, in a  $^{87}\text{Sr}$  uptake experiment in which the basic calcium content is changed by calcium amendments, there is also a proportionate change in the strontium concentration, thereby confounding the results by the unsuspected alteration of both important variables. Hegsted and Breenahan<sup>11</sup> noted the adventitious strontium in rat food, and observed that small additions of stable strontium to the diet enhanced the deposition of either  $^{45}\text{Ca}$  or  $^{87}\text{Sr}$ . Only with larger additions of stable strontium to the diet was a diluent effect of strontium noted.

Stable strontium added to the soil resulted in a similar effect on the uptake of  $^{87}\text{Sr}$  by ladino clover<sup>12</sup>. That the diluent effect of small amendments of stable Sr is generally ineffective was noted by Thompson<sup>13</sup>. The obvious correlative is that many organisms simply increase the total amount of strontium in their bodies when the environmental concentration is increased. In other words, the biological systems are not saturated with respect to strontium and only when the system becomes saturated does isotopic dilution become effective.

Much research on  $^{87}\text{Sr}$  and calcium in the environment has had an ultimate objective of obtaining information that would permit accurate prediction of  $^{87}\text{Sr}$  uptake by man or other organisms<sup>14,15</sup>. Ratios involving  $^{87}\text{Sr}$  and calcium<sup>1,9</sup> have been used frequently as a predictive tool. Since there is a large volume of information on the distribution of calcium in Nature, these ratios have the obvious feature of utilizing existing information along with new data on  $^{87}\text{Sr}$ . Because of the variability of these ratios, their limitations for prediction have been recognized<sup>16,17</sup>. A portion of the variability in  $^{87}\text{Sr}$  uptake by plants has been associated with the availability of calcium in soil<sup>18</sup>. An investigation of strontium and calcium uptake by algae<sup>19</sup>, under conditions similar to those of this experiment with snails, showed that uptake of either strontium or calcium was dependent primarily on the concentrations of the respective elements in solution. With either the algae, which have a rapid metabolic turnover of strontium, or the snail shell, which is a biogeochemical sink, it is clear that strontium uptake

is more closely associated with environmental strontium concentrations than with calcium concentrations. The results of these experiments show the advisability of considering stable strontium concentrations in an interpretation of the behaviour of  $^{87}\text{Sr}$  in biological systems.

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## GEOCHRONOLOGY OF THE LEWISIAN BASEMENT NEAR LOCHINVER, SUTHERLAND

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THIS article is a summary of part of a detailed investigation of the petrology, structural geology and isotopic geology of a small but complex area of Lewisian gneiss near Lochinver on the north-west coast of Scotland. The first systematic study of the Lewisian resulted in the comprehensive 1907 memoir by Peach *et al.*<sup>1</sup>, who identified three pre-Torridonian events, that is: (1) the formation of the fundamental pyroxene-bearing complex; (2) intrusion of north-west trending basic and ultrabasic dykes; (3) deformation and metamorphism of the dykes.

This chronological division of the Lewisian was enlarged on by Sutton and Watson<sup>2</sup>, who called the predyke (granulite facies) metamorphism 'Scourian' and the post-dyke (amphibolite facies) metamorphism 'Laxfordian'. The conclusions of these earlier workers have since been confirmed by the isotopic dating of Giletti *et al.*<sup>3</sup>, who concluded that the Scourian is older than 2,460 m.y. and that the Laxfordian is about 1,600 m.y. old. More recently, Evans and Tarney<sup>4</sup> have shown that the dykes were intruded between 2,200 and 1,900 m.y. ago.

**Isotopic results from Lochinver.** The strontium composition of whole-rock samples from each metamorphic facies in the Lochinver district is presented in Table 1. The extremely low rubidium content makes it impossible to determine the primary age of these rocks, but it does allow us to estimate the initial composition of the strontium. Since the present complex has a strontium isotopic composition comparable with the mantle<sup>5</sup>, it is unlikely that these rocks contained normal crustal abundances of rubidium for any great length of time, if ever. A normal crustal Rb/Sr ratio of 0.25 would result in a detectable increase in the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio in about 300 m.y. Similarly, if the acid gneiss represents pre-Scourian granite from which the potassium and rubidium have been removed by metasomatism, the time-interval between their original differentiation and later remobilization could not have been more than about 100 m.y. The pre-Scourian complex may thus represent either a basal layer in the Earth's crust which was depleted in volatile elements by a continuation of the same process by which it differentiated from the mantle, or it may have been a normal crustal series of rocks which lost its volatile elements during a profound metamorphism shortly after its original differentiation.

The existence of very old basement rocks with a strontium isotopic composition comparable with the present-

day mantle suggests that considerable caution must be used in applying Hurley's<sup>6</sup> strontium-87 model of continent formation. The addition of rubidium to such material during a subsequent metamorphic episode would produce a result isotopically indistinguishable from new crustal material derived from the mantle. Precisely such a metasomatic origin has been postulated<sup>7</sup> for the alkali-rich northern part of the Lewisian. If rocks of similar isotopic composition and Rb/Sr ratio to those of the Lochinver district composed a significant part of the early sialic crust, the proposed model dates the addition of rubidium to this material rather than the addition of large quantities of new sial to the continents.

**The Scourian episode.** Scourian granulite facies metamorphism produced a series of hypersthene-bearing rocks exposed to the south of Lochinver<sup>8</sup>. Potassium-argon dating of basic and ultrabasic granulite facies materials (Table 2, A) indicates a minimum age of at least 2,600 m.y. for this event. In interpreting the apparent ages obtained on different materials from these rocks, it must be remembered that they were formed under great pressure, and have been reheated to some extent by subsequent thermal events which caused complete amphibolization only a few kilometres away. Thus, some extremely retentive materials may contain excess or inherited argon<sup>9-12</sup>, while the least retentive minerals have probably lost argon.

Biotite and brown hornblende (samples 290 and 547) appear to be the least retentive minerals of the granulite facies suite. It is well established that biotite loses argon readily on reheating. Since ex-solution of dusty, opaque ore from brown hornblende is one of the first indications of retrograde amphibolization in this area, it is not surprising that it also has a poor retentivity. On the other hand, the pale green hornblende from ultrabasic rocks (494 and 610) appears to be an excellent mineral for potassium-argon dating. The two samples have potassium contents differing by a factor of two, yet give the same age to within 1 per cent, indicating little or no inherited argon. The apparent age of 2,620-2,630 m.y. obtained on these hornblendes is some 4 per cent greater than the oldest age obtained on biotite in this investigation.

Plagioclase and hypersthene each yield apparent ages of 2,860 m.y., but it is not known whether this represents the true age of their formation and cooling, or a spuriously high age due to excess argon. Augite from a biotite-augite pod (sample 546) clearly contains a great deal of excess argon, giving an apparent age of about 5,200 m.y.,

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as compared with 2,520 m.y. for the biotite. The calculated whole-rock age of this sample is  $2,600 \pm 60$  m.y., suggesting that much of the excess argon in the augite may have been derived from the biotite.

From the results obtained on the most retentive hornblende and biotite samples, it is concluded that Scourian, granulite facies metamorphism occurred at least 2,600 m.y. ago, and that it may be of considerably greater age.

**Intrusion of pegmatites.** The Scourian gneiss is intruded by alkali-rich pegmatites, which occur as dyke-like bodies. The pegmatite dykes south of Lochinver have been dated by Rb-Sr at  $2,250 \pm 50$  m.y. (ref. 7) and will be discussed in detail in a later communication. They appear to belong to the same generation of pegmatites that were dated by Giletti *et al.*<sup>8</sup>

**The Inverian episode.** Following the intrusion of pegmatites and prior to the intrusion of the north-west trending basic dykes, parts of the Lochinver district together with large portions of the central block of Lewisian gneiss underwent almandine amphibolite facies

metamorphism and structural deformation under a north-east-south-west principal stress. The name 'Inverian' is proposed for this previously unrecognized event, and the type area is specified as the lower three kilometres of the River Inver<sup>9</sup>.

Potassium-argon determinations on hornblende and biotite from amphibolite facies gneiss range from 2,100 m.y. to 1,560 m.y. (Table 2, B). Both hornblende and biotite from this suite are thoroughly recrystallized and readily distinguished petrographically from their counterparts in the pre-existing granulite facies suite. Since it is unlikely that any of the measured argon was generated prior to amphibolization, the 2,100 m.y. age obtained from the most retentive hornblende is taken as the best estimate of the minimum Inverian age.

The large spread in apparent ages of amphibolite facies materials is thought to reflect varying degrees of argon retentivity during the 1,580 m.y. Laxfordian reheating (see following). In this respect it should be noted that hornblende again appears to be more retentive than biotite.

Table 1. Sr 87/86 RATIOS IN LEWISIAN GNEISS OF THE LOCHINVER DISTRICT

Sample No.	Locality grid ref.	Rock type	Rb (p.p.m.)	Sr (p.p.m.)	87/86 observed	%E	86/88 observed	87/86 norm.	Est 87/86 2,600 m.y. ago
<b>A Pyroxene Gneiss (Scourian)</b>									
711	097210	Ultrabasic body	1.4 <sup>id</sup>	18 <sup>d</sup>	0.7213	0.20	0.1196	0.7227	0.7105
590	094201	Basic garnet gn.	bdl	20	0.7103	0.20	0.1196	0.7118	0.701
518	081207	Basic gneiss	9	247	0.7109	0.12	0.1194	0.7109	0.7070
545	078211	Acid gneiss	3	108	0.7077	0.21	0.1181	0.7035	0.7003
707	087191	Acid gneiss	bdl	390	0.7065	0.15	0.1202	0.7090	0.7063
Average Sr 87/86 ratio weighted for Sr content								0.7092	0.7065
<b>B Amphibolite Gneiss (Inverian and Laxfordian)</b>									
682	065242	Ultrabasic body	3	52	0.7073	0.21	0.1195	0.7076	0.7006
618	108237	Basic garnet gn.	1.5 <sup>id</sup>	41	0.7065	0.15	0.1196	0.7072	0.7029
498		Acid gneiss	bdl	503	0.7092	0.15	0.1189	0.7077	0.7073
499		Acid gneiss	bdl	483	0.7055	0.14	0.1190	0.7042	0.7086
Average Sr 87/86 ratio weighted for Sr content								0.7061	0.7063

%E, per cent standard deviation of the Sr 87/86 ratio based on 30-40 scans. Observed 87/86 ratios are corrected for fractionation in the mass spectrometer on the assumption that 86/88 = 0.1194. Average 86/88 = 0.11933 with a standard deviation of 0.00068. Average 87/86 = 0.7074 (present day) weighted average of Sr in both metamorphic facies. Average 87/86 = 0.7058 (2,600 m.y. ago). bdl: below detection limit (assumed to be  $2 \pm 2$  p.p.m.). <sup>id</sup> analysed by isotope dilution. Other Rb and Sr analyses are by X-ray fluorescence (G. Holland, analyst).

Table 2. K-Ar AGE DETERMINATIONS FROM THE LOCHINVER AREA

Sample No.	Locality grid ref.	Rock type	Material analysed	% K	Radiogenic <sup>40</sup> Ar%	<sup>40</sup> Ar/g std. a.c. $\times 10^{-4}$	Apparent age m.y.	Analytical error m.y.
<b>A Southern Pyroxene Zone (Scourian)</b>								
290	094201	Basic gar gn	W.r.	0.142	95	0.220	2,610	90
290	094201	Basic gar gn.	Hornblende	0.521	91	0.909	2,890	40
546	077212	Mafic pod	Biotite	7.22	98	15.55	2,820	40
546	077212	Mafic pod	Augite	0.089	92	0.444	5,240	340
546	077212	Mafic pod	Augite	0.059	87	0.435	5,200	350
547	077212	Mafic pod	Biotite	4.76	98	9.35	2,420	30
547	077212	Mafic pod	Hornblende	1.40	98	2.618	2,350	50
547	077212	Mafic pod	Plagioclase	0.186	97	0.420	2,560	50
494	064213	Ultrabasic	W.r.	0.451	96	0.792	2,370	120
494	064213	Ultrabasic	Hornblende	0.493	95	1.120	2,620	70
494	064213	Ultrabasic	Hypersthene	0.051	96	0.164	2,860	190
610	065214	Ultrabasic	W.r.	0.559	96	1.419	2,520	40
610	065214	Ultrabasic	Biotite	6.67	97	12.36	2,340	40
610	065214	Ultrabasic	Hornblende	1.10	100	2.509	2,630	50
711	097210	Ultrabasic	W.r.	0.062	88	0.301	3,790	360
<b>B Central Amphibolite Zone (Mixed Inverian and Laxfordian)</b>								
34	065216	Acid gneiss	Biotite	7.78	92	8.11	1,640	40
34	065216	Acid gneiss	Hornblende	1.41	96	1.88	1,570	40
85	095222	Mafic pod	Hornblende	0.696	96	1.07	2,100	30
182	096221	Basic gneiss	Biotite	7.20	95	7.77	1,680	30
182	096221	Basic gneiss	Hornblende	0.32	92	0.481	2,070	100
194	101221	Agmatite	Biotite	6.94	98	7.66	1,700	30
194	101221	Agmatite	Hornblende	0.655	97	1.11	1,880	30
672	121241	Mafic pod	Biotite	7.62	94	7.39	1,560	40
672	121241	Mafic pod	Hornblende	0.920	95	1.25	1,940	30
703 F	065242	Vein	Hornblende	0.158	90	0.220	1,980	70
714 F	065242	Vein	Biotite	7.25	98	7.62	1,630	40
714	065242	Ultrabasic	Biotite	7.05	99	7.29	1,630	30
714	065242	Ultrabasic	Hornblende	0.235	95	0.335	1,590	30
715	065242	Basic gneiss	Biotite	6.55	99	7.57	1,750	30
715	065242	Basic gneiss	Hornblende	0.39	87	0.431	1,710	70
<b>C Amphibolized Dykes and Shears (Laxfordian)</b>								
34D	067223	Metadolerite	Biotite	3.45	82	3.59	1,640	90
49D	096222	Metadolerite	W.r. 200	0.351	92	0.331	1,530	60
49D	096222	Metadolerite	W.r. 40-200	0.393	95	0.357	1,490	30
579	102227	Shear	Hornblende	0.260	87	0.242	1,540	70
648	108256	Sheared pod	Biotite	7.43	93	7.59	1,620	40
648	108256	Sheared pod	Hornblende	0.222	76	0.230	1,580	80

$$\lambda_1 = 0.584 \times 10^{-10} \text{ yr}^{-1}, \quad \lambda_2 = 4.72 \times 10^{-10} \text{ yr}^{-1}.$$

Table 3

Sample No.	Locality grid ref.	Rock type	Material analysed	Rb (p.p.m.)	Sr (p.p.m.)	<sup>87</sup> Sr/ <sup>86</sup> Sr now	Age m.y.	Intersection age m.y.	K-Ar age m.y.
O Country Rock Sr 87/86 initial = 0.706									
545	077212	Mafic pod	Biotite	433	15.2	2.432	1,400 ± 50	1,400 ± 60	2,520 ± 40
546	077212	Mafic pod	Aug.	Bdl	72*	0.714	—		5,240 ± 240
672	121241	Mafic pod	Biotite	110	10.0	1.390	1,450 ± 50	1,390 ± 60	1,560 ± 40
673	121241	Mafic pod	Hbe.	18.6	25.6	0.787	2,480 ± 160		1,940 ± 80
648	108256	Mafic pod	Biotite	74.2	7.23	1.334	1,420 ± 50	1,420 ± 50	1,620 ± 40
648	108256	(sheared)	Hbe.	Bdl	28*	0.709	—		1,580 ± 80
714	065242	Ultrabasic	Biotite	96.6	10.2	1.209	1,460 ± 50	—	1,630 ± 30

\* Analysed by emission spectrograph.  $\lambda = 1.47 \times 10^{-11}$  yr<sup>-1</sup>.

The possibility of excess argon in the hornblende samples is once more discounted due to the lack of any relationship between potassium content and apparent age. While the leakage of argon during the reheating of these older amphibolites casts some doubt on the proximity of the 2,100 m.y. minimum age to the actual event, this same phenomenon makes it possible to estimate a maximum age of 2,350 m.y. for the Inverian from its effect on the least retentive minerals in the neighbouring Scourian gneiss.

The rubidium-strontium age of the pre-Inverian pegmatites (2,250 m.y.) improves this inferred maximum by about 4 per cent. The minimum potassium-argon limit was improved by a similar amount by a rubidium-strontium age of 2,190 m.y. obtained on a relatively fresh picrite dyke which cuts across folded and amphibolized Inverian gneiss<sup>4</sup>.

**The Laxfordian episode.** While the central belt of Lewisian gneiss escaped the major metasomatic effect recorded in the Laxford area, Laxfordian deformation is clearly recorded in the post-Inverian dykes near Lochinver, where it takes the form of brittle shears accompanied by epidote amphibolite facies metamorphism.

This event was extensively dated as 1,500–1,600 m.y. in its type area by Giletti *et al.*<sup>3</sup>. Potassium-argon results on materials taken from amphibolized dykes and shears in the Lochinver area (Table 2, C) are in general agreement, with an average of 1,580 m.y. However, the rubidium-strontium results on metamorphic biotites (Table 3) are distinctly lower, indicating an age of about 1,420 m.y. Although little is known about the nature of this later event, it appears to have affected the rubidium-strontium age of Scourian biotite as well, causing extreme discordance between the potassium-argon age of 2,520 m.y. for sample 546 and the rubidium-strontium age of 1,400 m.y. for the same sample. Giletti *et al.*<sup>3</sup> attribute the spread in their Laxfordian ages to mild reconstitution later than 1,160 m.y., but Evans and Park point out that a spread in 'Laxfordian' ages might well be expected in view of the multiphase nature of this event in the Gairloch district to the south.

Analytical methods are those described by Evans and Tarney<sup>4</sup>.

**Conclusions.** (1) The pre-Scourian complex was derived from a source region with a strontium 87/86 ratio lower than most basic igneous rocks. If this complex ever contained normal crustal abundances of rubidium, it was removed within a short time after original differentiation from the mantle.

(2) Scourian granulite facies metamorphism occurred at least 2,600 m.y. ago.

(3) Potassic pegmatites were intruded about 2,250 m.y. ago.

(4) Inverian amphibolite facies metamorphism occurred about 2,200 m.y. ago.

(5) A period of dyke intrusion began immediately following Inverian metamorphism, and may have continued for as long as 300 m.y., during which time the country rock became progressively cooler.

(6) Parts of the area were affected by Laxfordian reheating about 1,580 m.y. ago.

(7) An event of unknown character appears to have purged the radiogenic strontium from metamorphic biotites about 1,400 m.y. ago.

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## FAR INFRA-RED FARADAY ROTATION IN A PLASMA

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**F**ARADAY rotation in a laboratory plasma has been previously observed both in the optical region<sup>1</sup> and at an infra-red wave-length of  $3.39\mu$  (ref. 2). Rotations of several degrees per kilogauss are reported here, observed using a pulsed water-vapour laser as a source. This laser, developed at Services Electronics Research Laboratory, Baldock, by Large and Hill<sup>3</sup>, gives monochromatic  $27.9\mu$  wave-length pulses of about 1  $\mu$ sec duration and 10-W peak power. The design is based on the observations of laser action in water-vapour by Crooker *et al.*<sup>4</sup>.

The Faraday rotation in radians, for propagation through a plasma of electron density  $n$  cm<sup>-3</sup> in a direction parallel to a constant superimposed magnetic field  $B$  gauss, is given by:

$$\psi = 2.63 \times 10^{-17} n B l \lambda^2 \quad (1)$$

where  $l$  is the path-length in the plasma and  $\lambda$  is the free-space wave-length of the radiation, both in cm. In the experiments described here,  $B$  and  $l$  are known and  $n$  is deduced from the measured value of  $\psi$ . Since  $\psi$  is



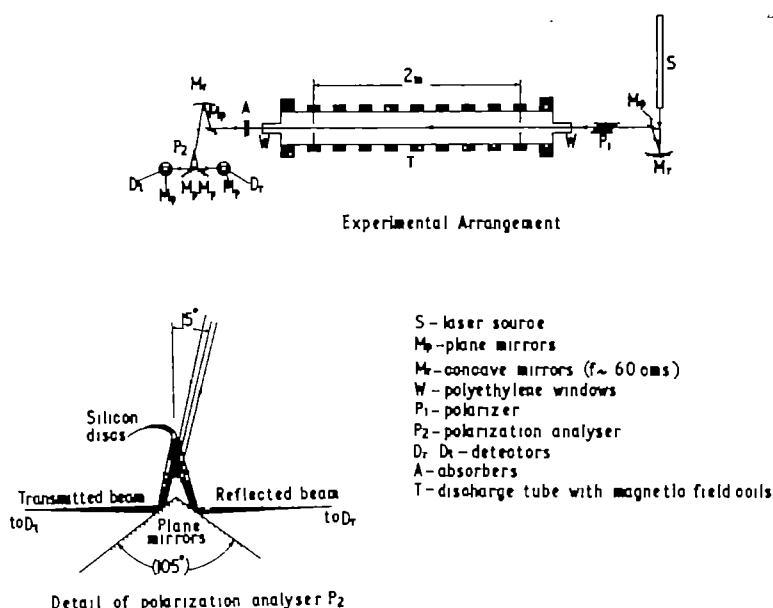


Fig. 1

proportional to  $\lambda^2$ , the rotation is 68 times that which would occur for  $\lambda = 3.39\mu$  and 1,950 times that for  $\lambda = 6328 \text{ \AA}$ , the wave-lengths at which rotation has been previously observed.

Fig. 1 shows the arrangement of the apparatus. The plasma, length  $l = 200 \text{ cm}$  between the end electrodes, has already been described in connexion with experiments on hydromagnetic wave propagation<sup>5</sup>. The uniform axial magnetic field of up to 6 kG varies negligibly during the 1-μsec laser pulse. Polyethylene vacuum windows were used at each end of the plasma column, their transmission loss being small at 27.9μ wave-length. The input polarizer, P1, consists of 10 sheets of 37μ thickness and 10 sheets of 52μ thickness polyethylene sheet, inclined at the Brewster angle (55°) to the incident laser radiation. Such polarizers were described by Mitsuishi *et al.*<sup>6</sup>. P1 can be rotated about an axis lying along the propagation direction, the angular setting  $\theta$  being registered on a scale. Tests with two identical polarizers showed the intensity of transmitted power in the crossed position to be 1.8 per cent of that in the aligned position.

The polarization analyser is P2 which consists of two vertical plates of high resistivity silicon, each of thickness

this value  $\theta_0$  is about 45° to the vertical. If  $\theta = \theta_0 + \phi$ , then:

$$V_i/V_r = \cot^2 \theta_0 \tan^2 (\theta_0 + \phi) \approx \tan^2 (45^\circ + \phi) \quad (2)$$

if  $\theta_0$  is near 45°, a relation which was verified experimentally over a 180° range of  $\phi$ . With a plasma present, P1 is rotated so as to keep  $V_i$  and  $V_r$  equal. The polarization of the radiation leaving the plasma is therefore still as before, the rotation of P1 giving the magnitude and sense of the Faraday rotation directly. For a ratio  $V_i/V_r$  not exactly equal to unity the small correction to give  $\theta_0$  is calculated from (2).

Oscillograms of  $V_i$  and  $V_r$ , with no plasma present, are shown in Fig. 2 ( $V_i$  is the upper trace in all the oscillograms). The three shots shown were taken with  $\theta = 47.5^\circ$  and the values of  $\theta_0$  are calculated as 46.5°, 48° and 48.5° for Figs. 2a, b and c, respectively. The initial small spike on these oscillograms is due to electrical interference, picked up from the laser power supply. With an argon plasma, initial gas pressure 37 mtorr, having an axial field of 4.6 kG, it was necessary to reset P1 to  $\theta \approx 33^\circ$  to restore equality of  $V_i$  and  $V_r$ . Fig. 3

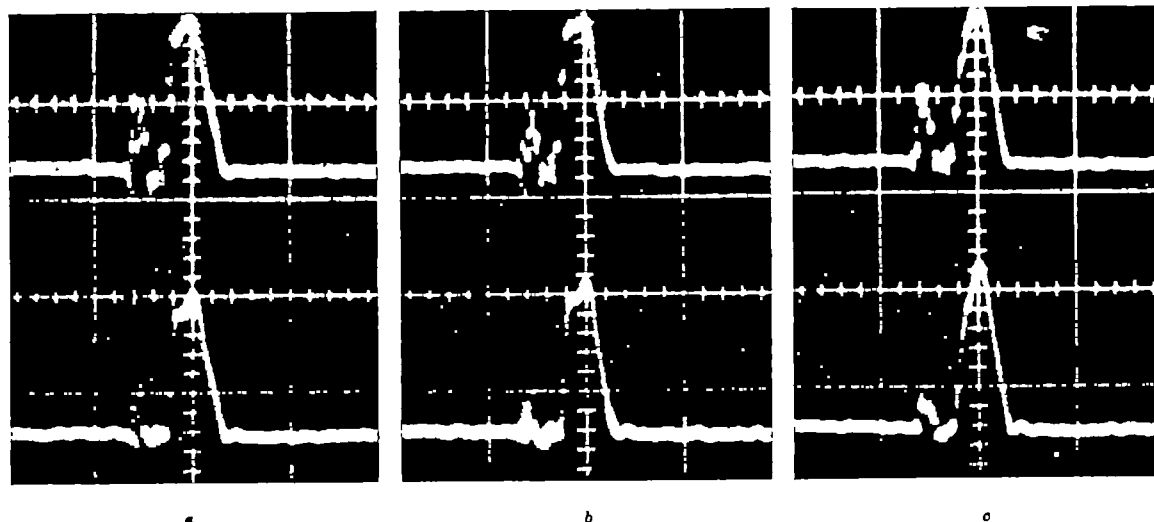


Fig. 2. Signals from the infra-red detectors at 2 μsec/cm sweep speed.  $V_i$  (upper traces) and  $V_r$  (lower traces) for  $\theta = 47.5^\circ$  with no plasma present

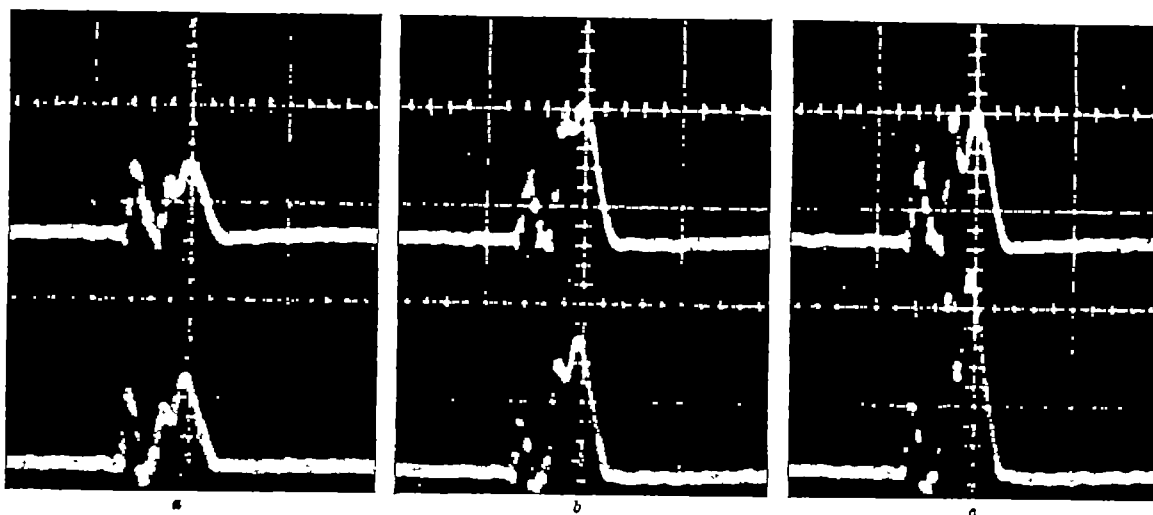


Fig. 3. Signals from the infra-red detectors at 2  $\mu\text{sec}/\text{cm}$  sweep speed.  $V_t$  (upper traces) and  $V_r$  (lower traces) for  $\theta$ ,  $30^\circ$  with an argon plasma.

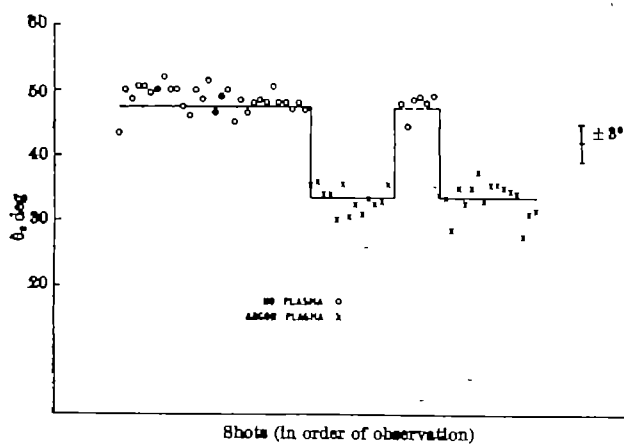


Fig. 4. Measured values of  $\theta$ , on successive shots.

shows three typical oscillograms, taken with this argon plasma, for  $P_1$  set at  $\theta = 30^\circ$ . The values of  $\theta$ , the angle to which  $P_1$  should have been set to make  $V_t = V_r$ , according to expression (2), are  $34^\circ$ ,  $30^\circ$  and  $35.5^\circ$ , respectively, for Figs. 3a, b and c. The time was 500  $\mu\text{sec}$  after the start of the current pulse.

Series of shots with plasma present were interposed between series with no plasma, and the resulting values of  $\theta$ , deduced as already described, are plotted against shot number in Fig. 4. Two definite values of  $\theta$ , should result for these two conditions, their difference being the magnitude of the Faraday rotation,  $\psi$ . In fact, a spread of values was observed, though with well-defined mean values, drawn as solid lines in Fig. 4. The standard deviation is about  $2^\circ$  so that  $\psi$  is taken as  $15^\circ \pm 3^\circ$ . Substituting the previously known values  $l = 200$  cm and  $B = 4.6 \pm 0.1$  kG in expression (1) yields:

$$n = (1.4 \pm 0.3) \times 10^{18} \text{ cm}^{-3}$$

The large spread observed in the values of  $\theta$ , is unsatisfactory, being much greater than the fundamental limit set by detector noise. The spread is thought to be due to the effects of fluctuations in polarization and mode pattern of the laser from shot to shot combined with cross-polarization leakage in  $P_1$  and non-identical optics in the two detector channels.

There is no measurable Faraday rotation, due to the vacuum windows, with a pulsed magnetic field of 6 kG but no plasma.

Using a helium-neon CW gas-laser to measure  $n$  interferometrically, by the method originally described by

Ashby and Jephcott<sup>7</sup>, though detecting the  $3.39\mu$  wavelength directly, a value:

$$n = (1.33 \pm 0.08) \times 10^{18} \text{ cm}^{-3}$$

was obtained for the same argon plasma. A time-resolved interferogram is shown in Fig. 5, the time at which the Faraday rotation was measured being indicated as  $t_F$ . The upper trace is a plot of the plasma gas current, the peak value being 20 kA. Fringes occurring at late times are not shown in this figure.

Faraday rotation was also measured in a hydrogen plasma in the same tube, the initial gas pressure being 50 mtorr. Magnetic field values 3.0, 4.0 and 6.0 kG, all  $\pm 0.1$  kG, were used. The results, taken at a time 100  $\mu\text{sec}$  after peak current (40 kA), are given in Table 1.

Table 1

$B$ (kG)	$\psi$ (degrees)	$n$ , $\text{cm}^{-3}$ calculated
3.0	$33 \pm 7$	$(4.7 \pm 1.0) \times 10^{18}$
4.0	$45 \pm 7$	$(4.7 \pm 0.8) \times 10^{18}$
6.0	$60 \pm 10$	$(4.3 \pm 0.6) \times 10^{18}$

A  $3.39\mu$  wave-length interferometer measurement was carried out for the condition with 6.0 kG and gave:

$$n = (4.3 \pm 0.2) \times 10^{18} \text{ cm}^{-3}$$

Faraday rotation at  $\lambda = 27.9\mu$  in a plasma has thus been clearly demonstrated. In the experiment described here, where the magnetic field strength was already known, it has resulted in electron density measurements in agreement with those derived from an interferometer

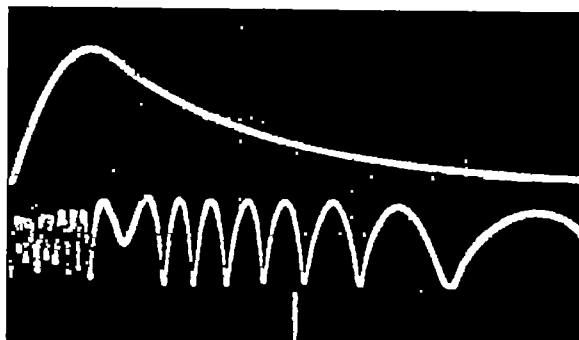


Fig. 5. Interferogram in the argon plasma at  $\lambda = 3.39 \mu$  (lower trace). The upper trace shows the gas current pulse. Sweep speed is 100  $\mu\text{sec}/\text{cm}$ .

working at  $\lambda = 3.39\mu$ . While the accuracy achieved was poorer than that of the latter method, considerable refinement of the accuracy should be possible. Moreover, the difficulties which arise in the interferometric method from the need to follow increases and decreases of density to a point where the density is known do not occur, and a spot density measurement can be made in a fraction of a microsecond without reference to the previous or subsequent behaviour of the plasma.

In the general case, however, when the magnetic fields are not known, the two methods are complementary; the Faraday rotation gives information on the magnetic field while the interferometer does not. Since the effect on an infra-red beam of transverse components of the magnetic field is normally negligible compared with that of very much smaller longitudinal components, the

Faraday rotation along a path-length  $l$  measures  $\int nB \cdot ds$

while the phase shift measured interferometrically is proportional to  $\int_0^l n \cdot ds$ .

We thank Dr. R. J. Bickerton for his advice, and Mr. B. Parham for help with the measurements. The experiments were made possible by the generous loan of the laser from Services Electronics Research Laboratory, Baldock, and by the supply of infra-red detectors from the Radar Research Establishment, Malvern.

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## EVIDENCE FOR AN UNUSUAL SOURCE OF HIGH RADIO BRIGHTNESS TEMPERATURE IN THE CRAB NEBULA

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RECENT observations using both the lunar occultation technique<sup>1</sup> and an interferometer of high resolution<sup>2</sup> have disclosed the presence of a component of small angular diameter in the Crab Nebula which becomes pronounced at low frequencies. The radio spectrum of this component has a steep gradient of  $-1.2$  and accounts for about 20 per cent of the total emission from the nebula at a frequency of 38 Mc/s. Further observations of the nebula, carried out in connexion with an International Quiet Sun Year programme during 1963-1964, have yielded the additional evidence that this component exhibits remarkable fluctuations of intensity which cannot be ascribed to ionospheric scintillation since other sources do not show similar effects. The occurrence of the fluctuations exhibits a pronounced annual variation which can be readily explained if the phenomenon is due to interplanetary scintillation.

Interplanetary scintillation, arising from the passage of radiation through irregularities of electron density in the interplanetary medium, is a diffraction effect which is confined to radio sources of exceedingly small angular diameter. Already it has been used to place upper limits on the angular dimensions of 'quasi-stellar' sources<sup>3</sup>, and the present observations may only be accounted for if the small component of the Crab Nebula has an angular diameter  $\sim 0''.1$ . This result raises interesting questions concerning the physical nature of the source since it implies an exceptionally high brightness temperature which cannot be accounted for by synchrotron emission.

The observations at 38 Mc/s were carried out with an interferometer comprising two corner-reflectors of length 800 ft. and 3,000 ft. on an east-west baseline of 10 km. At this separation the Crab Nebula is largely resolved and the response is entirely due to the component of small angular diameter. Some additional observations were made with an interferometer of low angular resolution consisting of two halves of the 3,000 ft. corner-reflector; in this case only 20 per cent of the response is due to the small-diameter component and the fluctuations were not as prominent on the records. For both systems similar phase-switching receivers were used with a time constant of 2 sec. Typical recordings of the fluctuation phenomenon are shown in Fig. 1a and c. It is seen that the Crab Nebula exhibits pronounced variations of apparent intensity while 3C123, which is separated in declination

by only  $7.5^\circ$  and transits roughly 1 h earlier, yields a smooth fringe pattern. Similar records are obtained on most occasions.

The fluctuations exhibit considerable day-to-day variations, but observations over a period of 12 months

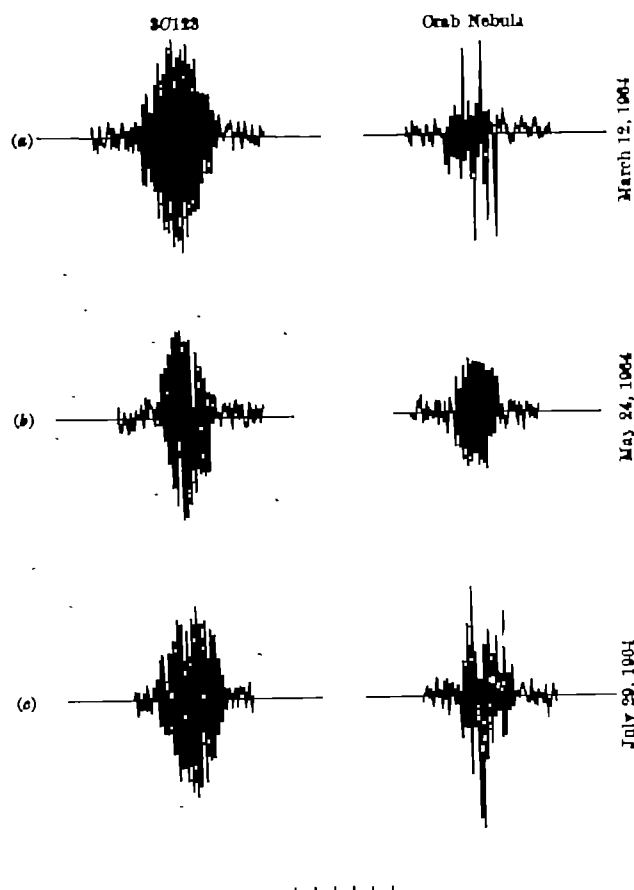


Fig. 1. a and c, Typical recordings of intensity fluctuations of the Crab Nebula; b, a record showing the marked decrease of fluctuation when the Crab Nebula was near the Sun. Time scale in minutes

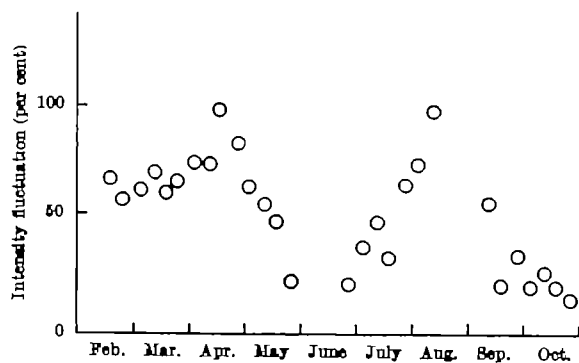


Fig. 2. Weekly means of the average intensity fluctuation (per cent) indicating a systematic annual variation

have disclosed a systematic annual variation. Weekly means of the average deviation of apparent intensity, expressed as a percentage of the mean intensity, are shown in Fig. 2. It is seen that the fluctuations are most pronounced in April and August, falling to lower values during October–February and vanishing almost completely over a period centred on mid-June. During the winter months, when the source is in transit during the night, ordinary ionospheric scintillation is of frequent occurrence, thus rendering the observations uncertain. On several occasions, however, free of ionospheric scintillation, recordings showing the absence of fluctuations were obtained.

The time-scale of the fluctuations is not easy to ascertain owing to the short interferometric fringe-period of 12 sec. Frequently successive positive and negative fringe maxima show unrelated fluctuations so that the time-scale must be of the order of a few seconds. Variations at an appreciably faster rate would have been smoothed by the 2-sec time constant.

An attempt to detect angular deviations associated with the intensity fluctuations was carried out during April when the fluctuations were most pronounced. An analysis of the time intervals between successive fringes indicated no significant fringe displacements, from which it was deduced that the direction of arrival of the radiation was unperturbed within  $\pm 25''$ . On the supposition that the fluctuations are due to random diffraction by phase-changing irregularities, considerably larger angular deviations would be expected unless the scale of the diffraction pattern was appreciably larger than the baseline of the interferometer. It may thus be deduced that the scale of the diffraction pattern is greater than 10 km. Independent evidence for a scale exceeding 10 km also comes from the high degree of correlation which is noticed for large fluctuation maxima observed simultaneously on the interferometers of high- and low-angular resolution.

In seeking an explanation of the fluctuations it is apparent that intrinsic variations of the source itself cannot reasonably account for the annual variation and for the fast fluctuation rate. Ionospheric scintillation can be excluded for several reasons; the fluctuations are most pronounced at times well removed from the usual nocturnal scintillation maximum and they are not observed on other radio sources. Again, large intensity variations in the diffraction pattern of phase-changing irregularities can only occur beyond a critical distance of the order  $L/4\phi$  where  $L$  is the scale of the irregularities and  $\phi$  the typical angular deviation<sup>1</sup>. Putting  $L \geq 10$  km, the minimum possible value, and  $\phi \leq 25''$ , we deduce that the irregularities must be situated at a distance of at least  $2 \cdot 10^4$  km, which places them far beyond the ionosphere.

The only satisfactory explanation which can be suggested is that the fluctuations are caused by interplanetary scintillation as has been observed for quasi-stellar radio sources<sup>2</sup>. The notable disappearance of scintillation during June, when the Crab Nebula passed within a small angular distance of the Sun, is then explained as a blurring

of the diffraction pattern due to the finite diameter of the radio source. This effect has already been predicted and suggested as a technique for angular diameter measurement<sup>3</sup>; it has also been observed<sup>4</sup> at 178 Mc/s on the source 3C2. It is important to note that the disappearance of the fluctuations is an effect which may be examined independently from the decrease of fringe amplitude which is observed when the source reaches a smaller angular distance from the sun, and which has been used previously to examine the outer corona<sup>5</sup>. Both phenomena are, indeed, manifestations of the same diffracting irregularities, but scintillation only occurs for a source having a sufficiently small angular diameter.

It is known, both from previous measurements<sup>6</sup> and from the decrease of fringe amplitude observed in 1964, that the angular scattering imposed by the interplanetary medium has a magnitude of about  $0.25''$  at a distance of 0.5 A.U. for a frequency of 38 Mc/s. Assuming a gaussian angular spectrum  $\exp(-\phi^2/\phi_0^2)$  it follows<sup>7</sup> that the autocorrelation function of the diffraction pattern in a plane perpendicular to the line of sight is given by  $\rho(\xi) = \exp[-(\pi^2 \xi^2 \phi_0^2/\lambda^2)]$  where  $\lambda$  is the wave-length. It may be shown<sup>8</sup> that the degree of scintillation is then reduced by one half when  $\lambda/\pi\phi_0\theta_0 H \sim 1$ , where  $\theta_0$  is the diameter (to  $1/e$ ) of a circularly symmetrical gaussian source and  $H$  is the distance of the diffracting irregularities from the plane of the observation. Putting  $\phi_0 \sim 0.25''$  and  $H = 0.8$  A.U., corresponding to the observations of mid-May when the scintillation was considerably reduced, we derive  $\theta_0 \div 0.1''$  for the angular diameter of the radio source in the Crab Nebula.

The physical size of a source subtending a diameter of  $0.1''$  at the distance of the Crab Nebula is approximately  $10^{-3}$  parsec and the brightness temperature required to account for the observed radio flux is then about  $10^{14}$  °K. Optical continuum radiation from the central portions of the nebula can be explained<sup>9</sup> as synchrotron radiation produced by relativistic electrons having energies up to  $10^{13}$  eV. Energies  $> 10^{13}$  eV are needed to account for the high radio brightness temperature in terms of synchrotron emission, but the magnetic field then has to be  $< 10^{-8}$  oersteds which is much smaller than the suggested value of  $10^{-4}$  oersteds. Brightness temperatures of the same order are, however, observed during intense radio outbursts associated with solar flares. It has already been noted that a continuous injection of energetic electrons is needed to account for the optical radiation, and the possibility has been suggested that these electrons are derived from an active remnant of the original supernova explosion<sup>10</sup>. In this case the remnant might be expected to show continuous flare-type phenomena on a sufficiently grand scale to account for the radio emission. Alternatively, there may be some connexion between the radio emission and the light-ripples which have been observed near the centre of the nebula.

It is clear that further measurements, particularly at longer wave-lengths, are needed to establish the spectrum of the source with greater accuracy. It might also be interesting to seek for secular variations of the radio emission since it would be surprising if such a physically small source radiated steadily for long periods. An examination of previous recordings of the Crab Nebula made during an investigation of coronal scattering in April 1962 has revealed similar fluctuations of intensity, so the source appears to have shown little change during at least two years.

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# LETTERS TO THE EDITOR

## ASTROPHYSICS

### Observations of the Sun in the Extreme Ultra-violet made from a Stabilized Skylark Rocket

THE successful launching of the first two stabilized *Skylark* rockets from Woomera, South Australia, in August and December 1964 was recently reported together with the results obtained by experiments prepared at Culham Laboratory<sup>1</sup>. These flights demonstrated accurate solar pointing and obtained photographic records of the extreme ultra-violet spectrum of the Sun and the distribution of extreme ultra-violet radiation from the solar disk. The third stabilized *Skylark* rocket was launched on April 9, 1965, at 0100 U.T. and carried two Culham experiments. These were particularly successful, giving new data on the chromospheric and coronal ultra-violet spectrum and new extreme ultra-violet spectroheliograms at shorter wave-lengths. This communication presents the latest results.

*New ultra-violet spectra of the solar chromosphere and corona.* The instrumentation prepared for the recent flight included a normal incidence grating spectrograph with a servo-controlled alignment system similar to that used on the first two payloads. An electromagnetic actuator developed by Sperry Gyroscope Co., Ltd., Bracknell, was used to control the mirror alignment and the spectrograph was set to cover the wave-length range 950–2950 Å. The alignment system stabilized an image of the Sun so that the spectrograph slit was set 10 sec of arc outside of the solar limb in order to view the chromospheric and coronal layers separately from the bright solar disk. The rocket attitude control unit and the optical alignment system both operated correctly and the solar image was stabilized at the spectrograph slit with an accuracy (root mean square) of  $\pm 2$  sec of arc. This coronagraphic type of experiment based on the accurate control of the image position enabled the spectrograph to record new emission lines originating in the chromosphere and corona at wave-lengths where the solar spectrum is normally dominated by emission from the photosphere.

Fig. 1 shows a part of the spectrum recorded during an exposure for 95 sec at a mean altitude of 150 km. The

2795 Mg II — 2803 Mg II

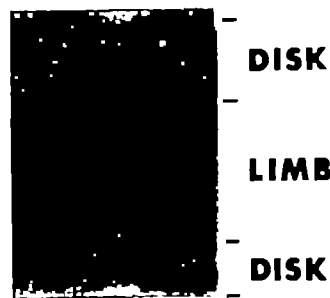


Fig. 2. Chromospheric emission of MgII doublet (*Skylark* SL 303, April 9, 1965)

central strip of the spectrum is produced by that region of the chromosphere and corona which was imaged on the centre of the slit, although there is some increase in the length of the spectral lines caused by astigmatism in the spectrograph. The spectrum recorded along the outer borders of the film results from photospheric radiation which is scattered from the primary concave mirror and enters the full length of the spectrograph slit. It therefore represents the spectrum of the total Sun and can be compared at any wave-length with the central strip of spectrum which represents the chromospheric and coronal emission. This is illustrated in Fig. 2, which shows a detail of the spectrum at 2800 Å. The resonance doublet ( $3s^1S - 3p^1P$ ) of MgII is seen in the photospheric spectrum as two strong absorption lines with faint emission cores, but the central limb spectrum shows the two emission lines as the dominant features. Many emission lines are recorded in the central spectrum which do not appear in the outer borders and have not previously been observed in the solar spectrum. A pure continuous spectrum is also observed for the first time in this region.

A preliminary analysis of the new spectra shows many features of interest. The strong line at 1908.6 Å is identified as the  $2s^1S_0 - 2p^1P_1$  intercombination line

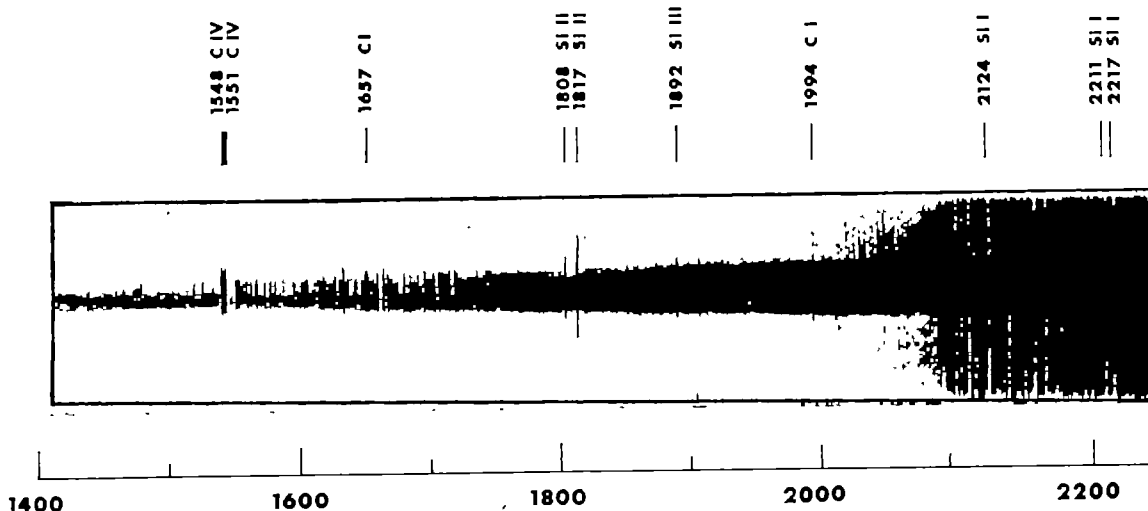


Fig. 1. Solar limb spectrum 1800–2300 Å (*Skylark* SL 303, April 9, 1965)

of CLII which will determine the  $2p\ ^3P_1$  term-level. The similar transition in SiIII produces the adjacent strong line at 1892 Å. Many strong emission lines between 2000 Å and 2400 Å cannot be identified with certainty and probably include forbidden transitions in highly ionized atoms related to the visible emission line spectrum of the corona. A complete analysis of the new solar spectra is now in progress and will be published in due course.

**Monochromatic extreme ultra-violet solar photography.** Photographs of the Sun in soft X-rays have been previously obtained using pinhole cameras carried in stabilized rockets<sup>1-3</sup>. The wave-length response of this type of camera is determined by the transmission of thin metal filters which cover the pin-hole aperture. These filters transmit broad wavebands of radiation and cannot provide monochromatic images of the Sun in particular spectral line wave-lengths. A concave grating spectroheliograph has been used by Tousey<sup>4</sup> to record monochromatic solar images in certain strong emission line wave-lengths, but this technique cannot be used at short wave-lengths because of the low reflectance of normal incidence optics. The need for suitable techniques to record spectroheliograms at wave-lengths below 260 Å became evident after the observation of a group of intense emission lines at 170–220 Å in the solar spectrum<sup>5</sup>. These lines have recently been produced in plasma devices at Culham Laboratory and have been classified as allowed transitions in ionized iron FeVIII to FeXII (ref. 6). Information about the intensity distribution of these spectral line emissions on the solar disk would be a valuable aid to our understanding of the structure of the solar atmosphere.

An instrument which can be used to record monochromatic images in the wave-length region below 400 Å has recently been developed at Culham<sup>7</sup>. The imaging properties of a pin-hole camera are combined with the dispersion of a plane diffraction grating used at grazing incidence to form a compact extreme ultra-violet spectroheliograph. This instrument was flown for the first time on the stabilized *Skylark* rocket launched on April 9, 1965. A single exposure on Kodak Pathé 'SO-7' film was obtained during 250 sec of stabilized flight, the peak altitude being 160 km. The attitude control unit stabilized the nose cone within the extremes of  $\pm 3$  min of arc relative to the Sun.

Fig. 3 shows the extreme ultra-violet spectroheliograms together with a comparison photograph obtained with the same instrument viewing an ionized helium plasma in a laboratory discharge tube. The only important spectral lines produced by the helium plasma in this wave-length region are the first two members of the HeII Lyman

series at 304 Å and 256 Å. Monochromatic images of the discharge tube window can be seen at these wave-lengths. Two series of solar images are reproduced together with the helium comparison spectrum. These were obtained using two different sizes of pin-hole aperture each covered by thin aluminium filters to exclude visible light. The upper pin-hole was 0.75 mm diameter giving 10 min of arc resolution. The lower pin-hole was 0.25 mm diameter to give less exposure but a greater resolution of about 3 min of arc (about one-tenth of a solar diameter). The zero order image in the upper spectrum is over-exposed but the HeII 304 Å image is correctly exposed and shows only slight limb brightening on a uniformly emitting disk image. The image formed mainly by FeIX 171 Å radiation is best seen in the centre spectrum. Equatorial limb brightening is marked, but the emission from active spots is less obvious than in soft X-ray images obtained with a simple pin-hole camera in the same flight. Initial considerations indicate that the limb brightened emission between 60 Å and 150 Å is real.

The results described here were obtained by the combined efforts of several groups and individuals as listed in reference 1. We particularly thank the groups at the Royal Aircraft Establishment, Farnborough, Elliott Brothers of Frimley, W. R. E. Salisbury and our colleagues in the Natural Plasma Group at Culham for their participation.

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## RADIO ASTRONOMY

### Spectrum of Low-frequency Radio Emission from NGC 1275

Two recent communications<sup>1,2</sup> have described an anomalous feature in the spectrum of the radio emission from the Seyfert galaxy NGC 1275 (3C84) (ref. 3) at frequencies above 3,000 Mc/s (Fig. 1). Between 178 Mc/s and 3,000 Mc/s, measured flux densities can be fitted with

a constant spectral index. At lower frequencies, measurements by Burke and Franklin<sup>4</sup> at 22.2 Mc/s and, more recently, by Erickson and Cronyn<sup>5</sup> at 26.3 Mc/s and by Williams<sup>6</sup> at 38 Mc/s, have given flux densities greater than would be predicted using this spectral index. This communication describes some preliminary results of observations at 10.03 Mc/s and 22.25 Mc/s made using the new pencil beam T-shaped arrays at the Dominion Radio Astrophysical Observatory. The observations at these frequencies also show excessive flux densities for the source.

The 22 Mc/s observations were made using a central portion of the final T now nearing completion. This smaller T measures 32λ east-west by 16λ north-south, giving a beam of 3.0° by 3.4° at the zenith. An absolute calibration of the system has not yet been attempted. The flux density scale has been calibrated with estimates for three sources; Virgo A, Hercules A, and 3C123, all of which appear to have a nearly constant

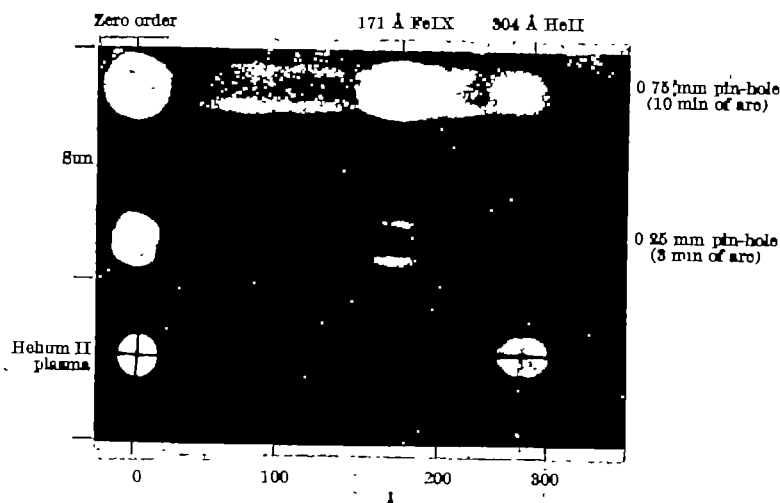


Fig. 3 Extreme ultra-violet spectroheliograms (*Skylark* SL 303, April 9, 1965)

Source	$S_m \times 10^{-26} \text{ W m}^{-2} (\text{c/s})^{-1}$	Probable error (P.E.)
Virgo A	5,900	$\pm 810$
Hercules A	5,330	$\pm 510$
3C123	835	$\pm 130$
NGC 1275	815	$\pm 155$

spectral index between 38 Mc/s and 1,000 Mc/s. Flux densities in this range were taken from a compilation by Kellerman<sup>7</sup> and fitted with least-squares parabolas to give estimates for each source at 22.25 Mc/s. Calibrations of the scale using the three sources have a root mean square variation about the adopted mean value of 10 per cent, or a probable error of the mean of about 5 per cent. Because of possible systematic errors in the flux densities used to derive these estimates an absolute accuracy of 15 per cent has been adopted for the calibration. Corrections for ionospheric absorption have been included, but are small, since most of the observations were made during the night.

The observations must be corrected for 'confusion' with 3C83.1, which is 30 min of arc from NGC 1275. This has been done using an estimate by Williams<sup>8</sup> of  $108 \times 10^{-26} \text{ W m}^{-2} (\text{c/s})^{-1}$  for the flux density of 3C83.1 at 22.25 Mc/s. The corrected flux density of NGC 1275 and the flux densities of the three standard sources determined using the adopted mean calibration are shown in Table 1.

The 10 Mc/s *T* measures 45λ east-west by 24λ north-south, giving a beam of 2.7° by 2.3° at the zenith. An absolute calibration of the system is not available at present and flux densities are measured relative to Virgo A. A linear extrapolation of measured values<sup>7</sup> yields a flux density of  $10,000 \times 10^{-26} \text{ W m}^{-2} (\text{c/s})^{-1}$  for Virgo A. This is in good agreement with a value based on computed antenna parameters and noise diode calibrations.

Ionospheric absorption, occurring mainly in the *F*-region, has been estimated from ionosonde data as 6 per cent and 27 per cent for the observations of Virgo A and NGC 1275, respectively (mean transit times at 0130 and 1815 L.T.). A correction for the 'confusing' source 3C83.1 has been made using an estimate by Williams<sup>8</sup> of  $173 \times 10^{-26} \text{ W m}^{-2} (\text{c/s})^{-1}$  for its flux density at 10 Mc/s.

The flux density obtained for NGC 1275 at 10.03 Mc/s is  $980 \pm 340$  (P.E.)  $\times 10^{-26} \text{ W m}^{-2} (\text{c/s})^{-1}$ . The major sources of error in this preliminary result are due to the effects of ionospheric scintillation and absorption.

The results are plotted as open circles in Fig. 1 together with a compilation of the results of other observers<sup>1,2,3,4</sup>. The points in the frequency interval 178 Mc/s to 3,000 Mc/s have been fitted with a straight line of slope  $-0.69$  and this has been extended as a dashed line to higher and lower frequencies. The remaining points have been fitted with a smooth solid curve.

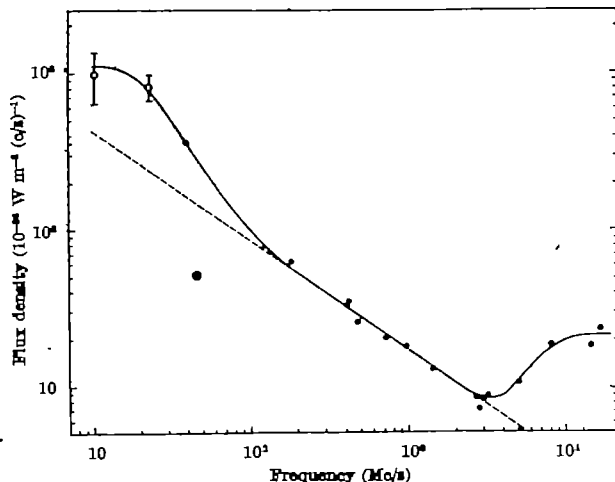


Fig. 1. The radio spectrum of NGC 1275. The flux densities reported in this letter are shown as open circles

The results presented here confirm the existence of a low-frequency spectral component in the radio emission from NGC 1275. It has a spectrum with a slope of about  $-2.0$ , which becomes less steep at frequencies below 20 Mc/s. These observations do not yield any information on the accurate position and angular diameter of the source. Measures of these parameters would be of considerable value in any discussion of the nature of this low-frequency component.

The 10 Mc/s project is a joint undertaking with the Mullard Radio Astronomy Observatory, Cambridge.

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## PHYSICS

### Spectrum of Laser Light Scattered from a Single Giant Pulse in a Laboratory Plasma

IN a previous communication<sup>1</sup> we reported the forward scattering of a laser beam in a 0-pinch plasma in which co-operative effects dominated the scattered spectrum.

The spectrum had been obtained from a number of separate shots; the Fabry-Perot etalon being adjusted after each shot to accept a different spectral element. These results suffered due to the lack of reproducibility of the laser from shot to shot as well as possible variation in the plasma properties of the discharge. Particularly damaging were the random shifts in the laser central frequency because of temperature variations as the ruby had to be refrigerated to obtain a sufficiently narrow spectral line breadth of the laser emission. As these random shifts were comparable with the spectral breadth of the ion peak itself the complete profile of the line could not be obtained.

To improve this situation a fourteen-channel photoelectric spectrum analyser was constructed using the Fabry-Perot etalon as the dispersing element. The basic operation of this type of instrument is described in another publication<sup>2</sup>.

Briefly, different portions of a Fabry-Perot fringe corresponding to different spectral resolution intervals are focused at various positions along the axis by an axicon or thin prism of revolution. An array of light guides placed along the axis conducts the light so focused to a series of photomultipliers. The number of channels is set equal to the finesse of the etalon which in our case was  $\sim 14$ .

In order to present the spectrum on a single oscilloscope, the photomultipliers were connected by lengths of 125-ohm cable so that the outputs of various channels were delayed 25 nsec with respect to each other. As the giant pulse of the laser was only 20 nsec long the signals of the different spectral channels were thus separated in time. The photomultipliers were matched to the cables by the use of two small inductances which with the anode capacitance of the photomultiplier formed a T-section with the same characteristic impedance as the cables. Reflexions were reduced to the level of the noise. One



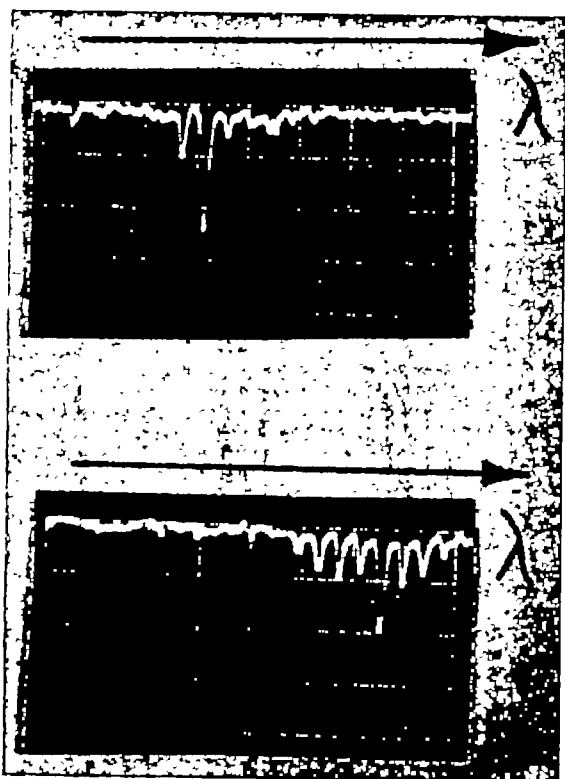


Fig. 1. Above, oscillogram giving spectrum of laser alone; below, oscillogram giving spectrum of laser and plasma together

end of the cable was terminated and the other passed to the input of a Tektronix 517 A oscilloscope. The photomultipliers were RCA type 7285. Variations in sensitivity among the channels due to the attenuations of the delay cable, different transmissions of the light guides and variations in the sensitivities of the photomultipliers themselves were compensated by adjusting the voltages applied to the dynodes of the different tubes. Because the shot noise arising from the light emitted by the plasma would combine from the different channels to give a noise level  $(14)^{1/2}$  times larger than that of each channel alone if the photomultipliers were continuously active it was necessary to include an electro-optic shutter which would admit light to the analyser only during the time of the giant pulse. A Kerr cell was thus placed in the system immediately before the etalon and pulsed open for 50 nsec at the time of the giant pulse. Except for this Kerr cell and the multichannel spectral analyser the apparatus and experimental conditions were essentially as described in the first reference.

The free spectral range of the etalon was 0.78 Å, hence the spectral interval between channels was 0.056 Å. Fig. 1 shows the spectrum of the laser pulse alone and the spectrum of the light in the plasma. The difference in position of the laser pulse between the two shots is an example of the troublesome fluctuations in laser emission frequency that plagued our earlier work.

It will be noted that the present spectrum is consistent with our previous results in so far as the errors in the other permit comparison. The ion temperature computed from the present profile is ~50 eV. The spectrum shows some evidence of the 'shoulders' or weak maxima predicted by the theory. A slight asymmetry toward shorter wave-lengths is observed, but this effect is not as strong as it appeared in the previous report, as the low-frequency wing of the ion spectrum was completely obscured by the shot-to-shot fluctuations of the laser central frequency.

The profile of the ion spectrum corresponds most nearly to that for equal electron and ion temperatures.

Using the estimate of density obtained from the magnitude of the total scattered intensity the resulting value of  $\alpha$  is well in excess of unity.

We thank Prof. B. Brunelli, director of the Laboratorio Gas Ionizzati, and other staff members of the Laboratory for their advice. We also thank R. Marchetti for his assistance. One of us (J. K.) was a EURATOM Research-Fellow during 1961-63 when this work was initiated.

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### Annealing Investigations In High Dose Graphite

AFTER irradiation, the electron microscope has been used to show that graphite contains a dense speckling attributed to interstitial aggregates<sup>1,2</sup>. This anneals into resolvable loops, and finally disappears between 1,700° and 2,000° C.

In the work recorded here, graphite crystals obtained from the Ticonderoga Lead Mines, New York State, have been cleaned in the usual manner and irradiated to a total nickel dose of  $5 \times 10^{18}$  n cm<sup>-2</sup> at 200° C. As received the crystals could not be cleaved into specimens suitable for transmission electron microscopy. After annealing at 2,500° C in a carbon tube furnace flushed with argon, the crystals were easily cleaved and were found to contain a high density of large loops about 1000 Å diameter, all sheared by a  $1/3 \langle 10\bar{1}0 \rangle$  displacement, and similar in appearance to those reported by Heerschap and Delavignette<sup>3</sup>. However, by tilting the foil to remove the ambiguity of the basal plane component of the Burgers vector, and observing the way the contrast changed from one side to the other of the dislocation line on tilting the specimen through small angles about a Bragg reflexion<sup>4</sup>, the loops were found to be of interstitial character.

A modified<sup>5</sup> specimen holder for the Philips EM 200 allowed a thin foil to be annealed in a furnace and a predetermined area to be re-examined. Hence it was possible to observe given loops after a series of 5 min anneals at 2,500° C and to measure their change in diameter with time.

It was found that all loops obeyed the equation<sup>6</sup>:

$$\frac{dr}{dt} = A \cdot \exp\left(-\frac{E}{kT}\right) \cdot \left[\frac{1}{r} - \frac{1}{\bar{r}}\right]$$

where  $A$  is a constant,  $E$  the energy of formation plus the energy of migration for the mobile point defects. Loops of radius  $r$  larger than a critical radius  $\bar{r}$  grew, and loops smaller than  $\bar{r}$  shrank. This is typical of a supersaturation controlled process. From a plot of  $dr/dt$  against  $r$  a value of 5.5 eV was obtained for  $E$ .

This result can only be taken as approximate at the moment because of assumptions made in evaluating the pre-exponential factor  $A$ . It is hoped to improve the accuracy by carrying out further isothermal anneals at different temperatures.

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### Maximum Growth Rate of Rayleigh-Taylor Instabilities due to an Electromagnetic Force

INTEREST has recently been shown in Rayleigh-Taylor instabilities such as might affect the flow of a striated fluid in a magnetohydrodynamic-generator duct. In such situations there will be an electromagnetic force of value  $J \times B$ , where  $J$  is the electric-current density and  $B$  is the magnetic-field strength, in addition to forces due to gravity and acceleration of the fluid.

Lemaire<sup>1</sup> considered this problem, and showed first that the stability of a two-fluid interface for wave-fronts perpendicular to the current lines (Fig. 1) depends on the sign of  $dB/dx$ . If  $dB/dx < 0$  the situation is stable, if  $dB/dx = 0$  the situation is neutral, and if  $dB/dx > 0$  the situation is unstable. It should be pointed out that if  $J$  or  $B$  is reversed in direction so that the force is directed from the less-conducting fluid to the more-conducting fluid, these conditions are also reversed, giving the possibility of instability in this case also. The reason for this behaviour is that the total current passing under a trough or a crest of a wave is the same, and the distribution of the field vector will therefore determine whether the total force due to the  $J \times B$  terms is greater under a trough or a crest.

Lemaire goes on to consider the stability of the interface for wave-fronts parallel to the current lines. The results in this case when the field is uniform can also be obtained by adding a term in the analysis of Bellman and Pennington<sup>2</sup>, since the force is now of constant direction and magnitude and can be treated as a gravitational force in the analysis.

Prof. J. A. Shercliff suggested the idea of conducting an experiment to investigate these instabilities. A trough about 1 in.  $\times$  1 in.  $\times$  2 in. deep was set in a magnetic field and partly filled with mercury. The trough was designed so that the current could be passed through the mercury at right angles to both the field and the vertical. The deflexion of the surface was measured with a capacitance probe at the suggestion of Dr. H. Marsh. The probes, which were made at the laboratories, were used in conjunction with a Wayne Kerr meter and gave a full-scale deflexion for a gap of about 0.020 in. Tests were run to obtain the maximum growth rate of instabilities for various values of the  $J \times B$  force when directed upwards. Under the prevailing conditions of uniform field the instabilities should be two-dimensional, and this appeared to be confirmed as at just unstable conditions the mercury rose in a two-dimensional wave with the crest running

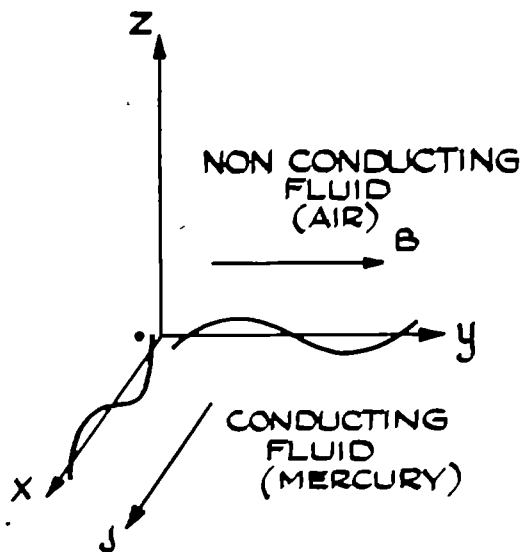


Fig. 1. Geometry of the problem

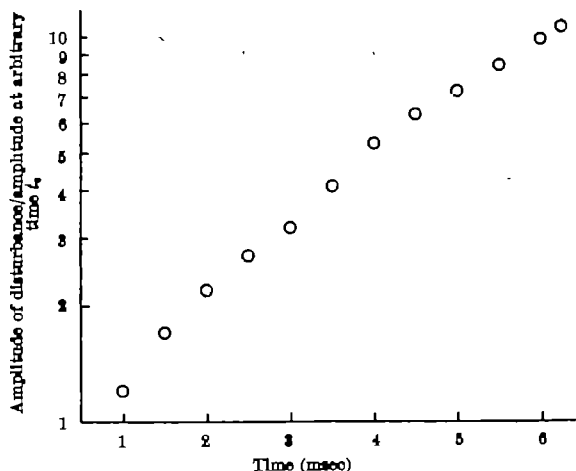


Fig. 2. Logarithmic plot of record of the growth of the instability for  $J \times B = 1.06 \times 10^4$  amp  $\times$  webers/m<sup>2</sup>

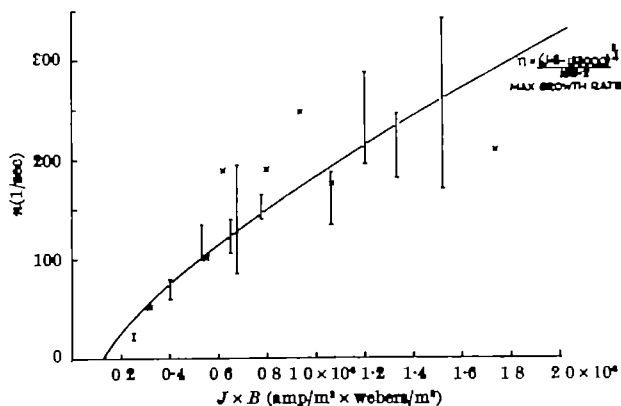


Fig. 3. Plot of experimental points obtained with 'Tufnol'-sided trough, with theoretical curve for comparison

from one electrode to the other and remained moderately stationary at a large deflexion. The maximum growth rate for these two-dimensional instabilities<sup>3</sup> for a mercury-air interface taking into account surface tension and neglecting viscosity, when modified to take account of the  $J \times B$  force, directed upwards, is:

$$n^2 = \frac{2}{3(3T)^{1/2}} \frac{(J \times B - g\rho)^{3/2}}{\rho} \text{ where } J \times B = |J| \times |B|$$

and the disturbance grows as  $\exp nt$ . In the foregoing equations  $g$  is the acceleration due to gravity,  $\rho$  is the density of the mercury,  $T$  is the surface tension at the mercury-air interface, and  $t$  is time.

The results obtained with a 'Tufnol'-sided trough are shown in Fig. 3. A logarithmic plot of a typical record of the growth of an instability is shown in Fig. 2. The uncertainty in some of the points in Fig. 3 is due to a decrease of slope with time on their logarithmic plots.

Further runs were performed with the trough lined with lead to alter the surface tension effects. In several of these traces the amplitude was found to vary very little before the trace disappeared due to the mercury making contact with the probe. The slopes, so far as they could be obtained from these records, all appeared to be low except for one in which the trace appeared similar to those obtained for Fig. 3. In this run the lead may have ceased to be effective.

The most likely explanation for the behaviour in this latter set of runs would seem to be that the lead lining sufficiently anchored the edges of the mercury that a whole number of wave-lengths was necessary to satisfy continuity, and hence at the mid-point of the trough there would have been a node and the probe situated at that point would have seen the surface changing in angle

only and not in height. This is to be contrasted with the case of 'Tufnol' sides in which the menisci would determine a symmetrical pattern of waves, and the lessened wetting of the side walls would remove the restriction on the number of wave-lengths.

When the force was acting downwards it was noticed that the mercury behaviour was extremely sensitive to anything which affected the uniformity of the current density. The insertion of any object obstructing the flow of current caused small fountains immediately next to it where the current density had fallen. The same effect was noticed when the mercury had not completely wetted the electrodes; particularly was this so in the trough corners.

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<sup>1</sup> Lesmaire, A. Commissariat à l'Énergie Atomique. Groupe d'Études de Magneto-hydrodynamique. Centre d'Études Nucleaires de Saclay, France. *Rapp. ITP7713 CEA/PAIGW/RT.150* (September 1962).

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Several fine colour photographs of this display were obtained; they show the characteristic bluish-white colour and band structure common to noctilucent clouds. These pictures appear to be the first photographic evidence of noctilucent clouds in the southern hemisphere. A black-and-white print of one of these is shown in Fig. 1.

Now that the existence of southern hemisphere noctilucent clouds has been proved, there are a number of questions relating to their characteristics remaining to be answered. Among these are their frequency of occurrence, spatial extent, height and velocity.

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## GEOPHYSICS

### Noctilucent Clouds over Punta Arenas, Chile

As pointed out in a recent communication<sup>1</sup> the existence of noctilucent clouds in the southern hemisphere has long been in doubt.

The observational data from the northern hemisphere, showing a maximum in the month of July, suggest that, if noctilucent clouds also occur in the southern hemisphere, they should most likely be seen in the month of January.

In order to settle the question about the existence of noctilucent clouds in the southern hemisphere, I went to Punta Arenas, Chile (53°1' S, 71° N), for the period January 8-17, 1965, when the activity should be at its maximum. During this 9-day period, one night was overcast, one had broken and five had scattered tropospheric clouds, and two were completely clear. During the seven nights when noctilucent clouds could have been seen if they were present, only one display was observed. This was on the night of January 9-10, 1965. The display was moderate in intensity and extended up to about 8° above the horizon and in azimuth from about south-west to south-south-east. It was first observed at 2200 L.M.T. and was obscured from the observer's view by low tropospheric clouds at 2245 L.M.T.



Fig. 1

## GEOLOGY

### Early Metamorphic Complex of the Lewisian north-east of Gairloch, Ross-shire, Scotland

THE Lewisian of the Gairloch area has been recently divided<sup>1</sup> into an older *Ialltaig* and a younger *Gairloch* complex, the former tentatively equated with the Scourian<sup>2</sup> of the Assynt-Scourie region and the latter with the post-Scourian sequence (the 'Laxfordian' of Sutton and Watson<sup>3</sup>) of the Laxford area. More recently, the post-Scourian sequence of the Assynt-Scourie region has been shown to include two separate metamorphic episodes, at 2200 m.y. (Inverian) and 1600-1500 m.y. (Laxfordian *sensu stricto*)<sup>4</sup>. Preliminary dating of rocks from Gairloch<sup>4</sup> indicates that recrystallization of the early *Ialltaig* complex occurred in the period 1600-1500 m.y. and that this is probably the age of the main phase of metamorphism and deformation of the later Gairloch complex—that is, equivalent to the Laxfordian, *sensu stricto*, of the north.

Detailed structural work in the Tollie area lying between Gairloch and Loch Maree (Fig. 1), which will be the subject of a separate paper, has shown that a granulite-facies metamorphic complex antedates and is affected by the main tectonic phase (Laxfordian) of Gairloch. The Tollie area was first described by Clough<sup>5</sup>, and the structure explained in terms of an early complex of gneisses cut by basic dykes and affected by later deformation and metamorphism. Sutton<sup>6</sup> has criticized the validity of my interpretation of the chronology of the Gairloch area<sup>1</sup> on the grounds that it is inconsistent with Clough's observations at Tollie. However, detailed structural or petrological work relating to this area has not hitherto been published nor a precise correlation made with better known and dated sequences. The purpose of this paper is to summarize the evidence relating to the age of the early complex at Tollie and the correlation with the structural sequence at Gairloch.

It will suffice to state here that the Lewisian of the Tollie area is made up of a complex of gneisses comprising both basic and acid varieties cut by a large number of basic dykes, now amphibolite<sup>7</sup>. The most common type of gneiss is a quartz-plagioclase-K feldspar-biotite rock with epidote, sphene, apatite and ore. The biotite-gneiss is frequently interbanded with a hornblende variety. Basic and acid hornblende-gneisses predominate in the north-east.

Correlation with the Gairloch structural sequence. The main tectonic phase in the

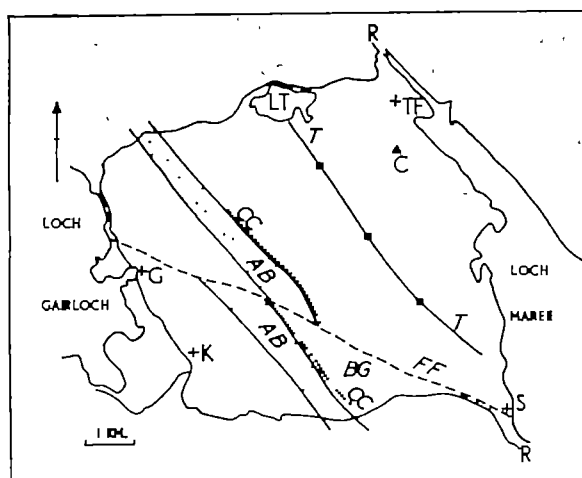


Fig. 1. Sketch map showing the location and certain geological features of the Tollie and Gairloch areas. G—Gairloch P.O.; LT—Loch Tollaigh; TF—Tollie Farm; C—Craig Mhor Thollaidh; S—Slattadale; K—Kerrydale; R-R—main road Poolawe-Gairloch-Achnasheen; T-T—trace of axial plane of Tollie antiform; AB—Aundray basite; BG—Buainichean gneisses; CC—Creag Bhan crush-belt; FF—Flowerdale fault.

Gairloch (post-Ialltaig) complex<sup>1</sup> is characterized by north-west-south-east steep or vertical foliation and strongly developed lineation with variable plunge. Folds are generally similar in style and have axial planes parallel or nearly parallel to the steep foliation. The main-phase deformation was accompanied by metamorphism of epidote-amphibolite facies in which both schists and gneisses were formed from the post-Ialltaig sediments.

The main-phase structure and metamorphism can be traced north-eastwards into the Tollie area where they are much more variably expressed. South-west of a north-west-south-east line through the west side of Loch Tollaigh (Fig. 1) the main-phase structure is indistinguishable from that of the Buainichean gneisses in the north-eastern part of the Gairloch area, but north-east of this line, along the crest and on the gently dipping north-east flank of an antiform (the 'Tollie antiform' of Clough<sup>2</sup>), the closely spaced steeply dipping main-phase foliation is replaced by a variably dipping, usually nearly horizontal foliation that is much less prominent, and the similar folds with steep axial planes are replaced by more open plastic folds with variably dipping axial planes (Fig. 2). The lineation, which is the dominant structural element and is parallel to the fold axes, corresponds to the intersection of the main-phase foliation  $S_M$  with the earlier gneissose banding  $S_G$  or to the intersection axis of variably dipping mica cleavages. On the south-west flank of the Tollie antiform,  $S_G$  is steeply dipping and becomes progressively obliterated and replaced by  $S_M$  in a south-westerly direction. On the crest of the Tollie antiform, and north-eastwards to Loch Maree,  $S_G$  is typically well developed and is modified rather than destroyed by the main-phase structure. The metamorphic fabric shows a corresponding variation across the area. In the eastern part, basic gneisses containing a relict granulite-facies mineral assemblage (plagioclase-hypersthene) are enclosed in the normal epidote-amphibolite-facies gneisses which are granoblastic with smoothly rounded, roughly equidimensional grains whose mean diameter is around 1 mm, the relict high-grade fabric being considerably coarser than 1 mm. In the central part of the area on the crest and north-east limb of the Tollie antiform, the mean grain-size is around 0.5 mm, but the fabric is otherwise similar to that in the east. On the south-western limb of the antiform, the fabric changes progressively and markedly. The mean grain-size decreases to around 0.1–0.2 mm; the grains are irregular in outline and vary considerably in size; large grains are frequently sutured and surrounded by smaller; lepidoblastic rather than granoblastic texture is pre-

dominant; and large quartz grains are strained. These differences are attributed to the effect of large-scale differential movement along the closely spaced foliation planes  $S_M$  during recrystallization on the flank of the antiform, contrasted with the comparatively static conditions in the crestal zone.

The pre-main-phase banding  $S_G$  is a coarse gneissose banding formed in a previous metamorphic episode that must, at least in parts, have reached granulite facies. The banding is very irregular in orientation and is affected by tight folds which are refolded by the main-phase folds (Fig. 2b). It is also cut by dykes which are themselves affected by the main-phase metamorphism, the main-phase lineation being represented in the dykes by hornblende alignment and by folded acid veins.

$S_G$  is affected by at least two sets of major folds (the Tollie antiform is a composite structure) which probably correspond to the two post-Ialltaig sets (early phase and early main phase) postulated in the Gairloch area. Late phase minor folds, pegmatites, and crush belts, similar to those in Gairloch<sup>1</sup> post-date the main-phase structures of the Tollie area.

**Age and significance of the early complex.** The Gairloch complex is considered to represent a series of sedimentary and basic igneous rocks younger than the granulite-facies Ialltaig complex which forms a small thrust-bounded wedge within the Gairloch rocks<sup>1</sup>.

If the early high-grade rocks of central and eastern Tollie are equivalent to the Ialltaig complex, as seems most likely, there must be a discontinuity (an unconformity, possibly tectonically obscured) between the rocks of the Gairloch complex and the Tollie gneisses. The nearest rocks that undoubtedly post-date the early high-grade metamorphism of the Tollie rocks are fine-grained schists on the north-east side of the thick Aundray basite (Fig. 1). The discontinuity thus must lie somewhere in the 1½ km-wide belt of gneisses separating these schists from rocks with definite early banding in the Tollie antiform. However, despite effectively continuous exposure, no change in the character of the gneisses can be detected within this belt—although such a change could well be completely obscured by the structural and metamorphic effects of the main tectonic phase. The most likely site for the required discontinuity is the line of the late-phase Creag Bhan crush-belt (Fig. 1) which lies within a zone of intense main-phase movement<sup>1</sup> at or near the schist-gneiss junction. This interpretation implies that the greater part of the Buainichean gneisses may be older than the Gairloch sediments. Although I previously grouped them with the sediments, I also suggested the possibility of these gneisses being much older than the adjacent rocks (ref. 1, p. 420).

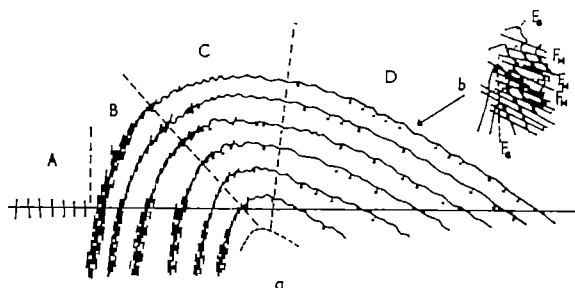


Fig. 2(a). Schematic diagram representing the main-phase structure of the Tollie antiform, section normal to antiform axis, looking north-west. Continuous lines represent  $S_G$  affected by main-phase minor folds; short lines represent main-phase foliation  $S_M$ ; dots represent main-phase lineation. In zone A,  $S_G$  is unrecognizable; zone B shows  $S_G$  dragged into a near-vertical attitude and intersected by sub-parallel  $S_M$ ; lineation is subordinate; in zone C, lineation is predominant and  $S_M$  has no consistent attitude; in zone D, lineation is again predominant and  $S_M$  is sub-parallel to gently-dipping  $S_G$ .

Fig. 2(b). Specimen of biotite-gneiss from the north-east flank of the Tollie antiform showing an early fold  $F_0$  affected by main-phase folds  $F_M$  with variable axial planes. The biotite has a variable planar orientation with a strong intersection lineation normal to the plane of the diagram, its growth is clearly associated with the formation of  $F_M$  and post-dates  $F_0$ .

The most probable age of the early high-grade complex is Scourian. However, the nearest dated Scourian rocks are 30 miles distant across the Laxfordian strike, and no detailed structural work has been published for the intervening ground. Alternatively, the early complex could be Inverian in age, or could correspond to some other metamorphic episode as yet unrecorded.

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### A New Carbonatite in the Legetet Hills, Kenya

As a result of recent laboratory examination of some specimens obtained while collecting rock-types in the Legetet area of the Kericho District of Nyanza Province, a new occurrence of carbonatite has been found. The outcrop, which is quarried for lime, is located 35° 15' 32" W, 0° 9' 7" S (first edition of the 1:50,000 sheet South A-36, F-1-NE). Later inspection of aerial photographs of the region suggests that the carbonatite forms a hill about 200 ft. high, and extends for about half a mile north from the above locality (Fig. 1).

The carbonatite was formerly thought to be equivalent to the Koru limestone of Miocene age, which forms the hill just west of the carbonatite and just north of Legetet (Fig. 1). The Koru limestone is itself problematic, since although it is interbedded with fossiliferous rocks<sup>1-3</sup>, some curious features have been recorded. Thus Pulfrey<sup>4</sup> gives its mineralogy as "calcite 85 per cent, hydrated iron oxides and manganese oxides (mainly iron) 9.5 per cent, apatite 3 per cent, silicates 2.5 per cent".

In a 1927 Survey report, Wayland<sup>5</sup> recognized two distinct limestones at Koru, a fossiliferous one, and underneath it an "apparently unstratified, essentially crystal-

line" grey limestone with "no trace of organic structures", which "does not display the characters one usually associates with the limestones of the archaean complexes". Wayland further mentions that the grey limestone "seems to contain about 2.5 per cent of calcium phosphate".

On this evidence, it seems possible that other parts of the Koru limestone may also turn out to be carbonatite.

Our own observations, which are restricted to the one locality examined, are that the Legetet carbonatite is a fine-grained dark-grey carbonatite (specimen U.1 which was mistaken in the field for a calcified volcanic tuff) cut by dyke-like bodies three to four feet wide of a creamy calcite rock with conspicuous octahedra of magnetite (specimen U.3). The carbonatite seems to pierce the tuffs of Tinderet which are Tertiary in age. The dyke-like bodies trend approximately north-east-south-west, which is similar to that of the nephelinitic and alnoitic dykes which locally cut the Tinderet tuffs and Koru limestone.

In thin section the grey carbonatite (specimen U.1) consists of small phenocrysts of twinned calcite, embedded in a matrix of anhedral calcite grains nearly all of which are coated with iron oxide. Occasional apatite and a few small wollastonite crystals occur. The rock is cut by a thin vein of a clear untwinned carbonate mineral with platy habit which proved, by X-ray powder photography, to be pure calcite.

The creamy carbonatite U.3 contains phenocrysts up to 4 mm long of a carbonate mineral. Staining techniques indicate that the matrix is pure calcite but the phenocrysts are slightly ferrous calcites. An examination of the rock in thin section indicates a mixture of two carbonatites. A coarse carbonatite rich in apatite and containing crystals of pyrochlore and cancrinite is invaded by a fine-grained carbonatite. The texture of the apatite suggests that it is being replaced by calcite. The phenocrysts in the fine-grained carbonatite appear to be derived from the coarser carbonatite.

Preliminary geochemical evidence from Mr. T. Deans of the Overseas Geological Surveys, London, suggests that specimens U.1 and U.3 contain amounts of strontium, barium, niobium and yttrium comparable to those found in other carbonatites, and in excess of those normally to be expected in sedimentary limestones.

That carbonatite should occur here is not surprising. The area lies on the south-west flanks of the Tertiary alkaline volcano of Tinderet described by Binge<sup>1</sup>. It is considered significant that these new outcrops and the fenitized rocks of Buru Hill, seven miles south-west of Legetet, as well as Tinderet itself should lie in the eastward extension of the Nyanza (formerly Kavirondo) rift-valley described by Shackleton<sup>2</sup>.

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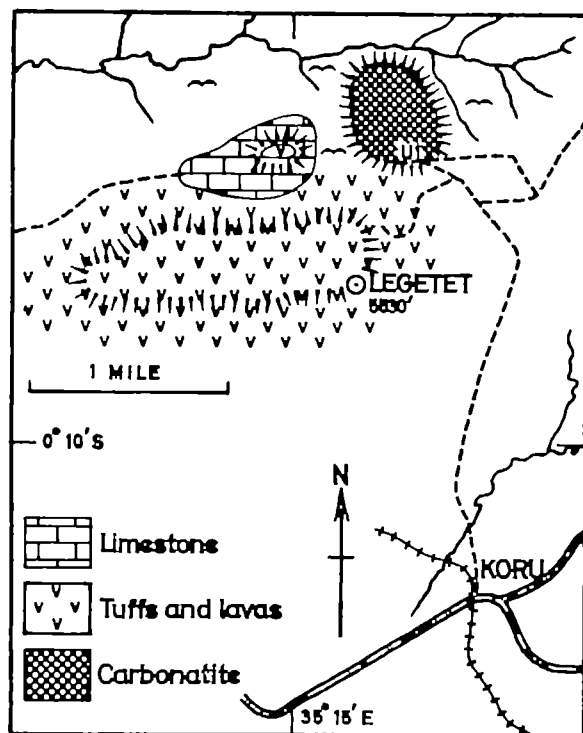


Fig.

### METALLURGY

#### Distribution of Grain Size in Annealed Metals

MUCH consideration has been devoted to the statistical distribution of grain size in a well annealed metal, as examples of which the work of Smith and of Beck<sup>1</sup> may be cited who, however, did not give a formula to represent the distribution curve. Recently, Papadakis<sup>2</sup> has published a paper concerning the relation between the volume distribution of grain size and the distribution as revealed by surface examination, but the measurements considered do not suffice to provide the basis for a precise, experimentally confirmed law.

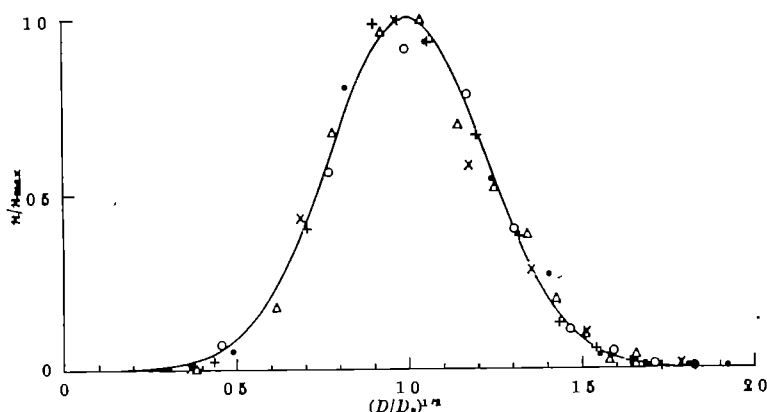


Fig. 1. Variation of  $n/n_{\max}$  with  $(D/D_0)^{1/2}$ .  $\times$ , magnesium at  $123^\circ\text{C}$ ;  $\bullet$ , zinc at  $22^\circ\text{C}$ ;  $\Delta$ , cadmium at  $35^\circ\text{C}$ ;  $+$ , cadmium at  $135^\circ\text{C}$ ;  $\circ$ , thallium at  $100^\circ\text{C}$ .

Our investigations have led us to believe that  $D^{1/2}$ , where  $D$  is the grain diameter, has a fundamental significance in problems of grain growth<sup>1</sup>. We have therefore been led to prepare distribution curves of  $n$  against  $D^{1/2}$ , where  $n$  is the number of grains for which  $D$  lies within a specified narrow limit. We have done this for the four close-packed hexagonal metals with which we have been working, namely, magnesium, zinc, cadmium and thallium, with specimens subject to long anneal at  $123^\circ$ ,  $22^\circ$ ,  $35^\circ$  and  $100^\circ\text{C}$ , respectively, and also with cadmium subject to long anneal at  $135^\circ\text{C}$ , for which the average grain diameter is about 6 times that for  $35^\circ\text{C}$ . The average grain size ranged from 125 grain/mm for magnesium to 4.8 for cadmium at  $135^\circ\text{C}$ .

If  $D$  is expressed in terms of the value for which  $n$  is a maximum and if this maximum is taken as the same in all cases, the points for  $n$  against  $D^{1/2}$  in all five cases lie on the same curve, as shown in Fig. 1. The curve in question is represented by:

$$n = n_{\max} e^{-\alpha^2(D/D_0)^{1/2} - 1}$$

where  $D_0$  is the value of  $D$  for which  $n$  has the maximum value  $n_{\max}$ .  $\alpha$  is a dimensionless constant, which has the value  $\pi$ . This constant measures the spread of the curve.

The number of grains measured was in each case about 1,000. We have shown, by successive etchings, that the grain size, as revealed by surface examination, is the same at all depths.

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## Neutron Microradiography of a Cadmium-Tin Alloy

X-RAY microradiography investigation of the internal structure of various alloys is a complementary tool to standard metallography. However, for alloys containing two or more elements with similar X-ray absorption coefficients, X-ray microradiography becomes difficult if not impossible. In an attempt to find a transmission method which could be used in its place, it was decided to use neutrons instead of X-rays.

The sample chosen for inspection was made from a furnace-cooled cadmium-tin casting containing 29.25 per cent cadmium. The thickness of the sample was  $125\mu$ . Cadmium and tin were chosen because of the large difference in neutron absorption cross sections. This gives the cadmium-rich areas of the alloy much more absorption capacity. The cross section of cadmium is 2,500 barns and that of tin is 0.63 barns.

The irradiation facility was in the University of Missouri at Rolla. This reactor is a 'swimming pool' type generating an average flux of  $8 \times 10^{12}$  n/cm<sup>2</sup> sec at 10 kW. The neutrons passed through 5 ft. of graphite before they intersected the sample.

The method used for neutron microradiography was an adaptation of Beck's procedure. In this process the sample is placed on an indium sheet and irradiated by a monoenergetic, unidirectional flow of neutrons. After irradiation, the indium is removed and placed against the emulsion of a Kodak 'Metallographic' plate. The indium will then expose the plate principally with  $\beta$ -radiation.

Fig. 1 is a photomicrograph of the specimen at a magnification of 6 diameters. The light tin-rich grains are in a dark cadmium-rich matrix. There are two types of grains, spheroid and ellipsoid. However, the spheroid grains are arranged in a line such that at low magnification their appearance is that of ellipsoid grains. Fig. 2 is a photomicrograph of the specimen of approximately the same magnification as Fig. 4 ( $\times 1.5$ ). Fig. 3 is an actual size neutron microradiograph of the specimen. The tin-rich areas are light while those of the cadmium are dark. In this sample the elongated tin grains and those of cadmium are easily distinguishable. This tends to indicate that the negative could be enlarged as much as 10 diameters. Fig. 4 is a neutron microradiograph magnified 2.5 diameters.

The white area is an indication of a flaw in the sample. The ellipsoid grains of the alloy are easily distinguishable.



Fig. 1



Fig. 2



Fig. 3



Fig 4

There are dark and light areas which are not evident in the photomicrographs. We believe these to be high concentrations of cadmium and tin, respectively. This indicates that the alloy was not thoroughly mixed.

We thank the reactor staff at the University of Missouri at Rolla, and Nelson Beck of Argonne National Laboratory, for their help. We thank the Indium Company of America and the American Smelting and Refining Company for materials.

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### Diffusion Creep in Polycrystalline Magnesium

LIGHTLY stressed polycrystalline metals may deform by the vacancy diffusion mechanism proposed by Nabarro<sup>1</sup> and Herring<sup>2</sup>. At high temperatures,  $\sim 0.8$  of the absolute melting temperature  $T_m$ , lattice self-diffusion is rapid and an appreciable diffusion creep may take place at a rate ( $\dot{\epsilon}$ ) given by<sup>3</sup>:

$$\dot{\epsilon} = \frac{B_1}{d^3} \frac{\Omega \sigma}{kT} D_l \quad (1)$$

where  $B_1$  is a constant,  $\sim 10$  for equiaxed polycrystals;  $\Omega$  the atomic volume of the material;  $\sigma$  the small applied stress;  $D_l$  the lattice self-diffusion coefficient;  $d$  the mean grain diameter; and  $kT$  has the usual meaning.

An equation for the contribution to diffusion creep by grain boundary self-diffusion (coefficient  $D_{gb}$ ) has been suggested by Coble<sup>4</sup>:

$$\dot{\epsilon} = \frac{B_2}{d^3} \frac{\Omega \sigma}{kT} \omega D_{gb} \quad (2)$$

where  $B_2$  is a constant,  $\sim 150$ ; and  $\omega$  is the grain boundary width.

As equation (2) has a stronger grain size dependence than equation (1) and the activation energy for grain boundary diffusion is smaller than that for lattice diffusion<sup>4</sup>, vacancy creep controlled by the grain boundary mechanism will be favoured by fine grain sizes and lower temperatures of deformation ( $\sim 0.6 T_m$ ).

This communication describes experiments which confirm that diffusion creep may be controlled by either the lattice or grain boundary mechanisms according to the test temperature. Magnesium was selected for these experiments since convincing metallographic evidence for creep has been obtained previously during

experiments on a magnesium alloy containing internal slip lines was observed on

the specimen surface and the marked stress dependence of the steady creep rate ( $\dot{\epsilon} \propto \sigma^3$ ) was typical of a dislocation climb process<sup>5</sup> in agreement with previous work<sup>6</sup>. Below this stress, however, and down to  $\sim 1.7 \times 10^6$  dynes/cm<sup>2</sup> no slip lines were observed, and the creep rate was a linear function of the stress. These creep rates agreed within a factor of two with those predicted from equation (1) using known values for  $\Omega = 2.32 \times 10^{-23}$  cm<sup>3</sup> and the lattice self-diffusion parameters<sup>7</sup> ( $D_l = 1.0$  cm<sup>2</sup>/sec,  $Q_l = 32.0$  kcal/mol). This must be considered good agreement in view of the approximations in the theoretical derivation of the value of  $B_1$ . In addition, at a stress of  $9.6 \times 10^6$  dynes/cm<sup>2</sup>,  $\dot{\epsilon} \propto 1/d^2$  for grains between 8 and  $40 \times 10^{-3}$  cm in diameter and direct determinations of the activation energy for creep by the temperature cycling technique<sup>18</sup> yielded values  $30.8 \pm 5.6$  kcal/mol at a stress of  $3.5 \times 10^6$  dynes/cm<sup>2</sup> and a temperature of  $412.5^\circ$  C. These results indicate that diffusion creep rates in magnesium at  $0.75 T_m$  follow equation (1) and are controlled by lattice self-diffusion.

In the second experiment, carried out at  $270^\circ$  C ( $0.59 T_m$ ) on specimens of  $\sim 5 \times 10^{-3}$  cm grain size,  $\dot{\epsilon} \propto \sigma^3$  for stresses in excess of  $2.8 \times 10^7$  dynes/cm<sup>2</sup> while at lower stresses  $\dot{\epsilon} \propto \sigma^1$ . Under these conditions of linear stress dependence the experimental rates were more than thirty times faster than those predicted from equation (1), suggesting the dominating importance of equation (2). In order to compare the experimental rates with equation (2), values for  $D_{gb}$  and  $\omega$  were required. Following work on zinc<sup>11</sup> and cadmium<sup>12</sup> we take for magnesium  $D_{gb} \sim 1.0$  cm<sup>2</sup>/sec with  $\omega \sim 5 \times 10^{-4}$  cm while the activation energy for grain boundary diffusion is  $Q_{gb} \sim 0.6 Q_l$ , giving  $Q_{gb} \sim 19.2$  kcal/mol. From equation (2)  $\dot{\epsilon}/\sigma = 3.5 \times 10^{-18}$  cm<sup>3</sup>/dyne sec at  $270^\circ$  C in good agreement with the experimental value of  $\dot{\epsilon}/\sigma \sim 8 \times 10^{-18}$  cm<sup>3</sup>/dyne sec. Some support is given to this calculation by direct determinations of  $Q_{gb}$  by the temperature cycling technique. Initial results are  $Q_{gb} = 20.0 \pm 1.9$  kcal/mol at  $6.9 \times 10^6$  dynes/cm<sup>2</sup> and  $285^\circ$  C, indicating control by the grain boundary mechanism.

The practical importance of vacancy creep in metals and alloys operating at high fractions of the melting temperature has been pointed out previously<sup>4</sup>. The foregoing results demonstrate, for the first time, that vacancy creep by a mechanism involving grain boundary diffusion can be of dominating importance in fine-grained metals stressed at lower temperatures ( $\sim 0.6 T_m$ ).

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### CRYSTALLOGRAPHY

#### Growth of Single Crystals of $\text{Ba}(\text{B}_{0.33}\text{Ta}_{0.67})\text{O}_3$ Perovskite-type Compounds

A REVIEW of the literature revealed that a considerable effort has been expended in the past few years on investigations of a large number of new compounds with the general formula,  $A(\text{B}'_x\text{B}''_{1-x})\text{O}_3$ , where  $\text{B}'$  and  $\text{B}''$  are two different elements in the octahedrally co-ordinated cation



the nickel spectrum is of a type similar to that observed by Cotton and Bannister<sup>4</sup>, for the corresponding triphenylphosphine oxide-perchlorate complex.

The structure of the cobalt-perchlorate complex has been shown by X-ray analysis to correspond to a tetragonal pyramidal arrangement of the groups around the metal ion, with the cobalt slightly out of the plane formed by the four oxygen atoms of the arsine oxide groups and a perchlorate group co-ordinated in the fifth position about the cobalt atom<sup>5</sup>. The second perchlorate group is considered to be ionic. The infra-red data are therefore in agreement with such a structure. The presence of co-ordinated perchlorate groups has previously been suggested for certain compounds of copper and nickel<sup>6,7</sup>.

The magnetic properties of the perchlorate complexes are of interest in view of this stereochemical arrangement. For the Mn(II) complex the observed moment, 6.1 B.M. (although a little high), is of the order expected for a spin-free  $d^5$  configuration, as the ground term is an orbital singlet and hence  $\mu_{\text{eff}}$  should be essentially independent of the stereochemistry of the molecule. For the Cu(II) compound the observed moment, 1.85 B.M., is in the general range reported for copper(II) salts with a planar or octahedral arrangement. However, the magnetic properties of the cobalt(II) and nickel(II) complexes are particularly interesting. For the cobalt complex the moment is 5.5 B.M. and this corresponds more closely to that anticipated for an octahedral environment with an orbital triplet ground state ( $^4T_{1g}$ ). This is the first reported example of a five co-ordinate cobalt(II) with the spin-free configuration. For an octahedral environment the maximum moment anticipated is 5.3 B.M.; the observed value can be accounted for on the basis of a distortion from octahedral symmetry which can lead to an increase in the moment<sup>8</sup>. It would appear from the magnetic data that the presence of the perchlorate group has a very significant effect on the composition of the ground term of the cobalt ion, and the magnetism indicates a considerable deviation from a planar arrangement. For the nickel complex the moment is of the order reported for the corresponding triphenylphosphine oxide complex<sup>4</sup>, notably higher than that normally observed for an octahedral arrangement. We are now investigating the magnetic properties of these complexes over a temperature range.

\*Note added in proof. Prof. Saccoccini and his co-workers have recently reported (*J. Amer. Chem. Soc.*, 87, 2057 (1965)), the structure of another example of high spin five-co-ordinated Co(II) derivatives in which there is a square pyramidal arrangement of the attached donor atoms.

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of manganese, iron, cobalt, nickel, copper and zinc. The magnetic properties of this series, together with their absorption spectra in the ultra-violet, visible and infra-red regions, are discussed by those authors. Since the structural implications of these physical properties were not entirely conclusive, we have undertaken a structure analysis of the cobalt compound by single crystal X-ray diffraction techniques.

Powder diffraction photographs show the several compounds to be isostructural and single crystal photographs of the cobalt complex show the crystals to be tetragonal, space group  $P4/n$ ,  $a = b = 16.33 \text{ \AA}$ ,  $c = 10.77 \text{ \AA}$ ; with  $\rho_{\text{obs}} = 1.52 \text{ g/cm}^3$ ,  $\rho_{\text{calc}} = 1.50 \text{ g/cm}^3$  for two formula units per unit cell. The structure was determined by three-dimensional sharpened Patterson methods and a series of observed and difference Fourier syntheses phased on an increasing number of atoms. Subsequent refinement of the atomic positional and thermal parameters was accomplished using an anisotropic least-squares procedure, the usual residual being 0.12 for the 1,100 independent reflexions.

The cobalt atom lies on a four-fold crystal axis: the co-ordination polyhedron is a distorted tetragonal pyramid consisting of four crystallographically equivalent oxygen atoms of the arsine groups and one oxygen atom of a perchlorate group. The cobalt atom is displaced from the plane of the oxygen atoms from the four equivalent arsine oxides by 0.32  $\text{\AA}$  towards the co-ordinated perchlorate. Moreover, the co-ordinated oxygen atom of the perchlorate does not lie directly above the cobalt atom but is displaced 0.72  $\text{\AA}$  to one side of the four-fold axis in such a way that the arsine oxide O—Co—(perchlorate)—O angles range from 85° to 95°. The chlorine atom of the  $\text{ClO}_4$  group does, however, lie on, or very nearly on, the four-fold axis through the cobalt. Since the perchlorate ion does not have  $C_4$  symmetry, there must be rotational disorder of this group in the crystal among four equivalent orientations. The perchlorate ion is tilted with respect to the four-fold axis so as to make the Co—O—Cl angle through the co-ordinated oxygen equal to 110°; the Co—O distance is 2.10  $\text{\AA}$  compared with 2.02  $\text{\AA}$  for the distance Co—OAsMePh<sub>3</sub>, the difference being statistically non-significant at the present stage of refinement.

The co-ordinated perchlorate ion is strictly tetrahedral within experimental error. The locations of the oxygen atoms have been determined only with some difficulty, since with the random occupation of four equivalent positions it is necessary to locate four sets of four peaks of two electrons each rather than one set of four peaks of eight electrons each. The standard errors of the structural parameters for these atoms are, therefore, high. It is nevertheless quite clear from our study of the observed and difference Fourier syntheses, as well as the observed and calculated structure factors, that the perchlorate ion is acting as a monodentate ligand. From similar considerations we conclude that there are no water molecules present, and in particular that the metal atom is not octahedrally co-ordinated.

The arsenic atoms are tetrahedrally surrounded by the oxygen, two phenyl and one methyl groups (Fig. 1) with As—O = 1.70  $\text{\AA}$ , As—O<sub>1</sub> = 1.95  $\text{\AA}$ , As—O<sub>2</sub> = 1.97  $\text{\AA}$ , and As—O<sub>3</sub> (methyl) = 1.90  $\text{\AA}$  (all  $\pm 0.05 \text{ \AA}$ ). The angles at the arsenic atom vary from 105° to 115° and the angle Co—O—As = 129°.

The second, crystallographically independent perchlorate group is uncoordinated and is located on a two-fold crystal axis in a 'large hole' in the lattice among the

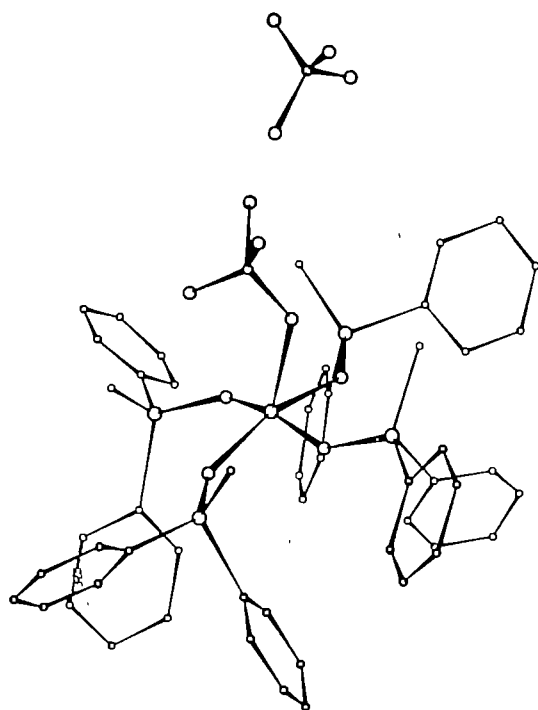


Fig. 1

crystal examination shows the dinitrate compounds  $(\text{Co}(\text{Ph}_2\text{MeAsO})_2(\text{NO}_3)_2)$  to be virtually indistinguishable from the diperchlorate. The nitrate group does not have point symmetry consistent with the 4 crystal symmetry of the two-fold position but, like the perchlorate, does have point symmetry consistent with the half-occupied four-fold position on the 2 axis.

While recent conductometric<sup>1,2</sup> and infra-red investigations, both in these laboratories and elsewhere<sup>3,4</sup>, have provided evidence for perchlorate co-ordination, it is nevertheless often assumed that the perchlorate group has negligible co-ordinating power, and direct structural evidence for perchlorate co-ordination in this series of complexes is therefore of interest. The orientation of the co-ordinated group must result from some compromise between the high symmetry (4) of van der Waals interactions (principally with the methyl groups) and the requirement of appreciable overlap between the bonding orbitals of the oxygen and the axially symmetric metal orbital.

A detailed discussion of the bonding in this complex and the relation of its stereochemistry to that of other 5-co-ordinate metal ions (for example, nitroso (dimethyldithiocarbonato) cobalt<sup>5</sup>) will be given elsewhere, as will full details of the analysis and refinement.

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### Conduction In Tantalum-doped Thorium Dioxide

As part of a search for conducting refractory oxides which can be heated directly from room temperature to temperatures in excess of  $2,000^\circ\text{C}$  by induced radio-frequency currents without the need for pre-heating arrangements, a study has been made of the behaviour of hot-pressed thorium dioxide doped with various amounts of tantalum pentoxide. These systems possess no apparent advantages in oxidizing atmospheres, but do exhibit striking properties when prepared in reducing conditions and operated in a neutral atmosphere.

Using the induction heated hot-pressing furnace described by Moore, Ubbelohde and Young<sup>1</sup>, pure  $\text{ThO}_2$  attained a density of only 92.9 per cent theoretical under 300 kg  $\text{cm}^{-2}$  at  $2,000^\circ\text{C}$ , though reagent grade  $\text{ThO}_2$  containing  $\sim 200$  p.p.m. of trivalent cations did densify at this temperature. The hot-pressing was done *in vacuo* using a tantalum-lined graphite susceptor, and under these conditions the product possessed a white, stoichiometric core with blackened outer regions, presumably containing free thorium. After intimate mixing with  $\text{Ta}_2\text{O}_5$ , hot pressing under these strongly reducing conditions resulted in ready densification with considerable grain growth at moderate temperatures. Densities are calculated assuming ideal solution.

Moles % $\text{Ta}_2\text{O}_5$	0.05	0.25	0.5	1.0	2.0	3.05	5.0
Hot-pressing temp. $^\circ\text{C}$	1,950	1,960	1,980	1,700	1,650	1,600	1,740
Density, % of theoretical	88.5	97.9	97.4	96.3	98.4	99.0	97.9

The materials so obtained ranged in colour from violet (0.5 per cent  $\text{Ta}_2\text{O}_5$ ) to black (5 per cent). It appeared that the outer layers were substoichiometric, thus measurements were made only on material sliced from the core. Electron probe microanalysis confirmed that the  $\text{Ta}_2\text{O}_5$ , which presumably had served as a liquid intermediate phase during the early stages of sintering, was in fact distributed uniformly throughout the sintered specimens and had not aggregated at grain boundaries. On heating in air at  $800^\circ\text{C}$  the colour of the reduced specimens discharged, giving a colourless translucent product. X-ray powder diffraction revealed no lattice parameter changes either in the oxidized or reduced forms, consistent with the small weight uptake on oxidation, which indicated that less than 1 per cent of the  $\text{Ta}^{3+}$  ions were reduced to  $\text{Ta}^{4+}$ . Barring evidence to the contrary, we assume that the tantalum is in solid solution, because it was found to be distributed uniformly in grains several hundred microns across in the product, even though the particle size of the starting materials was  $< 10\ \mu\text{m}$ .

Resistivity measurements were made by 4-point potentiometric methods using graphite current electrodes and platinum-5 per cent rhodium voltage probes at temperatures up to  $1,540^\circ\text{C}$ . In agreement with Rudolph<sup>2</sup>, oxidized material showed no useful decrease in resistivity, general agreement being achieved between d.c. and 50 kc/s. However, in the reduced condition the room temperature resistivity of the doped samples was found to have fallen by 6-10 orders of magnitude with respect to pure  $\text{ThO}_2$ . Representative values measured in argon are 3-15 ohm cm (5 moles per cent  $\text{Ta}_2\text{O}_5$ ), 3,000 ohm cm (0.25 mole per cent  $\text{Ta}_2\text{O}_5$ ). Irreproducibility between specimens originated from the extreme sensitivity of contact resistance to atmospheric oxidation rather than from variations in bulk properties. Thus all surfaces had to be prepared under oil, even light abrasion in air leading to the

mole per cent  $Ta_2O_5$  fell in one run from 2,850 ohm cm at 25° C to 670 ohm cm at 1,140° C for example, whereas the resistivity of a sample containing 1.0 mole per cent tantalum pentoxide fell from 24 to 18 ohm cm over the same temperature-interval. In later runs this latter sample reached bulk resistivities as low as 0.2 ohm cm. Such materials can be heated *in vacuo* to temperatures in excess of 2,000° C by a direct current of a few amperes, but cannot be heated by induction at 500 kc/s, though promising results have been obtained at 15 Mc/s.

The mechanism of conduction is at present obscure, though it appears to have an excitation energy of 0.05 eV. No magnetoresistance or Hall effect could be detected even at 17 kgauss. One accordingly presumes that a hopping process occurs.

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### Fluorine Shielding in Diatomic Molecules and the Lamb Term

Alexakos and Cornwell<sup>1</sup> have recently measured the  $F^{19}$  shielding constant  $\sigma_{\text{HF}}$  in chlorine mono-fluoride, ClF, and they found that the shielding is greater in ClF than in  $F_2$ . Three values of  $F^{19}$  shielding constants in diatomic molecules are now available, and they are shown

Table 1

System Shielding constant	Shielding constants (p.p.m.)		Relative to $F_2$	
	$F_2$ 0	HF 625	$F_2$ 676	ClF 875

In Table 1 together with the value<sup>1</sup> for free  $F^-$ . This value is subject to some uncertainty, most measurements obtained in compounds of  $F^-$  being somewhat lower<sup>2</sup>. The correct value is unlikely to exceed that shown in Table 1.

The shielding constant  $\sigma$  is usually regarded as the sum of the Lamb term  $\sigma^L$  and the paramagnetic term  $\sigma^P$ , which are of opposite sign. Saika and Slichter<sup>3</sup> suggested that the results for  $F^-$ , HF, and  $F_2$  could be explained purely on the basis of variations in  $\sigma^P$ , which would be zero for  $F^-$  and increase with the increasing covalency of the bond  $F-X$ . This view seems to have become accepted<sup>4</sup>, but if it is correct, the shielding should be greater in  $F^-$  than in any other species. The new value for  $\sigma_{\text{HF}}$  directs attention to possible variations in  $\sigma^L$ . In fact Saika and Slichter obtained a value of 2,000 p.p.m. for  $\sigma^P$  in  $F_2$ , which must either be an overestimate or an indication that there is a significant variation in  $\sigma^L$ .

The possibility of variations in  $\sigma^L$  for  $F^{19}$  has been mentioned only to be dismissed<sup>4</sup>. The value of  $\sigma^L$  increases with an increase in the electron density near the nucleus. Apart from the electronegativity, there are two other causes of a variation in this electron density. The first of these is an increase with the number of electrons. Thus the values for  $\sigma^L$  given by Dickinson<sup>5</sup> show an increase from 18 p.p.m. in hydrogen to 464 p.p.m. in fluorine and 1,150 p.p.m. in chlorine. In a negative ion, however, the greater diffuseness of the orbitals compared to an atom tends to cancel out the increase with the number of electrons. Thus, Sidwell and Hurst's value<sup>6</sup> for  $\sigma^L$  in  $F^-$  is only 2 p.p.m. higher than their value for fluorine. The increase in  $\sigma^L$  with the number of electrons is also shown for molecules, although the calculated values may be less reliable than those for atoms. With Ransil's

functions<sup>7</sup>,  $\sigma^L$  for the proton<sup>8</sup> in HF is 78 p.p.m. higher than the accepted value<sup>9</sup> for  $H_2$ , and  $\sigma^L$  for  $F^{19}$  is 48 p.p.m. higher in  $F_2$  than in HF<sup>9</sup>.

The second effect is the increase in electron density at the nucleus on molecule formation. This effect is well known for hydrogen, but it is often neglected for other molecules. It leads to an increase in  $\sigma^L$  of 14 p.p.m. for  $H_2$ <sup>8</sup> compared to  $H^+$ , and 51 p.p.m. for  $F_2$ <sup>8</sup> compared to  $F^+$ .

Both these effects will contribute to a greater value for  $\sigma^L$  in ClF than in  $F^-$ , and the increase might well be 100 p.p.m. or more. Although this does not account for the whole of the anomaly pointed out above, it is too large a contribution to be neglected. An experimental value for the shielding in BrF would throw much light on the relative importance of the various factors. The whole question is discussed in more detail elsewhere<sup>10</sup>.

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### Carbon Adsorbents with Molecular Sieve Properties

It has been found possible to obtain an adsorbent with typical molecular sieve properties by thermal processing of granulated carbonaceous material (prepared on the basis of pyrolysis of wood material at about 500° C) at 970–980° C in the atmosphere of gases preventing the oxidation of the carbon material. The adsorption of oxygen at –196° C occurs in its micropores, whereas nitrogen and argon are adsorbed at this temperature on the surface of transitional pores and macropores<sup>1</sup> only. The volume of micropores of this adsorbent is 0.15 cm<sup>3</sup>/g and the value of the specific surface of transitional pores and macropores is nearly 17 m<sup>2</sup>/g. Adsorption of nitrogen and argon is substantially increased by raising the temperature to –78° C. This effect is completely analogous to that observed in the adsorption of nitrogen on 'Zeolite NaA' (type 4A)<sup>2</sup>. At 20° C water vapour is adsorbed in the micropores of the adsorbent, whereas benzene vapour is adsorbed at this temperature only on the surface of transitional pores and macropores. The values of adsorption are presented in Table 1. From the adsorption data it is evident that the dimensions of openings of the micropores are close to 4 Å. The adsorbent may thus be considered the carbon analogy of the 'Zeolite NaA' (4A). The shape of the adsorption isotherm of water vapour is

Table 1. VALUES OF THE ADSORPTION OF GASES (m.moles/g) AT PRESSURES 15 AND 70 MM. MERCURY

Gas	Temperature °C	15 mm Hg	70 mm Hg
H <sub>2</sub> O	20	7.70	—
O <sub>2</sub>	–196	5.25	5.80
N <sub>2</sub>	–78	0.38	0.84
N <sub>2</sub>	–196	0.17	0.18
N <sub>2</sub>	–78	0.50	0.51
Ar	–196	0.10	0.12
Ar	–78	0.25	0.42
C <sub>2</sub> H <sub>6</sub>	20	0.00	0.12

characteristic for the system water-active charcoal<sup>1</sup>. The steep rise of the adsorption isotherm is observed in the region of relative pressures 0.4–0.5 and the isotherm reveals a typical hysteresis.

From the point of view of adsorption selectivity the carbon molecular sieves are comparable to the classical 'Zeolite' sieves. In some cases the selectivity is even better. The carbon molecular sieve prepared by thermal processing of 'Saran' charcoal at 1,750° C selectively adsorbs benzene from the benzene-cyclohexane mixture<sup>2</sup>. These hydrocarbons, according to Barrer<sup>4</sup>, belong to the same group of compounds separated by zeolites. Additional activation (by carbon dioxide) of the carbon molecular sieve prepared from the 'Saran' charcoal to a low burn-off (11 per cent) renders its microporous structure accessible for cyclohexane molecules<sup>1</sup>. It can therefore be concluded that the molecular sieve properties of carbon adsorbents on the basis of 'Saran' charcoal are caused by narrowing of the openings of micropores.

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### Structures of Oxodiperoxo-1:10-phenanthrolinechromium(VI) and Diperoxoquo-ethylenediaminechromium(VI)hydrate

As a part of an investigation of the structures of peroxochromates<sup>1–4</sup>, peroxochromium complexes with ethylenediamine and 1:10-phenanthroline have been studied, and that of  $\alpha, \alpha'$ -bipyridyl is under investigation.

The structures of  $[\text{Cr}(\text{O}_2)_2(\text{C}_{10}\text{H}_8\text{N}_2)]$  and  $[\text{Cr}(\text{O}_2)_2(\text{H}_2\text{O})(\text{C}_4\text{H}_{10}\text{N}_2)](\text{H}_2\text{O})$  have been solved by three-dimensional Patterson and Fourier methods, and refined some least-squares cycles. The *R*-factor has dropped to 0.13 and 0.18 respectively.

The unit cell dimensions of  $[\text{Cr}(\text{O}_2)_2(\text{C}_{10}\text{H}_8\text{N}_2)]$  were determined from rotation and Weissenberg photographs, and were refined from Guinier photographs using  $\text{CuK}\alpha$  radiation. The unit cell is orthorhombic with 4 molecules in the cell and with  $a = 10.554$  Å,  $b = 8.857$  Å and  $c = 16.239$  Å. The systematically absent reflexions were  $0kl$  with  $k + 1 = 2n + 1$  and  $h0l$  with  $h = 2n + 1$ . This is characteristic for the space groups *Pnma* and *Pna2*. During the structure investigation it became obvious that a plausible structure could be obtained based on space group *Pnma*.

The investigation has revealed that chromium is co-ordinated to two peroxo groups, one oxide oxygen atom and to the two nitrogen atoms of the 1:10-phenanthroline molecule, the geometrical configuration of the co-ordinated atoms being a pentagonal bipyramid with the oxide oxygen and one nitrogen atom at the apices. The O—O distance is  $1.42 \pm 0.02$  Å and the Cr—O<sub>oxo</sub> distance is  $1.58 \pm 0.03$  Å, values which are close to those obtained for similar compounds (1–4). Table 1 gives the atomic positions at the present stage of the refinement.

The unit cell dimensions of  $[\text{Cr}(\text{O}_2)_2(\text{H}_2\text{O})(\text{C}_4\text{H}_{10}\text{N}_2)](\text{H}_2\text{O})$  were determined from rotation and Weissenberg photographs and were refined from Guinier photographs using  $\text{CuK}\alpha$  radiation. The orthorhombic unit cell contains 4 molecules and has the dimensions:  $a = 7.594$  Å,  $b = 12.258$  Å, and  $c = 8.168$  Å. The systematically absent

Table 1. PARAMETER VALUES FOR  $[\text{Cr}(\text{O}_2)_2(\text{C}_{10}\text{H}_8\text{N}_2)]$   
Space group *Pnma*

Atom	Equivalent position	x	y	z
Cr	4c	0.245	0.250	0.173
O <sub>1</sub>	8d	0.239	0.519	0.153
O <sub>2</sub>	8d	0.180	0.434	0.128
O <sub>3</sub>	4c	0.337	0.250	0.235
N <sub>1</sub>	4c	0.412	0.250	0.100
N <sub>2</sub>	4c	0.175	0.250	0.043
C <sub>1</sub>	4c	0.531	0.250	0.123
C <sub>2</sub>	4c	0.631	0.250	0.079
C <sub>3</sub>	4c	0.624	0.250	0.223
C <sub>4</sub>	4c	0.490	0.250	0.263
C <sub>5</sub>	4c	0.393	0.250	0.013
C <sub>6</sub>	4c	0.473	0.250	0.875
C <sub>7</sub>	4c	0.351	0.250	0.847
C <sub>8</sub>	4c	0.242	0.250	0.901
C <sub>9</sub>	4c	0.258	0.250	0.966
C <sub>10</sub>	4c	0.110	0.250	0.877
C <sub>11</sub>	4c	0.027	0.250	0.966
C <sub>12</sub>	4c	0.046	0.250	0.017

reflexions were  $0kl$  with  $k = 2n + 1$  and  $h0l$  with  $l = 2n + 1$ , which is characteristic for the space groups *Pbcm* and *Pbc2*.

As there are only 4 molecules in the unit cell and as it was supposed from other evidence that the molecules did not contain a mirror plane, the correct space group was thought to be *Pbc2*. The fractional co-ordinates of the atoms, based on this assumption, are at the present stage of the refinement those given in Table 2 (*R* = 0.176). During the structure investigation it soon became evident that all atoms except the two carbon atoms were situated at positions that fitted space group *Pbcm* equally well (within the limits of error). With the carbon atoms assumed to occupying positions 8c of *Pbcm* with only half the equivalent positions occupied (evidently this means that in half the unit cells C<sub>1</sub> is above and C<sub>2</sub> below the 'mirror' plane, while in the rest of the unit cells the reverse is true), refinement was undertaken and the *R*-value became 0.183 after six cycles, the parameters being those given in parenthesis in Table 2.

Table 2. PARAMETER VALUES FOR  $[\text{Cr}(\text{O}_2)_2(\text{H}_2\text{O})(\text{C}_4\text{H}_{10}\text{N}_2)](\text{H}_2\text{O})$   
Space group *Pbc2*, assumed. In parenthesis are given the values obtained by assuming *Pbcm* to be the correct space group with O<sub>1</sub> and O<sub>2</sub> in 8d, C<sub>1</sub> and C<sub>2</sub> in 8c (half-filled), O<sub>3</sub> in 4c and all the others in 4c

Atom	x	y	z
Cr	0.241 (0.241)	0.013 (0.013)	0.251 (0.250)
O <sub>1</sub>	0.237 (0.237)	0.018 (0.020)	0.014 (0.017)
O <sub>2</sub>	0.233 (0.231)	0.013 (0.013)	0.063 (0.084)
O <sub>3</sub>	0.238	0.021	0.430
O <sub>4</sub>	0.278	0.013	0.413
O <sub>5</sub>	0.277 (0.278)	0.964 (0.963)	0.245 (0.250)
O <sub>6</sub>	0.255 (0.255)	0.241 (0.250)	0.000 (0.000)
N <sub>1</sub>	0.501 (0.500)	0.069 (0.068)	0.247 (0.250)
N <sub>2</sub>	0.204 (0.204)	0.183 (0.182)	0.250 (0.250)
C <sub>1</sub>	0.220 (0.219)	0.173 (0.173)	0.220 (0.219)
C <sub>2</sub>	0.269 (0.269)	0.234 (0.232)	0.202 (0.202)

This molecule can also be described as being co-ordinated to five ligands, namely, to two peroxo groups, one water molecule and the two nitrogen atoms of one ethylenediamine molecule as co-ordinating groups. The geometrical arrangement of the co-ordinated atoms is a pentagonal bipyramid. The water molecules are involved in hydrogen bonding, one with one peroxidic oxygen atom of each of two neighbouring  $[\text{Cr}(\text{O}_2)_2(\text{H}_2\text{O})_n]$  molecules and the other with two peroxidic oxygen atoms of one neighbouring complex molecule, forming chains through the whole crystal. The O—O distances are  $1.44 \pm 0.04$  Å and  $1.46 \pm 0.04$  Å, the Cr—O<sub>water</sub> distance is  $2.04 \pm 0.02$  Å, and the hydrogen bonding distances O<sub>peroxo</sub>...H...O<sub>water</sub> are 2.69 Å, 2.86 Å, 2.72 Å and 2.72 Å.  $\angle \text{O}_{\text{peroxo}} \dots \text{O}_{\text{water}}$  are 102° and 107° respectively.

The refinement will be continued to convergence, and complete reports will be published elsewhere.

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# BIOPHYSICS

## Comparison of Viscometric Behaviour of Suspensions of Polystyrene Latex and Human Blood Cells

THE viscosity of blood is a complex physical property determined by many factors. These factors include at least: (a) the physical characteristics of the blood cells (size, shape, deformability, etc.); (b) the concentration of the blood cells; (c) the composition of the plasma (especially the proteins) and the interactions between plasma and cells; (d) the shear rate. To examine the influence of cell shape and deformability on blood viscosity, we have compared, at low shear rates, the viscosities of suspensions of red blood cells and suspensions of rigid, spherical latex particles with diameters comparable to the major diameters of red cells in human blood. Polystyrene latex (Dow Chemical Co., Midland, Michigan) used for this purpose consisted of rigid spheres, with a mean diameter of  $9.2\mu$ , suspended in water with soap and stabilized with colloidal silica. Human red blood cells are biconcave disks with a mean major diameter of  $7.2\mu$  (ref. 1) and are deformable<sup>2-4</sup>. In the work recorded here, plasma was removed from human blood and the cells washed and suspended in a protein-free solution (Ringer's solution), thus eliminating the influence of plasma proteins on blood viscosity and making the cell suspensions more comparable to the latex suspensions. Because of the dependence of the viscosity of cell suspensions on cell concentration and shear rate<sup>5,6</sup>, viscosity measurements on cell suspensions and latex suspensions were made over a wide range of particle concentration and at shear rates varying from  $50 \text{ sec}^{-1}$  to  $0.02 \text{ sec}^{-1}$ .

The coaxial cylinder viscometer developed by Glinson, Dauwalter and Merrill<sup>7</sup> was used in these experiments. The outer specimen cylinder rests on a nitrogen-gas bearing set in the fields of an electromagnetic torque-sensing and torque-generating system. The torque-sensor is connected through a feedback amplifier system to the torque generator, producing torques of  $10^{-1}$ – $10^{+3}$  dyne-cm to prevent the specimen cylinder from rotating. The inner rotating cylinder is driven by motors, powered and controlled by tachometer and feedback amplifier systems (Servo-Tek Products Co., Hawthorne, New Jersey), through a gear train at any rate of  $10^{-2}$ – $82 \text{ r.p.m.}$  Shear rates were calculated from the rate of rotation of the inner cylinder and the annulus gap between the two cylinders, assuming uniform shear rate across the annulus. Viscosities were calculated from the quotient of the torque generated and the shear rate. Viscosities and shear rates calculated in this manner (without use of approximations to the unknown flow equations) are only approximate. Nevertheless, these approximate viscosities are useful in

describing the flow behaviour of these complex systems in a differential or quotient form, demonstrating graphically the large differences in flow behaviour among these suspensions.

Venous blood was drawn from healthy male subjects. Heparin was added to prevent clotting. Plasma was removed from the blood after centrifugation for 10 min at approximately  $10^4g$ , and the cells were washed three times with a modified Ringer's solution (NaCl 0.9 g, KCl 0.042 g, CaCl<sub>2</sub> 0.024 g, and NaHCO<sub>3</sub> 0.02 g per 100 ml. aqueous solution). Aliquots of cells were suspended in appropriate volumes of Ringer's solution to prepare series of samples with cell volume fractions ranging from 0 to 0.95.

The supplied latex suspension contained 0.435 volume fraction of polystyrene spheres. Sodium lauryl sulphate (1:200 in relation to the polystyrene content) was added to remove the protective colloid from the polystyrene particles. Samples with various volume fractions were prepared by the addition or removal of water. When the samples were mixed briefly without prolonged stirring (referred to as the unstirred samples), the particles were uniformly dispersed. Stirring gently for 30 min produced loose clusters involving some of the particles, while stirring for 4 h caused the latex particles to form compact clusters containing most of the particles (Fig. 1).

The stirring of the latex suspensions caused dramatic changes in viscometric behaviour. Fig. 2 shows the relation between viscosity and shear rate for three samples of latex suspensions (unstirred, stirred for 30 min, and stirred for 4 h), as well as for Ringer suspension of human blood cells, all at volume fraction of 0.435. The unstirred latex suspension shows a dilatant behaviour, that is, the viscosity decreases as the shear rate is reduced. The latex suspension stirred for 30 min becomes mildly thixotropic, that is, the viscosity increases as the shear rate is reduced below  $1 \text{ sec}^{-1}$ . The latex suspension stirred for 4 h shows increased thixotropy. As already pointed out here, stirring of latex suspensions causes marked changes in the state of aggregation of the particles (Fig. 1). Since the uniform monodispersion of latex particles in the unstirred suspension is associated with a dilatant behaviour, and the formation of clusters in the stirred suspensions is accompanied by a thixotropic behaviour, it seems that the appearance of thixotropy in the latex suspensions is the result of cluster formation. This explanation is in agreement with the finding that heparinized whole blood, with the presence of many aggregates and plasma-cell interactions, has a more pronounced thixotropic behaviour than cell suspensions in Ringer's solution<sup>5,6</sup> in which the cells are largely monodispersed.

The viscosities of the latex suspensions, whether stirred or not, are considerably higher than those found for the cell suspensions (Fig. 2). The difference in viscosity

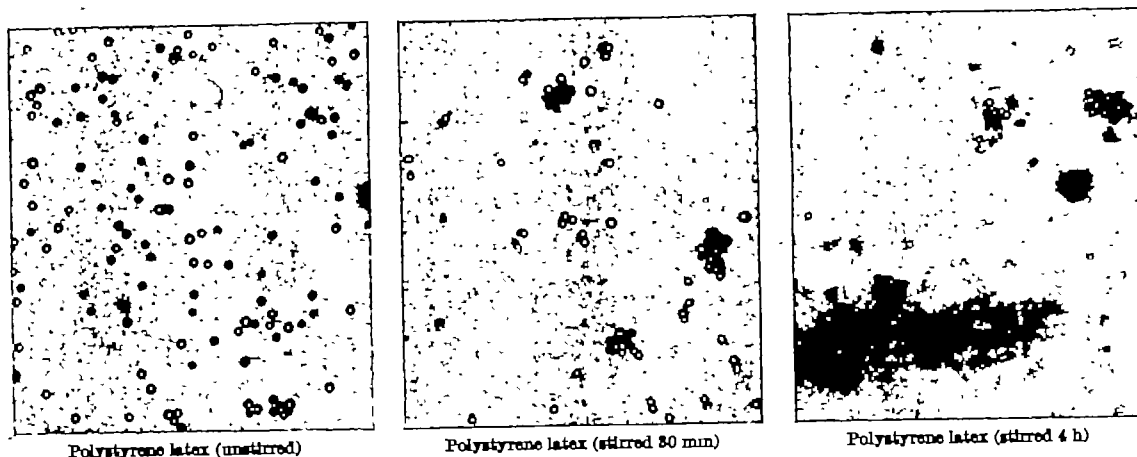


Fig. 1. Photomicrographs of diluted latex suspensions

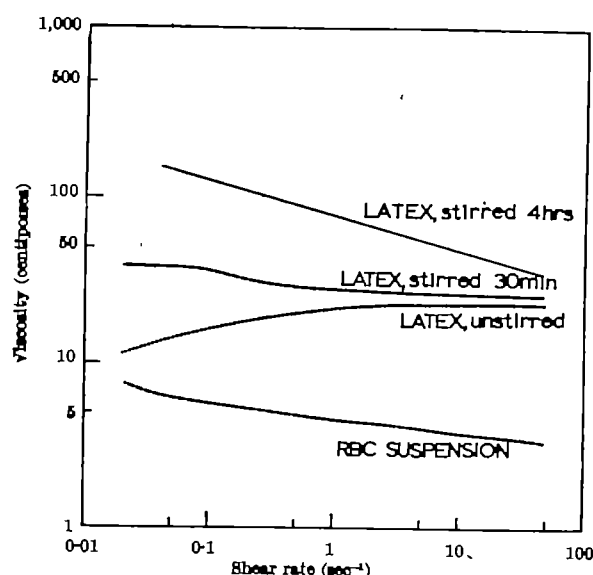


Fig. 2. Logarithmic plot of viscosity versus shear rate for latex suspensions and cell suspensions, all with a volume fraction of 0.436 at 37° C

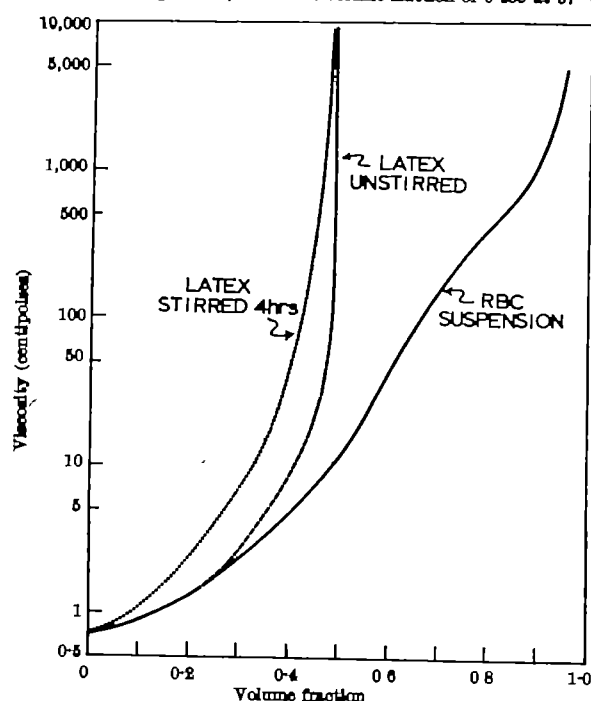


Fig. 3. Semilogarithmic plot of viscosity versus volume fraction of suspended particles for latex suspensions and cell suspensions at a shear rate of 0.05 sec<sup>-1</sup> at 37° C

between these two forms of suspension is especially marked at high particle concentrations. In Fig. 3, the log of viscosity at a fixed shear rate (0.05 sec<sup>-1</sup>) is plotted against the volume fraction of the suspended particles. At volume fractions below 0.3, the curves for unstirred latex and cell suspensions are essentially the same, whereas the stirred latex has a slightly higher viscosity. Above a volume fraction of 0.3, the viscosity of the latex suspensions increases sharply with rising particle concentration and becomes markedly higher than that of the cell suspensions. With particle concentration higher than 0.47, the viscosity of the latex suspensions becomes so high that the torque generated is above the limit of our instrument. As shown in Fig. 3, the viscosity of the latex suspensions rises sharply toward infinity. On the other hand, the viscosity of a cell suspension with a volume fraction as high as 0.95 is still within limits of the instrument and can be readily determined.

These results reveal striking differences in the viscometric behaviour between suspensions of rigid spherical particles (polystyrene latex) and those of deformable biconcave particles (red cells). We believe that these contrasting viscosity behaviours are evidence for the widely held idea that the ability of blood to flow at very high cell fractions depends on the deformability, and probably on the shape, of the cells.

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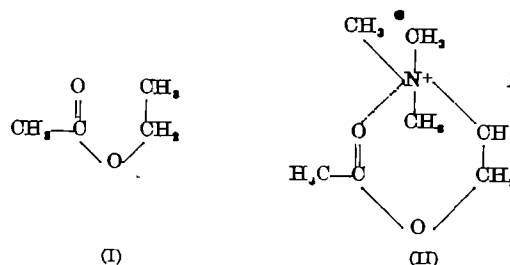
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## Conformation of Acetylcholine

THE development of the attenuated total reflexion (ATR) method<sup>1,2</sup> for the examination of aqueous solutions in the infra-red region has made it possible to re-examine the problem of the conformation of acetylcholine (ACh) in aqueous and mammalian Ringer solution.

In the published crystal structure of ACh bromide, Sorum<sup>3</sup> postulated the simultaneous existence of two types of ACh chains: the extended and the ring planar; the latter form had an N<sup>+</sup> - - - alkyl and carbonyl oxygen distances of 3.51 and 5.32 Å respectively.

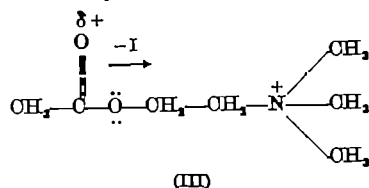
Three years later, on the basis of the infra-red study of ACh chloride dissolved in ethanol, it was suggested<sup>4</sup>, from the higher frequency of the carbonyl group ( $\nu_{C=O}$ ) in ACh than in ethyl acetate (I), that the former compound had a cyclic structure (II). The free base 2-dimethylaminoethyl acetate showed two  $\nu_{C=O}$  bands at 1,748 and 1,728 cm<sup>-1</sup> as did (I). In a later account the same authors<sup>5</sup> rejected the cyclic structure and suggested that there was an inductive effect from the quaternary nitrogen atom which would influence the carbonyl frequency.



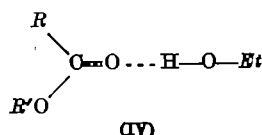
(I)

(II)

The arguments presented<sup>4</sup> to account for the higher frequency of the carbonyl group in terms of the inductive effect of the quaternary nitrogen atom are unsatisfactory. It has been shown (ref. 6, and unpublished work by E. F. M.) that the mesomeric and/or inductometric interaction between the alkoxy and the acyl groups are negligible, that is, the contribution from (III) is not expected to be important. Further, in Fellman and Fujita's paper<sup>4</sup> the  $\nu_{C=O}$  of methyl and substituted methyl acetates,  $CH_3CO_2X$ , where  $X = Me$ ,  $-CH_2CN$  and  $-CH_2CO_2Et$ , are within the range  $1,758 \pm 4 \text{ cm}^{-1}$  and similarly those of ethyl and substituted ethyl acetates  $CH_3CO_2CH_2X$ ,  $X = Me$ ,  $-CH_2NO_2$ ,  $-CH_2N^+Me_3$ ,  $-CH_2N^+(Pr)_3$ , and  $CH_2N^+(Bu)_3$ , are within the range  $1,754 \pm 6 \text{ cm}^{-1}$ . (See Tables 2 and 3 of ref. 5):



The presence of a group exhibiting a strong  $-E$  effect, namely, nitro, cyano or carbethoxy, does not appreciably decrease the carbonyl frequency. If the cyclic structure (II) was predominant it would be expected that the carbonyl frequency would be decreased due to the increased polarization of the  $(C=O)$  group on bonding; for example, in co-ordination studies it has been clearly demonstrated<sup>7-9</sup> that if the carbonyl oxygen acts as a donor atom it results in a decreased  $\nu_{C=O}$ . However, since the spectra were recorded<sup>4,5</sup> in ethanol, there was the possibility of intermolecular hydrogen binding occurring (IV), which would not be expected to compete with (II) if this latter was present, but would give rise to lower frequency carbonyl bands in other esters investigated.



In the present work, although the spectra of ethyl acetate and of Ach chloride and bromide have been recorded in ethanol solution by transmission, the majority of the spectra have been recorded in water solution by reflexion using the ATR method<sup>1,2</sup>. This method was especially chosen since in reflexion water is remarkably transparent and thus we have also been able to record the spectra of Ach in mammalian Ringer's solution (a medium approximating more closely to those found *in vivo*). The frequencies of Ach chloride in ethanol solution obtained in reflexion ( $1,748 \text{ cm}^{-1}$ ) and in transmission ( $1,751 \text{ cm}^{-1}$ ) are in fair agreement, but this latter value is lower than that given by Fellman and Fujita<sup>4,5</sup> (namely,  $1,760 \text{ cm}^{-1}$ ).

The effect of solvent on the carbonyl frequency of Ach has been investigated in ATR, and since the frequencies are dependent on solvent it would suggest that hydrogen bonding is predominant; this is further confirmed, as examining ethyl acetate and Ach chloride and bromide under high resolution in ethanol by transmission shows that the carbonyl bands are multiplets (Table 2) presumably corresponding to different associated forms.

From Table 1 it is clear that the carbonyl frequency of Ach is of the order expected for aliphatic esters, and since the frequency is so dependent on the nature of the solvent the cyclic structure previously suggested<sup>4</sup> is not in agreement with the experimental evidence.

Ring structures do, however, exist in the crystalline state, the electrostatic interaction being between the  $N^+$  quaternary group and the alkyl oxygen, and not the acyl oxygen.

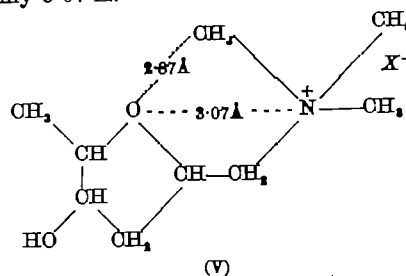
Table 1. CARBONYL FREQUENCIES OF ACH CHLORIDE IN DIFFERENT SOLVENTS —ATR METHOD

Solvent	$\nu_{C=O} (\text{cm}^{-1})$	Dielectric constant
MeOH	1,742	35.4/13°
EtOH	1,748	28.0/14°
EtOH: water (1:1)	1,753	—
Water	1,757	81.0
Ringer's solution	1,757	—

Table 2. CARBONYL FREQUENCIES OF ETHYL ACETATE AND ACH CHLORIDE IN ETHANOL SOLUTION (IN TRANSMISSION)

Ethyl acetate	Acetylcholine chloride	Acetylcholine bromide
1,745	1,751	1,754
1,750	1,748 (sh)	1,743
1,719 (sh)	1,742	1,737
	1,730	

The correctness of the foregoing interpretation is especially shown in the X-ray crystal structure<sup>10</sup> of muscarine iodide (V) where the  $N^+ \cdots$  ring ether oxygen distance, calculated with the published atomic co-ordinates, is only  $3.07 \text{ \AA}$ .



The van der Waals radius of oxygen being  $1.4 \text{ \AA}$ , this distance of  $3.07 \text{ \AA}$  has great significance as compared with the  $N^+ \cdots Cl^-$ ,  $N^+ \cdots Br^-$  and  $N^+ \cdots I^-$  distances of  $4.38$ ,  $4.37$  and  $4.56 \text{ \AA}$  calculated from the published atomic co-ordinates of the X-ray crystal structures of the tetramethylammonium halides<sup>11,12</sup>. Similarly, in muscarine and pentamethonium iodides<sup>13</sup> the closest  $N^+ \cdots I^-$  distances are  $4.49 \text{ \AA}$  and  $4.43 \pm 0.14 \text{ \AA}$  respectively.

The ATR spectra were recorded using a Research and Industrial Instrument Co. 'T.R.-3' unit with  $KR 5$  prism, but the prism holder and cell were gold plated to prevent attack of the metal by acids liberated during hydrolysis; a Perkin Elmer '137' spectrometer was used. The transmission spectra were recorded using a Grubb Parsons 'Spectromaster'. The ATR spectra will be submitted to *Documentation of Molecular Spectroscopy*.

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## BIOCHEMISTRY

### Crystallization of Arginase from Normal and Cirrhotic Human Liver

It has been demonstrated that patients with liver disorders are particularly susceptible to ammonia intoxication<sup>1</sup>. Since ammonia is detoxified by urea synthesis, study of the enzymes of the Krebs-Hensleit cycle in the



normal and abnormal liver might provide useful information. Decrease of liver arginase activity in patients suffering from cirrhosis and hepatic coma has been reported by Ugarte, Pino and Valenzuela<sup>3</sup>. In our study, arginase was crystallized from a 'normal' human and a cirrhotic human liver and the catalytic efficiency of the enzymes from the two sources was compared.

Liver samples were obtained within 12 h of death and preparation of the acetone powder was begun immediately. The purification procedure was that developed by Bach and Killip<sup>4</sup>. A unit of enzyme is defined as that amount which would catalyze the production of 1  $\mu$ mole of urea in 1 min at 25° C, pH 9.5, at an arginine concentration of 0.285 molar<sup>4</sup>. Protein was estimated by the following formula<sup>5</sup>: protein concentration in mg/ml. =  $(OD_{280\text{nm}} - OD_{280\text{nm}}) \times F$ , where  $F$  is a dilution factor. Extraction of the acetone powder yielded 38.8 g of protein from the normal liver and 21.4 g of protein from the cirrhotic liver. The arginase activities of the solutions were 0.515 and 0.466 units/mg, the normal being the higher.

The analysis of the normal liver homogenate revealed that under our conditions of assay 13.0 units of enzyme were present per g of wet tissue. In the case of the cirrhotic liver 7.5 units of enzyme per g wet tissue were found. This represents specific activities of 0.013 unit/mg and 0.008 unit/mg respectively.

The specific activity of the crystalline enzyme from the normal liver approached 150 units per mg, and that from the cirrhotic 80 units per mg of liver protein.

The results indicate that the enzyme in the cirrhotic liver is either: (a) partially inactivated; (b) absolutely reduced in amount; (c) a less efficient catalyst; or (d) is more susceptible to denaturing agents than the normal and thus loses activity in the isolation procedure. Whatever the mechanism an apparent difference exists between the enzymes crystallized from the two sources. Cellulose acetate electrophoresis (Barbital buffer, ionic strength 0.1 M, pH 8.6) showed that the enzyme from both sources migrated in a single band.

The subjects, aged 53 and 74 years, both died of heart failure. The cirrhotic, who was the older, had a history of moderate diabetes mellitus for ten years and at the time of autopsy appeared slightly malnourished. In the case of the 'normal' patient, no other factors influencing liver function were apparent. Definite conclusions must await further investigation.

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### Use of Light-scattering to Measure the Selective Interaction of a Macromolecular Solute with the Components of a Binary Solvent

A SOLUTION of a non-diffusible solute will in general contain the components of a mixed solvent in proportions different from those of an equilibrium diffusate, owing to non-ideal interactions between the solute and the solvent components. The distribution can be measured refractometrically<sup>1,2</sup> and can be expressed unambiguously for a binary solvent as the excess quantity of one of the solvent components (arbitrarily chosen) relative to unit quantity of the other, per unit quantity of solute. Direct refractometric measurement requires preparation of an equilibrium diffusate, and is limited with respect to the concentration of the solute by the sensitivity of the measurement. The use of diffusate can be avoided by measurement of

light-scattering, which also allows measurements to be extrapolated reliably to zero concentration of solute.

Casassa and Eisenberg<sup>1,2</sup> have shown that the excess scattering at zero angle  $\Delta R(0)$  of the macromolecular solute is expressed by:

$$k \frac{(dn/dc_2)c_2}{\Delta R(0)} = \frac{1}{M_2} + \frac{Bc_2}{M_2^2} \quad (1)$$

provided that: (1) the specific refractive increment of the solute  $(dn/dc_2)$  is measured against diffusate; (2) the scattering of diffusate is used in arriving at  $\Delta R(0)$ ; ( $k$  is constant;  $c_2$ ,  $M_2$  are the concentration and weight-average molecular weight of the solute and  $B$  the second virial coefficient). Conventionally equation (1) is used to determine  $M_2$  by measurement of  $(dn/dc_2)$ ,  $c_2$  and  $\Delta R(0)$ ; however, it may be used alternatively to measure  $(dn/dc_2)$  if  $M_2$  is known.

Consider a macromolecular solute (3) in a solvent consisting of a component (1) to which a second component (2) may be added.  $M_2$  is first measured by light-scattering of solutions of 3 in 1,  $(dn/dc_2)_1$  being measured refractometrically. Next, light-scattering measurements are made on a series of concentrations of 3 in a constant mixture of 1 and 2. Provided that the presence of 2 does not cause aggregation or disaggregation of 3, the value of  $M_2$  is unaffected.  $\Delta R(0)$  is obtained by subtracting the scattering of the solvent from that of solution, and the results are extrapolated to zero  $c_2$  in the usual way. Now, although these values of  $\Delta R(0)$  will be incorrect at finite  $c_2$ , they approach the correct value at zero  $c_2$ , since the compositions of solvent and diffusate then become identical; also at zero  $c_2$ , the term containing  $B$  vanishes (since a solute behaves ideally at zero concentration even if it reacts selectively with the components of a mixed solvent<sup>3</sup>) and equation (1) becomes:

$$\frac{L\lambda}{c_2 \rightarrow 0} k \frac{(dn/dc_2)c_2}{\Delta R(0)} = \frac{1}{M_2} \quad (2)$$

The value of  $(dn/dc_2)$  obtained is that which would be given by the difference of refractive index  $\Delta n$  of solution and diffusate at zero concentration of solute.

This value, which may be written  $(dn/dc_2)_{12}$ , is related to the excess quantity  $\alpha_2$  of (say) component 2 which is associated with unit quantity of component 3, since:

$$\frac{L\lambda}{c_2 \rightarrow 0} \Delta n = (dn/dc_2)_{12}c_2 + \alpha_2 c_2 (dn/dc_2)_1 \quad (3)$$

and:

$$(dn/dc_2)_{12} = \frac{L\lambda}{c_2 \rightarrow 0} \Delta n/c_2 = (dn/dc_2)_1 + \alpha_2 (dn/dc_2)_1 \quad (4)$$

where  $(dn/dc_2)_1$  is the specific refractive increment of 3 in 1.

In general  $\alpha_2$  is a function of  $c_2$  and may be positive or negative according as component 2 is 'bound' or 'excluded'<sup>4</sup> by component 3. For example, if 3 and 2 form a reversible stoichiometric complex, with dissociation constant  $K$  (expressed in molar terms), at  $c_2 = 0$  the degree of association  $\beta$  is given by:

$$K = \frac{(1 - \beta) 1000c_2}{\beta M_2} \quad (5)$$

and:

$$\beta = M_2/\alpha_2 \quad (6)$$

so that equations (4), (5) and (6) may be used to determine  $K$ . Again, if component 2 is 'excluded' by 3 (for example, a protein by hyaluronic acid<sup>5</sup>) from a volume  $\epsilon$  ml./g of 3, then:

$$\alpha_2 = -\epsilon c_2$$

The effect of adding a second solvent component on the excess scattering depends on the sign of  $\alpha_2(dn/dc_2)_1$ . If this is positive and increases with  $c_2$ , the excess scattering increases. If, however, it becomes increasingly negative with increase of  $c_2$ , the value of  $(dn/dc_2)_{12}$  and the excess scattering will at first decrease to zero, and

then increase again, provided that the solute is homogeneous with respect to  $(dn/dc)_1$  and  $\alpha_1$ ; if the solute is heterogeneous in either respect, a non-zero minimum of the excess scattering will be reached.

We have used this method to measure the selective interaction (in this case, exclusion) of hyaluronic acid (component 3) with bovine serum albumin (component 2) and have obtained results that agree with partition measurements<sup>6</sup> and with osmotic measurements<sup>7</sup>.

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Phosphorylase a/b Ratio In the Lamprey Heart

CYCLOSTOME branchial hearts are known to contain large amounts of endogenous catecholamines<sup>1</sup> and to be markedly insensitive to added adrenaline<sup>2,3</sup>. There is considerable evidence to suggest that the process whereby the sympathomimetic amines normally enhance the force of cardiac contraction involves the transformation of the enzyme phosphorylase from the b to the a form<sup>4,5</sup>. The lack of sensitivity of cyclostome hearts to added adrenaline or noradrenaline might therefore be due to the endogenous amines having already activated the conversion of the phosphorylase enzyme from the b to the a form so that even in the absence of any added adrenaline or noradrenaline the enzyme phosphorylase would exist mainly in the a form. This possibility was investigated using hearts isolated from freshly decapitated lampreys (*Mordacia*). After five minutes immersion in aerated (95 per cent O<sub>2</sub> + 5 per cent CO<sub>2</sub>) modified Ringer solution<sup>6</sup> at 22° C, under which conditions isolated hearts will beat spontaneously for many hours, the hearts were rapidly frozen and the phosphorylase activity determined as described previously<sup>7</sup>, using tissue dilutions of 1/150 (w/v) and rabbit liver glycogen (Nutritional Biochem. Lab.) which had been purified as described by Krebs *et al.*<sup>8</sup>. The reaction mixture was incubated at 25° C.

Typical results are displayed in Table 1 where it is shown that approximately half the phosphorylase enzyme present in lamprey hearts is in the a form. Comparison of these results with those obtained from other similar experiments in which freshly isolated toad (*Bufo marinus*) hearts were used indicates that the percentage of the enzyme phosphorylase in the a form is approximately the same in toad and lamprey hearts. These results are summarized in Table 1. Lamprey hearts are known to contain approximately 50 µg catecholamines per g<sup>9</sup>; toad hearts contain approximately 1.25–1.6 µg/g<sup>9</sup>. It therefore seems unlikely that the endogenous catecholamines of lamprey hearts participate to any marked extent in the natural regulation of the percentage of the enzyme phosphorylase which is present in the a form.

Perfusion of isolated toad hearts with Ringer solution containing 4 µg/ml. adrenaline ('Hermette', D. Bull and Co.) or noradrenaline ('Levophed', Winthrop Laboratories) produces a marked positive inotropic and chronotropic response. The data summarized in Table 1 indicate that these inotropic and chronotropic changes are accompanied by the conversion of almost all the phosphorylase enzyme to the a form. These same concentrations of adrenaline and noradrenaline failed to produce either a chronotropic

Table 1. PERCENTAGE OF PHOSPHORYLASE ENZYME PRESENT IN THE 'a' FORM IN ISOLATED LAMPREY AND TOAD HEARTS

Experiment	Lamprey % a Inotropic <sup>5</sup> action	Toad % a Inotropic <sup>5</sup> action
Control	53 54 57 55	58 62 55 63
Adrenaline 4 × 10 <sup>-4</sup>	60 62	88 96 ++++ ++++
Adrenaline 1 × 10 <sup>-4</sup>	83 86	96 96 ++++ ++++
Noradrenaline 4 × 10 <sup>-4</sup>	46 48	85 92 ++++ ++++
Noradrenaline 4 × 10 <sup>-4</sup>	61 62	98 98 ++++ ++++
Isoprenaline 4 × 10 <sup>-4</sup>	90 95	100 95 ++++ ++++
Isoprenaline 5 × 10 <sup>-4</sup>	92 92	+++ +++

Where '-' denotes the absence of inotropic activity; '+' denotes an arbitrary unit of positive inotropic activity. Results of individual experiments are shown.

or inotropic response from isolated lamprey hearts. Table 1 shows that 4 µg/ml. of either adrenaline or noradrenaline did not produce any marked change in the percentage of the phosphorylase enzyme which was present in the a form in lamprey hearts. 100 µg/ml. adrenaline and 400 µg/ml. noradrenaline produced small positive chronotropic and inotropic responses from isolated lamprey hearts. Table 1 shows that these same concentrations of adrenaline and noradrenaline increased the percentage of the phosphorylase enzyme present in the a form.

During the course of these experiments it was observed repeatedly that 4–50 µg/ml. isoprenaline ('Isuprel', Winthrop Laboratories) evoked a marked positive inotropic and chronotropic response from isolated lamprey hearts. In Table 1 it is shown that isoprenaline was more effective than either adrenaline or noradrenaline in converting the enzyme phosphorylase in lamprey hearts from the b to the a form.

In conclusion these results suggest that a parallelism exists between the ability of certain amines to exert a positive inotropic effect on isolated lamprey hearts and their ability to activate the conversion of the enzyme phosphorylase from the b to the a form. It seems unlikely that the endogenous catecholamines present in lamprey hearts participate in the natural regulation of the phosphorylase a/b ratio in this tissue, or that the insensitivity of these hearts to added adrenaline and noradrenaline can be accounted for simply in terms of the percentage of the enzyme which naturally occurs in the a form.

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### Isolation of Nucleic Acids from Gelatine

EVIDENCE has accumulated in recent years that the retarding action exerted by some gelatines in the photographic emulsion system is associated with the presence of small amounts of nucleic acids or their breakdown products.

Steigmann first suggested<sup>1,2</sup>, on the basis of the reaction of *p*-dimethylaminobenzaldehyde with gelatine, that pyrimidines and hence nucleic acids were present. However, this reagent is far from specific. The pioneer work in this field is that of Pouradier and Venet<sup>3</sup>, who applied several different tests to gelatine. They concluded from their results that gelatines contain 0.3–2 mg/g of RNA + DNA. Wood<sup>4</sup> and Gordon and Swann<sup>5,6</sup> published papers almost simultaneously on relatively specific methods of determining adenine in gelatine (the latter authors also detected guanine). These contributions have not settled the question in what form the nucleic acid fragments occur in the gelatine. Pouradier and Venet believed, since they observed good agreement between phosphorus and ribose or deoxyribose contents, that nucleic acids were present as such. Gordon and Swann were of the opinion that the phosphate is likely to be removed during gelatine manufacture and hence that the impurities of interest were likely to be present as nucleosides. Wood did not express an opinion. Both these latter authors, however, used hydrolytic procedures and hence detected adenine as such. In the present communication we wish to report work establishing that a high proportion of the adenine is present as high molecular weight, relatively undegraded nucleic acid.

We have been interested in studying the distribution of impurities in gelatine effected by various techniques of fractionation. The method mainly used so far is the alcohol coacervation procedure described by Stainaby<sup>7</sup>. For the nucleic acid work a particular gelatine was chosen. This was 'PG 334' (British Glues and Chemicals), a normal alkali-processed hide gelatine. The initial adenine content, as determined by Gordon and Swann's<sup>8</sup> polarographic technique, was quite high—161 p.p.m. The phosphorus content was determined after nitric-perchloric acid oxidation by King's method<sup>9</sup>; the original gelatine contained 292 p.p.m. These two determinations were done on most of the fractions as an index of the kind of fractionation that had occurred. When the conditions of fractionation were adjusted so that about 15 per cent of the gelatine was precipitated in the first fraction, the adenine content of this fraction (designated N1) was 417 p.p.m. and that of all subsequent fractions (four in all) remained fairly constant at about 20 p.p.m. The phosphorus content increased also in the first fraction but to a slightly less extent. Thus, almost 40 per cent of the adenine-containing impurity appeared in the first fraction. This suggested that the impurity might, like the gelatine itself, have a molecular weight distribution, that a proportion of it was relatively high molecular weight (or insoluble) and that it did not seem to be protein-bound.

Conditions were next adjusted to give a very narrow-cut first fraction—about 3 per cent by weight (designated N6). This was found to contain 3,330 p.p.m. adenine and 5,400 p.p.m. phosphorus. Ribose and deoxyribose were also present. In this case, 62 per cent of the adenine content of the gelatine has been concentrated into this narrow-cut fraction. Further concentration was effected by trypsin digestion of this fraction followed by precipitation with ethanol. In this way we have isolated about 200 mg of a fraction containing 6.3 per cent adenine and 7.5 per cent phosphorus. This fraction (designated N18) contained virtually all the adenine content of the N6 from which it was made, but only 70 per cent of the phosphorus. It is believed to be largely a mixture of DNA and RNA. It gives viscous solutions and is readily precipitated by acid. It is probably in the form of a calcium salt and contains 11.1 per cent moisture. From our

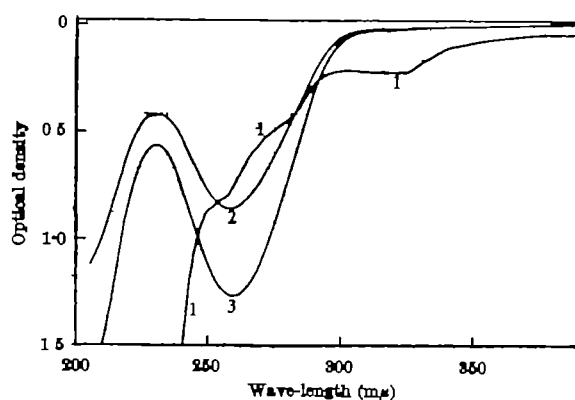


Fig. 1. Ultra-violet absorption spectra in pH 7 buffer. Curve 1, gelatin, 5 g/l; Curve 2, fraction N18, 46.8 mg/l; Curve 3, DNA (herring sperm), 45.8 mg/l.

results, we conclude that the material as isolated contains at least 90 per cent nucleic acids. The gelatine content, via hydroxyproline<sup>10</sup>, was 1.6 per cent. The ribose content was consistent with about 12 per cent RNA, the rest being largely DNA. Ion exchange<sup>10</sup> and paper<sup>11</sup> chromatography established the presence of thymine and cytosine (the first time these pyrimidines have been positively identified in gelatine), but uracil proved more difficult to detect. The identity of the bases was confirmed by their ultra-violet spectra.

Gel filtration on 'Sephadex'<sup>12,13</sup> in phosphate buffer, pH 7, gave a single sharp slightly asymmetrical peak, indicating a molecular weight of the order of 400,000. Ultra-violet spectra of the original gelatine, of fraction N18 and of an authentic sample of DNA are given in Fig. 1. The curves show clearly the similarity of the last two and the enormous difference in optical density between N18 and its source gelatine.

Further work is planned, and this, together with photographic assessment, will be described in fuller detail elsewhere. Thanks are due to the Directors of Minnesota 3M Research, Ltd., for permission to publish this communication.

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### Peptide Chains of Tropomyosin

THIS communication reports the determination of the molecular weight of rabbit tropomyosin by equilibrium sedimentation in a solvent consisting of 8 M urea, 0.2 M sodium chloride, 0.025 M sodium dihydrogen phosphate adjusted to pH 7.0, and 0.1 M  $\beta$ -mercaptoethanol. Columns 1 mm high were used and the speed was adjusted so that the weight-average molecular weight could be calculated from the refractive index gradient at the mid-point of the column<sup>1,2</sup>. The results are given in Fig. 1 as a plot of  $1/M_{app}$  versus concentration, expressed as  $\Delta n$ , the refractive index increment. For the molecular weight

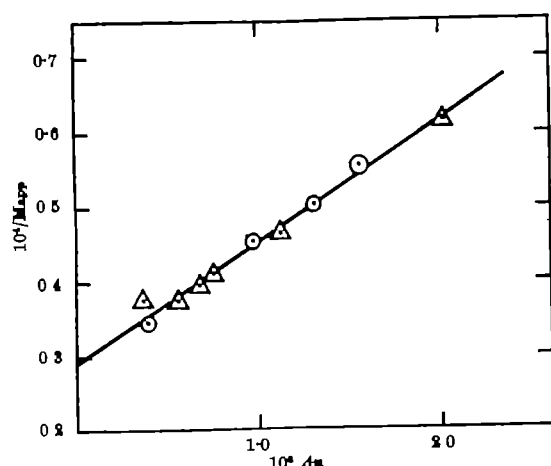


Fig. 1. Molecular weight of rabbit tropomyosin as a function of concentration. Rotor speed 14,290 r.p.m., column height 1 mm, temperature of runs 22°. Solvent was 8 M urea, 0.2 M sodium chloride, 0.025 M sodium phosphate, 0.1 M  $\beta$ -mercaptoethanol, pH 7.0. Two preparations (F117 ( $\Delta$ ) and F118 ( $\circ$ )) of tropomyosin were used.

calculations a partial specific volume of 0.728 c.c./g was used, the figure determined by Kay<sup>8</sup> for tropomyosin in 8 M urea. A least-squares line through the points extrapolates to a molecular weight of 34,000.

Rabbit tropomyosin has been shown to be a highly  $\alpha$ -helical protein<sup>4</sup> which exists as a monomer of molecular weight 54,000 (refs. 5 and 6) at pH 2, at pH 7 in high ionic strength buffers, and in 8 M urea. The particle length deduced from hydrodynamic measurements is approximately 400 Å. Since the length of a fully extended  $\alpha$ -chain of this molecular weight would be in the neighbourhood of 680 Å, a double helical structure seems probable. Previous attempts to demonstrate chain separation by performic acid oxidation were not successful<sup>7,8</sup>. Tropomyosin was therefore considered to be cyclic, branched, or to have a hairpin type of structure<sup>9-10</sup>.

The decrease in molecular weight to slightly more than half the monomeric value brought about by  $\beta$ -mercaptoethanol (Fig. 1) suggests that the two chains of tropomyosin are covalently linked by a disulphide bond or bonds, since urea alone does not decrease the molecular weight to this value. The presence of one —S—S— linkage per mole has been reported<sup>11</sup>. A more detailed sedimentation equilibrium study of the molecular weight of tropomyosin and its constituent polypeptide chains is in progress.

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### Time Factor in the Action of 5-Fluorodeoxyuridine on the Development of Crown-galls

It has been shown on numerous occasions that the transformation of a normal cell into a crown-gall cell takes place within a period of time which is limited to a few days after infection by *Agrobacterium tumefaciens*<sup>1</sup>. In earlier investigations I showed that the effectiveness of

bromouracil<sup>2</sup> and of 5-fluorodeoxyuridine (FUDR)<sup>3</sup> in inhibiting this transformation is also limited to this period. From these results I concluded that the inhibition applies exclusively to the process of transformation of the host cells and not to the growth of the tumour. In this connexion, strong evidence is provided by experiments in which the inhibition is eliminated by thymidine (TDR). Mennigmann and Szybalski<sup>4,5</sup> have shown that once an inhibition is completed by FUDR it is irreversible if FUDR is allowed to act unhindered for a sufficient length of time and hence for defective molecules of DNA to be formed. A similar mechanism also seems to be present in higher plants<sup>6</sup>. Thus if an inhibition produced by FUDR is no longer capable of being reversed by TDR after five days, it may be due to the fact that either the processes which can be blocked by FUDR have come to an end at this instant, or the inhibition produced by FUDR is no longer reversible. In order to decide which of these alternatives is the correct one it was necessary to choose an experimental approach which could show the selective action of FUDR on the development of a crown gall with the FUDR acting for a constant length of time during the process of transformation.

Leaves of *Kalanchoe daigremontiana* Hamet et Perr (= *Bryophyllum daigremontianum* Berger) were scarified at four places and at the same time inoculated with strain B6 of *Agrobacterium tumefaciens*. FUDR ( $4 \times 0.05$  c.c. of a  $10^{-3}$  M solution per leaf) was then injected into the leaves at 24, 48, 72, 96 and 120 h. At 0, 8, 24 and 32 h after each FUDR injection the same quantities of TDR (concentration  $5 \times 10^{-4}$  mol) were injected. Consequently, starting at different times, the FUDR could always act undisturbed for a constant period of time during the process of transformation. The results are plotted in Fig. 1. The point corresponding to the instant of time at which the FUDR-inhibition was neutralized by TDR has been entered in each case on the curves of the dry weights. In no instance has a treatment of 8 h produced an inhibitory effect, and the same applies when FUDR and TDR are given simultaneously (curve not shown in Fig. 1). In contrast to the simultaneous application the tumours are even promoted after an 8-h treatment. A 24-h treatment exerts a weak inhibitory influence, while one of 32 h, on the other hand, is distinctly inhibitory. Even then it is only the treatment which commences 72 h after infection that gives definite inhibition of crown-galls. Treatments commencing earlier give either weak inhibition or none at all. Similarly, 24- or 32-h treatments do not inhibit if they are applied 96 or 120 h after the infection. In this event, if no TDR after-treatment follows<sup>6</sup>, the inhibition is weak.

The experiment thus showed unequivocally that inhibition can be induced by a 32-h treatment with

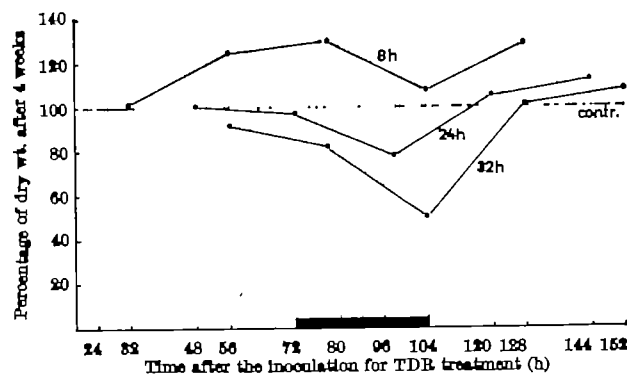


Fig. 1. Size of tumour after treatment with FUDR, which begins at different times and lasts for different periods. Ordinate, dry weight of tumour after 4 weeks expressed as a percentage of the control. Abscissa, time after inoculation at which the FUDR treatment was brought to an end by TDR. Each point on the curve represents the mean value from 4 experiments, each with 120 inoculations. —, Curves of the same durations of treatment with FUDR; —, time of inhibition of the transformation by FUDR.

FUDR, which is of limited duration and restricted to the period 72–104 h (4th day) after the infection. According to Braum<sup>7</sup> the crown-galls are completely altered about 4 days after the infection. The strongest action of the FUDR accordingly coincides with this period of time.

The curves in Fig. 1 indicate that the concentration of FUDR used (1) decisively affects only the last part of the process of transformation; (2) does not influence the growth of the tumour after the alteration.

D-Tryptophan is a very strong inhibitor of growth of *Agrobacterium tumefaciens*<sup>8</sup>. If the inhibition of the bacteria by D-tryptophan after infection is measured and plotted on a graph against time, the resulting curve shows no maximum for inhibition after 72 h. From this it can be concluded that FUDR does not take effect by way of the bacteria.

These experiments thus confirm that the process of transformation, limited in time, is inhibited by FUDR. The transformation only occurs when the whole of the DNA capable of functioning is propagated. This propagation of DNA occurs during the transformation<sup>9,10</sup> and probably before the first cell division of the tumour<sup>11</sup>. A proportion of DNA appears to be necessary for the transformation which is in excess of that required for the normal wound divisions and the growth of the tumour, since these proceed undisturbed.

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### Excitatory and Depressant Properties of Certain Brain Fractions

ALTHOUGH it is generally believed that at most synapses in the brain 'transmitter' substances are released from nerve endings to excite or inhibit the post-synaptic cells, the identity of the transmitters involved is still very much in doubt. Gray and Whittaker<sup>1</sup> have shown that a fraction of brain homogenates can be prepared containing mainly pinched-off nerve endings. More recently it has been possible to release apparently intact synaptic vesicles from such a nerve-ending fraction and to separate them from other membrane structures by density-gradient centrifugation<sup>2</sup>. The purified vesicle fraction contains acetylcholine but not the other components of the acetylcholine system. Since nerve endings can be expected to contain substantial amounts of various transmitter substances, we have examined the excitatory and depressant effects produced by various subfractions of guinea-pig brain, when applied from micropipettes to single units in the guinea-pig's cerebral cortex.

The fractions mainly used were the O and D fractions prepared from hypotonically disrupted cortical nerve endings by density-gradient separation as previously described<sup>3</sup>. Fraction O contained the soluble cytoplasmic constituents of the nerve endings. Fraction D consisted of a

suspension of synaptic vesicles. This fraction was treated in various ways to release the soluble constituents of the vesicles. All the material obtained was made up in sucrose solutions (approximately 0.5 M) and immediately inserted by centrifuging into multi-barrelled glass micropipettes of the type used in previous studies of mammalian cortical neurones<sup>4</sup>. When small electrical currents are passed through such solutions, which have a low conductivity, electro-osmotic movements of fluid can be expected to release a substantial amount of the solution from the tip of the pipette<sup>5</sup>. Using sucrose solutions labelled with <sup>14</sup>C or containing polystyrene beads, we have confirmed that such an electro-osmotic outflow does occur; under the conditions of our experiments, this reached a maximum of about 10<sup>-6</sup> ml./μcoulomb. Fractions O and D and various subfractions of D had a positive zeta potential with respect to the glass of the micropipettes, and they could therefore be expelled from the tip by making the lumen relatively positive. This method of application is much less reliable than iontophoresis, because the outflow is very susceptible to obstructions of the lumen.

All the D fractions and subfractions had a clearly depressant action on cortical neurones whereas simple solutions of sucrose or O fractions produced only minimal effects (Fig. 1). Spontaneous discharges, or firing evoked by the simultaneous application of L-glutamate or acetylcholine, were rapidly blocked by passing 60–80 nA through barrels containing D. This suggests that synaptic vesicles are associated with some substance capable of depressing neuronal activity. Excitatory effects were not seen even when the preparations were applied to cholinceptive cells; thus any effect of the acetylcholine present in the preparations was masked by the depressant effects.

Hypotonic or supersonic treatment of D gave subfractions of substantially greater potency, particularly in the supernatants obtained after sedimenting particulate material in the ultracentrifuge, presumably because of the release of soluble active factors from the vesicles. With the most active preparation enough was released by a current of 10 nA to cause a prolonged block of discharge. In this respect the suspension was comparable in depressant potency to neutral solutions of γ-aminobutyric acid<sup>6</sup>.

Amino-acid analyses of these fractions<sup>7</sup> revealed a substantial content of some amino-acids, including L-glutamate and aspartate, both of which strongly excite cortical neurones<sup>8</sup>, and γ-aminobutyric acid; it was shown, moreover, that similar mixtures of amino-acids prepared artificially also had mainly depressant effects on cortical neurones when applied in this way, presumably because the outward currents used would tend to prevent the release of the negatively charged excitatory amino-acids. Clear excitatory effects, which could be attributed to the release of these acids, have only been seen with extracts of

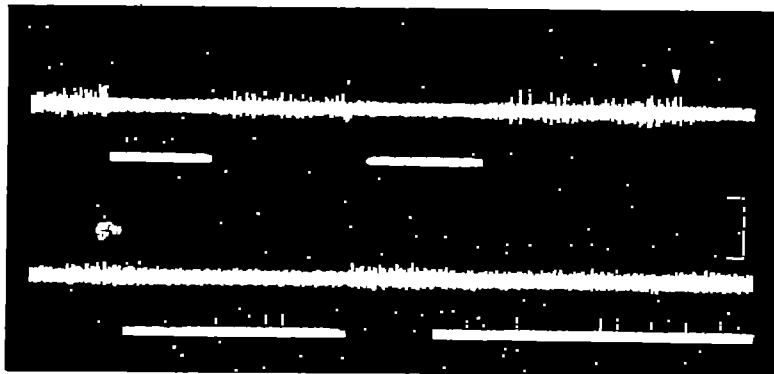


Fig. 1. Excitation of cerebral cortical unit of guinea-pig by (A) L-glutamate and (B) acetylcholine. In A, superimposed electro-osmotic applications of a suspension of synaptic vesicles in sucrose at 80 nA (D80, white bar) blocked the glutamate-induced discharge, whereas a fraction containing the soluble cytoplasmic components of the nerve endings applied in the same way (O80, between small arrows) had little or no effect. Similar results were obtained during firing induced by acetylcholine (lower trace). Dial anaesthesia.

a nerve-ending fraction (B), suspensions of which in sucrose had a negative zeta potential with respect to the glass of the micropipettes.

Although there is thus some reason for believing that enough  $\gamma$ -aminobutyric acid is present in D and its sub-fractions to cause a marked depression of cortical neurones, the subfractions of D in several instances had a strong and prolonged depressant effect on the amplitude of the neuronal spikes, which sometimes did not return to normal for several minutes after a short application. This kind of effect is never seen with  $\gamma$ -aminobutyric acid alone, and its occurrence suggests that some other potent factor or factors, with a different kind of action, may also be present in these fractions. Whether these play a part in normal function or whether they are artefacts arising from an unphysiological degradation of larger molecules cannot be decided at present.

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## PHYSIOLOGY

### Effect of Electrical Stimulation on Uptake and Release of Calcium by the Endoplasmic Reticulum

OVER the past decade the concept has been developed that  $\text{Ca}^{++}$  is a mediator of the so-called excitation-contraction coupling in muscle contraction and that the 'calcium pump' of the endoplasmic reticulum is responsible for relaxation<sup>1-3</sup>. According to this concept the electrical excitation of muscle membranes releases  $\text{Ca}^{++}$  from the endoplasmic reticulum and the released  $\text{Ca}^{++}$  in turn triggers myofibrils to contract. Relaxation then occurs when the released  $\text{Ca}^{++}$  is again taken up by the endoplasmic reticulum. However, as yet, no experimental evidence is available which directly supports the concept that following membrane excitation there is a release of  $\text{Ca}^{++}$ . In this communication, experimental results are presented which provide direct evidence in support of the concept that electrical stimulation releases stored  $\text{Ca}^{++}$  from the endoplasmic reticulum and that the cessation of electrical stimulation is followed by re-uptake of  $\text{Ca}^{++}$  by this system.

The investigation was concerned with the effect of electrical impulses passing through a suspension of microsomal relaxing factor on the uptake and release of  $\text{Ca}^{++}$  by the relaxing factor. The relaxing factor, which has been shown to consist principally of fragments of the endoplasmic reticulum, was prepared from rabbit skeletal muscle with some modification, according to the method of Weber *et al.*<sup>4</sup>. The uptake of  $\text{Ca}^{++}$  by the relaxing factor from the suspending medium was determined by the measurement of radioactivity of the medium filtered through a 'Millipore' filter, as described by Martonosi and Ferencsik<sup>5</sup>, following the incubation of the relaxing factor in the medium. The basic medium contained 3 mM  $\text{MgCl}_2$ , 1 mM ATP, 5 mM creatine phosphate (CP), 0.1 mg/ml. creatine phosphokinase, 50  $\mu\text{M}$  of total  $\text{CaCl}_2$  +  $\text{Ca}^{++}\text{Cl}_2$ , 0.08 M KCl and 0.01 M tris-maleate buffer, pH 6.5. Sets of

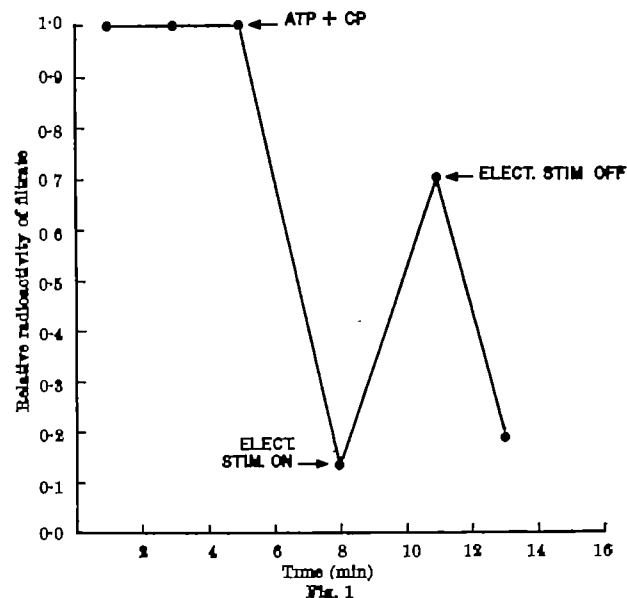
'Millipore' filters (25 mm diameter, type HS or GS, with 0.45 to 0.8  $\mu$  average pore diameter) were mounted on test tubes with side arms connected to a vacuum line which was closed during incubation of the mixture. One to one-half ml. of reaction mixture containing the relaxing factor (0.5-0.8 mg protein/ml.) was incubated in the tube above the filter disk. During the incubation period, a pair of flat platinum electrodes was immersed in the medium, and when electrical stimulation was desired, square wave pulses of 2 V and 10 msec duration were passed through the medium at a frequency of 80 impulses/min. The electrodes were 5 mm wide, and were separated by a gap of 4 mm. After the desired period of incubation, the medium was filtered through the 'Millipore' filter by opening the vacuum line connected to the side arm, and the radioactivity of the filtrate was measured. The amount of  $\text{Ca}^{++}$  bound to the microsomal relaxing factor was calculated from the difference between the radioactivity of the filtrates of relaxing factor-free incubation medium and of the relaxing factor-containing samples. The protein content was determined by the biuret method.

Table 1

Condition of incubation	Relative radioactivity of filtrate	$\mu\text{Moles Ca}^{++}$ taken up per mg protein relaxing factor
3 min in basic medium without ATP and CP	1.00	0.01
3 min without electrical stimulation	$0.13 \pm 0.08$	0.088
3 min with electrical stimulation	$0.66 \pm 0.1$	0.034
First 3 min without electrical stimulation	$0.71 \pm 0.09$	0.026
Second 3 min with electrical stimulation		
First 3 min with electrical stimulation	$0.21 \pm 0.07$	0.06
Second 3 min without electrical stimulation		

All incubations except the first were carried out at 25° in the basic medium (see text). Values in second column are mean values  $\pm$  S.E. calculated for six experiments.

The results are shown in Table 1 and Fig. 1. As can be seen in Table 1, the relaxing factor prepared by this method was found to take up  $\text{Ca}^{++}$  actively from the basic medium. Without ATP and CP, there was no significant  $\text{Ca}^{++}$  uptake. During the 3- or 6-min incubation periods in the presence of ATP and CP without electrical impulses passing through the electrodes (without stimulation), an average of 88 per cent of  $\text{Ca}^{++}$  present in the medium was taken up by the relaxing factor, leaving only 12 per cent in the filtrate. When electrical stimulation was passed through the mixture during the incubation period, only about 34 per cent of the  $\text{Ca}^{++}$  was taken up by the relaxing factor, leaving 66 per cent in the medium. This indicates that electrical stimulation inhibits  $\text{Ca}^{++}$  uptake by the relaxing factor. When a 3-min incubation period without electrical stimulation was followed by a 3-min incubation period with electrical stimulation, only 29 per cent of the



$\text{Ca}^{++}$  in the medium was taken up by the relaxing factor. This indicates that most of the  $\text{Ca}^{++}$  which was taken up by the relaxing factor during the first 3 min of the incubation period without electrical stimulation is released during the latter period with electrical stimulation. On the other hand, when a 3-min incubation with electrical stimulation was followed by a 3-min incubation without electrical stimulation, 79 per cent of the  $\text{Ca}^{++}$  was taken up by the relaxing factor. This indicates that the  $\text{Ca}^{++}$  which was not taken up during the first 3-min incubation period with electrical stimulation was afterwards taken up during the later incubation period without electrical stimulation.

The foregoing interpretation that  $\text{Ca}^{++}$  is taken up by the relaxing factor during the absence of electrical stimulation and is released from the relaxing factor during the stimulation is further supported by the results shown in Fig. 1. In this experiment, 2 mg protein of the relaxing factor was incubated in 4 ml. of the basic medium and every 3 min a 0.2-ml. sample was immediately filtered through a 'Millipore' filter and the radioactivity of the filtrate was measured. As can be seen in Fig. 1, following the addition of ATP and CP, the  $\text{Ca}^{++}$  content of the filtrate fell precipitously. When the medium was stimulated electrically, the  $\text{Ca}^{++}$  content of the filtrate increased, indicating that  $\text{Ca}^{++}$  taken up previously by the relaxing factor was released during electrical stimulation. The cessation of electrical stimulation was followed by re-uptake of  $\text{Ca}^{++}$  by the relaxing factor from the medium.

The results obtained are considered to demonstrate directly in an *in vitro* system that the passage of electrical impulses through the microsomal suspension releases  $\text{Ca}^{++}$  from the relaxing factor and the cessation of electrical stimulation is followed by uptake of  $\text{Ca}^{++}$  by the relaxing factor. Since the microsomal relaxing factor consists primarily of fragments of the endoplasmic reticulum of muscle, presumably the electrical stimulation of the endoplasmic reticulum occurring during the passage of an action potential would release  $\text{Ca}^{++}$  from this system *in situ* and the released  $\text{Ca}^{++}$  would initiate contraction of the myofibrils. After the termination of the electrical stimulation,  $\text{Ca}^{++}$  would again be taken up by the endoplasmic reticulum and relaxation would then ensue. The present experiments may be considered an *in vitro* demonstration of the  $\text{Ca}^{++}$  release from the endoplasmic reticulum which occurs *in vivo* during excitation-contraction coupling and the  $\text{Ca}^{++}$  uptake by this system which occurs during relaxation.

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### Electrophoretic Mobilities of Tissue Culture Cells in Exponential and Parasynchronous Growth

It has been reported<sup>1,2</sup> that tumour cells have a higher net negative charge than their normal counterparts and that the net negative charge of tumour cells increases with increased invasiveness. It was further reported that decreased mutual adhesiveness of cells is correlated with increased surface charge<sup>3</sup>. From observations of electrophoretic mobilities of rat liver cells<sup>4</sup> and mouse fibroblasts<sup>5</sup> it was suggested that increased negative charge is asso-

ciated with increased growth rate, and Eisenberg *et al.*<sup>6</sup> showed that cell populations with increased growth rate have increased heterogeneity in electrophoretic mobilities. Cook *et al.*<sup>7</sup> inferred from the small scatter of electrophoretic mobilities of individual Ehrlich ascites tumour cells that 'the electrokinetic properties of the tumour cell surface were independent of the state of the nucleus'. This communication describes an attempt to relate the electrophoretic mobility of cells to their stage in the mitotic cycle.

The electrophoretic mobilities of cells from cultures partially synchronized by double thymidine blocking<sup>8,9</sup> were compared with those of cells from unsynchronized cultures. Roswell Park Memorial Institute (R.P.M.I.) No. 41 cells, derived from a human osteogenic sarcoma<sup>10</sup>, cultured at 37° C in suspension in medium R.P.M.I. 906 plus 5 per cent calf serum<sup>11</sup>, and having a mean generation time of 18–20 h<sup>12</sup>, were used throughout. Thymidine was added to cultures which had been growing exponentially for at least 5 generations, to give a final concentration of 5–10 mM. After 14–24 h incubation the cells were washed twice and resuspended in fresh medium and 8–14 h later the treatment with thymidine was repeated. After a further 14–24 h, the cells were washed twice, resuspended in fresh warm medium, and sampled at hourly or half-hourly intervals for determination of cell concentration and mitotic index. The mitotic peak occurred 14–17 h after stopping the thymidine treatment, when a maximum of 15 per cent of the cells were in mitosis, compared with 2–3 per cent in unsynchronized cultures. The degree of synchronization<sup>13</sup> varied in different experiments between 65 and 80 per cent for the first generation, after which the degree of synchronization decayed with time as described by Petersen and Anderson<sup>8</sup>. No differences were detected between the number of viable cells as determined by uptake of trypan blue in partially synchronized (parasynchronous) cultures and control cultures in exponential growth. Parasynchronous cultures grew at the same rate as control cultures for at least 8 generations after release from thymidine. Thus thymidine at the doses used did not damage the cells.

Cell suspensions, removed either from control cultures or from cultures at various times after release from thymidine, were prepared for electrophoresis measurements in one of the following ways: (a) The cells were washed twice by centrifugation in Ca saline (0.145 M sodium chloride;  $5 \times 10^{-3}$  M calcium chloride;  $4 \times 10^{-4}$  M sodium bicarbonate) and resuspended in 4 per cent phosphate buffered formaldehyde solution (pH 7.2). After fixation for 20–24 h, the cells were washed twice in isotonic 5 per cent sucrose in half-strength Hanks's balanced salt solution at pH 7.7  $\pm$  0.2, and resuspended for electrophoresis in this solution. (b) 10 ml. buffered formaldehyde was added to 100 ml. cell suspension and left for 20–24 h. The cells were then washed and resuspended for electrophoresis in sucrose/Hanks's solution as in (a). (c) 100 ml. fresh cell suspension was washed twice and resuspended for electrophoresis in sucrose/Hanks's solution.

The times taken for individual cells to traverse 25  $\mu$  at 25° C, 50 V and 0.9 mamp, in a cylindrical chamber electrophoresis apparatus<sup>14</sup>, were measured, and the electrophoretic mobilities calculated. The results are shown in Table 1.

The significance of the difference in means between cells from control cultures and cells in various phases from parasynchronous cultures was determined by means of *t*-tests. Mitotic peak phase cells, comprising dividing cells, cells just before division ( $G_2$ ) and just after division ( $G_1$ ), have significantly higher mean mobilities than corresponding control cells. Cells during DNA synthesis (after release from thymidine) have mobilities similar to, or lower than, control cells and after division the mobility falls to the control value; thus increased mobility of cells during the mitotic peak phase is due to synchronization and not to thymidine treatment.



Table 1. ELECTROPHORETIC MOBILITIES OF FIXED AND FRESH R.P.M.L. No. 41 CELLS FROM CONTROL OR PARASYNCHRONOUS CULTURES

Treatment of cells after removal from culture	Type of culture growth	Phase of mitotic cycle	Number of cells measured	Mobility ( $\mu\text{sec}^{-1}\text{V}^{-1}\text{cm}$ ) $\pm$ S.E.	t-test, p-value. Controls v. parasyynchronous phase
Fixed method (a)	control (exponential)	—	118	$-1.46 \pm 0.027$	—
	parasyynchronous	mitotic peak	40	$-1.82 \pm 0.063$	<0.001
Fixed method (b)	control (exponential)	—	157	$-1.13 \pm 0.030$	—
	parasyynchronous	DNA synthesis	180	$-1.07 \pm 0.034$	<0.1, >0.05
	parasyynchronous	late DNA synthesis	181	$-1.13 \pm 0.033$	>0.9
	parasyynchronous	mitotic peak	117	$-1.37 \pm 0.037$	<0.001
	parasyynchronous	post division ( $G_1$ )	106	$-1.16 \pm 0.034$	>0.40
Fresh (c)	control (exponential)	—	150	$-1.06 \pm 0.023$	—
	parasyynchronous	mitotic peak	257	$-1.26 \pm 0.021$	<0.001
Fresh (d)	control (exponential)	—	200	$-1.05 \pm 0.020$	—
	parasyynchronous	DNA synthesis	145	$-1.00 \pm 0.022$	<0.1, >0.06
	parasyynchronous	mitotic peak	189	$-1.29 \pm 0.023$	<0.001
	parasyynchronous	post division ( $G_1$ phase)	168	$-1.12 \pm 0.032$	<0.1, >0.06

Control cells fixed in formalin by method (a) have a higher mobility than those fixed by method (b). Method (a) fixation is similar to that used by Cook *et al.*<sup>7</sup>, who also found increased mobility of fixed compared with fresh cells. It seems that the fixation procedure can affect the mobilities of the cells, but that comparatively gentle fixation, as in method (b), gives results similar to those obtained for fresh cells.

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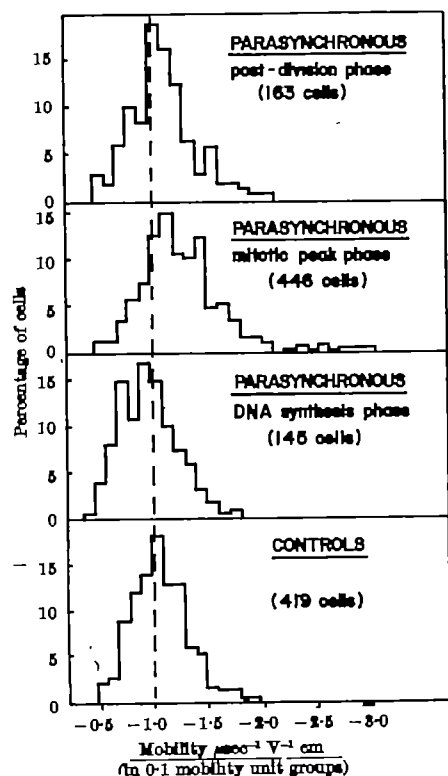


Fig. 1. Histograms of electrophoretic mobilities of fresh R.P.M.L. No. 41 cells from control and parasyynchronous cultures measured in sucrose/Hanks's solution at 25° C

Fig. 1 shows histograms of electrophoretic mobilities of fresh cells from control and parasyynchronous cultures. Cells from mitotic peak phase have a wider scatter in mobilities compared with those from the DNA synthesis phase, after division or controls, and there are a number of cells with mobilities 2 or more times greater than the mean.

If these results, which have been obtained on only one type of cultured cell, have a general validity, it may well be that the electrophoretic mobility of cells varies during the mitotic cycle, and indicates an increase in negative charge density (possibly due to an increase in negatively charged groups and/or a decrease in positively charged groups at the cell periphery). Changes in surface charge could possibly affect the adsorption of drugs etc.; this would possibly account for variation in sensitivity to cytotoxic drugs at different stages of the mitotic cycle<sup>18,19</sup>.

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## Effects of Tenotomy on the Growth of Muscle and its Tendon

THE immediate changes which occur in an adult muscle after section of its tendon have been summarized by Sunderland and Lavarack<sup>1</sup>. Regeneration of an experimentally transected tendon occurs within 2-3 weeks<sup>2,3</sup> even where the muscle has been denervated previously<sup>4</sup>, and some muscle function is thus soon restored. Because the dysfunction appears to be only temporary, the possibility of some permanent effect on the muscle has been overlooked even though it has been shown<sup>5,6</sup> that, six weeks after tenotomy, the repaired tendon is longer while its muscle remains both shorter and lighter than the control. Therefore, in an investigation of the relationship between the strength of a muscle and the collagen content of its tendon<sup>7</sup>, the operation of tenotomy was included to modify the normal muscle-tendon relationship during growth. The tendon of tibialis anterior was transected just distal to the muscle-tendon junction in the right hind-limb of five 4-week-old rabbits, the other limb serving as a control. The stumps were not sutured but allowed to retract. The animals made an uneventful recovery and were killed when 180 days old. No adhesions were present at the site of tendon repair.

Contrary to expectations the experimental muscle belly was not shorter but either the same length or even longer than the control and it was also significantly heavier ( $P=0.05$ , Table 1), thus having an increased girth in four of the five rabbits. There is no obvious explanation for this hypertrophy; but it has already been shown<sup>8</sup> that the diminution of electro-myographic activity four months after tenotomy is not due to atrophy since it is

Table 1

Rabbit No.:	1	2	3	4	5
Wet weight of control muscle belly (g)	2.80	2.80	2.19	2.30	2.52
Wet weight of experimental muscle belly (g)	2.51	2.80	2.78	2.46	2.49
Length of control muscle belly (mm)	72	79	72	75	76
Length of experimental muscle belly (mm)	73	86	79	75	76
Experimental muscle girth (weight/length) as % of control	108	109	117	107	99
Thickness of control tendon (mg dry weight/mm)	0.37	0.36	0.36	0.40	0.40
Experimental tendon thickness as % of control	117	123	114	114	97

normal or even increased during postural reflexes in the anaesthetized rabbit.

There was no significant difference between the mean increase of the girth of the muscle and that of its tendon when each was expressed as a percentage of the control. There were  $69.0 \pm 1.0$  mg collagen per 100 mg dry weight of the normal tendon and  $67.4 \pm 1.8$  of the experimental, distal to the tenotomy, estimated according to the method of Neuman and Logan<sup>8</sup>. At the actual site of regeneration the tendon was too small for its girth or collagen content to be assessed. The correlation between the increased girth of the tendon distal to the site of tenotomy and the increased girth of its muscle belly is in accordance with the similar relationship found when the muscle is stimulated to hypertrophy during growth by removal of synergic muscles<sup>10</sup>, by exercise<sup>11</sup>, or by immobilization at an extended length<sup>7,10</sup>.

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### Anoxia-Induced Release of Noradrenaline from the Isolated Perfused Heart

DECREASES in the catecholamine content of the myocardium have been found to occur in animals subjected to prolonged periods of hypoxia<sup>1</sup> and of myocardial ischaemia<sup>2</sup>. Results recently obtained in this laboratory<sup>3,4</sup>, however, suggest that release and subsequent loss of cardiac catecholamines from their storage site may be a more immediate response of the animal to arrest of the coronary circulation and to myocardial anoxia in general. In the work recorded here the isolated perfused heart was selected as a suitable object for approaching this problem experimentally and, at the same time, independently of the influence of nervous activity. Using the isolated perfused adrenal gland Bülbring *et al.*<sup>5</sup> and Cession-Fossion<sup>6</sup> had been able to demonstrate a direct mobilizing action of anoxia on the adrenaline stores of this organ.

The hearts of heparinized and anaesthetized adult rabbits and rats were rapidly excised and attached to a Langendorff apparatus for perfusion through the aorta and the coronary circulation with glucose and sucrose-containing Tyrode solution aerated with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide. The time elapsing between opening of the thorax and the start of perfusion did not exceed 1 min. After 30–35 min of perfusion at 34° C the hearts were left for 3 or 4 min in an anaerobic state, brought about in one series of experiments by arresting the perfusion, and in the other series by connecting the aortic cannula by means of a three-way

stopcock to a second infusion system in which the Tyrode solution was equilibrated with a mixture of 95 per cent nitrogen and 5 per cent carbon dioxide. Aerobic perfusion was then re-started. The perfusate was collected in consecutive fractions of 10–100 ml. in graduated shaking cylinders containing 0.1 volume of 1 N perchloric acid. Noradrenaline in the perfusate fractions, in the ventricular muscle, and in various sub-cellular fractions of this tissue was determined, after adsorption on aluminium oxide at pH 8.3 and elution with 0.1 N perchloric acid, by a modification<sup>7</sup> of the trihydroxyindole fluorometric method.

Both the interruption of perfusion and the shift to anaerobic Tyrode solution caused the discharge of considerable amounts of noradrenaline from the heart into the circulation. A representative experiment on a rabbit heart, illustrating the magnitude and time course of this response, is depicted in Fig. 1. During the steady aerobic state negligible amounts of the catecholamine appeared in the coronary outflow. A 3-min period of anaerobiosis, in this instance due to arrest of perfusion, led to the subsequent release into the circulation of a total of 5 µg of noradrenaline or, reckoned per g heart, of 0.7 µg/g. The mean loss of noradrenaline caused by 3 or 4 min of anaerobiosis from 11 preparations of rabbit hearts was 0.46 µg/g. This amount represents nearly one-fourth of the noradrenaline normally present in the rabbit's heart. The highest concentration of the catecholamine in the perfusate was found in the fractions collected during the second or third minute following return to aerobic conditions (Fig. 1). Thereafter the noradrenaline output fell rapidly toward or even below the pre-anaerobic level. A renewed output of the neurotransmitter was elicited in some preparations by a second exposure to anoxic conditions. At the end of these experiments the hearts were found to contain considerably less noradrenaline than hearts perfused for the same length of time without an intervening period of anoxia. No appreciable difference, however, was seen in the sub-cellular distribution of the amine as revealed by analyses of heart homogenate fractions separated by differential centrifugation.

Concomitantly with the anoxia-induced discharge of noradrenaline into the circulation there was a sharp rise in the lactic acid concentration in the perfusate. At its peak the lactic acid output by the heart approached 1–4 µmoles/g heart/min. It is conceivable that accumulation of lactic acid and other acid metabolites in the anoxic and post-anoxic myocardium may have significantly contributed, perhaps through an increase in the hydrogen ion concentration, to the liberation of noradrenaline<sup>8</sup>. This point is currently being investigated.

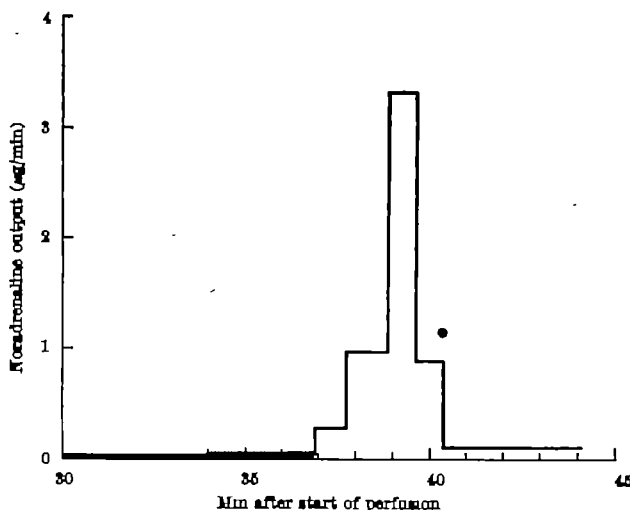


Fig. 1 Output of noradrenaline from the perfused rabbit heart before and after a 3-min arrest of perfusion. The period of arrest of perfusion is denoted by the row of oblique bars. Rabbit No. 15. Weight of heart at the end of the experiment, 7.1 g

When the isolated hearts of rabbits which had just been treated for 10 days with reserpine (0.1 mg/kg daily) were subjected to anoxia for 4 min, much less noradrenaline was released into the coronary bed than from the hearts of the untreated animals. The amounts released were proportional to the size of the rather severely depleted cardiac noradrenaline stores. No release of noradrenaline whatever was observed in preparations of rabbit hearts which had undergone only moderately severe losses of noradrenaline as a result of prior administration of tyramine (30 mg/kg rabbit given 30 min before excision of the heart). This finding suggests that the anoxia-sensitive fraction of the noradrenaline in the rabbit heart might form part of, or be identical with, the fraction releasable by tyramine.

Further characterization of the material in the perfusates at present determined as noradrenaline is provided by the following observations: (1) The fluorescence excitation and emission spectra of the alumina eluates of the perfusates treated by the trihydroxyindole procedure used<sup>7</sup> were indistinguishable from those of the lutine formed from authentic noradrenaline. (2) The blood-pressure-raising activity of the alumina eluates, assayed in the cat, corresponded roughly to that of the fluorometrically estimated quantities of noradrenaline in these eluates. (3) In heart preparations loaded with tritium-labelled noradrenaline a 4-min arrest of perfusion was followed by a sharp temporary rise in the rate of outflow of radioactive noradrenaline.

There is thus little doubt that myocardial anoxia can bring about, in the apparent absence of sympathetic nerve activity and with little delay, the release and loss of a good part of the noradrenaline stored in cardiac muscle. The liberation of the adrenergic transmitter may be held largely responsible for the initial outburst of phosphorylase activity and glycolysis in anoxic myocardium<sup>3,4</sup> and may conceivably play a part in the genesis of cardiac arrhythmias such as occur in the wake of an occlusion of a coronary vessel<sup>5</sup>.

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## PHARMACOLOGY

### Reserpine in a Tissue Culture of *Alstonia constricta* F. Muell

In a tissue culture study of certain members of the Apocynaceae, the plant *Alstonia constricta* F. Muell was investigated. The root and trunk barks of this tree, which is native to Australia, have been known to yield several indole alkaloids including reserpine<sup>1-4</sup>.

The callus tissue cultures were initiated from germinating seeds and maintained on a modified White's medium which included 2,4-dichlorophenoxyacetic acid, 1 mg/l., and 10 per cent coconut water<sup>5</sup>. The tissue was continuously cultivated for over two years and periodically it was gathered, lyophilized and stored for subsequent chemical analysis.

Recently, a preliminary chemical analysis was performed. Several grams of dried callus were reduced to a

number 20 powder and the powdered tissue was extracted for alkaloids according to a slight modification of the procedure reported by Svoboda<sup>1</sup>. This procedure involves the use of several solvents, the final one being chloroform. The chloroform extract was subjected to analysis by thin-layer chromatography. In this procedure the Deaga<sup>1</sup> apparatus (Brinkman Instrument Co., Westbury, New York) was used, including 200 mm square plates with a matrix of 'Silica Gel G' of Merck and a solvent system of ethyl acetate: ethanol 3:1. The solvent front was allowed to travel 100 mm. Each chromatogram was examined under ultra-violet light and its fluorescent pattern was recorded. The plates were then sprayed with modified Dragendorff's reagent<sup>6</sup>. Since reserpine had previously been reported to occur in root and stem bark of *Alstonia*, this compound was chromatographed alongside the callus extracts.

An examination of the chromatograms under ultra-violet light revealed the presence of five fluorescent spots. Four of these gave a blue fluorescence and one a yellow-green colour. The pure reserpine also fluoresced yellow-green and appeared at the same  $R_f$  as the yellow-green fluorescent spot from the callus extract. When the plates had been sprayed with modified Dragendorff's reagent three distinct positive spots appeared with  $R_f$  values of 0.19, 0.68 and 0.93. The  $R_f$  of reserpine under the same conditions corresponded to the middle value just listed, 0.68.

Further, to check the possibility that one of the unknown alkaloids was reserpine, the compound appearing at  $R_f$  0.68 as well as known reserpine was scraped from the plate before spraying with modified Dragendorff's reagent. The alkaloid-silica-gel matrix was eluted with chloroform by mixing in a centrifuge tube. The mixture was centrifuged and the supernatant chloroform extract removed. This extract was analysed spectrophotometrically in a Beckman DK-2 recording spectrophotometer.

The ultra-violet spectrum for the alkaloid at  $R_f$  0.68 was the same as for reserpine, namely a shoulder at 290 $\mu$  and a maximum at 267 $\mu$ . More tissue is being accumulated so that further investigation may be performed. From this preliminary work it appears that our tissue culture of *A. constricta*, which has been maintained for more than two years, has the capacity to biosynthesize reserpine.

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### Effect of Cold and Restriction of Movement on Mast Cells and Metachromasia of Rat Skin

SYSTEMIC administration of corticotrophin, cortisone and hydrocortisone causes degranulation and other changes in mast cells<sup>1-3</sup>. Exposure of rats to cold caused the number of mast cells in lung and thymus tissue to increase in rats weighing 185 g and decrease in rats weighing 380 g (ref. 4). Cold treatment of the heavier rats increased the number of dermal mast cells and decreased mesenteric (intervascular) mast cells<sup>5</sup>.

Fediay and Clay<sup>6</sup> reported that treatment of rats weighing 300 g for 2 days with cold and restriction of movement increased plasma corticosterone levels, decreased dermal

hyaluronic acid and chondroitin sulphate levels and decreased dermal spread of hyaluronidase-indian ink solution. After 14 days treatment corticosterone-levels remained elevated, hyaluronic acid and chondroitin sulphate increased, heparin decreased, and the spread of the hyaluronidase-indian ink solution increased. In the work recorded here samples of skin were taken from the rats to determine whether changes in the physical and chemical state of the skin can be correlated with changes in dermal metachromasia and mast cells.

Three groups of eight male Wistar rats (280–340 g) were treated as follows: group 1, control; groups 2 and 3, maintained in individual steel cages at  $12^{\circ} \pm 2^{\circ} \text{C}$  for 2 and 14 days, respectively. The dimensions of the cages (5.5 in.  $\times$  2.5 in.  $\times$  2.5 in.) permitted the rats to move only with difficulty. The rats were decapitated and full-thickness skin samples were cut from the shaved right flanks and fixed in 10 per cent neutral formalin. Frozen cross-sections (30–33 $\mu$ ) were cut and stained for 40 sec in a 0.5 per cent toluidine blue water solution. Intensity of ground substance metachromasia was recorded. Mast cells were counted by a modification of the method of Simpson and Hyashi<sup>7</sup> in 10 sections from each skin sample. Counts were made in square fields (0.25 mm  $\times$  0.25 mm) extending through the entire skin. Five such columns of fields were selected for each tissue section. Separate cell counts were made according to regional distribution or zone and cellular morphological characteristics. The zones are as follows: 1, immediately below the basal layer of the epidermis; 2, dermis; 3, subcutaneous tissue; 4, panniculus carnosus. The cell types are as follows: 1, heavily granulated, larger than 0.007 mm; 2, lightly granulated, larger than 0.007 mm; 3, heavily granulated, smaller than 0.007 mm; 4, lightly granulated, smaller than 0.007 mm; 5, mast cells with ruptured walls.

Table 1 shows an increase in the mean mast cell density (5 cell types, 4 zones) after 14 days of stress, which was due primarily to an increase in cell type 5, cells with ruptured cell walls (control, 4.812; 2-day stress, 4.561; 14-day stress, 12.465). The skin, after 14 days of stress, showed many areas containing individually dispersed extracellular metachromatic granules, cells with granules clumped together in irregular and angular shapes as well as areas of the ground substance which failed to stain metachromatically. The highest mast cell density in each of the 3 groups was found in zone 3, followed by zones 4, 2

Table 1. MEAN MAST CELL DENSITY PER MICROSCOPIC AREA IN THE SKIN OF NORMAL AND STRESSED RATS

Skin zone	Cell type	Animal treatment		
		Group 1 (control)	Group 2 (2-day stress)	Group 3 (14-day stress)
(1)				
Sub-epidermis	1	—	0.003 (0.008)	0.007 (0.010)
	2	—	0.009 (0.023)	0.010 (0.031)
	3	0.037 (0.068)*	0.109 (0.084)	0.047 (0.048)
	4	0.015 (0.043)	0.063 (0.106)	0.147 (0.249)
	5	—	—	0.007 (0.015)
Total 5 types		0.042	0.176	0.220
(2)				
Dermis	1	0.214 (0.150)	0.406 (0.231)*	1.201 (1.145)†
	2	0.084 (0.074)	0.143 (0.110)	0.223 (0.206)
	3	2.078 (0.043)	1.911 (1.246)	1.704 (1.368)
	4	0.310 (0.416)	0.273 (0.308)	0.514 (0.702)
	5	0.154 (0.155)	0.247 (0.232)†	1.858 (1.680)†
Total 5 types		2.840	2.980	5.690
(3)				
Subcutaneous layer	1	1.723 (1.420)	2.784 (1.118)	3.054 (3.227)
	2	0.541 (0.540)	0.931 (0.744)	0.330 (0.301)
	3	0.959 (0.531)	0.905 (0.333)†	0.308 (0.497)†
	4	0.115 (0.127)	0.192 (0.201)	0.043 (0.071)
	5	2.394 (1.007)	2.115 (1.020)†	6.843 (3.715)†
Total 5 types		5.737	6.917	10.596
(4)				
Panniculus carnosus	1	0.739 (0.753)	0.999 (0.977)	1.477 (1.183)
	2	0.237 (0.843)	0.593 (0.580)†	0.072 (0.101)
	3	0.204 (0.135)*	0.076 (0.080)	0.064 (0.068)†
	4	0.011 (0.017)	0.016 (0.037)	0.031 (0.061)
	5	1.764 (1.460)	2.199 (1.503)	3.744 (1.780)†
Total 5 types		2.955	3.861	5.380
Mean zones 2, 3 and 4		3.844 (1.552)	4.593 (2.061)	7.228 (2.924)

Figures in parentheses indicate standard deviation.

\* Significant difference between means. † Groups 1 and 2; ‡ Groups 2 and 3; § Groups 1 and 3.

Zone 1 is not equivalent to a full microscopic area.

Table 2. ORDER OF FREQUENCY OF MAST CELL TYPES ACCORDING TO EXPERIMENTAL TREATMENT AND SKIN ZONE

Treatment	Zone 1	Zone 3	Zone 4
Control	3-4-5-1-2*	5-1-2-3-4	5-1-2-3-4
2-day stress	3-4-5-1-2	1-5-2-3-4	5-1-2-3-4
14-day stress	5-3-1-4-2	5-1-2-3-4	5-1-2-3-4

\* Order of decreasing frequency.

and 1. Table 2 shows the relative uniformity in order of frequency of mast cell types in the 3 deeper zones of the 3 groups.

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## HAEMATOLOGY

### Separation of Factor VIII (Antihæmophilic Factor) Activity from Fibrinogen by Means of a Snake Venom

THE separation of Factor VIII activity from fibrinogen is of considerable importance. From the therapeutic point of view it would seem desirable when treating hæmophilias to use a protein fraction rich in factor VIII activity but as free as possible from other contaminating proteins, including fibrinogen. From the experimental point of view purified Factor VIII is required for studies of its chemical and physical properties.

Many attempts have been made to prepare plasma fractions rich in Factor VIII but free of fibrinogen<sup>1-7</sup>. Most of the procedures used have been only partially successful or have yielded inconsistent results.

In 1963 Reid, Chan and Thean<sup>8</sup> observed that human beings bitten by the Malayan pit viper (*Ancistrodon rhodostoma*) developed a prolonged defibrination state which was not associated with a severe bleeding tendency. Thromboplastin generation tests carried out on the blood of these fibrinogenopenic subjects was normal, suggesting that there was no loss of activity of the factors necessary for thromboplastin formation. We were particularly interested in the possibility that the venom did not reduce Factor VIII activity and might therefore be used *in vitro* to prepare plasma deficient in fibrinogen but containing Factor VIII.

Experiments were carried out in which citrated normal human plasma was incubated at 37° C with different concentrations of the crude venom for different periods of time and the residual Factor VIII activity in the plasma assayed (Table 1). The assay method used was that of Biggs, Eveling and Richards as modified by Biggs and Macfarlane<sup>9</sup>. After incubation for 1 h with final venom concentrations of 1–2  $\mu\text{g/ml}$ , the plasma fibrinogen clotted but without any loss of Factor VIII activity. On the contrary there was an apparent increase of activity in these samples. With a venom concentration of 4  $\mu\text{g/ml}$  there was slight loss of Factor VIII activity at 1 hr and with higher concentrations of venom and prolonged incubation the loss of activity became more marked. With venom concentrations above 1  $\mu\text{g/ml}$ , defibrination of the plasma seemed to be complete, since subsequent addition of a strong solution of thrombin ('Thrombin Topical', Parke Davis) produced no further clotting in the plasma. As a further check on the completeness of fibrinogen conversion by the venom, samples of plasma were clotted by the addition of either viper venom (1–4  $\mu\text{g/ml}$ ) or M/40

Table 1

Final venom concentration in plasma (μg/ml)	Residual Factor VIII activity after incubation for:		
	1 h	2 h	4 h
0.5	150	120	—
1	135	100	75
2	115	78	70
4	62	56	33
5	58	—	—
10	<10	—	—
Buffer	100	100	100

Incubation system: 3.6 ml. plasma + 0.4 ml. glycylalane buffer or solution of venom in buffer. After 1, 2 and 4 h incubation at 37° C, 1 ml. incubation mixture was removed from each tube, adsorbed with Al(OH)<sub>3</sub>, and assayed for Factor VIII activity. The residual Factor VIII activity was expressed as a percentage of the buffered plasma sample incubated for the same length of time.

CaCl<sub>2</sub>. After incubation at 37° C for 1 h the fibrin clots formed were washed in saline, digested and assayed for total nitrogen content by the micro-Kjeldahl method. It was found that the amount of fibrin formed by the action of the venom was the same as the amount formed when the plasma was clotted by recalcification.

With alumina-adsorbed fibrinogen-free plasma as starting material, attempts were made to precipitate Factor VIII activity by the alcohol fractionation procedure of Cohn<sup>10</sup>. Whereas in normal plasma Factor VIII is precipitated along with fibrinogen in Fraction I at a final ethanol concentration of 8 per cent, we found that in the defibrinated plasma maximal precipitation of Factor VIII activity took place at a final ethanol concentration of 15 per cent. It is well recognized that the precipitation characteristics of plasma proteins are altered in the absence of fibrinogen, and Gobbi<sup>11</sup> has shown that higher concentrations of alcohol are required to precipitate Factor VIII activity from the plasma of patients with congenital afibrinogenemia than from normal plasma.

The action of the venom in clotting plasma fibrinogen and yet sparing Factor VIII is consistent and reproducible and it should be possible by its means to prepare fibrinogen-free Factor VIII for experimental purposes and possibly also for therapeutic use.

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## Inhibition of Adenosine Diphosphate-Induced Platelet Aggregation by Histamine

It is now well known that adenosine and adenosine monophosphate (AMP) strongly inhibit the aggregation of human and rabbit platelets which occurs in the presence of adenosine diphosphate (ADP). Adenosine and AMP are effective at a concentration of approximately 10<sup>-7</sup> M, and are the most potent naturally occurring inhibitors known. O'Brien<sup>1</sup> has shown that antihistaminics, anti-malarials, and local anaesthetics, all at approximately

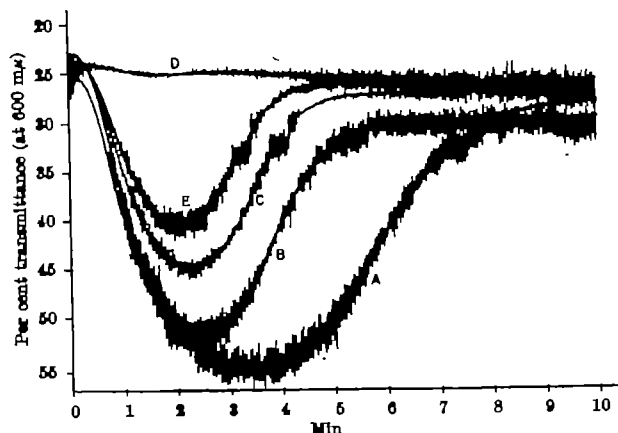


Fig. 1. Inhibition of ADP-induced platelet aggregation. At zero time 10<sup>-4</sup> M ADP was added to rabbit platelet-rich plasma which had been incubated 1 min with: A, 0.9 per cent sodium chloride solution; B, histamine, 5 × 10<sup>-3</sup> M; C, histamine, 10<sup>-3</sup> M; D, histamine, 10<sup>-4</sup> M; E, adenosine, 10<sup>-3</sup> M.

10<sup>-3</sup> M, inhibit ADP-induced platelet aggregation. It has recently been reported by Salzman and Chambers<sup>2</sup> that substituted amino-acids, at a concentration of 1.5 × 10<sup>-4</sup> M, inhibit ADP-induced platelet aggregation.

It has now been found that histamine inhibits ADP-induced aggregation of rabbit platelets. Changes in light transmittance of platelet-rich plasma on addition of ADP were recorded at 23° C, as described by Born<sup>3</sup> and O'Brien<sup>4</sup>. Fig. 1 shows the inhibitory effect of various concentrations of histamine when this was added to the plasma 1 min before the addition of ADP; all concentrations are final concentrations in the test system. The results were similar when histamine remained in contact with the plasma for 10 min prior to the addition of ADP; but when histamine was incubated with plasma for 30 min at 37° C, then cooled rapidly to 23° C, there was no inhibition of ADP. This may be explained by the action of plasma histaminase during the 37° C incubation period; in addition, it has been shown that there is increased platelet responsiveness to ADP in platelet-rich rabbit plasma which has been incubated at 37° C, and then cooled rapidly to 23° C<sup>5</sup>. Imidazole (10<sup>-3</sup> M) did not inhibit ADP-induced platelet aggregation. A series of substituted imidazoles, each tested at 10<sup>-3</sup> M, were ineffective as inhibitors of ADP:

2-aminoimidazole      β-(N,N-dimethylaminoethyl)imidazole  
2-methylimidazole      4-(β-n-propylaminoethyl)imidazole  
2-mercaptoimidazole      4-(β-isopropylaminoethyl)imidazole  
4,5-imidazoledicarboximorpholide      4-(β-butylaminoethyl)imidazole  
2-n-butylmercaptoimidazole      4-(β-di-n-butylaminoethyl)imidazole

O'Brien<sup>6</sup> has reported that histamine does not inhibit ADP-induced aggregation of human platelets, and this may be yet another example of species-determined differences in the behaviour of platelets toward both aggregating agents and their inhibitors. There is no obvious explanation of the mechanism by which histamine inhibits ADP-induced platelet aggregation. Butcher and Sutherland<sup>7</sup> have shown that imidazole stimulates cyclic phosphodiesterase, the enzyme which converts cyclic 3',5'-AMP to AMP. It is possible, therefore, that histamine might stimulate cyclic phosphodiesterase and so result in the production of an inhibitory level of AMP. Evidence against this concept is the lack of inhibition to ADP-induced platelet aggregation by imidazole.

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## Molecular Support of Rheumatoid Factor and Anti-Gm(a) Activity

A SPECIFIC serological activity was found several years ago in the serum of patients suffering from rheumatoid arthritis.

This rheumatoid factor (RAF) has been localized in the 19 S- $\gamma$ -globulin<sup>1</sup>. Grubb and Laurell<sup>2</sup> found a second serological activity in certain arthritis sera (Ragg) causing the agglutination of human red blood cells coated by selected incomplete anti-D sera. This factor has been called anti-Gm activity as it allows the detection of the different  $\gamma$ -globulin (Gm) groups. The anti-Gm activity is also associated with the  $\gamma$ -19 S group, so the question arises whether Ragg-serum containing both RAF- and anti-Gm(a) activity has 12 different serologically active populations or whether these activities are associated with the same molecular support.

Steinberg<sup>3</sup>, while studying this problem with the F II-latex-fixation test, stated that Ragg-serum with both RAF and anti-Gm(a) activity contains two distinct  $\gamma$ -19 S populations: one with RAF activity alone and the other with both RAF- and anti-Gm(a) activity. Harboe<sup>4</sup>, on the other hand, postulated that Ragg-serum with both serological activities contains two populations: one with RAF activity and the other with anti-Gm(a) activity. These results were obtained by the specific adsorption of RAF activity on sheep red blood cells coated with rabbitamboceptor.

While working on the isolation of the molecular support of anti-Gm(a) activity we found that anti-Gm(a) activity from normal serum can be adsorbed specifically on diazotized *p*-aminobenzylcellulose coupled with Gm(a<sup>+</sup>)- $\gamma$ -globulin<sup>5</sup>.

The same support has been found suitable for the dissociation of anti-Gm(a) and RAF activities in Ragg.

The chemical attachment of human  $\gamma$ -globulin to the insoluble cellulose derivative gives a better reagent than the simple adsorption of the protein on latex particles (polystyrene resin) which is more or less reversible. Pure  $\gamma$ -globulin of Gm(a<sup>+</sup>) as well as Gm(a<sup>-</sup>) serum has been prepared by a technique combining the precipitation of most of the serum proteins with rivanol and adsorption of the remaining impurities on DEAE cellulose.

The following complexes have been prepared: (A) PAB- $\gamma$  Gm(a<sup>+</sup>); (B) PAB- $\gamma$  Gm(a<sup>-</sup>); (C) PAB- $\gamma$  Gm(a<sup>+</sup>), heated; (D) PAB- $\gamma$  Gm(a<sup>-</sup>), heated. In the case of the heated preparations, the solution of  $\gamma$ -globulin (1 per cent) was heated for 10 min to 63° C before combination with the diazotized PAB-cellulose.

The unspecific adsorption of RAF on heat-aggregated  $\gamma$ -globulin is a well-known phenomenon. Initially we supposed that anti-Gm(a) activity would only be adsorbed by complex (A) and eventually by complex (C), whereas RAF would be adsorbed by (C) and (D).

However, we soon realized that these complexes have a considerable non-specific affinity for both activities. We therefore enquired whether there exists an important quantitative difference between the specific and the non-specific adsorption of anti-Gm(a) activity by adsorbing different Ragg sera with complexes (A) and (B). Under optimal conditions, using a minimal amount of complex, about 90-100 per cent of anti-Gm(a) will be adsorbed to complex (A), and less than 10 per cent to complex (B), the latter being used as a control to evaluate 'non-specific adsorption'. Such small differences cannot be detected by ordinary serological techniques and we had to do this quantitation by photometric titration<sup>6</sup>. It was evident that the non-specific adsorption of anti-Gm(a) to PAB- $\gamma$ -Gm(a<sup>-</sup>) could be very small (less than 10 per cent) under appropriate conditions.

However, the titration of RAF activity in the supernatant of complex (A) showed a loss of about 25 per cent. It is conceivable that RAF has a greater non-specific affinity for the cellulose-protein complexes than have the

anti-Gm molecules, and the loss of 25 per cent of its activity could be explained by non-specific adsorption. However, complex (B), which adsorbed about 10 per cent of anti-Gm(a) activity (non-specific) did not modify the titre of RAF activity. It is therefore probable that the part of RAF adsorbed together with the total activity of anti-Gm(a) shares a common molecular basis with anti-Gm(a) and that its adsorption is mediated by the specific adsorption of anti-Gm(a) to PAB-Gm(a<sup>+</sup>). The observations therefore suggest that there are two different populations, one of which has both activities, whereas the other has RAF activity alone, as 75 per cent of RAF remains in the adsorbed serum. We found that anti-Gm(a) has a greater affinity (non-specific adsorption) for all the complexes used, so it was not possible to use this system for the adsorption of RAF leaving anti-Gm(a) in the supernatant.

Evidence of a third population, with anti-Gm(a) activity alone, was provided by the adsorption of RAF activity on human red cells sensitized by rabbitamboceptor. Using the photoelectric titration method we found that 50 per cent of anti-Gm(a) activity disappears together with all the RAF activity.

The concomitant adsorption of 50 per cent of the anti-Gm(a) activity, however, was not due to non-specific adsorption. Indeed, the serum devoid of RAF activity and of 50 per cent of anti-Gm(a) activity after this first adsorption has been reabsorbed by an identical quantity of sensitized red cells and the anti-Gm(a) titre was unchanged (50 per cent of the original titre) after the second adsorption. These observations thus revealed two populations: one having RAF activity as well as anti-Gm(a) activity, the second having anti-Gm(a) activity alone. Different Ragg-sera submitted to both tests gave similar results. The exact quantitation for one of our sera revealed that 25 per cent of the RAF activity had a common basis with about 50 per cent of anti-Gm(a) activity whereas 75 per cent of RAF and 50 per cent of anti-Gm-activity had an independent molecular support. Further details will be published elsewhere.

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## Apparent Interaction Between the Xg<sup>a</sup> Blood Group System and the Sex Ratio: A Note of Explanation

PROF. J. H. BENNETT has called to our attention a possible source of confusion in our recent letter<sup>1</sup>. We left implicit the assumption that the expected sex-ratios in the two classes, 'Before and including the first female', and 'After the first female', were equal, and we failed to make clear that the numbers of children in our Table 1 included those from all-male sibships. If such sibships had been omitted, the sex ratio (male:female) would indeed have been expected to be higher in the first class than in the second, but when they are included, as was done, the expected ratios are equal. Other explanations of our

observation, such as selective family limitation, do not account for its limitation to the single Xg—Xg<sup>a</sup> mating type where the postulated 'incompatibility' could occur.

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## HISTOCHEMISTRY

### Fixation In Enzyme Histochemistry

Use of unfixed frozen sections is confined to enzymes extremely sensitive to denaturation such as the dehydrogenases. Most authors on the subject state or imply that the method introduces the disadvantages of (1) mechanical disruption by freezing and thawing, (2) reduction in quality of tissue detail, (3) uneven section thickness, (4) diffusion of soluble enzymes and co-factors leading to loss of reproducibility and false localization, and (5) inability to cut serial sections<sup>1-4</sup>. These disadvantages are said to outweigh the advantages of high specificity and activity, and simplicity of preparation. The alternative method of briefly fixing sections overcomes many of these disadvantages at the expense of possible alteration in specificity and activity.

The method described and evaluated here is suitable for all enzyme histochemical procedures and makes a better compromise between the conflicting demands of activity, freedom from artefact and quality of section.

Fresh-frozen sections were cut on a cold knife freezing microtome by a modified Adamstone-Taylor technique<sup>5</sup>. The section was manipulated with a squirrel-hair brush (previously dipped in a 5 per cent dimethyl polysiloxane solution—Dow Corning Corp. '1107'—dried and wiped free of excess siloxane), picked directly from the microtome blade onto a slide, and allowed to dry. The siliconed brush overcame the tendency of bristles to stick to tissue, resulting in easier handling. Serial sections of constant

thickness could be readily cut in the high ambient temperature of an Australian summer. Paired sections of rat and mouse tissue prepared by this technique were treated as follows: (1) One of each pair was incubated in standard substrate solutions for a wide variety of enzymes; it was then passed directly for 1 h to a fixing solution in which the dye precursor was insoluble, and staining was completed by the appropriate methods. Details of methods and results are set out in Table 1.

The quality of detail in the sections, and the staining, was satisfactory, and clouding of the substrate solutions, indicating diffusion of enzyme from the incubating section, was not seen. It was therefore assumed that diffusion of this type had not occurred. (2) The other section of each pair was fixed in an accepted histochemical fixative for periods of 5, 10, 20, 40 or 60 min, rinsed and then incubated and stained as in procedure (1). Details of methods and results are set out in Table 2. Enzyme activity was judged by eye against that of the unfixed sections in Table 1.

Sections fixed after incubation were in all respects better than those fixed before. They were free of stretching, shrinking or other artefacts of handling and showed no tendency to lift from slides even during 2 h incubation. Efficiency of subsequent steps was not disturbed and diffusion beyond the cell or into the nucleus did not occur. There was nothing to suggest false localization of hydrolysed products. All results were reproducible. No effect of mechanical disruption by freezing could be detected at the histological level. The optical quality of sections was comparable with those cut from paraffin blocks.

In contrast, most sections fixed before incubation showed significant loss of activity when fixed for the minimum time necessary to ensure even penetration of fixative. Diffusion of enzyme, giving false localization, also occurred. Optical quality of sections was inferior except when aqueous formalin had been used as fixative. These changes in activity, diffusion and optical quality were individual and not distributed in any pattern. No fixative could be described as satisfactory for the range of enzymes tested against the parameters used. Fixation after incubation in substrate is therefore recommended for general use in enzyme histochemistry.

Table 1. DETAILS OF METHODS USED ON FROZEN SECTIONS FIXED AFTER SUBSTRATE INCUBATION

Enzyme and method of incubation	Fixation (for 1 hour)	Subsequent handling
Alkaline phosphatase <sup>1,2</sup> (glycerophosphate)	10% formalin in absolute alcohol	Wash, Co(NO <sub>2</sub> ) <sub>2</sub> bath, (NH <sub>4</sub> ) <sub>2</sub> S rinse and counter stain <sup>10</sup>
Alkaline phosphatase <sup>11</sup> (naphthyl phosphate)	10% formalin in 0.1 M phosphate buffer pH 7.2	Wash and counter stain <sup>10</sup>
Acid phosphatase <sup>12</sup> (glycerophosphate)	10% formalin in absolute alcohol	Wash, (NH <sub>4</sub> ) <sub>2</sub> S rinse and counter stain <sup>11</sup>
Glucose 6 phosphatase <sup>13</sup>	" " " "	" " " " "
Adenosine triphosphatase <sup>14</sup>	" " " "	" " " " "
Adenosine diphosphatase <sup>14</sup>	" " " "	" " " " "
5 Nucleotidase <sup>15</sup>	" " " "	" " " " "
Non-specific esterase <sup>16</sup>	10% formalin in 0.1 M phosphate buffer pH 7.2	Wash, and counter stain <sup>14</sup>
Succinate dehydrogenase <sup>17</sup>	" " " "	" " " "
Lactate dehydrogenase <sup>17</sup>	" " " "	" " " "
6 Phosphogluconate dehydrogenase <sup>17</sup>	" " " "	" " " "

Table 2. DETAILS OF METHODS USED ON FROZEN SECTIONS FIXED BEFORE SUBSTRATE INCUBATION AND RESULTS

Fixation	Loss of enzyme activity— Time (min) to 25% loss					Diffusion of enzyme	Quality of section detail and staining
	Alkaline phosphatase (glycerophos.)	Acid phosphatase (glycerophos.)	Glucose 6 phosphatase	Adenosine triphosphatase	Non-specific esterase		
10% formalin in 0.1 M phosphate buffer pH 7.2	40	5	40	5	5	None	Satisfactory
10% formalin in absolute alcohol	20	10	5	5	5	Minor in non- specific esterase and glucose 6 phosphatase	Poor
60% acetone	40	>60	5	5	5	Gross in adeno- sine triphospha- tase	"
60% acetone in citrate buffer pH 7.2 <sup>17</sup>	10	5	5	5	5	"	"
20% formalin with chloral hydrate <sup>18</sup>	>60	5	20	5	5	None	Satisfactory



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## HISTOLOGY

### Demineralization of Bone

DEMINERALIZATION of specimens for the preparation of histological sections is a time-consuming process. Mineral acids demineralize the specimens faster than chelating agents or organic acids, but the resultant distortion in cellular morphology is greater when mineral acids are used. The rate of demineralization depends mainly on the temperature and concentration of the acid, but since cellular definition and enzymes are adversely affected if the temperature is raised, or when the concentration of the acid is increased, demineralization is usually carried out below or at room temperature and a concentration of mineral acid above 5 per cent of commercial nitric acid (55 per cent) is seldom used. Under these conditions even small specimens of bone must be immersed in acid for days before they are completely demineralized. For teeth, the period of time runs into weeks.

The whole process of demineralization can be reduced to hours, even minutes, if ultrasonic energy is propagated through the demineralizing solution. Subsequent sectioning of the specimen with a freezing microtome, in a cryostat, or as paraffin-embedded sections all produce preparations in which the cellular detail is superior to those seen in similar specimens treated with the same mineral acid for longer periods of time. All soft tissue detail is improved, but the most significant improvement in cellular morphology is seen in the chondrocytes and osteocytes.

In these preliminary experiments a Disontegrator system 80' with a frequency of 90,000 per sec was used. The temperature of the water in the tank was controlled by a constant exchange of the tank water from the cold-water supply. The best results were obtained when the demineralizing fluid and fixed specimens were placed in a large beaker immersed in the partially filled tank directly over the crystal. To prevent damage to the tank, sodium bicarbonate was added to the water in it and the beaker was covered to prevent spattering of the agitated acid.

Nitric acid at a concentration of 2.5, 5 or 10 per cent was used in these experiments and the acid solutions were changed every half-hour. Although it is not possible to

indicate an optimum concentration of the nitric acid at this stage, the more rapid decalcification in the 10 per cent solution of nitric acid seemed to produce less cellular distortion in the sections.

The time taken to demineralize a specimen is affected not only by the degree of mineralization, but also to a marked degree by the amount of investing soft tissue. When an adult rat femur is divided into three segments and 10 per cent acid is used, decalcification can be accomplished in less than 2 h. Without the investing soft tissue demineralization is complete within 1 h.

When haematoxylin and eosin are used the time taken to stain sections decalcified in this manner is considerably reduced and the differential staining of the various tissues is more selective.

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### Histology of the Posterior Chamber of the Swimbladder of *Argentina*

*Argentina* was the first genus among the salmonoid fishes in which the swimbladder was reported to lack an open communication with the oesophagus, typical for the sub-order Salmonoidae<sup>1,2</sup>. This has since been found to apply also to the families Microstomidae and Opisthoproctidae<sup>3</sup>. A more detailed description of the structure of the swimbladder of *Argentina silus*<sup>4</sup> revealed the occurrence of a large number of flat bundles of blood vessels, which constitute a counter-current system. The name micro-retia mirabilia<sup>5</sup> has been established for this type of rete mirabile. In *Argentina* a gas gland tissue was also detected<sup>6</sup>, being in contact with capillary loops from the micro-retia. From these findings it was concluded that the swimbladder of *Argentina silus* has a capacity for gas secretion comparable to the swimbladder with a rete mirabile of the compact type.

A thin sac at the caudal end of the swimbladder of *Argentina sphyraena* has been briefly described and suggested to be a posterior chamber<sup>7,8</sup>. Observations also argue that it might have a resorbent function<sup>9</sup>. The draining vessels were seen to be a part of the hepatic portal system<sup>9</sup>.

The present investigation was intended to produce histological evidence for a supposed resorbent function of the posterior chamber of the swimbladder. Several specimens of *Argentina silus* and *A. sphyraena* were caught off the west coast of Norway at the Biological Station of the University of Bergen. The animals were brought to the surface (200 metres depth to surface in 20 min). The swimbladder in most of them had not burst—an occurrence often reported in captured specimens of *Argentina*. The animals could be kept in aquaria for 6–10 h after capture. The posterior sac of the swimbladder was observed. Longitudinal and transverse sections, fixed in Bouin's fluid, were stained with Azan, and the morphology and histology of the posterior part of the main chamber and the posterior thin-walled sac were investigated. Swimbladders from some specimens were, immediately after killing, freeze-dried, treated with formaldehyde gas for 1 or 3 h at 80° C, and then infiltrated with paraffin *in vacuo* in order to condense catecholamines and 5-hydroxytryptamine to fluorescent products<sup>10</sup>. They were then investigated under a fluorescent microscope in dark-field illumination.

It was confirmed that the posterior sac constitutes a posterior chamber of the swimbladder in communication with the anterior part of the organ. It is not separated from the anterior chamber by a membrane with a central opening and possessing a radial muscle, as is normal in

other two-chambered swimbladders. The lumen of the anterior chamber gradually decreases in diameter and passes into the opening that leads to the posterior chamber (Fig. 1). The opening is surrounded by a circular muscle, which apparently constitutes a sphincter. This muscle is coherent with a rather thick circular muscle layer, which is an important part of the wall of the posterior chamber. It is in turn surrounded by a longitudinal muscle layer formed by more or less separate muscle bundles. The anterior parts of these longitudinal muscle bundles were seen to be attached to the sphincter muscle. Outside the muscles is a layer of connective tissue, lodging about ten big vessels, both arteries and veins. The arteries originate in one single vessel—a branch of the intestinal artery—and enter the organ at its posterior, forward-bent end. The veins leave the organ as 5–8 separate vessels but soon join into one, which is a branch of the hepatic portal system. No connexion could be traced between the vessels of the posterior chamber of the swimbladder and those of the anterior chamber.

The arteries in the submuscular connective tissue layer of the posterior chamber branch into a number of smaller vessels that traverse the muscle layers and enter a layer of loose connective tissue inside the muscles. By repeated division, the arteries there give rise to a capillary network in close contact with an epithelium, composed of flat to cubical cells, that forms the lining of the lumen. The capillaries recombine into venous vessels, which unite into the big veins mentioned above.

An investigation of the occurrence of catecholamines and 5-hydroxytryptamine, in order to localize the sympathetic nerves of the organ, revealed the following: After treatment for 1 h at 80° C, a green fluorescence was seen in

the anterior chamber between the muscle layer and the underlying gas gland tissue (Fig. 2). This indicated the presence of a primary catecholamine, possibly noradrenaline. It was located in a network of longitudinally arranged, smooth, preterminal nerve fibres, which were seen to give rise to intensely green-fluorescent, fine, varicose nerve-endings. No fluorescent fibres were seen to intermingle with the muscle fibres or with the gas gland cells. This fluorescent network was distributed over the whole anterior chamber. In the posterior chamber intensely green-fluorescent, varicose, terminal nerve fibres were abundant in the muscle layers. In the border region between the longitudinal muscles and the submuscular connective tissue, longitudinal bundles of smooth, preterminal nerve fibres were seen. In this region, there was also a relatively abundant occurrence of ganglion cell bodies with a moderate green fluorescence, giving rise to the preterminal and terminal fibres already mentioned. A number of bundles of smooth, preterminal nerve fibres were seen to reach the opening to the anterior chamber. They most probably give rise to the network of fibres found in this chamber.

There seems to be no doubt of the accuracy of the opinion of earlier authors<sup>1,2</sup> that the caudal, thin-walled sac is a posterior, resorbent chamber of the swimbladder. The present investigation shows that this sac has all the morphological characteristics of an organ with a gas-resorbent function. It is connected with the anterior chamber by an orifice that possesses a muscular sphincter which is apparently able to close the opening completely. The sphincter muscle is part of the muscular coat of the posterior chamber, which muscle system will when contracted decrease the luminal volume of this chamber and also close the orifice. When relaxed, this muscle will allow the orifice to open, give the chamber its maximal volume, and allow a maximum of blood to reach the base of the epithelium. The mucosa is very richly vascularized, and the vessels have no connexion with those of the anterior chamber. The sphincter muscle and the muscle layers of the posterior chamber seem to be sympathetically innervated, which is not the case with the musculature of the anterior, secretory chamber. Concerning the fluorescent nerves found in the anterior chamber, there is reason to believe them to innervate some part of the gas-gland tissue, since the musculature of this chamber is presumably antagonistically innervated to the muscles of the posterior chamber. Further investigation, however, is necessary to clarify this point.

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## PATHOLOGY

### Polyuridylic Acid Stimulation of Phenylalanine in Human Ribonucleoprotein Particles

THE discovery that synthetic polynucleotides can direct the incorporation into protein of specific amino-acids in cell-free bacterial systems<sup>1,2</sup> has been extended to include sub-cellular systems in rat liver<sup>3</sup>, and mouse plasma cell tumour<sup>4</sup>, where coding units for amino-acid incorporation were found to be similar to the *Escherichia coli* system. Weinstein and Schechter<sup>4</sup> also reported polyuridylic acid (poly U) stimulation of phenylalanine incorporation into crude cell-free fractions of normal mouse and rat livers,



Fig. 1. *Argentinus silus*, swimbladder. The posterior end of the anterior chamber (right) and the anterior part of the posterior chamber (left). The section is not exactly median and the circular muscle of the sphincter is visible where the two chambers meet ( $\times 630$ ).

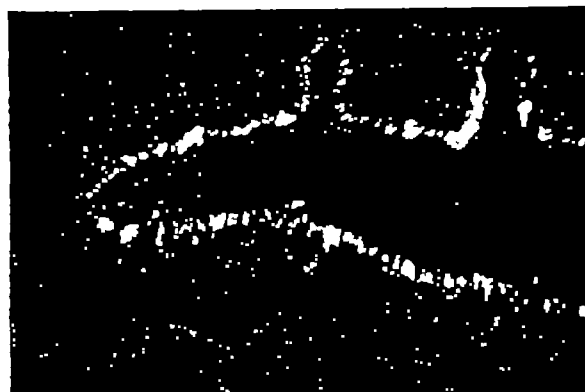


Fig. 2. *Argentinus silus*, swimbladder. Green-fluorescent nerve fibres in the anterior chamber. The nerves form a plexus between the muscle layer (next to the black lumen) and the gas gland tissue ( $\times 820$ ).

a rat liver tumour, rabbit reticulocytes and a human plasma cell tumour. The experiments reported here support the applicability of this type of study to various human tissues. Poly U was found to stimulate the incorporation of phenylalanine into ribonucleoprotein (RNP) particles of human liver, ovary, lung and prostate. Rat liver RNP preparations are similarly stimulated.

Seventy-five g human liver was obtained from each of two autopsies. The liver samples were available 4-5 and 16 h post-mortem and were free of gross and microscopic pathology. Comparable results were obtained with each of the two liver samples. Human lung (33 g), human ovaries (8 g), and human prostate (15 g) were obtained within 1 h of surgical removal in various operative procedures. The lung sample was from an area far removed from the proximal segment of lung involved with epidermoid carcinoma. Our sample was microscopically free of tumour involvement. The ovaries were part of a total hysterectomy block in which the only pathology was uterine fibroids. The prostatic tissue was from a patient suffering from benign prostatic hypertrophy. The rats were Columbia Sherman males weighing approximately 150 g and the mice were 6-week-old C57BL males.

RNP particles were prepared by a modification of the procedure of Rendi and Hultin<sup>8</sup>. Tissues were trimmed of capsule where necessary and then minced and rinsed 3 times with equal amounts (w/v) of ice-cold homogenizing solution (tris-HCl 0.034 M, pH 7.8, potassium chloride 0.02 M, magnesium chloride 0.01 M, and sucrose 0.2 M).

All subsequent procedures were performed at 4°C. The human tissues were homogenized with 1.5 volumes of homogenizing solution with a 'Model 43' Virtis homogenizer for 2 min at 40,000 r.p.m. The rat and mouse livers were homogenized with 1.5 volumes of homogenizing solution in a glass homogenizer fitted with a 'Teflon' pestle for 45 sec at approximately 1,500 r.p.m. All homogenates were centrifuged at 12,000g for 10 min. Aliquots of the resulting supernatant (S-12) were centrifuged for 2 h at 105,000g to obtain the supernatant (S-105) fraction. The remaining S-12 supernatant was added to 1/20 volume of 10 per cent 'Lubrol W'. A volume of 10 per cent sodium deoxycholate equal to the 'Lubrol W' was added with stirring. The resulting suspension was layered in 'Lusteroid' centrifuge tubes on an equal volume of layering solution (tris-HCl 0.034 M, pH 7.8, potassium chloride 0.6 M, magnesium chloride 0.01 M and sucrose 0.3 M), and centrifuged for 2 h at 105,000g. The resulting clear pellet of RNP (P-105) was rinsed 3 times with suspending solution (tris-HCl 0.01 M, pH 7.8, potassium chloride 0.2 M, and magnesium chloride 0.01 M). The outer layers of the RNP pellets were carefully dislodged and suspended in a few ml. of suspending solution and homogenized in small all-glass homogenizers for 15 sec. Protein was determined by the biuret method<sup>9</sup>. 'Lubrol W' was obtained from Arnold Hoffmann and Co., Providence, R.I., and the homopolymers from Miles Laboratories. The *in vitro* reaction system and method of handling of samples for isotopic counting is given in Table 1.

Poly U results in up to a fifty-fold stimulation of phenylalanine incorporation by RNP of rat liver, and human ovary, prostate, lung and liver (Table 1). With the exception of the rat and human liver RNP preparations, those experiments in which poly U stimulation was noted, included a heterologous source of amino-acid activating enzymes and soluble nucleic acid fractions (S-105 fraction). Autologous and homologous S-105 fractions were inactive in these experiments. Poly U enhanced phenylalanine incorporation by human liver RNP preparations regardless of the S-105 source. Other homopolymers did not stimulate phenylalanine incorporation into the human liver RNP system.

Apart from a brief report on a human plasma cell tumour<sup>4</sup>, in which relatively crude supernatant fractions were used, the experiments reported here are, to our

Table 1. POLYURIDYLIC STIMULATION OF PHENYLALANINE INCORPORATION INTO RAT AND HUMAN RIBONUCLEOPROTEIN PARTICLES

Ribonucleoprotein (P-105)	Polynucleotide addition	Supernatant (S-105)	cpm of alanine/mg RNP protein
Rat liver	None	Rat liver	80
Rat liver	+40 µg Poly U	Rat liver	147
Human lung	None	Mouse liver	8
Human lung	+40 µg Poly U	Mouse liver	89
Human lung	None	Human lung	3
Human lung	+40 µg Poly U	Human lung	2
Human ovary	None	Mouse liver	4
Human ovary	+40 µg Poly U	Mouse liver	204
Human ovary	None	Human ovary	4
Human ovary	+40 µg Poly U	Human ovary	2
Human ovary	+40 µg Poly U	Human prostate	4
Human prostate	None	Mouse liver	0
Human prostate	+40 µg Poly U	Mouse liver	54
Human prostate	None	Human prostate	0
Human prostate	+40 µg Poly U	Human prostate	0
Human prostate	+40 µg Poly U	Human ovary	0
Human liver	None	Rat liver	18
Human liver	+40 µg Poly U	Rat liver	53
Human liver	+40 µg Poly U	Rat liver	18
Human liver	+40 µg Poly U	Rat liver	20
Human liver	+40 µg Poly U	Rat liver	20
Human liver	None	Human liver	10
Human liver	+40 µg Poly U	Human liver	125

The complete reaction mixture was contained in a total volume of 0.65 ml. It contained the following components (in  $\mu$ moles unless otherwise specified): Tris-HCl buffer, pH 7.8, 22.4; MgCl<sub>2</sub>, 8.0; KCl, 50.0; mercaptoethanol, 5.0; ATP, 0.8; creatine phosphate, 8.0; creatine phosphokinase, 40 µg; GTP, 0.2; 'Lubrol', 0.8 mg; a mixture of <sup>14</sup>C-L-amino acids excluding phenylalanine, 0.004 of each, and <sup>14</sup>C-L-phenylalanine ( $\phi$  alanine), 25  $\mu$ moles, sp. act. 10-3 mCi/mM (about 25,600 c.p.m.).

As specified, 40 µg of the potassium salts of polyuridylic (poly U), polyadenylic (poly A), polycytidylic (poly C), polynucleic (poly I) acids and the equivalent of 1-4 mg of S-105 protein and 0.5-1.5 mg of P-105 protein were added per reaction mixture.

Samples were incubated at 37°C for 45 min, deproteinized with 10 per cent trichloroacetic acid (TCA). The precipitates were washed once with 10 per cent TCA, and heated twice for 15 min each time with 5 per cent TCA at 90-95°C, plated and dried with ethanol and ether on a 'Tracerlab' precipitation apparatus and counted in a gas flow counter with an error of less than  $\pm 2$  per cent. Samples were corrected for self-absorption and for reagent control blanks.

knowledge, the first to demonstrate polynucleotide-stimulated amino-acid incorporation by RNP preparations from human tissue. The nature of this stimulation in human tissue supports the concept of universality for the genetic code previously surmised from work in other mammalian<sup>3,4</sup> and bacterial and viral systems<sup>7-10</sup>. We are now defining the human tissue RNP system in terms of its requirements and inhibitors and studying the effect of homologous and heterologous ribonucleic acid preparations on amino-acid incorporation by normal and diseased human tissue RNP preparations<sup>11</sup>.

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### Electron-microscopic Demonstration of Bacteria in the Skin of Patients with Lichen Ruber Planus

THE aetiology of lichen ruber planus is uncertain. A number of hypotheses have been advanced<sup>1</sup>. Lichen ruber planus eruptions have been observed in connexion with the use of different drugs, for instance atabrin. In

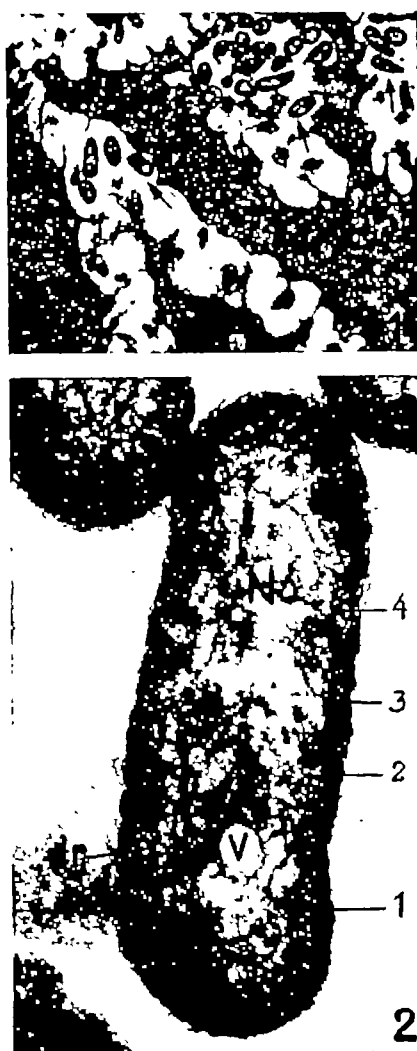


Fig. 1. Groups of bacteria (arrows) within the dilated interspace in the deeper part of the epidermis ( $\times 4,500$ )

Fig. 2. Higher magnification of longitudinally and transversely sectioned bacteria. 1 and 2, outer membrane and intermediate layer, respectively, of the cell wall; 3, inner membrane or plasma membrane; 4, less opaque layer between the plasma membrane and the granular cytoplasm; r, ribosomes; N, nuclear apparatus with fibrous material; v, vesicle ( $\times 84,000$ )

these cases, however, it has not yet been determined whether it is a matter of a genuine lichen or of a lichenoid dermatosis. In cases in which a relation to drugs is out of the question two main possibilities have been put forward, namely, those of an infectious or a nervous aetiology. Conclusive proof for any of these theories has not yet been presented. As infectious agents, viruses and spirochaetes have been suggested. The therapeutic success attained in some cases with chemotherapeutics and antibiotics has been adduced as an argument in favour of an infectious aetiology<sup>1</sup>.

During the course of electron-microscopic investigations of skin eruptions in lichen ruber planus, pictures have been obtained which indicate the presence of bacteria within and around capillaries in the stratum papillare of the dermis and between the cells in the deeper part of the epidermis. A report on this finding will be given below.

In skin affected by lichen ruber planus from four patients cells of the type seen in Fig. 1 were found. They appear singly or in small groups, irregularly distributed throughout the tissue. The cells are rod-shaped and have a length of approximately  $1.3\mu$  and a breadth of approximately  $0.4\mu$ .

The cells are bounded by an envelope having a total thickness of  $240\text{--}280\text{ \AA}$ . Three sub-layers are distinguished within the envelope: an outer and an inner membrane, displaying a triple-layered pattern, and an intermediate, single layer (Fig. 2). The outer membrane has a wavy course and usually shows a higher opacity than the inner membrane. Its total width is  $90\text{--}100\text{ \AA}$ . The intermediate layer has a low opacity and varies between  $60$  and  $100\text{ \AA}$  in breadth. The inner membrane encloses the protoplasm of the cell and is  $80\text{--}90\text{ \AA}$  wide.

The protoplasm consists of a granular and a fibrous component (Fig. 2). The granular substance is found mainly within a zone along the periphery of the cell. This zone is separated from the inner membrane of the envelope by a narrow, less-opaque layer (Fig. 2). The granular substance contains fairly opaque particles ranging between  $80$  and  $200\text{ \AA}$  in size.

The opaque, fibrous material occupies for the most part the central area of the cells, which displays a low electron density (Fig. 2). This area is not bounded by any membrane, but there is direct contact between the fibrous and granular substances. Fine filaments with a thickness of  $20\text{--}30\text{ \AA}$  and thicker fibrous strands varying between  $100$  and  $250\text{ \AA}$  are observed in the same cell. Occasionally filaments of the finer diameter may be discerned within the strands. The filaments and fibrous strands form a continuous, irregular network.

Within the area of low electron density a varying number of vesicles occur. They vary from  $840$  to  $1580\text{ \AA}$  in length and from  $730$  to  $1580\text{ \AA}$  in breadth. The vesicles are bounded by a triple-layered membrane (Fig. 2).

The cell described here is with respect to size, shape and ultra-structure consistent with the description of bacteria. The envelope of the cell shows the same breadth and ultra-structural arrangement in sub-layers as has been described for several Gram-negative bacteria<sup>2-4</sup>. Thus, in accordance with these investigations on Gram-negative bacteria, the inner membrane of the envelope will here represent the plasma membrane of the cell, whereas the intermediate layer and the outer membrane together form the cell wall. As is the case with bacteria belonging to the family Caulobacteraceae<sup>5</sup>, the outer membrane of the cell wall in the present study displays a wavy course.

A narrow, less-opaque layer between the plasma membrane and the granular substance of the protoplasm has been observed by me. In *Staphylococcus aureus* a similar layer has been described, being attributed to the cytoplasm as the cytoplasmic matrix<sup>7</sup>.

The protoplasm of the cell reported here exhibits an arrangement characteristic of bacteria with a nuclear apparatus consisting of a network of fibrous material within a less electron-dense area and in direct continuity with the granular cytoplasm<sup>2-4</sup>. The fibrous substance of the nuclear apparatus is considered to represent the deoxyribonucleic acid portion of the chromatin material<sup>2,4,8,9</sup>. The opaque particles of the granular cytoplasm are of the same size as has been reported in earlier studies and are thought to represent ribosomes<sup>2,4</sup>.

The observations reported here may thus justify the interpretation of the occurrence of bacteria in lichen ruber planus eruptions. No bacteria have hitherto been described in electron micrographs of skin<sup>10-12</sup>. The demonstration of bacteria in skin biopsies from four out of five lichen ruber planus patients appears to rule out the possibility of an incidental finding. Furthermore, the tissue lodging the bacteria exhibits consistent dermal and epidermal changes in all cases (to be published).

In frozen histological sections of lichen ruber planus eruptions Lennhoff<sup>13</sup> showed structures which he assumed to be spirochaetes. The bacteria found in the present investigation are considerably shorter in length than the structures described by Lennhoff.

Further investigations are being carried out to elucidate the frequency of the bacteria in a larger case material, to

attempt to identify the type here observed and to ascertain whether it plays any aetiological role and, if so, whether it alone may be responsible for eruptions of lichen ruber planus type or whether more factors are implied.

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## IMMUNOLOGY

### An Alpha<sub>2</sub>-globulin Component Absent in Serum of Human Foetuses

WHEN studying specific antigenic properties of sweat proteins using rabbit immune serum against sweat proteins, we have found sweat protein to consist of several components, some of which correspond to serum components whereas the others are most probably of a specific origin<sup>1,2</sup>. In an attempt to define the serum protein components in sweat, immunoelectrophoresis of normal human serum with rabbit-immune serum against sweat proteins was one of the methods used. In these experiments we observed qualitative differences in the immunoelectrophoretic precipitation pattern of a normal serum of adults in comparison with that of foetuses.

The immunoelectrophoretic precipitation pattern of a normal serum of adults using rabbit immune serum against

sweat proteins is marked by several precipitation lines, the strongest of which is localized in an alpha<sub>2</sub>-globulin zone; this precipitation line was found not to be present in the serum of foetuses (six foetuses, gestation age 18-24 weeks, Fig. 1).

On saturating the rabbit immune serum against sweat proteins with normal serum from adults, no precipitation reaction was found in the latter (Fig. 2); when the rabbit immune serum was saturated with a serum of foetuses, only the alpha<sub>2</sub>-globulin component was marked in the immunoelectrophoresis precipitation pattern of a normal serum of adults (Fig. 3).

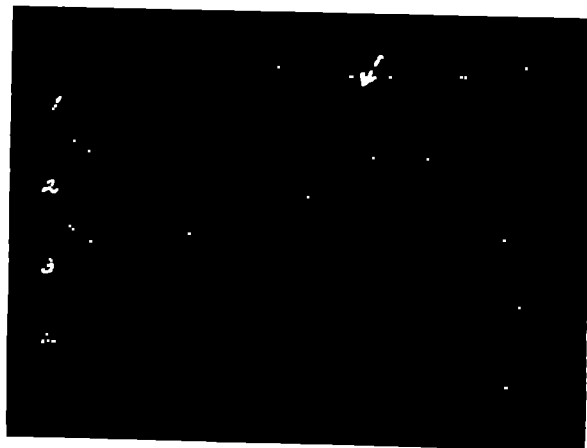


Fig. 2. Immunoelectrophoretic patterns of normal serum of adults (1, 2, 3, 4) using rabbit immune serum against sweat proteins with (in the second and fourth longitudinal basin) and without saturation with normal serum of adults.

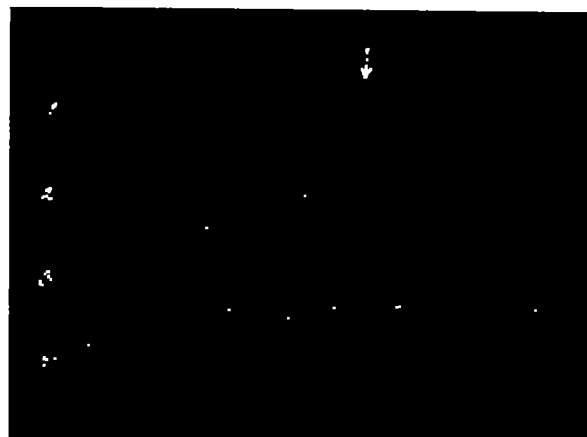


Fig. 3. Immunoelectrophoretic patterns of normal serum of an adult (1, 3) and of that of a foetus (2, 4) using non-saturated rabbit immune serum against sweat proteins with serum of foetuses (in the first, third and fifth longitudinal basin) and saturated with serum of foetuses 10:1 (in the second longitudinal basin) and 10:2 (in the fourth longitudinal basin).



Fig. 1. Immunoelectrophoretic patterns of normal serum of an adult in comparison with that of a foetus. 1, Normal serum of an adult; 2, serum of a foetus (in longitudinal basins horse immune serum against normal human serum); 3, serum of the same adult; 4, serum of the same foetus (in longitudinal basins rabbit immune serum against sweat proteins).

Using a double-diffusion technique in Ouchterlony gel<sup>3</sup>, a weak deviation of the end of the precipitation line corresponding to alpha<sub>2</sub>-globulin component in serum of adults was observed in foetal serum.

At present we are studying in detail the character of this alpha<sub>2</sub>-globulin component in serum, which, according to its immunoprecipitation intensity with anti-sweat protein immune serum, does not seem to correspond to any other alpha<sub>2</sub>-globulin component present in immunoelectrophoretic precipitation patterns of normal human serum, when a polyvalent immune serum against serum proteins is used. The details of the occurrence of this alpha<sub>2</sub>-globulin component in human serum during ontogenesis

(especially in new-borns and in babies in the first weeks after birth) will be published later.

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## RADIOBIOLOGY

### Modification of Radiation-Induced Injury in Barley by Pre-treatments with Solutions of different Osmotic Potential

It has already been shown<sup>1,2</sup> that the growth of the coleoptile and first leaf from barley seeds soaked prior to irradiation in 0.5 molar solutions of sodium and alkaline-earth chlorides is greater than that of controls soaked in distilled water. This radioprotection is provided by different salts not specially recognized as inducing such effects (magnesium chloride excepted)<sup>3</sup>. It has therefore been assumed that the mechanism of this reduction of X-ray damage is an indirect one. The physiological effects relating to the osmotic potential of the soaking solutions were proposed to explain the results obtained.

This communication presents new evidence in favour of this hypothesis which will be considered with increasing osmotic potential of a solution of calcium chloride in which seeds were soaked.

The barley seeds (caryopses of *Hordeum sativum* Joss. var. *pirolina*) were provided by the Institut agronomique de Gembloux. Before the irradiation the seeds are soaked for 12 h at room temperature in the solutions of calcium chloride (Calcium chloratum fustum sur Elementaranalyse, Merck), neutralized by hydrochloric acid (pH about 6.9), washed three times with distilled water and rapidly blotted. The molecular concentrations of calcium chloride solutions are 0.05, 0.25, 0.5, 1, 1.5, 3 and 4 M. The X-rays generated from a Siemens 'Stabilipan', operated at 140 kV and 20 m.amp are filtered through 2 mm of aluminium. The seeds lie in a single layer in an uncovered Petri dish on a rotating turntable 15 cm from the target; the dose rate is 1,000 r/min. In different experiments the total dose varied from 0.25 to 12 krad. Immediately after irradiation, all seeds were placed on moist germination paper in Petri dishes and grown in a controlled-environment room (16 h photoperiod;  $24^{\circ} \pm 1^{\circ} \text{C}$ ). Groups of 50 seeds were used for each dose replicated three times. For each treatment the lengths of the coleoptile and the first leaf were measured, averages taken and expressed in per cent of the non-irradiated controls. The osmotic potentials of the soaking solutions were calculated from the osmotic coefficient of calcium chloride tabulated by Robinson and Stokes<sup>4</sup> after transformation of molecular concentrations in molar concentrations.

There is a linear relationship between the osmotic potential of the calcium chloride solutions and the moisture content of the barley seeds, except for the lowest concentration (Fig. 1). The irradiation reduces the length of the coleoptiles and the first leaves: typical sigmoidal curves against dose are obtained. There is no difference between the seedling issued from seeds pretreated in 0.05 M calcium chloride solutions or soaked in distilled water. But with increasing molar concentration, the damage by X-rays for a given dose is reduced. The maximum protective effect is afforded with 1.5 molar solution. The concentrations above this value are not more efficient.

In Fig. 2, the relative heights of the first leaves for 4, 8 and 12 krad are plotted against the osmotic potential

of the solutions. For each dose, an increase of the osmotic potential from  $-2.5$  to  $-7.5$  joules  $\text{cm}^{-3}$  (ref. 5) brings a rapid augmentation in length of the leaves. The X-ray sensitivity of seeds particularly decreases only in a relative narrow range of concentrations.

The experimental evidence suggests that the protective effects of different salt solutions are related to their osmotic properties since they are not specific.

Two separate mechanisms involving osmotic effects can be considered as operative to explain this increase of resistance to X-rays. The consequences of the decreasing difference in the water potential between the more and more concentrated solutions and the cells of the seeds can to some extent make the latter less susceptible to irradiation.

However, our results involving reduction of the pre-irradiation humidity of the seeds by osmotic pretreatment differ from those obtained after soaking the seeds for different periods of time, or conditioning them at various constant humidity systems. A striking diminution of the growth above and below an optimum humidity was observed<sup>1,2</sup>. As this is not the case with our results, it

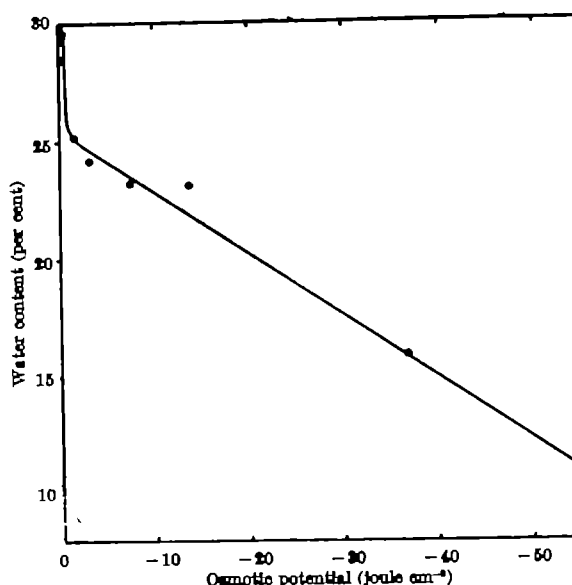


Fig. 1. Effects of the osmotic potential of calcium chloride solutions on the water content of barley seeds

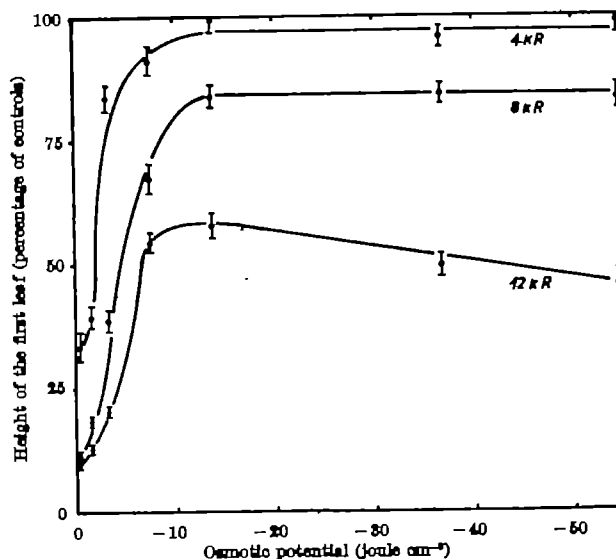


Fig. 2. Influence of osmotic potential of calcium chloride soaking solutions on the first leaf growth from barley seeds treated by different X-ray doses (S.D. is indicated)

is possible that induced modification of the water content of the seeds by various osmotic concentrations is not a determining factor.

A decrease in the amounts of ninhydrin positive substances leached out of the barley during soaking<sup>2</sup> and an activation of the hydrolysis of polysaccharides (anatonosis) result from increasing osmotic concentration of the external medium. Thus in the cells of the embryo an increased concentration of natural chemical protectors may be expected from a reduced loss of protides and increased content in sugars. This hypothesis explains the fact that beyond a certain osmotic potential the protection does not increase any further, since, as shown by Kamra *et al.*<sup>3</sup>, a plateau is reached in the loss of ninhydrin-positive substances at approximately the level of osmotic concentration which is optimal for radioprotection.

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### Use of Gamma-radiation for Quarantine Control of Imported Edible Beans

APPROXIMATELY 4,000 tons of edible beans, mainly *Phaseolus vulgaris* varieties, are imported into Australia annually. Under the quarantine regulations<sup>1</sup>, the majority of these beans are treated on entry to destroy their viability, thereby preventing the introduction and transmission of various plant diseases. Methyl bromide is commonly used for this purpose. It was decided to investigate the possibility of using  $\gamma$ -radiation as an alternative method of treatment, as it is well known that low doses of  $\gamma$ -radiation inhibit seedling growth<sup>2-4</sup>. The radiation dose required to destroy viability in several different varieties of imported beans was therefore determined, as well as the effects of radiation on various chemical constituents of one type of bean, the baby lima.

Permission was obtained from the Commonwealth Department of Health to import unfumigated beans of the following varieties: sallugia, ohtenashi, borlotti, baby lima, red kidney, Michigan pea, and black-eye. Large quantities of navy beans and smaller quantities of butter beans are also imported, but for these tests locally grown seeds were used. Packets of approximately 500 g of beans, enclosed in double polythene bags, were irradiated, using either spent fuel elements from the reactor *Hifor* or cobalt-60. Dosimetry was by the ferrous sulphate method<sup>5</sup>, three dosimeters being included in each packet.

After the beans were treated with a mould inhibitor ('Coversan'), laboratory germination tests were carried out either on sand or on paper towelling which had been sterilized by a radiation dose of 2.5 Mrads. The seeds were incubated for 9-20 days at 25° C. Although 9 days is the recommended time<sup>6</sup> for germination tests on *Phaseolus vulgaris* seeds, this period was found to be too short for an accurate assessment of germination of irradiated seeds. After incubation for 9 days, seedlings grown from irradiated seeds may show all the structures

considered essential for normal growth, although the seedling height is greatly reduced, and the seed coat may still be attached. Further incubation results in very little difference in the appearance of these seedlings: the seedling height remains unchanged and, even if the seed coat comes away, the cotyledons remain closed. If the mean seedling height of the irradiated beans, after 14 days testing, failed to exceed 10 per cent of that of the controls, it seemed reasonable to assume that these beans would never reach maturity.

Based on these considerations, Table 1 sets out the radiation dose needed to meet the quarantine requirements for various edible beans, as estimated by laboratory germination tests conducted over 14 days. At these doses the maximum seedling height would be 3 cm; there would be no protrusion of possibly infected leaves, and no possibility of growth to maturity. A limited amount of evidence obtained in preliminary tests using French bean seeds indicated that irradiated seeds may show a higher percentage of germination under laboratory conditions than under field conditions. Hence, it may be possible to reduce the radiation dose required for quarantine control.

Table 1. ESTIMATED RADIATION DOSE REQUIRED FOR QUARANTINE CONTROL (Calculated from laboratory germination tests)

Type	Dose (rads)
Red kidney	<40,000
Michigan pea	40,000
Navy	>40,000
Sallugia	50,000
Ohtenashi	50,000
Borlotti	55,000
Baby lima	55,000
Butter	75,000
Black-eye	80,000

The average radiation dose required to inhibit laboratory germination is about 50,000 rads. It was expected that the chemical changes in beans given this dose would not be great, and therefore the effects of a dose of 1 Mrad on the nutrients of baby lima beans were examined. Only when there were considerable differences between the values for control and irradiated beans was the effect of the 50,000-rad dose-level investigated.

Before analysis, baby lima beans were ground to pass through an 85-mesh sieve. Moisture (vacuum oven method), ether extract (indirect method), and ash (direct method) were estimated as described by the Association of Official Agricultural Chemists<sup>7</sup>. Total nitrogen was determined using the micro-diffusion technique<sup>8</sup>. Thiamin (thiochrome method) and riboflavin (fluorimetric method) were examined by methods outlined by the Association of Vitamin Chemists<sup>9</sup>. Sulphydryl and disulphide groups were determined directly on the flour by an automatic amperometric titration method using silver nitrate<sup>10</sup>, and the radiometer titrator recording equipment. All determinations were carried out at least in duplicate.

The results are set out in Table 2. Apart from the sulphur-containing constituents, little change was apparent in the constituents after irradiation. Riboflavin content appeared to increase. However, in view of the sensitivity of riboflavin to  $\gamma$ -irradiation<sup>11</sup>, it seems possible that the higher concentration of riboflavin in the irradiated seeds was due to fluorescent artefacts which were not being extracted during the procedure. An apparent increase in

Table 2. EFFECT OF  $\gamma$ -RADIATION ON CONSTITUENTS OF BABY LIMA BEANS

Constituent	0	Dose (Mrads) 0.05	1
Moisture: g/100 g wet wt.	10.69		10.20
Ether extract: g/100 g wet wt.	1.13		1.27
Total nitrogen: g/100 g wet wt.	2.97		2.96
Ash: g/100 g wet wt.	3.80		3.80
Thiamin: $\mu$ g/100 g wet wt.	860	970	760
Riboflavin: mg/100 g wet wt.	0.083	0.104	0.093
Sulphydryl groups: $\mu$ mole/g	6.53	4.65	3.73
Disulphide group: $\mu$ mole/mg N	0.22	9.16	0.13
	11.50	2.22	2.22
	0.39	0.23	0.07



riboflavin has been noted in other products when treated by irradiation or by other processing methods<sup>12</sup>.

Sulphydryl and disulphide groups were determined, not so much because of their nutritional importance, but because it is generally considered that these groups are particularly sensitive to radiation<sup>13</sup>. Although the majority of experiments elsewhere have involved only solutions containing low concentrations of these groups, similar results were obtained in these experiments using bean flour. There was a considerable decrease in both groups following irradiation.

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## BIOLOGY

### Far Southern Animals and Plants

IN recent years the southern limits of known natural occurrence of animals have been pushed farther south, and during the 1964-65 Antarctic season several new southernmost records were established. Records of the most southern plant collections made this season are also included here.

In 1959, Tyndale-Biscoe<sup>1</sup> found one species of mite (Acarina) and two species of springtails (Insecta), with lichens and moss, between the Hood and Beardmore Glaciers, 83° 45' and 83° 55' S. The mite is *Nanorchestes antarcticus* Strandtmann, 1963 (Prostigmata: Pachygnathidae) and the springtails *Bischoia subpolaris* Salmon, 1962 (Collembola: Hypogastruridae), *Anurophorus subpolaris* Salmon, 1962 (Collembola: Isotomidae).

Recently, Wise<sup>2</sup> recorded *N. antarcticus* and *A. subpolaris* from near the lower Shackleton Glacier at 84° 35' S.

The preliminary records listed here are presented before complete identification of the groups concerned, as some identifications must await opportunity for detailed systematic work. Records presented here are listed in latitudinal order from south to north. They represent, in most cases, the most southern collections made, of the various elements of the biota, during the 1964-65 season. These are probably also, in each case except for the mosses, the southernmost known occurrences for these groups.

Lichens: Ridge west side unnamed glacier west of Wisconsin Range, Horlick Mts. area; 86° 09' S., 131° 14' W.; alt. 1980 m; 15.I.1965; Wise (site HM18).

Mite, *Nanorchestes antarcticus* Str.: Pt. Durham area, east side mouth of Robert Scott Glacier; 85° 32' S., 153° W.; alt. 975 m; 13.I.1965; Wise (site RS3).

Rotifers; Algae (macroscopic): Swithinbank Moraine, west side Shackleton Glacier; 85° 09' S.; 176° 50' W.;

alt. 1,600 m; 1.XII.1964; Gressitt (algae, rotifers) (site S25), Wise (algae) (site S1).

Algae (microscopic); Bacteria: South side McGregor Glacier, near Shackleton Glacier; 85° 09' S., 174° 57' W.; alt. 1,737 m; 17.XII.1964; Wise (soil) (site S8); cultured by J. Shoup.

Springtails, *Anurophorus subpolaris* Salm., *Tullbergia* sp. (Onychiuridae); Mites, *N. antarcticus* Str., ? *Stereotylus mollis* Wom. and Str., ? *Proteronides* sp.: east side Shackleton Glacier; 84° 47' S., 176° W.; alt. 762 m; 13.XII.1964; Wise (site S16).

Moss: Garden spur, east side Massam Glacier, east of Shackleton Glacier; 84° 35' S., 174° 52' W.; alt. 700-762 m; 13.XII.1964; Wise (site S20). Point east side Barrett Glacier, east of Shackleton Glacier; 84° 35' S., 173° 35' W.; alt. 457 m; 13-14.XII.1964; Wise and J. Shoup (site S21). (E. V. McGregor, N.Z. Antarctic Research Programme, collected moss at both these sites in 1963-64, and apparently also at Cape Smith, 84° 42' S., 170° W.)

The map (Fig. 1) indicates the areas mentioned in the foregoing list and also inland Antarctic areas which have been found, by Bishop Museum personnel, to be negative for fauna<sup>3</sup>. The latter include the Queen Maud Mts. above 1,600 m, the Ellsworth Mts. (77°-80° 30' S.), the Whitmore Mts. (82° 20'-82° 45' S.), Pagano Nunatak and Hart Hills (88° 40' S.), and the Horlick Mts. (84° 35'-86° 20' S.). Lichens have been found in some of these ranges. The most southerly area of bare ground is about the Robert Scott Glacier. This and other large areas between Beardmore-Shackleton-Robert Scott Glaciers still remain to be investigated. Much of the bare ground lies at relatively low altitudes even though these areas appear to be far inland. Actually, the landward edge of the Ross Ice Shelf must be considered coastal, the sea and shores being covered with ice a few hundred metres thick.

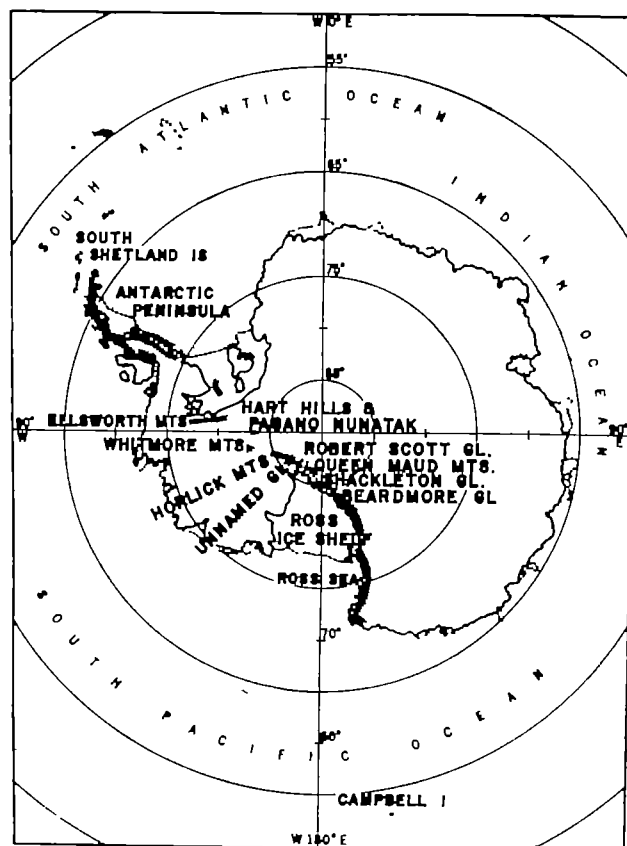


Fig. 1. Map of Antarctica, showing areas positive and negative for biota in inner Antarctica, as mentioned in text. Heavy hatched lines are principal mountain ranges pertinent to the discussion.

Biotic environments occur mainly on northward facing slopes with snow patches, on flats below permanent snow-ice slopes, on cliffs with snow patches, on ice-cored moraines, or on flats above glacier levels where snow-clouds, blown snow or clay beneath the substrate provide a source of moisture. The obvious key factor to a faunal environment is the daily rise of soil temperatures above freezing point together with the presence of available moisture. In the far south, and at higher altitudes farther north, snow and ice recede, in the summer, through sublimation. Even though soil temperatures may rise above 0° C the soil may still be quite dry. It is only where there is local melting of snow or ice that fauna can survive.

The southern records noted here are of interest not only for the actual geographical positions but also for the ecological factors involved, particularly length of continuous light and dark periods, and length of periods when soil temperatures rise above freezing point, as well as the distributional factors involved in placing biota in such areas. So far as is known at present there is no indication, in the fauna, of species in the central (southern) area of Antarctica linking species in the peripheral (northern) areas of the Ross Sea sector with those of the Antarctic Peninsula, but the recently discovered *Tullbergia* species has yet to be examined particularly in regard to its relationship with Campbell I.<sup>4</sup> and South Shetland Is. species.

These are partial results of work on the U.S. Antarctic Research Programme, supported by grant GA-131 to Bishop Museum from the U.S. National Science Foundation.

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### Differences in Regulation in the Silk Glands of the Spider

It has been found that the production of fibroin from the ampullate glands of the spider, *Aranus sericatus*, can be stimulated both by cholinergic agents and by emptying the gland<sup>1</sup>. However, as will be shown here, the cylindrical glands do not have a cholinergic regulatory mechanism.

The basic work on the structure and function of the silk glands of *Aranus diadematus* was carried out at the end of the nineteenth century<sup>2,3</sup>, by workers who carried out careful investigations based on gross dissection, observation of which spinneret was used, the presence or absence of certain types in certain families or, in the case of the egg-cocoon glands, presence only in the female. It was postulated that the ampullate glands produced the fibroin for the scaffolding of the web, the aggregate glands the viscid thread, and the cylindrical glands the egg-cocoon fibroin. By serial sectioning the spider after it had performed a certain action which involved the use of silk and checking the state of filling of the glands, it was possible to determine with certainty which glands are involved in producing fibroins for a given purpose<sup>4</sup>. It has been demonstrated by these means that the scaffolding of the web and the safety thread come from the ampullate glands and that the aggregate glands are involved in the formation of the catching spiral. If thread is drawn from



Fig. 1. Sections showing the cylindrical glands (C) before (a) and after (b) construction of the egg-cocoon. Some aggregate glands (A) are also visible. Note the full glands as normally found compared with the empty lumen after the construction of the cocoon (haematoxylin and eosin,  $\times c. 200$ ).

the spider by winding it on to a rod which is turned by a small motor, it is the ampullate glands alone which are emptied<sup>4</sup>. The cylindrical glands are normally full of protein (Fig. 1a), but after the completion of the egg-cocoon they are found to be empty (Fig. 1b). Our observations on *Aranus diadematus* and *Aranus sericatus* have revealed no differences in any of the respective silk glands of these two species.

It has been shown by two methods that the rate of incorporation of amino-acids into the ampullate gland is stimulated by cholinergic agents both *in vivo* and *in vitro*<sup>1,5</sup>. The amount of incorporation of alanine labelled with carbon-14 into the fibroin was measured by determining the radioactivity of the fibroin pulled from the spider. Also the course of the incorporation of <sup>14</sup>C-alanine into the ampullate glands was followed by means of autoradiographs. Both methods showed that physostigmine and carbachol both increased the rate of incorporation to approximately double the normal rate. The ampullate glands can also be stimulated by emptying out the fibroin. This stimulation, unlike the cholinergic mechanism, is not blocked by atropine. Thus there is evidence for the existence of two, more or less, independent mechanisms to regulate the protein production in the ampullate gland. The cylindrical gland is different from all the other silk glands of the spider in that it is not in daily use. Egg cocoons are normally made only once or twice in the life of a spider<sup>4</sup>. Autoradiographs show no stimulation of the activity of the cylindrical gland after the administration of physostigmine. This is shown in Fig. 2, where it can be seen that there has been no incorporation into the cylindrical gland although there is considerable radioactivity in the aggregate gland. It can also be seen

from the photograph that the epithelium of the cylindrical gland is narrow and contains very little DNA or RNA, as would be expected in an inactive gland.

Thus it is found that the cholinergic mechanism is present only in those glands which are used daily and is absent in the cocoon-producing glands which are used infrequently.



Fig. 2. Autoradiographs of aggregate (a) and cylindrical (b) glands. Both photographs are from the same section the glands are 0.006 mm apart. The spider was fixed 8 h after oral administration of  $^{14}\text{C}$ -alanine and 1 mg/kg physostigmine. The black dots, formed on the photographic emulsion covering the slide, show the location of the labelled alanine. Considerable activity is found in the lumen of the aggregate gland (a) (two dots are indicated by arrows) showing the presence of freshly synthesized silk whereas the cylindrical glands (b) show no activity (azure B,  $\times$  c. 500).

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## ENTOMOLOGY

### Finding Metarchons for Pest Control

SALMOTIVE control of pest insects by behaviour-controlling scents or other metarchons<sup>1</sup> promises to become a preferred method, but if, as seems probable, the scents to which insects respond are in most cases blends of several distinct chemical substances, the problem of isolating and identifying the active materials becomes very difficult<sup>2</sup>. This control method is based on the assumption that by sufficiently permeating the environment with a particular scent, such as a sex attractant, either the males will be unable to perceive the small increment released by the female or, what is effectively the same thing, their receptors will become fatigued and temporarily cease to register any signal.

The question now arises whether, in order to block the response, it is necessary to disseminate all the components of a complex scent, or whether it is sufficient to raise the background concentration of one or possibly a few constituents of the blend to which the creature normally reacts.

A large wind tunnel, 2 x 2 x 10 ft. and containing some hundreds of fruit-flies (*Drosophila melanogaster*), was used as an olfactometer<sup>3</sup>. The up-wind end of the main observation chamber was closed by a wire screen behind which could be concealed an attractive bait of fermenting bananas which was shown by vapour-phase chromatography to consist of at least ten components. The fruit-flies had no difficulty in locating and congregating on the part of the screen through which the scent was emerging. Up-stream from the screen and the attractive bait, the tunnel narrowed to a 6-in. inlet pipe. A porous polyurethane sponge 2 in. thick could be fitted into this inlet and moistened with various liquids so as to permeate the incoming air before it passed over the bait and through the screen.

When the sponge was treated with 30-50 ml. of ethyl alcohol, the fruit-flies continued to locate the attractive area without difficulty, but when sponges similarly treated with ethyl acetate or amyl acetate were used, the insects' ability to find the lure disappeared entirely. Other substances which were effective in hiding the bait when added to the air stream were acetone and glacial acetic acid (the vapour of which also produced a considerable mortality); while propyl alcohol and 2,3-butanedione ('Biacetyl'), like ethanol, had little if any ability to hide the scent. Geraniol, which has a powerful smell and is strongly attractive to the Japanese beetle but not to *D. melanogaster*, was likewise completely effective in preventing the fruit-flies from congregating in the scent stream from the fermenting fruit.

While these experiments were made with a food odour, there is no reason to suppose that a sex-attractant scent or oviposition lure would not act similarly. If so, the search for practically useful metarchons should be greatly simplified. In place of the tedious process of isolation, identification and synthesis of the components of the natural scent, or the too-often unrewarding mass screening of thousands of organic compounds for signs of attractiveness, all that is required is a direct test of various materials for their ability to interfere with the perception of a specific scent by a particular insect. This could be done, for example, by placing male insects in a wind tunnel,

concealing females behind the inlet screen, and adding the test substances to the air intake. Not only would this provide a direct route to metarhcon formulation, but it would throw considerable light on the olfactory process itself.

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### Damage to Polythene by Aquatic Moths

RECORDS of damage to polythene by insects are now fairly frequent. The larva of the common household pest *Hofmannophila pseudospretella* Stainton (the Brown House or False Clothes moth) is known to eat its way through many man-made products, including polythene, polystyrene, and nylon.

Recently the larvae of an aquatic lepidopteran species were found damaging the polythene linings of ponds (1,000 gauge, approx. 0.25 mm thick). The damage to the inner lining of the pond was caused by the larvae of the moth eating holes through the polythene to the rough paper outer-lining. The larvae then attached themselves to this outer-lining paper before pupating. After the moths emerged the pupal cases were soon washed away, leaving holes of 1–3 mm diameter. This damage occurred both in a greenhouse where the pond was a wood-framed structure with a paper underlay and a polythene lining, and out-of-doors where soil was underneath the polythene. All the holes were at or just above water-level.

It is possible that the very smooth polythene failed to provide a suitable surface on which the larvae could anchor themselves before pupation and that they therefore chewed through to a rougher surface. Perhaps if the polythene surface was rougher the larvae would attach themselves without burrowing through it. The damage was first noticed in 1964 although the polythene had been in use for 7–8 years.

Specific determination of the moths was not possible in the sample examined, as all the pupal cases were empty. However, it is certain that the moths concerned were a species of *Hydrocampinae* (*Pyralidae*) and almost certain that the species was *Nymphula nymphaea* Linnaeus, the Brown China-Mark moth.

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### MICROBIOLOGY

#### Variation in the Chemical Composition of the Cell Walls of *Bacillus subtilis* during Growth in Different Media

DURING recent years the chemical composition of the cell wall has been used as an aid in the classification of micro-organisms<sup>1</sup>. Although differences in the composition of the cell wall have been demonstrated during growth of organisms with: (a) variable morphological forms<sup>2</sup>; (b) complex auxotrophic requirements<sup>3</sup>; (c) addition of D-amino-acids<sup>4</sup>, many investigations have not considered the variations which occur in the amino-acid and amino-sugar content of more stable strains of bacteria during the growth cycle and in response to different growth media. The results presented here demonstrate that the amino-acid and amino-sugar content of the cell wall of *Bacillus subtilis* varies not only with growth but is also

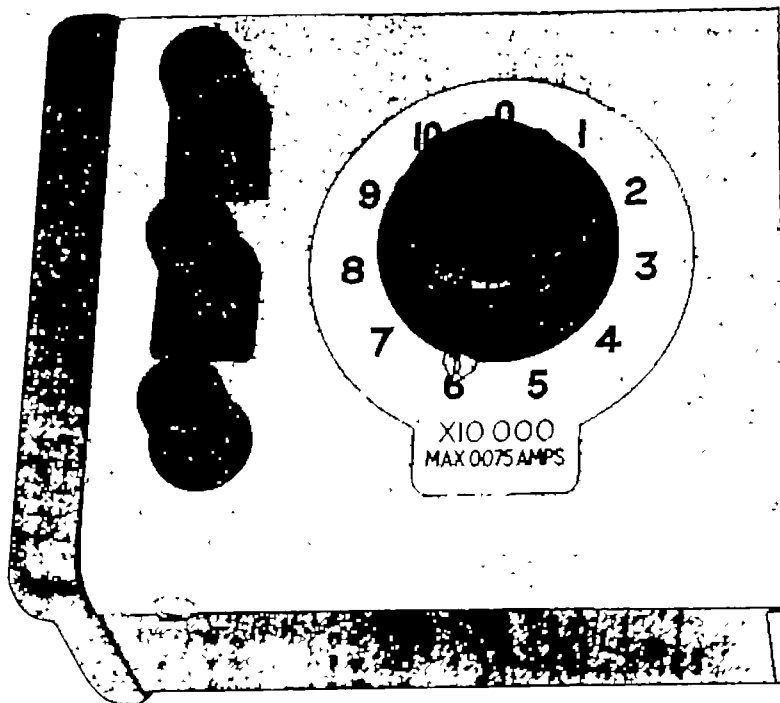
significantly influenced by the composition of the growth medium. Furthermore, galactosamine, one of the major components of the cell wall of the highly transformable strain *B. subtilis* 168 I-O<sup>+</sup>, is consistently decreased when this strain is grown under conditions which do not permit the development of the capacity to bind deoxyribonucleic acid (competence). A marked decrease in galactosamine is also observed in three poorly transformable strains of *B. subtilis*.

*B. subtilis* 168 I-O<sup>+</sup> was grown for various intervals at 37° C with vigorous aeration in 800-c.c. cultures contained in 2-l. flasks. Three media were used: (A) the standard growth medium containing 0.5 per cent glucose, 0.005 M MgSO<sub>4</sub>, 50 µg/ml. L-tryptophan, and 0.02 per cent casein hydrolysate<sup>5</sup>; (B) the previous medium supplemented with 0.4 per cent casein hydrolysate; (C) 'Pen Assay' broth (Difco). *B. subtilis* 168 does not sporulate in these media under the experimental conditions used. The organisms were collected by centrifugation, washed and disrupted by mechanical disintegration<sup>6</sup>. Samples of cell walls were dried over phosphorus pentoxide, hydrolysed at 105° ± 1° C for 11 h in 4 N hydrochloric acid, and evaporated *in vacuo* to remove the hydrochloric acid. The amino-acid and amino-sugar content was determined on the Beckman Spinco amino-acid analyser<sup>7</sup>.

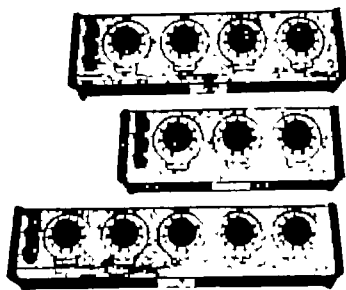
Previous experiments have shown that logarithmic growth in medium A occurs 1.5–5 h after incubation<sup>8,9</sup>. The culture then remains in a stationary period for 3 h before a marked decrease in the number of viable organisms occurs. As shown in Table 1 the major amino-acids and amino-sugars in the cell wall of *B. subtilis* are alanine, glutamic acid, diaminopimelic acid, muramic acid, glucosamine, and galactosamine. During the logarithmic and early stationary phases of growth (1.5–8 h) the amounts of glutamic acid, diaminopimelic acid, and glucosamine do not vary beyond the limit of the accuracy of this method (± 5 per cent on repeated analyses of the major components in the same sample of cell walls). On the other hand, the content of alanine within the cell wall increased during incubation. A consistent decrease in muramic acid was observed after 5 h of growth. The highest concentration of galactosamine occurred after 5 h of incubation which coincided with the time that the population developed maximal competence. Since the galactosamine is confined to the teichoic acid fraction of the cell walls<sup>10</sup>, this two-fold increase in galactosamine might significantly alter the structure of the cell wall. After 24 h of incubation there was an increase in all the major components except galactosamine. The small differences in the minor components (aspartic acid to arginine, Table 1) are not significant.

Previous investigations have demonstrated that growth in nutrient media such as media B and C inhibit the development of competence<sup>8</sup>. As shown in Table 2, there is an increase in alanine and a decrease in galactosamine under these conditions of growth. This decrease in galactosamine was observed after both 3 and 5 h of incubation in 'Pen Assay' broth.

Two additional poorly transformable strains of *B. subtilis* were examined. *B. subtilis* 23 is an excellent donor strain in transformation experiments, but a very poor recipient. *B. subtilis* H is essentially not transformable. As shown in Table 3, the most outstanding difference was in the galactosamine content of the cell wall. At present it is not known whether galactosamine is an integral part of the teichoic acid polymer or is in another polymer which is fortuitously isolated with the teichoic acid fraction during purification. Nevertheless, it is clear that galactosamine varies during the growth cycle and is decreased when the transformable strain is grown in more complex media. In addition, the concentration of galactosamine is markedly decreased in the two poorly transformable strains used in this work and in a poorly transformable mutant of *B. subtilis* 168 (ref. 6).



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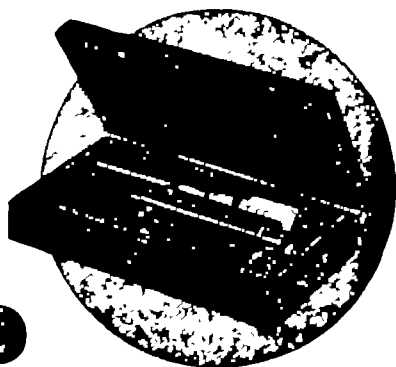


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These results demonstrate that changes occur in the structural mucopolysaccharide of the cell wall during growth and in various growth media. Although such differences are not as marked as those observed in organisms with

pleomorphic growth patterns, they are of sufficient magnitude to warrant the use of a variety of cultural conditions in studies of the structure of the cell wall of micro-organisms.

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### Prevention of Competitive Suppression in Microbial Plating Experiments

An assumption inherent in back-mutation experiments is that at the cell concentrations used 'background' prototrophs in a substantially auxotrophic population will be expressed quantitatively in the form of colonies or turbid culture when the mixed population is incubated on or in the basal medium. This is not always valid<sup>1,2</sup>. Fewer prototrophs may appear than expected when high cell concentrations are used, because the growth of prototrophs is suppressed by the presence of sufficiently large numbers of auxotrophs. This is referred to as 'competitive' suppression<sup>3</sup>. On diluting a suspension of auxotrophic cells to different degrees in basal medium the frequency of back-mutant colonies which arises may increase as the dilution rate increases and the suppression decreases.

Competitive suppression occurs as a result of the removal of carbohydrate from the selective medium by the non-growing (auxotrophic) population so that it is not available for the growth of the mutant or back-mutant species being selected for<sup>1,2</sup>. This starvation can be prevented if an energy source is used which the bulk of the population cannot utilize but which the mutant being selected can. Such a situation is achieved (a) if the microbial strain can adapt to use some particular energy source, for example, a *lac*<sup>+</sup> (*tyr*<sup>+</sup>) strain of *Escherichia coli* on lactose medium, and (b) if the mutation being studied is a back-mutation from an amino-acid requirement to a non-requirement, that is, prototrophy. If the *lac*<sup>+</sup> strain and lactose are chosen as the strain and substrate respectively the bacteria should be grown under conditions which prevent adaptation to lactose utilization, and starved of the required amino-acid to exhaust the metabolic pool. Back-mutants can then be selected on medium containing lactose as the energy source. Since the latter would be able to synthesize the required amino-acid they could make  $\beta$ -galactosidase and hence could utilize lactose for growth. The auxotrophs, on the other hand, would be unable to make the enzyme and thus could not utilize the sugar.

Should it be desirable to allow several replications of the microbial population on or in the selective medium, sufficient glucose should be added to the lactose medium with the amino-acid supplement to leave an excess of glucose after the complete utilization of the amino-acid to ensure that adaptation to lactose is repressed.

Table 1 presents the data of two experiments to illustrate the effectiveness of this experimental design in eliminating competitive suppression. Overnight cultures of *trp*<sup>-</sup> (*wp*, Witkin<sup>4</sup>) bacteria grown to exhaustion of glucose were incubated in a glucose basal medium for 3 h to exhaust their amino-acid pool of tryptophan. Aliquots of the washed bacteria were spread on glucose and lactose

Table 1. INFLUENCE OF DURATION OF GROWTH ON THE CHEMICAL COMPOSITION OF THE CELL WALLS OF *Bacillus subtilis*

Component	0	15	Duration of growth (h)					24
			3	5	6	8	10	
			(μmoles/mg)					
Alanine	0.58	0.70	0.78	0.74	0.86	0.83	0.81	0.83
Glutamic acid	0.37	0.34	0.36	0.33	0.36	0.37	0.39	0.51
Diaminopimelic acid	0.40	0.32	0.33	0.30	0.30	0.32	0.33	0.45
Muramic acid	0.22	0.26	0.29	0.18	0.23	0.23	0.24	0.25
Glucosamine	0.34	0.32	0.31	0.28	0.32	0.32	0.31	0.40
Galactosamine	0.12	0.12	0.17	0.23	0.20	0.17	0.15	0.17
Aspartic acid	0.04	0.04	0.05	0.05	0.06	0.04	0.06	0.07
Threonine	0.02	0.02	0.02	0.03	0.03	0.03	0.03	0.04
Serine	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.04
Proline	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Glycine	0.05	0.04	0.04	0.04	0.04	0.03	0.05	0.08
Valine	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.03
Isoleucine	0.02	0.02	0.03	0.03	0.02	0.02	0.02	0.03
Leucine	0.04	0.03	0.04	0.03	0.03	0.03	0.03	0.05
Tyrosine	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.02
Phenylalanine	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.02
Lysine	0.06	0.04	0.04	0.04	0.04	0.03	0.04	0.04
Histidine	0.01	0.01	0.01	0.01	0.03	0.02	0.02	0.02
Arginine	0.03	0.01	0.01	0.02	0.02	0.02	0.01	0.02
Ammonia	0.61	0.52	0.55	0.49	0.51	0.51	0.48	0.52

*B. subtilis* 168 I-C<sup>+</sup> was grown for various intervals in medium A. Maximal competence occurs after 5 h of growth. The frequency of transformation usually varies from 0.4 to 0.8 per cent at this time. An additional 3-8-fold increase in transformation can be obtained if the cells are centrifuged and resuspended in the transformation medium described previously<sup>4</sup>.

Table 2. INFLUENCE OF MEDIUM ON THE CHEMICAL COMPOSITION OF THE CELL WALLS OF *Bacillus subtilis*

Components	Medium B		Medium C	
	5 h	3 h	5 h	5 h
		(μmoles/mg)		
Alanine	0.82	1.11		1.02
Glutamic acid	0.30	0.25		0.33
Diaminopimelic acid	0.29	0.24		0.30
Muramic acid	0.25	0.23		0.23
Glucosamine	0.26	0.28		0.25
Galactosamine	0.16	0.12		0.10
Aspartic acid	0.04	0.06		0.06
Threonine	0.02	0.04		0.03
Serine	0.02	0.03		0.04
Proline	0.01	0.01		0.01
Glycine	0.03	0.05		0.06
Valine	0.02	0.03		0.03
Isoleucine	0.02	0.02		0.02
Leucine	0.02	0.03		0.03
Tyrosine	0.01	0.01		0.02
Phenylalanine	0.01	0.01		0.02
Lysine	0.03	0.05		0.06
Histidine	0.01	0.01		0.01
Arginine	0.01	0.02		0.02
Ammonia	0.49	0.68		0.48

*B. subtilis* 168 I-C<sup>+</sup> was grown for 5 h in the minimal medium containing 0.4 per cent casein hydrolysate (medium B) and in the 'Pen Assay' medium (medium C) for 3 h and 5 h. Under these conditions of growth the frequency of transformation is 100-1,000-fold lower than that obtained after 5 h of growth in medium A.

Table 3. VARIATION IN COMPOSITION OF THE CELL WALLS IN OTHER STRAINS OF *Bacillus subtilis*

Component	<i>B. subtilis</i> 23 (μmoles/mg)	<i>B. subtilis</i> H (μmoles/mg)
Alanine	0.68	0.84
Glutamic acid	0.30	0.36
Diaminopimelic acid	0.28	0.34
Muramic acid	0.21	0.21
Glucosamine	0.32	0.28
Galactosamine	0.01	0.01
Aspartic acid	0.02	0.06
Threonine	0.02	0.06
Serine	0.02	0.06
Proline	0.01	0.01
Glycine	0.03	0.06
Valine	0.03	0.03
Isoleucine	0.01	0.04
Leucine	0.02	0.06
Tyrosine	0.01	0.01
Phenylalanine	0.01	0.01
Lysine	0.03	0.06
Histidine	0.01	0.01
Arginine	0.01	0.01
Ammonia	0.52	0.60

*B. subtilis* 23 and *B. subtilis* H were grown for 5 h in medium A. The frequency of transformation is 5,000- and 50,000-fold lower respectively in these strains than the highly transformable strain at a comparable period of growth.



Table 1. NUMBER OF *tryp*<sup>+</sup> REVERTANT COLONIES DETECTED AFTER SPREADING *tryp*<sup>-</sup> BACTERIA ON GLUCOSE AND LACTOSE MINIMAL AGAR

	Glucose (0.5%)	Lactose (0.5%)	
Experiment 1	17*	25	
	27*	38	
	16*	25	
	10*	32	
	19*	28	
	80	146	
<i>tryp</i> <sup>+</sup> /plate	17.8 ± 2.7	28.2 ± 2.1	
<i>tryp</i> <sup>-</sup> bacteria/plate	1.06.10 <sup>10</sup>	1.06.10 <sup>10</sup>	
Experiment 2	3*	283	25
	20*	277	17
	24*	269	33
	68*	267	39
	125	1,096	199
<i>tryp</i> <sup>+</sup> /plate	31.25	274.0	28.4
<i>tryp</i> <sup>-</sup> bacteria/plate	1.34.10 <sup>10</sup>	1.34.10 <sup>10</sup>	1.84.10 <sup>9</sup>

\*Minute colonies.

basal media and the numbers of prototrophs scored. In one experiment different numbers of bacteria were spread on lactose medium to look for a dilution effect.

In experiment 1 (Table 1) significantly more *tryp*<sup>+</sup> colonies appeared on the lactose than on the glucose medium. Moreover, the *tryp*<sup>+</sup> colonies in the latter were minute in size. When the number of *tryp*<sup>-</sup> bacteria was increased by 30 per cent in experiment 2, the discrepancy between the numbers of *tryp*<sup>+</sup> colonies observed became considerably greater—presumably more were suppressed. Also the variance of the mean number of prototrophs was much higher on the glucose medium group. This is a common feature of the suppression effect when the number of suppressing bacteria reaches a threshold value<sup>2</sup>.

On the lactose plates a strict proportionality between the number of *tryp*<sup>-</sup> bacteria plated and *tryp*<sup>+</sup> colonies observed was found (exp. 2, Table 1), that is, there was no 'dilution effect' and hence no competitive suppression.

Besides avoiding minor or major errors in plating experiments the use of this selection technique, by preventing competitive suppression, has made it safe to use high densities of bacteria in selection experiments. It should be of general applicability in a modified form to other microbial species.

It has the further particular advantage that in any experiment requiring bacteria to remain on the selective medium for a relatively long period the use of non-adapted cells and lactose as the energy source almost entirely prevents cell death<sup>3</sup> and the complication this can introduce in assessing viabilities.

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## VIROLOGY

### Replication Cycle of an Insect Granulosis Virus

WHEN a lepidopterous larva such as a cutworm (Noctuidae) in a late stage of infection with a granulosis virus is shaken up in distilled water, as much as 10 ml. of a



Fig. 1. Section through two granules, side by side in the same cells; note the apparent extrusion and elongation of the virus rod, the outer membrane now encloses only half the rod (arrows), note also an apparent membrane surrounding the granules. ( $\times 82,500$ )



Fig. 2. Low power electron micrograph of a section through a mass of branching virus threads inside the cell. ( $\times 40,000$ )

fluid of the consistency of thin cream is obtained. This consistency is due to the immense numbers of protein crystals, the granules, each containing a short virus rod, which are liberated from the insect's tissues. Centrifugation of this fluid for 30 min at 5,000 r.p.m. causes the granules to form a sediment and gives a clear supernatant with a greenish opalescence. When this opalescent fluid is examined with the electron microscope it is found to contain large numbers of long virus-like threads branched in an intricate manner.

This communication is concerned with the origin of these threads and the part they are thought to play in the replication cycle of the virus.

In a previous communication<sup>1</sup> the suggestion was made that the short thick virus rod emerged from its occluding crystal, lengthened in the process and commenced to branch. Since these observations were all made on purified preparations of the virus threads and their concomitant granules, the criticism could be made that some of these phenomena were artefacts arising from the negative staining with phosphotungstic acid. The peculiar

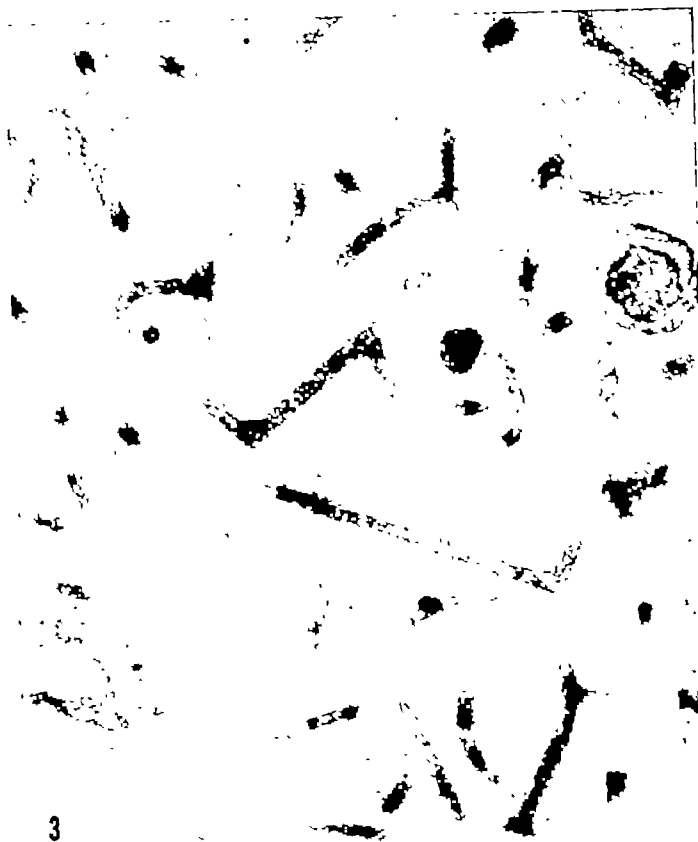


Fig. 3. Similar to Fig. 2 but at higher magnification; the branching of the threads is clearly visible. ( $\times 100,000$ )



Fig. 4. A long virus thread negatively stained with phosphotungstic acid, note the internal helix (arrow). ( $\times 100,000$ )

branching of the virus might also be attributed to adsorption of the various lengths.

Further investigations have now been made, but this time on thin sections of infected tissue fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. The changes described here all occurred inside the cell itself. Based on these results, an explanation is offered of the origin of the long virus threads and of their role in the replication of the virus. The cycle proposed is a tentative one, but it appears to fit most of the observed phenomena.

As previously suggested<sup>1</sup>, the initial step in the replication cycle seems to be the extrusion of the short thick virus rod from its occluding protein crystal, the granule. To this end there appears to be a cap on one end of the crystal which lifts up to allow exit of the virus particle. In addition to being occluded in the crystal, the virus is also completely enclosed in an outer membrane.

Numerous examples have now been observed of this extrusion process occurring inside the cell. As the rod emerges from the crystal it increases in length and decreases in diameter; the outer membrane in which the particle was originally completely enveloped is sometimes pushed out with it. In Fig. 1 are sections of two crystals side by side in the same cell; in one is the short thick virus rod with its enveloping membrane. In the other the virus rod is in process of emergence, and it is noteworthy that the outer membrane, pushed out by the emerging rod, now envelops only half its length. Empty membranes and the U-shaped empty granules can be found loose in the cell.

It is of interest to speculate on the nature of the stimulus which causes the virus rod to emerge from the crystal.

Perhaps the most interesting phenomenon in this replication process is the apparently unlimited and uncontrolled branching of the long virus threads, a phenomenon that, so far as we know, is unique among viruses. That the branching is a genuine phenomenon and that it occurs inside the cell cytoplasm are shown in Figs. 2 and 3. The reason for the branching is at present obscure, unless it is a means of increasing the DNA which must be inside the long thread.

High-resolution electron micrographs of the virus threads suggest that they are composite with an inner helical core. Fig. 4 shows one of these threads negatively stained with phosphotungstic acid. The stain has apparently entered, possibly by a small break in the outer coat, and delineated the internal helix.

Presumably the next step in the replication process is the break-up of the long thin virus threads into the short rods. Lying free in the cell cytoplasm there occur single short rods, some with, and some without, their outer membrane. The last stage in the replication process seems to be the deposition on the finished virus rod of the protein to form the occluding granule as originally suggested by Hughes<sup>2</sup>.

We thank Dr. Hilton Mollenhauer for assistance with the electron microscopy.

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<sup>1</sup> Smith, Kenneth M., Trondl, Z. M., and Frist, R. H., *Virology*, **24**, 508 (1964).  
<sup>2</sup> Hughes, K. M., *J. Bact.*, **64**, 375 (1952).

## GENETICS

## Inheritance of a Correlated Day-length Response in Spring Wheat

In the course of genetic and physiological studies of developmental processes leading to flowering in spring wheats a major gene conditioning a positive response to vernalization was detected in the variety 'Insignia 49' (ref. 1). Further investigations have now led to the recognition of a second gene governing the photoperiodic response of the Canadian variety 'Selkirk'.

The work was based on two varieties—'Triple Dirk', a back-cross derivative of the Australian variety 'Dirk 48', and 'Selkirk'. I have found (in unpublished work) that 'Triple Dirk' is not responsive to vernalization, and although no tests have yet been undertaken, it seems likely that 'Selkirk' would be unresponsive as are so many Canadian varieties.

When grown at Wagga Wagga (latitude 35° 5' S) during the long days of summer (day-length 13.5–14.5 h) 'Triple Dirk' and 'Selkirk' are comparable in maturity. Sown on December 26, 1963, the two varieties came into ear in 52 days. When sown in the greenhouse in the autumn and grown throughout the cooler short days of winter (day-length 10.5–11.5 h), flowering of both is delayed, with 'Selkirk' much more sensitive than 'Triple Dirk'. Sown on April 15, 1964, the number of days to ear emergence for 'Triple Dirk' and 'Selkirk' was 94 and 132 respectively.

An *F*<sub>1</sub> and *F*<sub>2</sub> of the cross 'Selkirk' × 'Triple Dirk' and the *F*<sub>1</sub> of the back-cross ('Selkirk' × 'Triple Dirk') × 'Selkirk' sown at the same time showed: (a) almost complete dominance of earliness; (b) an *F*<sub>2</sub> ratio of 34 early : 7 late, (c) an *F*<sub>1</sub> back-cross ratio of 23 early : 22 late. The

two segregations fit 3:1 and 1:1 ratios with *P* values > 0.2 and > 0.8 respectively. The distribution of the segregating populations is set out in Figs. 1 and 2.

It is of interest to report a complete association of maturity with the number of leaves on the primary tillers at ear emergence and also with the number of spikelets on the same primary tillers. The relevant data for the parents, the *F*<sub>1</sub> and the two segregating populations are set out in Table 1.

Table 1. CORRELATED BEHAVIOUR OF TIME TO EAR EMERGENCE AND NUMBER OF LEAVES AND SPIKELETS ON PRIMARY TILLERS

	Range in number of days to ear emergence	Range in number of leaves on primary tillers	Range in number of spikelets on ears of primary tillers
'Selkirk'	130–134	9–10	25–27
'Triple Dirk'	92–97	7–8	16–17
'Selkirk' × 'Triple Dirk', <i>F</i> <sub>1</sub>	93–96	7–8	16–18
'Selkirk' × 'Triple Dirk', <i>F</i> <sub>2</sub> early segregates	85–120	7–8	17–23
'Selkirk' × 'Triple Dirk', <i>F</i> <sub>2</sub> late segregates	131–138	9–10	23–27
('Selkirk' × 'Triple Dirk') × 'Selkirk', <i>F</i> <sub>1</sub> early segregates	93–112	7–8	17–23
('Selkirk' × 'Triple Dirk') × 'Selkirk', <i>F</i> <sub>1</sub> late segregates	125–133	9–10	23–27

The early and late classes of both segregating populations were separated by gaps of 18 days for the *F*<sub>2</sub> and 11 days for the *F*<sub>1</sub> back-cross. There was no inter-class overlap with respect to the leaf number and spikelet number classes.

As far as the writer is aware this is the first occasion in which a clearly defined monohybrid segregation governing a correlated response to short days has been reported in *Triticum*. This finding is significant in both its basic and applied aspects. The presence of a single gene pair conditioning the behaviour of wheat varieties to short days would suggest the operation of a simple physiological process leading to ear initiation. Similarly, the simple mode of inheritance makes possible a much greater degree of control by the plant breeder in fashioning varieties adapted to specific day-length environments. Maturity control may now be achieved through the use of specific major genes operative through either a vernalization or a day-length control mechanism.

Just as the spikelet number on the primary ear of a given variety may vary with the day-length to which the plants are exposed prior to ear initiation, so too is it under genetic control in a segregating population such as that described herein. Furthermore, the association of spikelet numbers with maturity differences has many implications in wheat improvement programmes as well as in agronomic practice.

Unpublished investigations by me suggest that additional genes may be involved in other crosses between sensitive and less sensitive varieties.

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<sup>1</sup> Pugley, A. T., *Austral. J. Agric. Res.*, 14, 622 (1963).

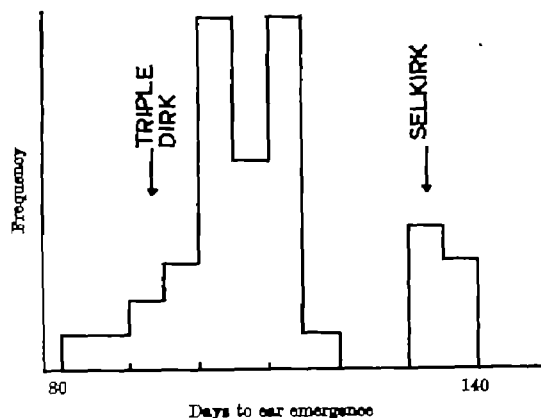


Fig. 1. Distribution of *F*<sub>2</sub> segregates from the cross ('Selkirk' × 'Triple Dirk') with respect to days from sowing to ear emergence

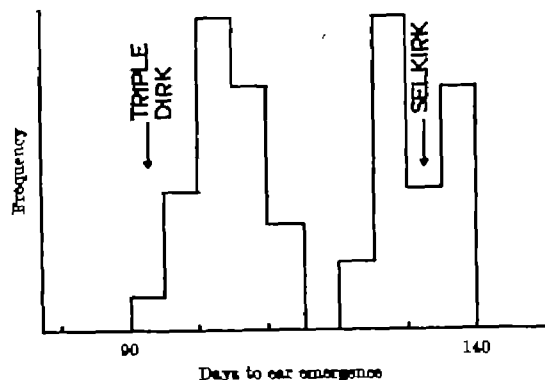


Fig. 2. Distribution of segregates from the back-cross ('Selkirk' × 'Triple Dirk') × 'Selkirk' with respect to days from sowing to ear emergence

## ABO Blood-groups in Leukaemia

It has been shown that the blood group A is of some importance in the multifactorial genetic system of sarcooidosis<sup>1,2</sup>. It may be postulated that the prevalence of one blood group is similarly important in other pathological conditions under multifactorial genetic influence. Some examples are set out in Table 1.

Genetic factors are known to play a part in the incidence of leukaemia, and it seems to me to be quite conceivable that they are of a multifactorial nature. I therefore studied the incidence of the ABO blood-group system in

Table 1. ABO BLOOD-GROUP IN CONDITIONS WITH MULTIFACTORIAL GENETIC SYSTEMS

	No. sample control	No.		Incidence	$\chi^2$	P Probability	P Heterogeneity
		Patients	Comparison				
Tuberculosis*	10	4,506	A:O	1:186	18.19	5.10 <sup>-2</sup>	0.06
Sarcoidosis†	1	518	A:O	1:143	18.23	10 <sup>-2</sup>	—
Periculous anaemia‡	9	1,498	A:O	1:25	16.84	6.10 <sup>-2</sup>	0.2
Diabetes mellitus*	5	2,450	A:O	1:14	10.8	8.10 <sup>-2</sup>	0.04
Ulous duodenit‡	9	8,272	O:A	1:35	144.53	10 <sup>-18</sup>	0.004
Ulous ventriculit‡	9	8,999	O:A	1:16	18.84	1.5.10 <sup>-2</sup>	0.5

\* Collected by Jørgensen (1963).  
† By Jørgensen and Wurm (1963).  
‡ Collected by Roberts (1967, 1956).

Table 2. RELATIVE INCIDENCE OF ABO BLOOD-GROUP IN LEUKAEMIA

Type of leukaemia	No. of Patients	No. of Controls	Comparison	Relative Incidence Type A (m=1)	$\chi^2$	P Probability
Acute leukaemia	78	694	A:O	1.032	0.0162	0.92
Chronic granulocytic leukaemia	104	694	A:O	1.106	0.2247	0.63
Collection of acute and chronic types	182	694	A:O	1.119	0.0425	0.83

leukaemia. No statistically significant relation was found between leukaemia and the ABO blood groups (Table 2).

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<sup>1</sup> Jørgensen, G., *Hæbl. Schr.* Göttingen (1963).  
<sup>2</sup> Jørgensen, G., and Wurm, K., *Nature*, **203**, 1095 (1964).  
<sup>3</sup> Roberts, J. A. F., *Brit. Med. Bull.*, **15**, 129 (1959).

## PSYCHOLOGY

### Effects of Age on the Recall of Dichotic Words

A RECENT report by Inglis<sup>1</sup> appears to demonstrate unequivocally that a short-term storage mechanism of the type postulated by Broadbent<sup>2</sup> is required if an individual is to recall correctly a series of dichotic digits. Furthermore, this storage mechanism becomes less efficient as age increases. Briefly, Broadbent has suggested that both perceptual and storage mechanisms are necessary in a dichotic situation. There is sequential recall, and one half of the dichotic series is held in temporary storage while the other half is being recalled. Once the first half-set has been reproduced, the stored, second half-set becomes available for recall. It is this second half-set which is subject to deterioration with advancing age. This phenomenon has been previously demonstrated<sup>3,4</sup>, but the possibility existed that these findings might also be explained in terms of other age-related changes in the nervous system. The report by Inglis<sup>1</sup> appears to preclude these possibilities. His subjects were 120 people aged 11-70. By specifying the order of recall, either before or after presentation of the stimuli, he was able to show that the results could not be attributed to age changes in perception and/or attention. That is, there was no evidence of failure to hear or to attend to the stimuli.

We have carried out a further experiment in an attempt to extend the generality of these findings. The preliminary results are reported here. The problem was to determine if non-numerical stimuli are processed in the same way as digits. That is, if an individual is presented with dichotic words, will these be recalled sequentially and, if so, will the ability to do this be related to age?

Subjects were 120 people aged 11-70. There were 20 (10 male and 10 female) in each age decade. The series of stimulus words, for example, floor, hair, grass, girl, ranged in length from one to four per half-set. The subjects were free to recall first whichever half-set they wished. This procedure was identical to earlier investigations<sup>3,4</sup>, the only difference being the nature of the stimuli.

The results were virtually identical to those using digits<sup>1,3,4</sup>. There was no significant impairment in the

ability to recall the first half-sets. There was, however, progressive and significantly greater difficulty in recalling the second half-sets as age increased. In addition, the longer the series to be recalled, the greater the overall difference between first and second half-sets.

These results are in remarkably close accord with those already cited and reinforce the notion that there is an age-related decline in the efficiency of a short-term memory storage process and this is true whether the material to be remembered is digits or words.

This work was supported by a Federal-Provincial Mental Health Grant (project No. 609-5-145). The testing was carried out by Mr. R. P. Jones.

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<sup>1</sup> Inglis, J., *Nature*, **204**, 103 (1964).  
<sup>2</sup> Broadbent, D. E., *Perception and Communication* (London: Pergamon Press, 1958).  
<sup>3</sup> Inglis, J., and Caird, W. K., *Canad. J. Psychol.*, **17**, 98 (1963).  
<sup>4</sup> Mackay, H. A., and Inglis, J., *Gerontologia*, **3**, 193 (1963).

### Hippocampal Ablation in Rats: Effects of Intertrial Interval

DEFICITS in recent memory following damage to the hippocampus have been reported in man<sup>1</sup> and in the monkey<sup>2</sup>. In a recent investigation<sup>3</sup>, we tested the hypothesis that the hippocampus is involved in recent memory functions by using different intertrial intervals in runway acquisition and extinction. The method consisted of administering acquisition and extinction trials to rats under either massed or distributed practice conditions. It was found that the temporal spacing of trials during acquisition was not important but that distributed practice trials served to increase resistance to extinction for hippocampal-damaged rats. The purpose of the work reported here was to clarify the earlier results by determining whether the extinction phenomenon was due to the spacing of trials during acquisition or during extinction. In this work, each subject received both massed and distributed trials during acquisition so that, by the end of this phase of training, all subjects had equal experience with each of the two intertrial intervals. At that time the subjects were extinguished under either massed or distributed practice conditions.

Twenty-four male rats of the Long-Evans strain were used. Age of the rats at the time of operation was 100-120 days. Twelve subjects had the hippocampus and overlying cortical tissue removed bilaterally by aspiration and 12 had cortical tissue overlying the hippocampus removed and thus served as operated cortical controls. Unoperated control subjects were not included since results of the previous investigation indicated similar performance for both operated and unoperated control subjects.

Twelve days after operation the subjects were placed on a deprivation schedule of 10 g of laboratory chow daily. Preliminary training was started eight days later and consisted of exploratory trials in the runway without food. After two days of preliminary training the subjects were

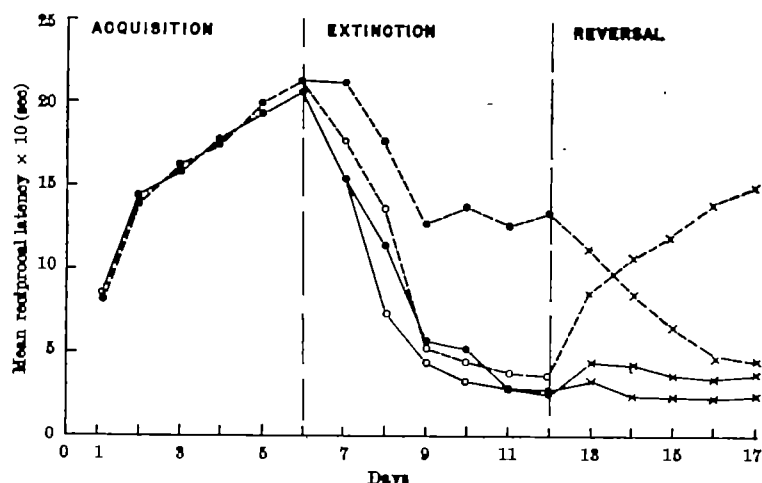


Fig. 1. Mean reciprocal latency in seconds for groups on successive days of acquisition, extinction, and reversal training. —○—, Hippocampal massed; - -○- -, hippocampal distributed; —●—, cortical control massed; - -●- -, cortical control distributed

given 10 acquisition trials on each of 6 consecutive days. Response latencies and running times were measured by timers activated and stopped by photoelectric cells. The two intertrial-interval conditions of 10 sec and 10 min were administered to the subjects on alternate days in a counterbalanced order. Thus, at the end of six days of acquisition all subjects had run an equal number of trials with each of the two intertrial intervals.

After 60 acquisition trials, the hippocampal and cortical control groups were each divided into two sub-groups which were equated for performance during acquisition. These sub-groups were assigned to 10 sec and 10 min intertrial-interval conditions, respectively. Ten extinction trials a day were carried out for six days, the intertrial interval for an animal being the same throughout this extinction period. If a subject did not reach the food cup within 60 sec, it was removed from the runway, and latency and running times for the trial were recorded as 60 sec.

It was originally planned to terminate the investigation at the end of the extinction period. However, after inspection of acquisition and extinction data we decided to continue running the rats without food but with intertrial-interval conditions for the animals reversed from those under which they had been extinguished. The rats were given 10 trials on each of 5 days under these reversed intertrial-interval conditions.

The data were converted to speed scores by taking the reciprocal of each measure for each subject. Means of the 10 daily reciprocal latency and running time measures were then computed and used in the various analyses. Analysis of variance was used with acquisition and reversal data, and analysis of co-variance with the extinction data. A level of significance of  $P = 0.05$  was used throughout. The most important results are summarized in Fig. 1 where mean reciprocal latencies in seconds are plotted for successive days. Analysis of both latencies and running times indicated that acquisition was similar for the different groups. However, during extinction hippocampal subjects showed significantly faster latencies than cortical control subjects. Further analysis indicated that those hippocampectomized animals which received distributed extinction trials showed significantly faster latencies than both massed-practice-hippocampal subjects and control animals. An analysis of extinction running times did not show any differences between the various groups.

During the period of reversed intertrial intervals, hippocampal subjects showed faster latencies than controls, but running times did not differ. Further analysis of the latency data indicated that animals with hippocampal destruction which were changed from massed to distributed

trials were significantly faster than similar animals changed from distributed to massed trials on reversal days 3, 4 and 5 but not on days 1 and 2. Thus, a significant reversal in latencies accompanying the change in intertrial intervals was not immediate but developed only after two days of running. Inspection of Fig. 1 indicates that the latencies for distributed-practice-hippocampal subjects on extinction day 6 and reversal day 5 are at approximately the same level.

Histological examination of the brains revealed extensive bilateral lesions involving the hippocampal formation and overlying neocortex in all subjects in the hippocampal group. Lesions varied in size from 50 to 85 per cent destruction of the hippocampus. Examination of sections made from animals with only neocortical lesions revealed more neocortex destroyed in these animals than in the hippocampal subjects although the total amount of brain destruction was slightly greater in the hippocampal-lesioned group.

(Further anatomical information will be supplied on request from one of us (L. E. J.).)

The results recorded here agree with our previous findings which showed no differences between hippocampal ablated and partial neocorticate subjects in runway acquisition but an increase in resistance to extinction (as indicated by latency scores) for those hippocampal-damaged animals which received distributed extinction trials. Since the procedure in the present investigation was altered so that all subjects had equal experience with the different intertrial intervals during acquisition, the slower extinction of hippocampectomized animals under distributed practice must be due to processes occurring during the extinction period. Reversing the intertrial intervals after six days of extinction resulted in an exchange of latency scores between groups of hippocampal-damaged subjects: those hippocampals which had received distributed trials during extinction developed significantly slower latencies and those hippocampals which had received massed extinction trials (and had been 'extinguished') began quickly moving out of the start box again. It is significant that the switch in latencies did not occur immediately after the reversal of intertrial intervals but appeared only after several days of running under the reversed conditions.

The present results clarify the earlier findings by showing that the slower 'extinction' of distributed-practice-hippocampal subjects is determined by partially reversible effects of a variable operating during extinction training. Further investigations will be needed to determine the specific nature of this variable. However, the present findings suggest that the temporal spacing of trials for hippocampal-damaged subjects is an important variable that should probably be taken into consideration in future studies involving this and related limbic structures.

This work was carried out while the first author was in a National Science Foundation Research Participation Programme at the University of Michigan, 1964. Additional funds were supplied by grant DA-MD-49-193-64-6120 from the Office of Surgeon General, U.S. Army, to R. L. Isaacson. We thank Sheldon Zeck for his assistance.

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<sup>1</sup> Milner, B. and Penfield, W., *Trans. Amer. Neurol. Assoc.*, 80, 42 (1955)

<sup>2</sup> Steplen, L. S., Cordeau, J. P., and Rasmussen, T., *Brain*, 83, 470 (1960).

<sup>3</sup> Jarrard, L. E., Isaacson, R. L., and Wickelgren, W. O., *J. Comp. Physiol. Psychol.*, 57, 442 (1964)

## FORTHCOMING EVENTS

Wednesday, July 7

ROYAL SOCIETY OF MEDICINE (at 1 Wimpole Street, London, W.1), at 5.30 p.m.—Annual Meeting.

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER IN PHARMACOLOGY—The Registrar, The University, Manchester, 12, quoting Ref. 127/65 (July 7).

ASSISTANT LECTURER IN THE DEPARTMENT OF GEOGRAPHY—The Secretary, University College, Gower Street, London, W.C.1 (July 7).

COLLEGE RESEARCH FELLOWSHIP tenable in one of the following subjects: PURE MATHEMATICS; APPLIED MATHEMATICS; PHYSICS; CHEMISTRY; GEOLOGY; GEOGRAPHY; BOTANY; ZOOLOGY; METALLURGY; ENGINEERING (Mechanical, Civil, Electrical, Industrial); and CHEMICAL ENGINEERING—The Registrar, University College of Swansea, Singleton Park, Swansea (July 10).

LECTURER IN PHYSICAL CHEMISTRY—Prof. R. M. Barrer, F.R.S., Chemistry Department, Imperial College of Science and Technology, London, S.W.7 (July 10).

LECTURER or ASSISTANT LECTURER (with experience and interest in any branch of physical research, but some preference will be given to those with interest in low temperatures, N.M.R. or elementary particles) IN PHYSICS—The Deputy Secretary, The University, Southampton (July 10).

ASSISTANT (honours graduate) IN THE DEPARTMENT OF GEOGRAPHY—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (July 11).

ASSISTANT LECTURER IN STATISTICS IN THE DEPARTMENT OF MATHEMATICS—The Registrar, The University, Nottingham (July 12).

CLINICAL PSYCHOLOGIST (Principal Grade) to take charge of the Department—The Medical Superintendent, Rampton Hospital, Retford, Nottingham (July 12).

HYDROCARBON RESEARCH GROUP STUDENT (man or woman) IN THE CHEMISTRY DEPARTMENT for research in infra-red spectroscopy—The Secretary, Royal Holloway College (University of London), Englefield Green, Surrey (July 12).

PIG INDUSTRY DEVELOPMENT AUTHORITY POSTGRADUATE SCHOLAR (graduate in veterinary science, agriculture, or a biological science) IN THE SUB-DEPARTMENT OF ANIMAL HUSBANDRY at the University of Liverpool's Veterinary Station, to participate in a research programme on the social behaviour of pigs (applications from students who expect to qualify in July, 1965, will be considered)—The Registrar, The University, Liverpool, quoting Ref. CV/135/W (July 12).

LECTURER or ASSISTANT LECTURER (with research interests in psychobiology, in personality and individual differences, or in some area of social psychology) IN PSYCHOLOGY—The Deputy Secretary, The University, Southampton (July 14).

RESEARCH ASSISTANT (with a good degree in agriculture or agricultural science, and an interest in or experience of experimental work) IN THE DEPARTMENT OF AGRICULTURE, to carry out experimental work in crop production—The Assistant Bursar (Personnel), The University, Reading, Berkshire (July 14).

ADMINISTRATIVE OFFICER (with postgraduate experience in experimental physics) to be responsible to the Professor of Physics for general laboratory administration, including academic planning and the design of the new laboratories—The Secretary, University of Lancaster, Bailrigg House, Lancaster (July 15).

RESEARCH ASSISTANT (with a good honours degree in botany and a special interest in ecology) to undertake investigations on the ecology and hydrology of Borth and Tregeon bogs—The Registrar, University College of Wales, Aberystwyth (July 15).

SENIOR DEMONSTRATOR IN PHYSICAL CHEMISTRY—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (July 15).

ASSISTANT LECTURER IN STATISTICS (in the Unit of Biometry) for duties which will involve teaching, consulting and research—The Registrar (Room 22, O.R.B.), The University, Reading (July 16).

CHAIR OF PHILOSOPHY; CHAIR OF EDUCATION; CHAIR OF GEOGRAPHY; and CHAIR OF GEOLOGY or GEOGRAPHY at Macquarie University, Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, July 16).

COMPUTER MANAGER (with organizational ability and considerable experience of computer systems) to be responsible for the day-to-day operation of a large time-sharing computing system scheduled for installation in December 1965 and for the provision of programming assistance to computer users—The Deputy Secretary, The University, Southampton (July 17).

SENIOR LECTURER and a LECTURER IN THE DEPARTMENT OF CHEMICAL ENGINEERING, University of the Witwatersrand, Johannesburg, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, July 17).

ASSISTANT CHIEF POLLUTION AND FISHERIES OFFICER (qualified engineer or chemist, with wide practical experience of sewage works management and design) IN THE POLLUTION AND FISHERIES DEPARTMENT of the Trent River Authority—The Chief Pollution and Fisheries Officer, Trent River Authority, Meadow Lane, Nottingham (July 19).

LECTURER (graduate in medicine, microbiology, or biochemistry) IN BACTERIOLOGY—The Secretary to the College, Trinity College, Dublin, 2, Republic of Ireland (July 19).

RESEARCH ASSISTANT (preferably with experience of digital computation) IN THE DEPARTMENT OF MATHEMATICS, to work on the Murrup differential equation approach to nuclear structure, under the direction of Prof. H. A. Jahn—The Secretary and Registrar, The University, Southampton (July 19).

RESEARCH ASSISTANT and a RESEARCH OFFICER (preferably graduates with experience in computer programming in) THE COMPUTING OFFICE, to assist in computer programming arising from research in all departments of the University—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (July 20).

SCIENTIFIC OFFICER (with an honours degree in botany, agriculture or forestry, preferably supplemented by additional study of plant pathology) IN THE PLANT PATHOLOGY DIVISION of the Ministry of Agriculture, mainly for forest pathology work—The Secretary, Civil Service Commission, Stormont, Belfast, Northern Ireland (July 20).

HEAD OF THE CHEMICAL AND ANIMAL NUTRITION DIVISION IN THE MINISTRY OF AGRICULTURE; and CHAIR OF AGRICULTURAL CHEMISTRY IN THE FACULTY OF AGRICULTURE of Queen's University of Belfast (combined post)

—The Secretary, Civil Service Commission, Stormont, Belfast, Northern Ireland (July 23).

HEAD OF THE PLANT PATHOLOGY DIVISION IN THE MINISTRY OF AGRICULTURE; and CHAIR OF MYCOLOGY AND PLANT PATHOLOGY IN THE FACULTY OF AGRICULTURE of Queen's University of Belfast (combined post)—The Secretary, Civil Service Commission, Stormont, Belfast, Northern Ireland (July 23).

ASSISTANT EXPERIMENTAL OFFICER (with a degree or equivalent qualifications) with the Soil Survey of England and Wales, to assist in mapping soils. Duties involve describing soils in the field, collecting agricultural and other data—The Secretary, Rothamsted Experimental Station, Harpenden, Herts, quoting Ref. 1062/70 (July 24).

CHAIR OF CHEMISTRY—The Registrar, The University, Leeds, 2 (July 25).

READER IN CLINICAL PHYSIOLOGY; and a LECTURER IN PHYSIOLOGY at Makerere University College (University of East Africa), Uganda—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (July 28).

SENIOR LABORATORY TECHNICIAN IN THE DEPARTMENT OF AGRICULTURAL BOTANY—Prof. A. H. Bunting, The University, Reading, Berkshire (July 30).

SENIOR LECTURER or LECTURER (preferably with experience in geophysics, ionospheric physics, geomagnetism, atmospheric physics, X-ray crystallography, X-ray diffraction and the analysis of molecular structure) IN PHYSICS at the University of the West Indies (Jamaica)—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (July 30).

ASSISTANT LECTURER (with appropriate qualifications and experience and specialised in physical chemistry) IN PHYSICAL CHEMISTRY at the University of Hong Kong—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, July 31).

DEAN OF THE FACULTY OF MEDICINE at Makerere University College Medical School, Kampala—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (July 31).

LECTURER or ASSISTANT LECTURER (medically qualified or non-medically qualified candidate with a special interest in immunology) IN THE DEPARTMENT OF BACTERIOLOGY—The Registrar, The University, Manchester, 13, quoting Ref. 127/65 (July 31).

LECTURER or SENIOR LECTURER IN HISTOLOGY at Massey University of Manawatu, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, July 31).

SENIOR LECTURER or LECTURER (with a degree in either science or veterinary science and experience in either chemical pathology or biochemistry) IN CHEMICAL PATHOLOGY at Massey University of Manawatu, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, July 31).

TEACHING FELLOW (with a good honours degree in zoology) or DEMONSTRATOR IN ZOOLOGY at the University of New England, Armidale, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, July 31).

LECTURER/SENIOR LECTURER (preferably with research interests in control, non-linear mechanics or the dynamics of machines) IN MECHANICAL ENGINEERING at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 6).

SENIOR LECTURER/LECTURER IN EDUCATION at Rhodes University, Grahamstown, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, August 6).

LECTURER (with an honours or higher degree and preferably some teaching experience) IN INORGANIC and/or PHYSICAL CHEMISTRY—The Director, The Swinburne Technical College, John Street, Hawthorn, Victoria, Australia (August 14).

MYCOLOGIST (graduate with special interests in the taxonomy of microfungi)—The Director, Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey (August 15).

SCIENTIFIC/SENIOR SCIENTIFIC OFFICER (with an honours degree and preferably a Ph.D. or other postgraduate research experience) at the Welsh Plant Breeding Station, to take part in a research programme concerned with the development of compatible varieties of grasses and clovers, and to undertake research on associated fundamental problems—The Registrar, University College of Wales, Aberystwyth (August 31).

SENIOR LECTURER (Crystallography) IN THE DEPARTMENT OF PHYSICS, University of Cape Town—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (South Africa and London, August 31).

THIRD CHAIR OF EXPERIMENTAL PHYSICS (candidates should have considerable research experience in nuclear physics)—The Registrar, The University, Liverpool (September 1).

ASSISTANT CONSERVATORS OF FORESTS (nationals of the United Kingdom or the Republic of Ireland, under 45, with a degree in forestry) in Malawi, to carry out the general duties of an Assistant Conservator of Forests—The Appointments Officer, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting RC 824/134/01.

ASSISTANT LECTURER or LECTURER (or, in special cases, Senior Lecturer) IN APPLIED MATHEMATICS—The Assistant Registrar (Establishment), University of Sussex, Stanner House, Stanner, Brighton, Sussex.

ASSISTANT PHYSICIST (preferably with an honours degree)—The Secretary, Royal Free Hospital, Gray's Inn Road, London, W.C.1.

ASSISTANT PROFESSOR/LECTURER (with an appropriate degree, or higher degree, from a university in Britain and relevant teaching experience) IN VERTEBRATE ZOOLOGY at the University of Libya, Tripoli—Recruitment Division, The British Council, 65 Davies Street, London, W.1, quoting Ref. 65/UNI/67.

CURATOR (with an honours degree with geology as one of the principal subjects) OF THE DEPARTMENT OF GEOLOGY AND GEOGRAPHY—Sir John Cass College, Jewry Street, London, E.C.3.

ENTOMOLOGIST (national of the United Kingdom or the Republic of Ireland, with an honours degree in zoology or a relevant subject, plus two years post-graduate study in agricultural entomology) in Uganda, to carry out surveys and conduct research into pests of crops and stored produce, especially cotton, coffee and cocoa and to advise on control measures—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1.

EXPERIMENTAL OFFICER IN THE PHYSICS DEPARTMENT, to accept responsibility for the design, construction and development of instruments, involving both electronic and mechanical components, required in the Department's research programme—Prof. J. M. Bruckshaw, Geophysics Department, Imperial College of Science and Technology, London, S.W.7.

**LECTURER** (preferably with research interests) in **BIOCHEMISTRY** to teach the subject to the standard required for H.N.C. and H.N.D. in applied biology, and to assist in the development of biochemistry as part of the proposed M.Biol. and honours degree courses—The Principal, South-East Essex College of Technology, Longbridge Road, Dagenham, Essex.

**LECTURER/SR. LECTURER in FOOD TECHNOLOGY**—The Acting Principal, Royal Melbourne Institute of Technology, 124 La Trobe Street, Melbourne, Australia.

**MASTERS** to teach Physics to "O" and "A" levels and to take charge of the Department (two laboratories)—The Headmaster, Seaborough College, Yorkshire.

**RESEARCH ASSISTANT** (with a good honours degree in microbiology or biochemistry, or related qualifications, for example Diploma in Technology—Applied Biology) in the **DEPARTMENT OF CHEMISTRY AND BIOLOGY** for work in chemical microbiology—The Registrar, Hatfield College of Technology, Hatfield, Hertfordshire.

**RESEARCH ASSOCIATE** (with a good honours degree) in the **DEPARTMENT OF MEDICAL PHYSICS**, to work on the physical aspects of ultrasonics in medicine—The Secretary, Queen Elizabeth Hospital, Birmingham.

**RESEARCH STUDENTS** (with a good honours degree, or equivalent, in science or technology, though not necessarily in microbiology) in **MICROBIOLOGY**—Head of the Department of Biological and Health Studies, Battersea College of Technology Annex, 14-16 Falcon Road, London, S.W.11.

**SENIOR ANALYST** (with a degree or equivalent qualification in chemistry, agricultural chemistry or some similar subject) for the **ANALYTICAL SERVICE OF THE FACULTY OF AGRICULTURE**—The Assistant Bursar (Personnel), University of Reading, Berkshire.

**SILVICULTURIST** (national of the United Kingdom or the Republic of Ireland, with a degree in forestry, considerable research experience, and preferably a knowledge of soils and plant ecology) in Ghana, to initiate and carry out research programmes in forestry, and to train subordinate staff in forestry research—The Appointments Officer, Room 301, Ministry of Overseas Development, Bland House, Stag Place, London, S.W.1, quoting EO 824/70/1.

**TECHNICIAN** (with a I.M.L.T. qualification or City and Guilds certificate in laboratory technology, and preferably a knowledge of electronics) in a **NEW DEPARTMENT OF CARDIOLOGY**, to assist in routine work and research—The Regius Professor of Medicine, Radcliffe Infirmary, Oxford.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

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- Bulletin of the British Museum (Natural History). Entomology. Vol. 16, No. 10: Revision of the Family Psephenidae (Orthoptera: Acridoidea). By V. M. Deth. Pp. 323-366. 20s. Supplement 1: The Types of Proctotrupidae (Hymenoptera) in the British Museum (Natural History) and in the Hope Department of Entomology, Oxford. By L. Masner. Pp. 54-60s. Geology. Vol. 10, No. 4: Silurian Polyzones from Benllyn Ridge, Shropshire. By Dr. D. R. Owen. Pp. 99-117+plates 1-6. 27s. Zoology. Vol. 13, No. 3: The British Museum (Natural History) Expedition to East Nepal 1961-63—Introduction and Lists of Localities. By J. G. Sheals and William G. Inglis. Pp. 95-114+plates 1-6. 12s. Vol. 13, No. 4: A Preliminary Revision of the Indo-Pacific Acanthinae (Pisces: Clupeidae). By P. J. P. Whitehead. Pp. 115-166. 10s. Vol. 13, No. 5: A Critical Review of the Marine Genus *Genus* *Macrurus* de Man, 1886. By John W. Coles. Pp. 157-194. 15s. (London: British Museum (Natural History), 1965.) [65]
- International Nickel Limited. Mond Chemical Products. Pp. 10. (London: International Nickel Limited, 1965.) [65]
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- British Medical Bulletin. Vol. 21, No. 2 (May, 1965): Symposium on "Transplantation of Tissues and Organs". Pp. 97-183. (London: The British Council, 1965.) 30s. [65]
- National Society for Clean Air. 35th Report 1964. Pp. 4. (London: National Society for Clean Air, 1965.) [65]
- Astronomical Contributions from the University of Manchester. Series III, No. 118: Isophotometry Applied to the Shadow Analysis of Plato and Archimedes. By M. T. Jones. Pp. H+26. Series III, No. 119: Orbits in the Mare Humorum. By P. V. Sudbury. Pp. H+22. (Manchester: The University, 1964.) [65]
- The British Coal Utilization Research Association. Annual Report 1964. Pp. 90. (Leatherhead: The British Coal Utilization Research Association, 1965.) [65]
- General Register Office. The Registrar General's Quarterly Return for England and Wales—Births, Deaths and Marriages; Infectious Diseases; Weather; Population Estimates, Quarter ended 31st December 1964 (No. 464, 4th Quarter 1964). Pp. 28. (London: H.M. Stationery Office, 1965.) 2s. 6d. net. [65]
- Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 41: Blackleg Disease of Cattle. Pp. 2. Leaflet No. 52: "Redwater" Disease in Cattle. Pp. 2. Leaflet No. 151: Variations in the Composition of Milk. Pp. 7. (Belfast: Ministry of Agriculture, 1965.) [65]
- University of Oxford. Ashmolean Museum—Report of the Visitors 1964. (Supplement No. 6 to the *University Gazette*, March 1965.) Pp. 87. (Oxford: The University, 1965.) 2s. 6d. [65]
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Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Paper 64-15: Field and Laboratory Methods Used by the Geological Survey of Canada in Geochemical Surveys. No. 6: Determination of Hydrocarbons in Soils by Gas Chromatography. By A. H. Debnam. Pp. IV+17. 75 cents. Paper 64-17 (Part 1): Age Determinations and Geological Studies. Part 1: Isotopic Ages, Report 5. By R. K. Wanless, R. D. Stevens, G. R. Lachance, and R. Y. H. Hemsley. Pp. IV+125. 75 cents. Paper 64-43: Geological Reconnaissance of the Precambrian of Northwestern Baffin Island, Northwest Territories. By R. G. Blackadar. Pp. VI+25. 75 cents. Paper 64-44: The Munkor Drilling Project. By D. O. Windley and C. H. Smith. Pp. VII+170 (11 plates). 75 cents. Paper 64-55: Triassic, Jurassic, and Lower Cretaceous Spores and Pollen of Arctic Canada. By D. O. McGregor. Pp. 32. 75 cents. Paper 64-57: A New Approach to X-ray Spectrochemical Analysis. By R. J. Traill and G. R. Lachance. Paper 64-58: Reinforcement of Oceanic Grinding Disks with a Steel Annular Ring. By P. J. Lavigne. Pp. III+3. 35 cents. (Ottawa: Queen's Printer, 1965.) [106]

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## GOVERNMENT POLICY AND INDUSTRIAL SCIENCE

IN his eighth Leverhulme Memorial Lecture to the Society of Chemical Industry on May 6, since published in *Chemistry and Industry* for May 22, 1965 (pp. 864-870), Mr. P. Chambers, discussing the place of the chemical industry in the development of the world's economy, was concerned mainly with the contribution of the chemical industry to the improvement of production, and only to a limited extent with the contribution which the industry could make in dealing with the problems of rising populations. Here, he was content to point out that it was desirable on grounds of self-interest, as well as on moral grounds, that the underdeveloped countries should progress more rapidly than in the past and that their standards of living should be brought with some speed nearer to those of the developed countries. Concerning productivity, he insisted on the growing scale of production and the necessity for larger economic groupings, and this was the main emphasis of the lecture.

The chemical companies with a complex of manufacturing operations starting from the petrochemical end will, in Mr. Chambers's view, tend to get larger and more international in character, because of the continuing need for very high-grade research and development on a large scale and on a co-ordinated basis. This is only economic if the costs can be spread over a correspondingly large turnover which is international rather than national in size—a point which is well illustrated by current arguments over pharmaceutical research and the cost of drugs. This view is fully supported by Prof. J. W. Mitchell in a Jubilee Memorial Lecture of the same Society on the organization of basic research for the British chemical industry. The main emphasis in this lecture, delivered in Manchester on February 12, and published in *Chemistry and Industry* for May 29, 1965 (pp. 908-935), is on the contribution of pure and applied research in increasing the value and volume of exports from those industries which depend on advances in the physical sciences for innovation and diversification. Although his theme is thus ostensibly narrower than that of Mr. Chambers, what is lost in breadth is more than gained in depth, for in an outstanding lecture Prof. Mitchell gives a most stimulating analysis of the present organization of basic research in Britain, including some penetrating criticism of educational, social and economic factors, and of Government policy and the reason for the emigration of British scientists. The whole is illuminated by a comparison of the situation in Britain with that in the United States in the light of his own considerable experience on both sides of the Atlantic.

Prof. Mitchell begins by reiterating that new knowledge is essential for industrial progress and challenging any complacency about the importance of the growth of creative science and technology for the national economy. He also insists that the vitality and attitude of mind of the research worker, his competence, and his understanding and mastery of the accumulated knowledge in his field, constitute the decisive factor for advances in scientific knowledge wherever he works. Prof. Mitchell believes that it is most desirable to separate expenditure on research from that on development, and that the administration of research should be separated from the administration of development. He gives a timely

warning that development costs can be grossly excessive if investment in preparatory and supporting basic research is inadequate, and that such inadequate research expenditure is often concealed by large total research and development costs.

Prof. Mitchell's definition of basic research is wide enough: it includes all research which leads to new knowledge. His analysis, moreover, is as clear and convincing as was that of Prof. I. D. Rattee in his inaugural lecture at the University of Leeds on "Discovery or Invention", and in this part of his lecture he seems to be thinking along similar lines. Stressing the complexity of the research problems of an industrial laboratory, Prof. Mitchell points out that careful and penetrating work on applied problems by outstanding scientists in industry has often led to discoveries of fundamental scientific importance. Here, again, he insists on the overriding importance of a stimulating and satisfying environment in which such men can work, and of enabling them to mix continuously with scientists and technologists in related fields at home and abroad.

This small creative minority of scientists and technologists, which is responsible for most new discoveries and inventions, is the key factor if we are to increase our volume of basic research relevant to the invention of new products and new processes for industry. Here Prof. Mitchell believes that Britain lags behind other countries and that this is one prime cause of difficulties in her export trade. Others arise out of the competition encountered in selling existing well-established products abroad, and here Prof. Mitchell points out that such difficulties are bound to increase as the rapidly developing countries build modern factories to supply their needs. Scientists and technologists can do no more than mitigate some of this competition, and the small size of many British firms is a further adverse factor. Nor is the position helped by limited productive capacity, long delivery times and a reputation for failure to adhere to promised schedules.

Prof. Mitchell then surveys the organization of basic research for industry and the respective contributions of the universities and colleges of technology, divisional and central industrial laboratories, Government and national laboratories, and independently sponsored research laboratories and research institutes. Each of these four groups has its own particular contribution to make, and despite the sub-title of this section of his lecture, it never suggests that academic research should be directed towards industrial objectives. On the contrary, he insists on the importance of a correct balance between creative research and teaching and that routine research should be the direct responsibility of industrial, Government or sponsored research laboratories. He is as emphatic that here, at the frontiers of knowledge, lies the distinctive contribution of the university in research as he is on the importance of the university as providing the gifted research worker and on the need for a higher staff-student ratio.

Apart from his warning against rashly dispersing a vigorous or outstanding research team, Prof. Mitchell's discussion of the function of industrial research laboratories is scarcely as pertinent to the present situation in

Britain as is his penetrating discussion of the functions of Government and national laboratories. First, Prof. Mitchell insists on the importance of the wise selection of research projects. He argues that sound advice on the selection and financial support of basic research projects can only come from outstanding scientists who are actively and continuously working in laboratories with dynamic and flourishing research programmes, which maintain their vitality and interest in advances in science and keep them aware of the level of support needed for effective work. These functions, he holds, cannot be performed by Civil Servants working alongside administrative staff in a Department of Education and Science or a Ministry of Technology, even if they have previously had some university training in science.

Prof. Mitchell does not entirely reject the idea of the part-time adviser, but late in his address he gives clear warning that a high proportion of the senior staff of the science departments of the universities cannot continue to engage in extensive external activity without seriously jeopardising the whole future of the nation, so numerous are the pressing academic problems which demand their undivided energies for urgent solution. This argument finds substantial support in any reflective reading of Mr. R. W. Clark's recent book, *Ticard*. Prof. Mitchell's own solution of the problem of advice is to put national research laboratories under the leadership of distinguished senior scientists, so staffed that they can divide their time between research and advisory and administrative work. These senior scientists would also be responsible for the objective evaluation of scientific matters of concern to the Government, based on thorough and penetrating analysis of problems and situations. One of his main reasons for challenging the decision to abolish the post of Director of the National Chemical Laboratory is that it involves the disappearance of a post for a senior scientist who might have taken a keen interest in the organization of research for British industry, stimulated by his responsibilities for running the laboratory.

Prof. Mitchell's judgment that this surprising decision is retrogressive is further substantiated by the functions and responsibilities which, towards the end of his lecture, he suggests the National Chemical Laboratory might be expected to perform. These are quite apart from three general reasons which he gives for maintaining Government and national laboratories: they can make an important contribution to continuing the education of graduates from the universities by giving them opportunities to work on research problems of national importance in contact with competent research workers while acquiring sufficient experience for independent work; they provide facilities for basic research, leading to the understanding of important national problems, which would be unprofitable for industry; and their work in establishing national standards, providing samples of chemical compounds of ultra-high purity, and measuring and evaluating their properties to provide national reference data.

Prof. Mitchell believes that greater differentiation of general functions is desirable among scientists and that it is not disadvantageous to have several groups working competitively on related problems of basic research. He is convinced that the senior members of the staffs of Government laboratories have an important and unique contribution to make to the balanced growth of science and technology, and, as already noted, he deprecates Britain's excessive reliance in the past on part-time services

of men from the universities and industry, whether in a personal capacity or as members of advisory committees. In this connexion, it should be observed that Prof. Mitchell repeatedly emphasizes the value of interchange of experienced scientists between the Scientific Civil Service and the universities and industry, but he is convinced that it is essential that there should be a sufficient nucleus of senior members from the staffs of Government institutions participating with scientists from other groups of the scientific community in Government consultations on scientific and technological matters.

This is a factor that appears to have received little attention in all the discussions on the organization of civil science during the past three years or so. He is also timely in directing attention to the social responsibility of the scientific community, and expresses the opinion that a strongly reacting scientific community has not yet been developed in Britain. Most outstanding scientists are concentrated in the universities and in the administrative sectors of the larger industrial firms, and there is little interchange between the universities and industrial laboratories. He finds none of the strong interactions and interchanges between scientists in the universities, in industry and in Government and sponsored laboratories that are found in the 25-45 age-group in the United States. The strength of the scientific community, however, is determined largely by the strength of such creative interactions as well as the quality and number of its members. Moreover, the present tendency is to increase the concentration of scientists in the universities, and neither the Robbins nor other reports on the plans adopted by the Government for the expansion of higher education appear to have directed attention to the need for balanced and co-ordinated growth in the scientific community.

Prof. Mitchell is also critical of some aspects of university expansion in Britain to-day, believing that a proper balance between teaching and research is threatened, that excessive burdens tend to be placed on senior staff and that, as regards students, the present system of examinations and selection tends to place a premium on possession of a good memory, which is no guarantee of creative ability in research. He is critical of the extent to which basic research is being undertaken in Britain to meet the needs of the medium-sized and smaller firms, and challenges Britain's concern to contract her industrial research effort when she should be reorganizing her industrial science and opening and equipping research laboratories. His comments on sponsored research and the industrial research associations may be deferred for consideration in that separate context, but his observations on the critical size and on the vitality of a research laboratory are of general validity and are highly pertinent to some recent Government decisions.

It is undeniable that responsible scientists have misgivings as to the effect of at least some of the changes already made, and Prof. Mitchell gives valid reasons for such misgivings which the Ministers concerned would do well to ponder. Moreover, he is not concerned to perpetuate any institution as such: the only true measure of the vitality of a research laboratory is its output of work of high quality, and this alone justifies the continued acceptance of the laboratory. But his insistence on the importance of a minimum critical size if a research laboratory is to be effective and productive is also related to the question of quality, on which the main stress is laid throughout the report. It is obvious that he sees real danger in a certain element of parsimony that marks

present policy. Care must be taken to ensure that the heavy burdens on senior scientists in the universities—and elsewhere—do not become insupportable for the creative minority with outstanding abilities. We can ill afford to quench their enthusiasm or diminish their creative power under the distractions of multi-functional activity.

Comparing the position of basic research in the United States and in Great Britain to-day, Prof. Mitchell attributes the greater strength of science and technology in the United States to the greater strength of the scientific community there. Federal assistance in various ways has been an important factor, together with the development of a system of graduate education in science and business management, and the consequently higher proportion of leading scientists employed in the industrial laboratories and with a wider influence. Moreover, he suggests that this greater strength of the American scientific community, and the higher standing of younger scientists, are important factors attracting many of the abler scientists from the United Kingdom, and he thinks that this continued emigration is regarded with too much complacency in Britain, especially the emigration of students from the group of Ph.D.s of average ability. Here, in Prof. Mitchell's view, the lack of openings for research with opportunities for continuing education in British industry is the decisive factor. This has been accentuated in the past two years by the closure of some industrial research laboratories and the incorporation of the National Chemical Laboratory in the National Physical Laboratory. There are, he believes, many British scientists in the United States who would return to Britain, regardless of the salary differential, if they could find non-academic posts for research which were professionally satisfying.

Prof. Mitchell does not overlook the benefits which the British scientist may derive from a period of work in the United States, but his comments on this question indicate that to some extent Britain's present difficulties are the direct outcome of Government practice and policy over the past two decades, as well as shortsightedness in industry, and that such policies and practices still persist. Policy in scientific matters cannot be guided primarily by political theory and prejudices, even when the ultimate decision is political. Nor does he omit to point out that all the efforts of scientists and technologists to resolve economic difficulties will be of little avail unless management, and those engaged in market research, and in sales, production and transport, play their full part in increasing the volume and value of Britain's exports.

The politician will scarcely find Prof. Mitchell's address comfortable reading. He rejects outright the assumption of the present Labour Government and of its Conservative predecessors that any deficiencies in basic research which are significant to the chemical industry can be rectified by supplying the universities and colleges of technology with all the laboratories and equipment and funds they need for research. This, he holds, could lead ultimately to a disastrous decline in the position of the industries based on science. The need is much deeper, and plans for university expansion must be accompanied by plans for employing the increased outflow of graduates. The place for basic research for industry is primarily in industry itself, and for this academic research is no substitute.

What is wrong is the lack of appreciation of implications of public policy where science and technology are concerned. This, as well as a lack of information, has characterized successive British Governments and their

supporters. Such information is the basis of any constructive criticism, and while this is not a specific objective of the recently established Office for Scientific and Technical Information, the new Office could assist the process, especially if the Parliamentary and Scientific Committee uses its opportunities. Prof. Mitchell refers to evidence of a widespread feeling that the formulation of policies affecting scientists in Britain is determined by small minorities, and that the scientists who will be concerned with implementing those policies are excluded from the preliminary consultations and discussions.

No effective structure for scientific and technical advice can be built up on such practices, nor are scientists of high ability and standing likely to be willing to serve under such conditions. Even if those conditions are only imagined to exist it is difficult to obtain co-operation, and the dissipation of any such atmosphere of distrust or uneasiness is a first essential. It should be followed, as Prof. Mitchell urges, by the establishment of some accepted procedure which brings into consultation the active leaders and representatives of scientists, and their professional societies, and gives them an opportunity of expressing their views before final and irrevocable decisions are made. On this point, Prof. Mitchell's analysis of the structure of advice needs to be taken deeper, and it is to be hoped that this will be done both by the Royal Society in its present examination of the situation, and by the Parliamentary and Scientific Committee. Prof. Mitchell welcomes this Committee's proposals that important matters of scientific and technological policy should be subject to public hearings by Select Committees of the House of Commons. Meanwhile, his challenge to the practice of making irrevocable decisions on the basis of secret reports and recommendations by anonymous advisers, and by confidential committees, is clear and unequivocal and in line with an important recommendation of the Commoner Committee on the integrity of science. It is not merely reasonable but vital that irreversible decisions should only be taken after the directors of research concerned have had full opportunity of expressing their own opinions and making their own recommendations on policies which would affect them. This is not claiming the right to determine policy but the right to ensure that the implications of policy are given adequate consideration. It is also the condition of responsible and dynamic leadership, and where it is not observed, policy, as well as those who dictate policy, is rightly suspect.

## HUTTON LOOKS BACK

### Recollections of a Technologist

By Prof. R. S. Hutton. Pp. 203+8 plates. (London: Sir Isaac Pitman and Sons, Ltd., 1964.) 25s. net.

PROF R. S. HUTTON'S recollections of his long and varied career have been published at a time when, apart from their interest to the numerous colleagues who have at one time or another worked or been associated with him in his several occupations, they are of some public interest in at least two connexions. First, *Recollections of a Technologist* illustrates most aptly the value of interchange between university, industry and the Civil Service which we are now belatedly trying to encourage. Prof. Hutton himself sandwiched some twenty-five years in industry in one capacity or another between two periods of academic teaching and research. Besides this, he was a founder-member of the Institute of Metals, and his recollections of his work, in and for this Institute, show the

important part which such learned societies or professional bodies can play in the advancement of technology. Secondly, the book should help to give a much more accurate and stimulating picture of the technologist in the mind of the reader and, all too short as it is, it should at least do something to assist in that urgent task of education if a sufficient number of the ablest students are to be attracted to such a career.

But to say no more would be unfair to Prof. Hutton, for the book derives added interest from his discerning comments on technical education, on the work of research associations, on the place of technical libraries and information services, and on other subjects. There is a sketch of the work of the Physics Laboratory in Manchester in the days of Schuster and Rutherford, and some more specific recollections of Schuster, Rutherford and Tizard are given in a separate chapter. As would be expected of Prof. Hutton, the references are full and well arranged at the end of each chapter.

Anyone, however, who has known and worked with Prof. Hutton will also certainly wish for more; but, suggestive as the book is and full of comments which, like those on scientific writing and the art of communication, are topical as well as wise, to those who have not known Prof. Hutton it can scarcely convey any real idea of how much we owe to him in such fields as those of technical education and the development of special libraries and information services. One may well be amazed at the range of his interests and at his untiring energy, but his work was never superficial. Prof. Hutton delved deeply in all his work and it remains for another writer to complement his own story by revealing the thoroughness and foresight of his work in the field of libraries and information services, for example, and the unfailing courtesy and deep understanding of the help he so readily and freely gave to those who turned to him for help and guidance. Inevitably in a book covering so wide a field there are points where opinions expressed might be challenged somewhat, or where small additions might seem desirable. These, however, are minor, and it is to be hoped that this readable and well-printed book will find many readers not only among scientists themselves but in industry, in Parliament and among the general public.

R. BRIGHTMAN

## NUCLEAR REACTOR PHYSICS

### Neutron Physics

By K. H. Beekurts and K. Wirtz. Pp. x + 444. (Berlin: Springer-Verlag, 1964.) 68 D.M.

### Introduction to Neutron Distribution Theory

By L. O. Woods. (Methuen's Monographs on Physical Subjects.) Pp. xii + 182. (London: Methuen and Co., Ltd.; New York: John Wiley and Sons, Inc., 1964.) 28s. net.

IT is now twenty-two years since the first nuclear reactor was built, and in that time reactor physics has become an established technological subject. These books represent the second generation of text-books in this field, and both can provide a very suitable basis for a university course. In fact, both books originated from such courses.

*Neutron Physics*, by Beekurts and Wirtz, is somewhat misnamed. Neutron physics as a subject is about thirty-two years old and has become diversified into several distinct areas. These include reactor physics, some parts of solid- and liquid-state physics, and some parts of nuclear physics. This book mainly covers the reactor physics side of neutron physics. With the advent of nuclear power there has been a substantial growth of interest in this area, and a number of text-books have been written. *Neutron Physics* is more broadly based than some and gives a clear and comprehensive introduction to the field. The depth of coverage has inevitably to be

limited, but adequate bibliographies are provided at the end of each chapter. The translation from the German has been carried out competently, so that almost everywhere the presentation is in good clear English. The book starts with a discussion of neutron sources, detectors and various types of experimental equipment for neutron cross-section work. It continues with the reactor physics theory and experiment, including neutron thermalization, diffusion and transport theory. The concluding section covers many of the experimental techniques of reactor physics. In the theoretical part of the book the mathematical presentation, while not particularly difficult, does require a graduate knowledge of mathematics. On the other hand, very little background of nuclear physics or knowledge of reactor physics is required. On the whole it can be recommended as an excellent introductory text and of value to university courses on reactor physics, particularly those concerned with needs of the experimental reactor physicist.

The monograph by Woods is based on a specialist course covering neutron distribution theory. Again it includes neutron thermalization, diffusion and transport theory; the mathematical presentation is slightly more difficult than in the book by Beekurts and Wirtz. For those requiring the theoretical side of the subject only, it forms an excellent introduction leading naturally to the more sophisticated theoretical treatments. Its scope is indicated by its division into three chapters; the first covers the basic equations of distribution theory, the second deals with neutron moderation, while the third covers multi-group theory. *Introduction to Neutron Distribution Theory* is a useful addition to the Methuen series and to the range of reactor physics text-books.

P. A. EGGLSTAFF

## MAGNETIC STORMS

### Solar Plasma Geomagnetism and Aurora

By Sydney Chapman. (Documents on Modern Physics.) Pp. 141. (London and Glasgow: Blackie and Son, Ltd., 1964.) 32s. 6d.

THIS monograph is one of a new series, entitled *Documents on Modern Physics*, in which the editors intend to specialize in presenting selected reviews, lecture notes, conference proceedings, and important collections of papers in branches of physics of special current interest. The contents of the book are based on lectures given by the author at the twelfth annual session of the Les Houches Summer School of Theoretical Physics in July 1962. The entire course of lectures at the School has been published previously in book form in 1963 under the title *Geophysics: The Earth's Environment*. It is rather surprising that the editors of the present monograph have not complied with the author's wishes regarding its title, as stated in his preface.

Prof. Chapman is an eminent geophysicist who has made outstanding contributions to the theory of geomagnetism. His purpose in the present book has been to describe the evolution of knowledge and theory concerning magnetic storms—the name given to intense transient disturbances in the geomagnetic field. The book may be regarded as a supplement to *Geomagnetism* (Chapman and Bartels, 1940) but, unlike the earlier treatise, the exposition is selective rather than comprehensive. Special emphasis is placed on the investigations of the author and his colleagues, notably S.-I. Akasofu and V. C. A. Ferraro. The discussion is confined primarily to researches which originated before the advent of satellites and space probes, and the reader is referred to the publications of other writers for an account of the many developments during the past decade.

The first chapter of the book outlines those aspects of geomagnetism and solar activity which are fundamental to an understanding of magnetic storm phenomena. The geometry and kinematics of the passage of solar plasma

from the Sun to the Earth are considered in Chapter 2. Several of the results obtained by the author some time ago have acquired new significance in view of present-day ideas on the extension of solar magnetic fields into inter-planetary space. Chapter 3 contains an excellent account of the early theoretical work on the interaction of solar plasma with the geomagnetic field and the more recent studies of the shape of the steady-state hollow formed in the solar plasma by the Earth's magnetic field. This chapter is undoubtedly the best available summary of the detailed investigations in this branch of the subject. The main features of magnetic disturbances are described briefly in Chapter 4. In the final chapter the terrestrial ring current is discussed in relation to the steady-state motion of a distribution of energetic charged particles trapped in a dipole field. This chapter summarizes one of the very few precise investigations of the geomagnetic effects of a symmetrical distribution of trapped radiation.

Much of the progress in the theory of magnetic storms has arisen from the formulation and solution of idealized problems in plasma dynamics. Prof. Chapman has been one of the chief exponents of this line of enquiry, and his present book provides an exemplary account of those basic concepts which form the foundations of the theory of geomagnetic storms. The book is a very valuable addition to the literature and is especially welcome at a time when speculation tends to outpace accepted theory.

D. M. WILLIS

## DESTINY OF PROTEIN

### Mammalian Protein Metabolism

Edited by H. N. Munro and J. B. Allison. Vol. 1: pp. xv + 566. 132s. Vol. 2: pp. xiii + 642. 150s. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.)

ONE of the earlier biochemical monographs in the pioneering series, edited by Plimmer and Hopkins, was the well-known book on protein metabolism written in 1912 by E. P. Cathcart. He defined his subject as "the processes in the animal organism which are concerned with the destiny of the protein, whether of the food or of the tissues". This definition is broad enough to cover the scope of the present book, but knowledge of the destiny of protein has advanced so much that it has now taken 27 authors and 2 author-editors to deal with the subject. The whole work is divided into three parts. The first part occupies all of Volume 1 and is concerned with the biochemical aspects of protein metabolism. It opens with an historical introduction by H. N. Munro, beginning with the discovery of nitrogen and its biological consequences. This is followed by chapters on protein digestion and absorption in non-ruminants (C. Gitler) and in ruminants (A. T. Phillipson), and on free amino-acids and peptides in tissues (H. N. Christensen). H. A. Krebs deals fully with the metabolic fate of the amino-acids individually and with the ornithine cycle of urea formation. He also discusses the cause of the specific dynamic action of proteins. A. Korner deals with the mechanism of protein synthesis and A. Neuberger and F. F. Richards discuss protein turnover in the whole animal, including the problems arising in the interpretation of turnover data obtained with isotopes. This is followed by a chapter on plasma proteins (A. S. McFarlane) and there are two chapters on interrelationships between hormones and protein metabolism by J. H. Leatham and by H. N. Munro. Volume 1 ends with a chapter on nitrogen excretion by J. B. Allison and J. W. C. Bird.

Volume 2 contains parts 2 and 3 of the work, covering respectively nutritional aspects and pathological aspects of protein metabolism. Much of part 2 is concerned with protein requirements. H. N. Munro contributes an introduction and this is followed by a chapter by J. B. Allison on the nutritional value of dietary proteins and one by A. E.

Harper on amino-acid imbalances. D. M. Hegsted writes on protein requirements in general, and this is followed by chapters on protein metabolism and requirements in pregnancy and lactation (K. L. Blaxter), in the new-born (R. A. McCance and E. M. Widdowson), and in the elderly (D. M. Watkin). Part 3 opens with a general survey of pathological changes in protein metabolism (H. N. Munro), followed by a most useful chapter by L. E. Holt, jun., and S. E. Snyderman dealing with the many different congenital anomalies and acquired disturbances of amino-acid metabolism which have been described, particularly in recent years. More than 40 conditions are now known to be associated with an increased excretion of amino-acids in the urine. D. P. Cuthbertson contributes an article on physical injury and its effects on protein metabolism; then come chapters on protein metabolism in tumour growth (G. A. J. Goodlad), experimental protein-calorie deficiency (B. S. Platt, C. R. C. Heard and R. J. C. Stewart), clinical aspects of protein malnutrition (F. Viteri, M. Béhar, G. Arroyave and N. S. Scrimshaw) and, finally, one on protein deficiency and infective disease (N. S. Scrimshaw).

This outline of the contents of the different chapters gives some indication of the wide scope of the work. The list of names of the authors is enough to indicate the high quality of the book, and the editors deserve congratulation on having persuaded so many distinguished workers to join them in this enterprise. The editors have done their work of planning and co-ordination admirably, and such overlapping between chapters as they have allowed, on infant nutrition for example, is helpful rather than otherwise. They have done much to unify the whole work by inserting cross-references where necessary. It seems surprising that they have not persuaded all the authors to use the internationally recommended coenzyme abbreviations NAD and NADP (rather than the old-fashioned DPN and TPN). Only a few misprints were noticed, but one of them ('possibly' in mistake for 'possible') unfortunately makes nonsense of the quotation at the beginning of the book. There are extensive lists of references at the ends of the chapters which greatly add to the usefulness of the book. Each of the two volumes is self-contained, with its own author index and subject index. The publication of this splendid monograph is of special importance at the present time when so much effort is being devoted to studying ways of improving the quality and quantity of food protein in the many countries where there is severe shortage. It is likely to remain the standard work on the subject for a long time to come. The book will interest a wide variety of people including workers in biochemistry, nutrition, agriculture and clinical science. Its publication must have created a problem for many librarians: not whether they should order a copy, but whether the library budget will allow them to order enough copies to satisfy the demand from scientists in so many different departments.

D. C. HARRISON

## TROPICAL AND SUB-TROPICAL PLANT ECOLOGY

### Die Vegetation der Erde In öko-physiologischer Betrachtung

Von Prof. Heinrich Walter. Band 1: Die Tropischen und Subtropischen Zonen. Zweite, veränderte und erweiterte Auflage. Pp. 592 + 9 tafeln. (Jena: Veb Gustav Fischer Verlag, 1964.) 68.10 D.M.

THE first edition of this book was published in 1962 and reviewed in the April 6, 1963, issue of *Nature*. The second edition is enlarged by 54 pages; new matter consists of additional sections on competition and adaptation in Chapter 1, part of a new Chapter 3 on rain-forest of tropical mountains, a new section on the savannah

concept, a revision of two sections on the Egyptian-Arabian desert and a supplementary chapter dealing mainly with grassland and swamp vegetation in Africa.

As was noted in the previous review, Prof. H. Walter's main interest is evidently in the drier parts of the tropics, and the map showing his very extensive travels shows that he has seen little of the main areas of equatorial rain-forest. His account of that forest is based largely on von Faber's revision of Schimper, greatly reduced but with retention of many excellent photographs; only 120 pages are devoted to the ever-wet tropics. This condensation is also in part due to Prof. Walter's interest in those aspects of ecology which have been the subject of physiological measurements (transpiration, cell-sap concentration, etc.) and to his natural inclination to report his own observations of this nature; such experimental evidence is probably more abundant from the drier than the wetter tropics, where the much greater complexity of vegetation makes it difficult for the would-be ecological observer to know where to start. But, though Prof. Walter could not devote so much space as von Faber to the ever-wet regions, he could have given references to recent publications. For example, in the chapter on mangrove he describes in detail his own observations on the peculiar zonation of species on the east coast of Africa (where the salt content of the soil-water is highest on the landward side) without giving adequate information as to the very different zonation in Malaya, or any reference to Watson's careful study of it; and he fails to mention the excellent summary by van Steenis of the ecology of mangrove prefaced to the account of the family Rhizophoraceae in *Flora Malesiana* (1958). His treatment of epiphytes is brief, and in connection with the role of ants in promoting epiphytic vegetation he refers to a work of 1905, not to van Leeuwen's much later study in Java. Prof. Walter does not mention the experimental evidence that gregarious one-day flowering of some common epiphytic orchids is temperature-controlled; and he is evidently unaware of the experimental study of velamen by Dyeus and Knudson (1957) in which they demonstrate that its function is protective, not absorptive. He appears to think that *Nepenthes* are normally epiphytes, but such a condition is rare, and it is very doubtful whether most *Nepenthes* absorb much water from rain collected in their pitchers. In referring to the fern thickets which often form on poor soil abandoned after cultivation, the author mentions only *Pteridium*, whereas the important thicket-forming ferns are several members of the family Gleicheniaceae. He cites nothing later than 1910 on the biology of Loranthaceae.

Because of Treub's foresight in establishing a laboratory for visiting scientists, more experimental work has been done at Bogor (Buitenzorg) in Java than anywhere else in the Malayan region, and von Faber's work was also based on Bogor. Thus the idea has been accepted that conditions in Java, and especially at Bogor, are normal for the region, whereas the volcanic soils of Java are very different from those of Malaya, Borneo and most of Sumatra, and (for example) Gleichenia-thickets, though abundant in Malaya, are not so in Java; and the diurnal rhythm of sun and rain at Bogor is different from the very irregular onset of rain in Singapore. Von Faber also saw little of dipterocarp forest, the characteristic primary vegetation of Malaya. Prof. Walter refers to Prof. P. W. Richards on this subject, but the forest in Sumatra which is mentioned very briefly on p. 90 of *Die Vegetation der Erde in öko-physiologischer Betrachtung* is probably more representative of dipterocarp forest in general than the sample investigated in Sarawak by Richards. A good deal of work has been done since the Second World War in the countries now united in the Federation of Malaysia, and Prof. Walter could find much that is relevant to his purpose if, according to his plan, he produces a regional study of that part of the world in a separate volume.

R. E. HOLTTUM

## ELEMENTARY PARTICLE PHYSICS

### Group Theoretical Concepts and Methods in Elementary Particle Physics

Edited by F. Gürsey. (Lectures of the Istanbul Summer School of Theoretical Physics, July 16–August 4, 1962.) (Quantum Physics and Its Applications, Vol. 1.) Pp. vii+425. (New York and London: Gordon and Breach, Science Publishers, 1964.) 19.50 dollars.

*GROUP Theoretical Concepts and Methods in Elementary Particle Physics* is the first volume of a series of texts edited by R. Smoluchowski (Princeton University) on *Quantum Physics and Its Applications*. It is of a high standard and augurs well for the future of the series.

The classification of elementary particles according to group-theoretical principles is well known to be a most profitable way of correlating and understanding their properties. This has been particularly evident during the past year or so with the introduction of the  $SU(3)$ ,  $SU(6)$  and  $U(12)$  classification schemes. For this reason, the publication of this collection of lectures by many of the world's experts in the field is to be applauded. The lectures were given at the Istanbul Summer School of Theoretical Physics during July 16–August 4, 1962—before the discovery of the  $\Omega^-$  had confirmed the essential correctness of the classification of elementary particle states according to unitary symmetry. However, all the basic theory is included together with many interesting tit-bits not directly related to the main stream of advance in this subject.

The basic mathematics of Lie groups is first introduced in a masterly fashion by G. Raosh. E. P. Wigner next lectures on unitary representations of the homogeneous Lorentz group including reflexions; here, the concern is not with the symmetry classification of particles, but with the symmetry properties of the space-time continuum in which physical events occur. N. Burgoyne gives an elegant exposition of the relation between spin and statistics approached from the point of view of axiomatic field theory. A refreshing breath of down-to-earth experimental information is included in the lectures by O. W. Greenberg, who concerns himself with the bounds and relationships between total cross-sections for different elementary particle processes at high energies. L. Michel, in his lectures, stresses the importance of the study of group extensions—the relationship between different invariance sub-groups. D. Speiser lectures on the theory of compact Lie groups and makes use of the global approach due to Hopf; he also considers the problems and consequences of global symmetry in elementary particle physics.

The "Unitary Symmetry Model" is discussed in considerable and useful detail by S. L. Glashow but, perhaps even more here than elsewhere, it could be regretted that the lectures were given before the higher symmetry schemes were mooted. Y. Nambu, in an interesting set of lectures laced with a certain amount of speculation, deals with the implications of Chiral symmetries for weak and strong interactions. A very brief lecture by A. Salam looks into some of the implications of broken symmetries. Next, F. Gürsey gives an introduction to the De Sitter group with particular reference to the implications of a De Sitter space for elementary particle physics. The volume ends with three short contributions by E. Inönü, O. W. Greenberg and E. R. Caianiello dealing respectively with the contraction of the Lie groups, parastatistics and the renormalization group.

The notes presented in this volume all seem to be carefully prepared, and it is clear that in many cases the presentation and content have greatly benefited from discussion and criticism during the Summer School itself. Each contribution is well worth-while and the overall impression given by the volume is excellent. One can only regret that it could not have been produced a little more cheaply.

R. J. BLIN-STOYLE



**New Methods of Analytical Chemistry**

Second edition. By R. Belcher and C. L. Wilson. In association with T. S. West. Pp. xv+366. (London: Chapman and Hall, 1964.) 60s.

THE United Kingdom can boast of only three university professors of analytical chemistry, and when they all collaborate in the revision of a well-known book it is certain that the work will possess that *sortor resartus* quality which signposts an improvement in its scientific and didactic value. This expectation is fully borne out by this second edition.

The first chapter deals individually with the more common titrimetric acid-base standards and then considers those of more recent origin, for example 4-aminopyridine for acids and sodium hydrogen diglycollate for bases. Oxidation-reduction standards such as cupric oxide or barium thiosulphate are also briefly considered.

Indicators of all types, chemiluminescent, screened, redox, metallochromic, etc., are all lucidly dealt with in the second chapter. A detailed account of titrant usages then follows, for example the use of sodium chlorite; ferricyanide procedures; potentiometric titrations of hydrazine; use of *N*-bromosuccinimide, etc.

Newer organic and inorganic precipitants form a most valuable section of this book, although they can only receive brief mention here. Selected spectrophotometric methods from recent literature are presented in much detail, for example, the arsenazo method for hafnium; and the pyrogallol red procedure for silver ion. Precipitation from homogeneous solution receives adequate treatment, four specific procedures being mentioned. The powerful techniques of solvent extraction receive full coverage, simultaneous extraction and direct analysis being sometimes possible.

The final chapter presents a survey of miscellaneous analytical methods which will titillate the palates of both the classical and the more sophisticated analyst. The gravimetric determination of fluoride and the titrimetric determination of barium sulphate are typical of the many analyses lucidly covered in this section.

This edition of *New Methods of Analytical Chemistry*, even more than its predecessor, will be a welcome addition to the libraries of analysts everywhere. The lecturer, teacher and practising analyst will all derive much instruction and help from this up-to-date authoritative discussion and presentation of the newer reagents and of recent methods.

D. T. LEWIS

**Coulomb Wave Functions**

By A. R. Curtis. (Royal Society Mathematical Tables, Vol. 11.) Pp. xxxv+209. (Cambridge: At the University Press, 1964. Published for the Royal Society.) 80s. net.

COULOMB wave functions arise in the solutions of Schrödinger's equation for an electron in a central field. The U.S. National Bureau of Standards published tables of these functions in 1952 (Applied Mathematics Series No. 17), and other tables are also known.

In 1950, the late Prof. D. R. Hartree and others proposed to the Royal Society Mathematical Tables Committee the preparation of tables of these functions, and the present volume is the outcome of their proposals. An attempt was made to choose solutions of the differential equation which are not only mathematically linearly independent but also numerically distinct, and at the same time to produce tables which are readily interpolable in the energy parameter. These two requirements are not entirely compatible, and the final choice of solutions is to some extent a compromise.

*Coulomb Wave Functions* contains a mathematical introduction, a useful collection of formulae concerning the selected standard solutions, instructions for the use of the tables, and a bibliography. The tables themselves give values of the selected solutions of the differential equation  $y'' + [a + 2x^{-1} - L(L+1)x^{-2}]y = 0$  for  $L = 0$ ,

1, 2;  $-2 \leq a \leq 2$ ; and  $0 \leq x < \infty$ . For  $x \geq 10$ ,  $x^{-1}$  is used as the tabular argument. The tables are reproduced photographically from sheets prepared on a card-operated typewriter.

A. EBDÉLYI

**Chemotaxonomie der Pflanzen**

Eine Übersicht über die Verbreitung und die Systematische Bedeutung der Pflanzenstoffe. Von Prof. R. Hegnauer. Band 3: Dicotyledoneae: Acanthaceae—Cyrillaceae. (Chemische Reihe, Band 18.) Pp. 743. (Basel und Stuttgart: Birkhäuser Verlag, 1964.) 123 Sw. francs.

WITH Volume 3 of *Chemotaxonomie der Pflanzen* begins the treatment of the large group of Dicotyledoneae which is covered as far as Cyrillaceae. The first few chapters contain a survey of the chemistry of alkaloids common in this group. They are subdivided into protoalkaloids or biogenic amines, pseudoalkaloids like terpenes, purines, and derivatives of nicotinic acid, and into alkaloids themselves. The pathways of biosynthesis of larger structures from simple amino-acids are schematically outlined.

The Dicotyledoneae include many plants of great medical and toxicological interest, of which a few examples may be mentioned. To the Apocynaceae belong compounds of the yohimbine-, reserpine-, strychnine-, and cantharidin-types, and strophanthin. Quaternary ammonium bases with curariform action are present in Cappariaceae, and salicin is found in Caprifoliaceae. Hallucinogens are discussed in connexion with several families; *Lophophora williamsii*, the Mexican peyote, is a well-known example, and for *Cannabis sativa* the chemical relationship between antibiotic and bactericidal actions and intoxicating effects is outlined. The pathways of synthesis of norephedrine in *Catha edulis* may be similar to those of ephedrine in Ephedra. Chinese and Japanese medicine uses plants of the Asarum family, the identity of which is not fully established. Derivatives of the anthelmintic pelletierine have been found in the Crassulaceae.

Of general interest are the Asclepiadaceae which contain arrow poisons, and the Annonaceae in the seeds, leaves and roots of which substances of an unknown type act as contact poisons against aphides.

Though the literature is considered until the end of 1963, the author states that Volume 3, unlike its predecessors, does not contain a complete survey of the known facts, since for some families, like the Apocynaceae, the phytochemical literature is very large and readily available in modern standard works. Even so, the volume presents in a clear manner a wealth of information on a field of wide interest.

L. WISLICKI

**Scientists as Writers**

Edited by James Harrison. Pp. 206. (London: Methuen and Co., Ltd., 1965.) 21s. net.

*SCIENTISTS as Writers* is essentially an annotated anthology of prose passages written by scientists, mostly of the present century, arranged around eleven themes, including the nature of the universe, of matter, of life, of mind and of science, evolution and man, the laws of chance, science and art, and science and religion. They are linked by explanatory notes and comments, and there are a few suggestions for further reading. The book is intended for use in general courses in sixth forms and elsewhere, to further both the command of English and the broader understanding of science. While it illustrates admirably that scientists can and do write about their work lucidly and in impeccable English, it seems to achieve its second purpose of interpretation even more effectively than the selection of similar excerpts edited by S. Rapport and H. Wright and published two years ago under the title *Science: Method and Meaning*.



## IMPOTENCE AND ACHIEVEMENT IN PHYSICS AND TECHNOLOGY\*

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AMONG the most lasting of Lord Cherwell's many interests was the principle of indeterminacy. And among the most permanent of his dislikes was any suggestion of defeatism. When I became one of his students in 1930, he was applying the indeterminacy principle with great enthusiasm; and I was alongside him in 1940 at the climax of his pre-war fight against defeatism in national defence. My own outlook was enriched by both these experiences, which were all the more fascinating because of the apparently different attitudes of Lord Cherwell—in one case the close ally of Winston Churchill in utter rejection of defeatism, and in the other the ready acceptor of the limits that Nature appears to place on human powers of observation.

Let me also acknowledge my debt to Sir Francis Simon. Just as Lord Cherwell had become engrossed with one postulate of impotence, the principle of indeterminacy, so Simon was fascinated by another, the third law of thermodynamics; and Simon, an artillery officer against Britain in the First World War, stood very firmly with Britain in the Second. His name was among those of British citizens to be rounded up by the Nazis if "Operation Sea-Lion" had succeeded; and I must now confess that this may have been due not only to his own eminence but also to a hoax that I extemporized on an agent of the German Secret Service whom I found wandering around the old Clarendon Laboratory in 1935, and whom I persuaded (in an effort to distract his attention from some air defence equipment) that there was a great anti-Nazi organization in Britain with Simon at its head. I hope that Simon forgave me—especially since I stand indebted to him for support at important stages in my own career.

In *The Physical Significance of the Quantum Theory*, Lindemann<sup>1</sup> quotes the futility of trying to describe the smell of nitric oxide, which is changed to nitrogen peroxide when it is drawn with air into the nose of the observer. Such a process is a self-frustrating one, and it can have similar results to impotence; and so, before considering principles of impotence, let us look at self-frustration. It is an important aspect of modern life, especially in human relations; and, had we thought about it more, we might have avoided many errors of administration.

Lord Cherwell, for example, during the War held a pass that was intended to allow him to enter any Government establishment. Such a universal and vital pass could only be issued to persons of extreme responsibility; most people held passes which would admit them to only one specific establishment. Since there were few persons of comparable responsibility to Lord Cherwell, there were very few of his kind of pass; and its appearance was kept confidential, so that it could not easily be forged. It followed that very few guards at the entrances to establishments had ever seen it, and so Lord Cherwell had great difficulty in persuading them that it was genuine, and to let him in.

Self-frustration can arise very easily in military security. An example which I remember particularly well occurred in the German Air Force towards the end of 1940, when Goering realized that by some means we often knew in advance where his night bombers were going to attack. He therefore ordered an intense search throughout the Luftwaffe for the means by which information was

leaking out. Fortunately for us, the search failed—the radio beam stations for guiding the bombers, which were the actual source of the leak, were at that time so secret that they were specially exempted from the scrutiny of the investigating officers.

In war, one sometimes tries to deceive the enemy; and in doing so may give away the very thing one is trying to hide. In the First World War, for example, British Army radio messages were encoded by a system which was changed daily, but which was used not only for operational signals but for practice messages as well. So that the latter could be distinguished from the former, and not taken seriously, each practice message was prefixed by the name of some animal. This step provided the German cryptographers with what they needed—a 'crib'. All they had to do was to examine our practice messages and try one animal name after another until they found a consistent encoding system: they could then simply read our operational signals.

As an example in which the roles were reversed, I may perhaps mention our estimates of German rocket production before the V2 campaign in 1944. One vital step in our argument was to estimate the numbers of rockets which the Germans intended to store in forward areas in France. Now these stores were extremely difficult to locate and we found only a few of them; in addition, the Germans had built dummy storage sites to divert our attention. The dummies were, of course, made easy to spot from the air, and we found them all quite quickly. Then, by luck, we captured a map which showed the genuine sites west of the Seine, with their capacities. Most of the genuine sites, however, lay east of the Seine, and we did not know where they were; but we did know the numbers of dummies both east and west of the Seine. Thus, assuming that the Germans had disposed the dummies in consistent proportion to the genuine sites, we could estimate the number of genuine sites east of the Seine. This reasoning produced easily the closest estimate<sup>2</sup> of the intended rate of fire: we could never have made it if the Germans had not tried to deceive us.

Notorious as producing a result opposite to the administrative intention has been the 'costs plus' system of paying firms for Government work. 'Costs plus ten per cent' appears to be a very reasonable price to pay, until one realizes that the manufacturer is automatically tempted to inflate his costs by any legitimate device, since he is thereby improving his profit. And if his nominal profit margin is unreasonably small—at the point, for example, where he would do better to sell his plant and invest his shareholders' money in another industry—then the Civil Servants who have to supervise the contract, and who know that the job must be done and the manufacturer of vital defence equipment kept in business, will turn a lenient eye on over-estimates of the manufacturer's costs. Thus, a system which at first sight appears fair and economical works out in practice to be extravagant and demoralizing.

We are becoming painfully acquainted with self-frustrating situations in education. A subject such as physics can prove vital to national survival, both in defence and in industry. When this happens, more physicists are demanded by Government establishments and industry, and so there are fewer left to staff the universities and the schools. Teaching then becomes more unattractive at all levels, owing to the increased

\* Substance of the sixth Cherwell-Simon Lecture delivered in the University of Oxford on May 18.

load, and so more men leave teaching; the output of graduates thus tends to fall in those very subjects in which the national need is greatest, while it rises in those subjects which are less vital. An educational system in this phase is like an internal combustion engine starting from cold—if the throttle is opened too quickly, the mixture gets too weak in fuel, and the system stalls.

Another way in which self-frustration can occur in education was exemplified by the French, when they set their academic standards very high. The difficult examination standards frightened away students, even those who might have attained them, with the result that the subjects with high standards were weakened instead of strengthened.

Even when all academic examinations are past, there remain further dangers. One of these is election to learned societies, where in some instances the candidate has to be supported by as many as six existing members. While this is apparently a very healthy check on the standing of the candidate, it gives an advantage to candidates who work in centres where there are already six members, and this can make for inbreeding rather than health.

It could be salutary to extend this catalogue of self-frustrating actions. The University of Oxford Union "King and Country" motion of 1933 made it more likely rather than less likely that those who voted against fighting would ultimately have to fight<sup>3</sup>. In Intelligence, it is almost as dangerous to watch one's enemy too closely as not to watch him at all—the watchers can become bored, or gradually acclimatized to the posture of the enemy, so that they fail to detect the imminent blow. This happened to us in the escape of the *Scharnhorst* and *Gneisenau* through the Straits of Dover, and it nearly happened again in June 1944 when, after a year of watching the flying bomb, we only just succeeded in overcoming the boredom of routine in time to detect the forward passage of the bombs to the launching sites<sup>4</sup>.

At the very least, an essay on self-frustration would be a valuable addendum to *Microcosmographia Academica*, since it provides one of the few lines of effective argument in the misconduct of academic business that was overlooked by F. M. Cornford<sup>5</sup>. The phenomenon usually arises because the administrator has applied the wrong criterion to regulate the situation with which he has to deal.

There is often peculiar humour about self-frustration. Humour itself is generally associated with incongruity<sup>6</sup>, and the element of incongruity in this case springs from the fact that we subconsciously recognize that true achievement requires both thought and work—the greater the achievement, the more the thought and work that have to go into it. When, instead, the extra thought and work result in frustration of the original aim, then we have an incongruity, and the rudiment of a comic situation.

Consider, for example, a train of events which started outside the old Clarendon Laboratory, Oxford. I came across a dirty beaker full of water just when I happened to have a pistol in my hand. Almost without thinking I fired, and was surprised at the spectacular way in which the beaker disappeared. I had, of course, fired at beakers before; but they had merely broken, and not shattered into small fragments. Following Rutherford's precept I repeated the experiment and obtained the same result: it was the presence of the water which caused the difference in behaviour. Years later, after the War, I found myself having to lecture to a large elementary class at Aberdeen, teaching hydrostatics *ab initio*. Right at the beginning came the definitions—a gas having little resistance to change of volume but a liquid having great resistance. I thought that I would drive the definitions home by repeating for the class my experiments with the pistol, for one can look at them from the point of view of the beaker, thus suddenly challenged to accommodate not only the liquid that it held before the bullet entered it,

but also the bullet. It cannot accommodate the extra volume with the speed demanded, and so it shatters. The experiment is rather a messy one to do in a lecture room; but, as Clerk Maxwell had said of his own hydrostatic experiments to the predecessors of this same class many years before, "We were not very wet"<sup>7</sup>.

The experiment became duly public in Aberdeen, and inspired the local Territorial contingent of the Royal Engineers, who used sometimes to parade on Sundays to practise demolition. One task that fell to them or, more accurately, refused to fall to them, was the demolition of a tall chimney at a local paper works. There are various standard procedures for this exercise, one of the oldest being to remove some of the bricks of one side, and to replace them by wooden struts. This process is carried so far as to remove the bricks from rather more than half-way round the base of the chimney and to a height comparable with the radius. A fire is then lit in the chimney, to burn through the struts and cause the chimney to fall.

The Royal Engineers, however, decided this time to exploit the incompressibility of water as demonstrated by my experiment. Their plan was to stop up the bottom of the chimney, fill it with water to a height of 6 ft. or so, and simulate the bullet by firing an explosive charge under the water. Since diversions on the Sabbath were rare in Aberdeen, the exercise collected a large audience and the charge was duly fired. It succeeded so well that it failed completely. What happened was that, as with the beaker, every brick in contact with the water flew outwards, leaving a slightly shortened chimney with a beautifully level-trimmed bottom 6 ft. up in the air. The whole structure then dropped nicely into the old foundation, remaining upright and intact—and presenting the Sappers with an exquisite problem.

Here again, in technology as well as in administration "the best laid schemes . . . gang aft a-gley" through some element built into the original plan. As a passing example, we may note the failure of all attempts to make a golf ball attain maximum range by polishing its surface. There is a story that P. G. Tait, then professor of natural philosophy in the University of Edinburgh, calculated the maximum range of a golf ball, and that his son then took him out on the links and showed him that the ball could be driven much further. It was also said that old balls went further than new ones. This was probably true, because the surfaces of old balls were chipped and roughened; and, based on this observation, new balls have ever since been intentionally dimpled. The then unknown factor which later came to light in the wind tunnel was that the roughness encouraged the onset of small-scale turbulence around the ball, and—over the useful range of velocities—this forestalled and obviated the large-scale and more dissipative turbulence which would occur when the laminar flow around the smooth ball ultimately broke down. Rough balls thus have less resistance, paradoxically, than smooth ones<sup>8</sup>. There is probably a lesson for the administrators here as telling as anything in classical politics.

As an antithesis to the self-frustrating regulation, we might examine the self-fulfilling prophecy. When, for example, Winston Churchill said in 1940 that "we shall never surrender" he did by his very words make our surrender more unlikely. But this avenue would lead away from our main course, which must head past Sancho Panza, pondering over the self-negating prophecy of the man who came to be hanged on the bridge<sup>9</sup>, towards principles of impotence.

The phrase is a relatively new one, invented by Sir Edmund Whittaker<sup>10</sup> in his presidential address to the Royal Society of Edinburgh in 1941; but the axiomatic method of thought that it implies is very old, going back at least to Heraclitus with his "You cannot step into the same river twice" because the water in the river will be different on the second occasion.

There is great fascination in the axiomatic approach, especially when the axiom is one of renunciation and when what follows from it is a gain in understanding comparable with what has been voluntarily renounced. It is almost like the battle between the angel and Robert of Sicily<sup>11</sup>, with the humble and meek being exalted after the final act of submission. Perhaps it was this aspect that appealed to Aristotle so much, for he was prepared to admit several principles of impotence; it was impossible, he held, to create a vacuum, an infinite universe, or a plurality of worlds<sup>12</sup>. The last two of these were largely a matter of metaphysics, but the first was a good working approximation to reality. In fact, in the old Clarendon Laboratory we sometimes thought that "Nature abhors a vacuum" should be our Laboratory motto, so much time did we spend looking for leaks. But Aristotle used the principle far more elegantly in disposing of an uncomfortable paradox which upset ancient dynamics. Based on the observation that bodies stop moving if the pushing agent were removed, a law was formulated that "what-ever is moved is moved by another". This was convincing enough so long as it was applied to bodies being moved over rough surfaces, but it encountered trouble when applied to the flight of an arrow. What could be moving the arrow, once it had left the bow? The ingenious answer was that the arrow and the air interacted; the head of the arrow pushed the air into motion, and this air then circulated around the arrow, blew up its tail, and so drove it along. On this theory, the air was essential to the flight of the arrow, which should thus cease to fly if it were in a vacuum. At the same time, there was also the matter of experience that the less the resistance, the faster a body moved. Since a vacuum could be expected to have no resistance, it followed on this basis that a body should move through it very fast indeed. Faced with this paradox of a plausible hypothesis producing exactly contradictory results through two different chains of argument, Aristotle skilfully argued that this showed that a vacuum could not exist<sup>13</sup>. He was here, incidentally, approaching the modern idea of it being sensible to talk only of phenomena that could be observed.

The Aristotelian principles of impotence survived until A.D. 1277, when they were rejected by a Council of Paris<sup>14</sup>; and in the following six centuries the rejection was increasingly justified by events. It was found possible to make a good working vacuum; and science was refounded on the much sounder and more positive basis of Newton's Laws.

Gradually, however, principles of impotence have come back, starting most clearly with the second law of thermodynamics, first formulated by Clausius and Kelvin about 1850. This law codified a wide range of practical experience, such as the impossibility of increasing the brightness of a source of light simply by means of an optical system<sup>15</sup>. This fact was evidently unknown to two workmen in Portsmouth dockyard who, in my time with the Admiralty, sent to the Inventions Branch a proposal to make a very bright searchlight by focusing the image of a carbon arc successively from one ellipsoidal mirror to another until the final image fell back on the arc, and so made it twice as bright as before. Then the enhanced light was re-focused around the system back again on to the arc, the brightness of which was therefore redoubled. When the process had gone far enough, the light was to be let out of the system into the searchlight beam. A formal letter was sent to the workmen from the Admiralty, thanking them for their proposal, and begging to state that Their Lordships considered that it would not work since it contravened the second law of thermodynamics. There came back an apologetic letter saying the proposers had had no idea that they had been breaking any of Their Lordships' regulations, but they still believed that their invention would work. The issue was neatly settled by the Admiralty making a grant of £50 to the workmen to try their ideas out, this measure being held cheaper and better for

morale than a prolonged correspondence. From the subsequent silence, the second law eventually triumphed.

While the second law, as formulated either by Clausius or Kelvin, was a definite postulate of impotence, it was perhaps not at first clear that the first law could be expressed in a parallel way. But in 1877 Maxwell<sup>16</sup> put it in the following form: "The total energy of any material system is a quantity which can neither be increased nor diminished by any action between parts of the system . . .", and this is sometimes popularly expressed as "it is impossible to create or destroy energy". Since we thus have a great conservation law re-expressed as a postulate of impotence, it follows that other conservation laws (including the newest ones in high-energy physics) could be expressed in a similar way. For example, the conservation of momentum can be re-expressed as 'it is impossible to change the total momentum of a system by any action between its parts' or 'it is impossible to move the centre of mass of a system by any action between its parts'. It is sometimes helpful to have the conservation laws framed in this negative way, although it is more likely that they arose in the minds of their discoverers in searching for quantities that were positively conserved.

An important quantitative law which was first deduced from a negative experiment was that of the inverse square law in electrostatics. This experiment, first reported by Franklin and appreciated by Priestley<sup>17</sup>, showed that it was impossible to detect any electrostatic force on an isolated charged body inside a charged hollow conductor. Whittaker<sup>18</sup> has stated that this, along with another postulate of impotence ("it is impossible to detect a uniform translatory motion, which is possessed by the system as a whole, by observations of phenomena taking place wholly within the system"), can be used to deduce the inverse square law for charges at rest, and then (by considering these charges in motion relative to the observer) the existence of magnetic force, and thence Maxwell's equations.

Historically, however, things did not evolve that way. Maxwell found his equations by quite different arguments; and, forty years later, Einstein was troubled by the asymmetry to which they apparently led in the actions between a magnet and a conductor, depending on which of the two was supposed to be in motion. Einstein<sup>19</sup> later explained in his autobiography:

"... I despaired of the possibility of discovering the true laws by means of constructive efforts based on known facts. The longer and more despairingly I tried, the more I came to the conviction that only the discovery of a universal formal principle could lead us to assured results. The example I saw before me was thermodynamics. The general principle was there given in the theorem: the laws of nature are such that it is impossible to construct a perpetual mobile (of the first and second kind). How then could such a system be found? After ten years of reflection such a principle resulted from a paradox that I had already hit at the age of sixteen. . . ."

Following Hume and Mach in examining the nature of observations, Einstein thrust his way to a new postulate of impotence, the impossibility of detecting, in a system in uniform motion, any change in the velocity of light *in vacuo* whatever the speeds of the source and observer. Combining this with the independence of the laws of mechanics from the choice of inertial system, he arrived at the special theory of relativity. By accepting man's impotence to observe absolute uniform motion, he grasped extraordinary prizes, such as an understanding of the increase of mass with velocity and, above all, the discovery of the equivalence of mass and energy. Incidentally, he also showed that this equivalence followed from Maxwell's equations, coupled with momentum conservation when expressed as an impotence to move the centre of mass of a system by action between its parts.

Shortly before Einstein's work, Planck had found that it was at last possible to understand the black-body radiation curve if one assumed that radiation took place

in jumps, governed by his mysterious quantum of action  $\hbar$ . Twenty-five years later, Heisenberg<sup>19</sup> showed that this constant expressed an apparently inevitable uncertainty in any simultaneous measurements of conjugate coordinates, such as the momentum and position of a particle, so that the product of the two uncertainties was itself, on average, equal to  $\hbar$ . Thus, if one accepts man's inability to perform experiments of unlimited precision in this respect, and acknowledges an inevitable degree of imprecision defined by the uncertainty relation, explanations for many of the mysteries of atomic physics can be found, and puzzles in the behaviour of observing instruments understood. Similarly, once the impossibility of distinguishing one electron from another is admitted, the nature of the homopolar bond in chemistry becomes evident; and theoretical chemistry has benefitted from the postulate that interested Simon so much, the unattainability of the absolute zero.

The determinacy arguments, enthusiastically developed by Lindemann, made much impression on those of us who were his students around 1930. And it was the good fortune of some of us to be actually present at the birth of another postulate of impotence, at a colloquium by E. A. Milne in Wadham College. In preparing a talk on the red shift, he was struck by the possibility of a completely new cosmology, starting from the assumption that there was no observation by which any man could tell that he was not at the centre of the universe; in this cosmology<sup>20</sup> all other observers would appear to be receding radially away from him, at distances proportional to their velocities. This principle can be more simply stated as "it is impossible to tell where one is in the universe"; and to this Bondi and Gold<sup>21</sup> in 1948 added the further postulate that "it is impossible to tell the cosmic time"—from which the hypothesis of continuous creation results. While these last two postulates have scarcely given rise to any 'useful' results, they have stimulated a great deal of thought; and Milne's method of distance measurement by light signals in a way anticipated radar.

A postulate of impotence, to have value, must state something much more than is self-evident—it is of little profundity, for example, to say that it is impossible to find an integral power of three that is divisible by two. (It may be of intermediate profundity to say that no perfect model of a new phenomenon can be made, and that all 'explanations' must be partial only.) A valuable postulate usually codifies, and extrapolates from, a great deal of practical experience in which something has been attempted by many routes, and all of them have resulted in failure. The postulate supposes that this failure is due to something inherently impossible about the thing being attempted, such as the detection of drift through the ether. The value of the postulate arises in two ways: it can then act as our guide to save us wasting further time on impossibilities such as perpetual motion engines, and it can enable us to think intelligently about other phenomena the explanation of which is otherwise obscure. It may even enable us to predict them as, for example, the uncertainty principle tells us at once to expect that long decay times will be associated with very sharp transitions.

The successes of postulates of impotence have made physicists the readier to formulate them, and have encouraged the axiomatic approach. E. T. Whittaker, indeed, looked forward "to a time in the future when a treatise in any branch of physics could, if so desired, be written in the same style as Euclid's *Elements of Geometry*, beginning with some *a priori* principles, namely, postulates of impotence, and then deriving everything else from them by syllogistic reasoning".

While Whittaker thought that this would apply especially to branches of physics "in a highly developed state", there has been an increasing readiness to set up postulates of impotence in advance of experimental evidence. The logical consequences of a postulate are

then evaluated, and experimental checks made in order to test its validity. This is a legitimate enough procedure, especially since it may stimulate experiments that would otherwise not be known; but the too ready postulation of impotence can be a danger to progress, especially in technology. It is this aspect that I now want to discuss.

My first cautionary example, curiously, is the very one which Whittaker cited as the prototype of postulates of impotence, and concerns the possibility of recognizing the two components of a double star with an astronomical telescope. It was found in the eighteenth century that this problem presented experimental difficulties due to the broadening of the individual images, and in 1823 Fraunhofer showed that the size of the star image in a telescope is inversely proportional to the aperture of the object glass. The blurring of the image is, of course, due to diffraction, and Fraunhofer's work led to the theory of the resolving power of optical instruments.

The subject was investigated—above all—by Rayleigh, who showed that the theory could be applied to the sharpness of images produced by any optical system; and, among other aspects, he evaluated the possibility of detecting very small rotations of a galvanometer by observing the reflected image from a mirror attached to the suspension. He came to the conclusion that a rotation of the mirror could be recognized by a movement of the image, provided that the rotation of the mirror was enough to move its edges through a distance equal to one quarter of the wave-length of the light which was being used to form the image. It was, incidentally, at the meeting of the British Association for the Advancement of Science in Aberdeen in 1885 that he announced this result<sup>22</sup>. It has been much quoted since.

So here we have a postulate of impotence, that it is impossible to detect the rotation of a mirror, unless its edges move by at least a quarter of a wave-length. In practice<sup>23</sup>, it is not difficult to observe rotations of the mirror that correspond to movements of one-millionth of this value. The reason is that the postulate is false. Of course, there is something behind it. The formula for resolving power is reasonably near the truth, but resolving power is a criterion that applies to recognition of two images simultaneously, when both are present, in an optical system. This, however, is not the problem involved in observing the rotation of a mirror. In the former case, we have to observe the presence of two diffraction patterns simultaneously, and to recognize that there are two present, whereas in the second case—the rotation of a mirror—we have to establish the centre of the diffraction pattern before the mirror is moved, and compare its position with that of the centre of the pattern after the mirror has moved. This is a very much easier problem, and the postulate of impotence of resolution no longer applies—we can do a million times better.

The confusion between resolution and precision extends over a wide field. I even encountered it in 1940, when one of our most distinguished physicists could scarcely believe that the Germans could set up a radio beam system over Britain with the precision that I had stated. His argument was that the resolving power of the aeriels used by the Germans would not allow the accuracy of alignment that would be needed. Fortunately, the problem was almost exactly the same as that of the galvanometer mirror, and I was able to convince him.

Radio has indeed been a field where false postulates of impotence have flourished, from the days when Marconi astonished many men of science by signalling across the Atlantic so far around the curvature of the Earth that he appeared to defy diffraction theory. The theory was, of course, reasonably correct—but in practice there was a new phenomenon that few had foreseen, reflexion by the ionosphere. Much later, it was difficult to make authorities in Britain take seriously the radio-navigational means of blind bombing being developed by the Germans—again

because it was thought that these means would have a shorter range than in fact they had.

We had in 1940, incidentally, almost given up hope of developing an airborne radar equipment for detecting towns, because we believed that it would require centimetric wave-lengths to give the necessary accuracy (which was correct) but that metric wave-lengths would be necessary to differentiate between the buildings in a town and the incidental irregularities of open country. We were therefore almost leading up to a postulate that it would be impossible to detect towns by radar from the air. Actually, there is always moral comfort when one concludes that some form of military nastiness is excluded by Nature; but for this very reason one needs to be very careful before concluding that it is indeed so. One must apply what in English studies<sup>24</sup> is known as 'Crow's Law', after Mr. John Crow, of King's College, London: 'Do not think what you want to think until you know what you ought to know', and which was anticipated by Pasteur's<sup>25</sup> 'I should like to see these profound words inscribed on the threshold of all the temples of science: 'The greatest derangement of the human mind is to believe in something because one wishes it to be so'".

To return to radar bombing: it was quite by accident that we found that towns showed up very well in centimetric airborne radar. The missing factor in our previous thinking was the presence of many corner-reflectors constituted by the vertical and horizontal surfaces in a typical town.

The development of centimetric radar was itself a triumph over another false postulate of impotence: that it was impossible to generate much power at wave-lengths shorter than about fifty centimetres. This was a doctrine when I was first concerned with air defence in 1935. The argument ran thus: the period of the shortest waves which it was possible to generate must be longer than the time that it took electrons to cross from the cathode to the anode of the generating valve. Now this argument was quite bogus—once again the wrong criterion was being watched. I first realized this through a comic incident at Farnborough, when a long-range loudspeaker was being demonstrated to the Air Staff. The origin of the demonstration was a desire for a more humane way of policing the tribesmen of the North West Frontier than bombing their villages. The idea was that instead an aeroplane should fly over the village and address the inhabitants through a loudspeaker. It was hoped that, both aircraft and loudspeakers being new weapons, the tribesmen might be awed into submission by the voice of God. Such a powerful loudspeaker was novel not only to the tribesmen—it did not exist at all, and had to be specially made. It was set up on one side of the aerodrome at Farnborough, and actuated by a microphone on the other side. You could thus speak into the microphone, and two or three seconds later your voice would come booming back to you from the other side of the aerodrome. On the day of the demonstration, the Air Staff representatives were as much impressed by the novelty as were the prospective victims. Before long, one of them, on hearing his own voice for the first time in this curious way, laughed. After the due interval, the loudspeaker laughed back. This time, the whole delegation laughed, and the loudspeaker duly roared with laughter. Next time, there was so much laughter from the loudspeaker that the microphone picked it up, and so made the loudspeaker laugh again. The air around Farnborough resounded with the laughter from this self-amusing system.

Now that demonstration showed that the argument about not being able to generate centimetric waves was false—for here we had a system which was capable of generating laughs at rates much higher than once in three seconds (the transit time of the sound) and could indeed generate sounds with periods of less than one thousandth of a second. What mattered was not the duration of the transit time, but its constancy, so that sounds did not get

blurred in the transit. I actually used this analogy to persuade the Admiralty that centimetric wave generation should be possible; and the fact that the Clarendon Laboratory was asked to work on this problem in 1939 was in part due to this event.

Another piece of false doctrine at about the same time was that it was impossible to make a bullet-proof fuel tank. Our bombers in the early raids on Kiel proved very vulnerable because a single shot through a fuel tank would stop them returning across the North Sea. By contrast, German aircraft proved difficult to shoot down—they went on flying despite several shots through the tanks. When we ultimately shot one down near the Firth of Forth, we saw that it had self-sealing tanks. The subsequent enquiry into our failure to fit similar tanks revealed a cautionary tale. It turned out that there were ideas about bullet-proof self-sealing tanks before the end of the First World War. In peace, of course, the ability to resist bullets was of little advantage: what would be an enormous comfort would be a tank that would not rupture in the event of a crash. The specification was therefore amended, to call for a tank which was both bullet-proof and crash-proof. Between the Wars, various inventors produced tanks, including some very like those ultimately used by the Germans. But each tank was duly taken to Farnborough, filled with fuel, and heaved over the side of a building, so that it fell some 60 ft. on to concrete. All the tanks burst, and so the doctrine grew up that fancy tanks were no good; we thus entered the Second World War without bullet-proof tanks because we could not make a crash-proof tank. Once again trouble had arisen through applying a criterion subsidiary to the operational problem.

The history of bombing in the Second World War illustrates how doctrine can develop from early disappointments to false postulates of impotence. At the beginning, we deluded ourselves that we could hit small targets in Germany; and in this phase we aimed at some crucial targets such as oil-production plants. This effort failed, and it became a doctrine of impotence that we could not expect to knock Germany out by attacks on what were called 'panacea' targets; such targets were held to be false concepts. The real reason for the failure was not the concept but the inaccuracy of our bombing, which was only good enough for hitting area targets such as towns. This therefore became our policy. In the meantime, our bombing accuracy sharply improved—but only in the last few months of the War was it switched back to oil targets. It then proved strikingly effective; but it could probably have been similarly effective several months earlier if it had not been inhibited by the doctrine of the impossibility of knocking out oil that had sprung from our previous disappointment.

Almost all the foregoing examples have this in common. Someone tried to do something and failed. He then invented a false reason for his failure, and this was usually a postulate of impotence when it should have been an admission of incompetence; but it was enough to obscure the true possibilities for some years, and to discourage others from making the attempt.

When I was an undergraduate it was still being debated whether a steam locomotive could be built to run at more than 100 m.p.h. The famous *City of Truro* had once been reported, shortly after 1900, at rather more than 100 m.p.h. downhill, but in the nineteen-twenties this figure could not be equalled in deliberate efforts with more powerful engines, even when streamlined. One cause of failure, Sir William Stanier told me, was that engineers had forgotten to allow for the expansion of steam in the super-heater tubes, which ought to have been made with gradually widening bores to keep down the steam velocities at the exit end, and thus reduce the superheater resistance. When this measure was taken, along with others, speeds easily went beyond the 100 m.p.h. figure; and those of us concerned with elementary physics have for a century

taught that because of linear expansion it is necessary to have frequent gaps in the rails; Kelvin calculated that at least two hundred yards of gaps were needed between London and Edinburgh. We have now to explain why, contrary to this doctrine, we are using great lengths of long-welded rail. The answer is that, if care is taken, the expansion forces, although great, need not be dangerous: these forces—which are along the length of the rail—can exert only a small component at right angles, and this can be contained by adequately securing the rail to its twin and to the ground.

Lest we laugh at the engineers, let us remember that pure science has had its own follies through refusal to face non-conforming evidence. The French Academy about 1780 denied the possibility of stones falling out of the sky: almost every astronomer and every physicist during 1932–1940 took no notice of Janaký's remarkable discovery of radio waves from the Milky Way: many geologists held for a long time that it was impossible for the continents to move: the very word 'atom' postulates an impotence that has been spectacularly shattered: and the supposed inability of Nature to distinguish between mirror-symmetrical arrangements has apparently been dispelled by the experimental violation of parity.

With such examples, we may wonder whether any of our postulates of impotence accepted to-day will be challenged. Shall we, for example, ever be able to go behind the limitations of electromagnetic quanta as means of observation? How far shall we be able to see into the structures of what we call elementary particles, or into the mysterious processes of life? Niels Bohr<sup>24</sup>, in his *Atomic Physics and Human Knowledge*, has exemplified that same reservation towards living systems that Kelvin and others placed on the second law of thermodynamics. They were careful to point out that they did not know whether this law held for living systems; and Bohr (p. 100) has held that "an account, exhaustive in the sense of quantum physics, of all the continually exchanged atoms in the organism not only is not feasible but would obviously require observational conditions incompatible with the display of life". But it will be interesting to see how far we can go.

Where, then, do we stand after this ramble through theory, experiment, and technology, and through genuine and false impotence? We have seen the philosophical fascination of arguing from impotence: each postulate describes a boundary condition for human achievement

and understanding. The assembly of postulates provides a framework of external anchor-points inside which the whole of physical knowledge can be fitted and 'explained'. But good postulates are not to be cheaply won, and, like good anchors, they must be well tested before employment. The price of the test is often a hard experimental beating against the bounds of experience, and a penetrating theoretical search for an explanation of the experimental failure in terms of what is already known. Only when theory and experiment have jointly and exhaustively failed can we be reasonably justified in trusting a new principle of impotence. Otherwise, we shall invent false reasons to delude ourselves away from achievements that could be within our competence. Principles of impotence, like other hypotheses, are not to be multiplied without necessity.

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## RESEARCH IN INDUSTRY IN INDIA

By S. H. M. HUSAINI, A. GHOSAL and A. RAHMAN

Survey and Planning of Scientific Research Unit, Council of Scientific and Industrial Research, New Delhi

A SURVEY of research as carried out in industrial establishments in India has been made by the Survey and Planning of Scientific Research Unit of the Council of Scientific and Industrial Research. The interest of business and industrial establishments in scientific research is of recent origin; even two decades ago they were sceptical of the utility of research in augmenting production. The survey was directed towards estimating research efforts made by the industrial establishments and also towards assessing the contribution of research towards growth of production. It was intended primarily to have a quantitative analysis of the situation so far as possible. A country-wide survey of all the establishments was planned; but it was found that only bigger establishments maintained research departments and others primarily made use of research results.

It was, therefore, decided to make a detailed study of research efforts in twenty-five top industrial establishments over the period 1955–62; the main observations of the survey of these establishments have been incorporated in this article. Out of the twenty-five establishments, four were in the public sector, and twenty-one in the private sector. A little more than half these establishments are engaged in the production of chemicals and pharmaceuticals products, whereas the other half included firms manufacturing rayon, textiles, iron and steel and electrical machinery and metallurgical products. Two of the big firms engaged in the manufacture of chemicals were subsidiaries of their parent companies in Britain, whereas one foreign firm collaborated with the Government of India in manufacturing products. Out of two steel plants considered, one was in the public sector and the other in the



private sector. All these establishments had fully equipped research departments.

### Personnel

Detailed statistics of personnel employed in these establishments were available. For all the twenty-five establishments together, production, maintenance and sales accounted for about 60 per cent of the total staff strength, administration about 18 per cent, routine technical and auxiliary jobs about 17 per cent and research and development for only 5 per cent of the total.

There was, however, variation from industry to industry. Those producing chemicals (including paint and varnishes), dyestuffs, insecticides, and rayons employed relatively more research staff than other industries. It was interesting to find that very few research personnel were employed by pharmaceutical industries covered in the survey. One metallurgical firm in the survey employed as much as 63 per cent of its total staff in research and development (for this firm, research is an adjunct for production).

The personnel employed only in research and development departments have been analysed under three headings: (a) scientists and technologists; (b) auxiliary technical staff; (c) administrative and other staff. The growth of personnel under all these heads has been considerable during the period 1956-62. It was found that in all the twenty-five establishments together the staff strength in research and development departments increased seven-fold during the period 1956-62; out of them the number of research personnel (scientists and technologists) increased seven-fold; the auxiliary technical staff increased about eight-fold. The growth was greatest during 1956-60. During the period 1960-62 the increase in the strength of research personnel attained 25 per cent. These norms, however, vary from industry to industry. A few characteristics for particular industries were observed regarding the distribution of personnel in research and development departments. In 1962 in drug and pharmaceutical establishments, for every two scientists one supporting staff was employed; in two steel plants, for every three scientists two supporting staff were employed; in insecticides, for every two scientists one supporting staff was employed; and for chemical industries, for every three scientists there was only one supporting staff.

An analysis of personnel by fields of specialization was also made for the years 1956-61. It was found that in the pharmaceutical establishments covered most scientifically qualified personnel were science graduates who did not specialize in any particular branch of science; these were employed mainly in production and maintenance departments. Steel plants included in the survey have started recruiting more science graduates not specializing in any particular discipline since 1961, primarily due to the fact that they did not get specialized engineers and technicians at the moment they required them; they planned to train these young graduates for various specialized jobs. Among the specialists, chemists exceed others in numbers; chemists were employed in large numbers particularly in a rayon factory, chemical factories and dyestuffs factories. The chemical engineers are also increasing in numbers in the industries, especially in steel plants, chemical and paint factories. Mathematicians, physicists and statisticians are conspicuous by their absence in most of the industrial establishments. In 1962, however, a large number of physicists were recruited in steel plants.

Analysing the distribution of scientists and technologists in various departments of all the industrial establishments covered, we found that about 35 per cent were employed in production, 14 per cent in maintenance, about 7 per cent in sales, 10 per cent in quality control and routine technical jobs, and 11 per cent in research and

development. The distribution, however, varies from industry to industry. Examining the age distribution of the scientific personnel in industries, we found that in rayon, lignite and metallurgy establishments covered in the survey more than 70 per cent were less than thirty, whereas in drugs, pharmaceuticals, dyes and dyestuffs and electrical machinery only 10-17 per cent were less than that age. In electrical machinery a little less than 60 per cent of all scientists and technologists were in the age-group 30-40.

A study of movement of scientific personnel to and from research and development departments has been made over the period 1958-63. Four types of changes were considered; transfer on promotion to other departments, transfer without promotion to other departments, resignations from the establishment and death or illness. It was found that most of the exits were due to transfer on promotion to other departments within the establishment, though during the period 1960-63 a number of transfers to other departments were effected without any promotion. Resignations from the establishments were comparatively small.

### Research Expenditure

An analysis of expenditure in the firms surveyed has been made. A few firms were hesitant in giving the details of their expenditure; others, however, gave the expenditure figures in great detail. The significant observation made from the survey was the small amount of expenditure by the industrial establishments on research and development. One pharmaceutical establishment spent only 1.5 per cent of a total recurring expenditure of Rs. 105 millions on research and development during 1958-62; four enterprises in chemical industry together spent only 0.8 per cent of their total expenditure of Rs. 224 millions; in two steel plants together, out of a total recurring expenditure of Rs. 1,472 millions over the same period, a little less than 0.1 per cent was spent on research and development. Comparatively high proportionate expenditure on research and development was observed in an insecticide establishment (about 5 per cent of the total).

It was originally intended to relate research expenditure to the value added (or to the value of net sales) in the establishments studied. The figures obtained so far are only approximate, though a few firms have also given detailed figures for these. A detailed investigation of the ratio of research expenditure to net sales (or valued added) is to be made; efforts are also being made to examine the elasticity of net sales with respect to research expenditure or vice versa. From rough analysis it is found that the rate of increase in the research expenditure is in many cases greater than the rate of increase in the total expenditure; but since the total research expenditure is on a very low level it appears that adequate research is hampered in the industry.

### Utilization of Research

Even though research efforts are not substantial in most of the industrial establishments surveyed, it is encouraging to find that most of the establishments exploited the processes which were developed internally. Four chemical firms adopted more than 80 per cent of the processes developed internally, whereas the rest were brought from outside sources; in the dyestuff establishment all the fifty-one processes exploited during 1958-62 were developed internally. This points to the fact that research in the field of applied chemistry and dyes has a fairly high standing in India, so that the respective firms can afford to compete in the market by developing their processes. The same conclusion holds good for the new designs used during 1958-62 by the chemical firms. It should, however, be borne in mind that the conditions regarding research and development prevailing in the big industries covered



in the survey do not hold true for numerous smaller establishments, most of which do not have their own research laboratories but depend on buying processes either from abroad or from other sources. The detailed investigation of all industries, which has been taken up by the Unit, will lead to important conclusions which will be representative for India at large.

### Contributions

The contributions by research departments of industrial establishments in terms of research papers, patents and inventions have also been examined. Most of the research papers were published by the research departments of drug and pharmaceutical and chemical firms. The research department of one steel plant also published a number of research papers during the period 1955-62. During the same period the number of research papers and technical reports published by the active departments of the establishments examined increased from 41 to 313. It is, however, generally observed that very few contributions to basic research were made by the research departments. It is expected that in the near future managers will become more research-conscious and more scientists will carry out basic research along with the problems of direct interest to the firms concerned.

From the nature of the papers published by research departments we find that very little engineering research was performed in the firms during the period 1955-62.

### Other Aspects

From the investigation carried out during the period 1955-62 it was found that development in the industries surveyed was, to a great extent, due to foreign collaboration both in terms of employment of foreign experts and also of the equipment. In four chemical firms 82 per cent of the money invested by the foreign collaborators was for purchase of specialized equipment; this percentage was 71 for the electrical industry; in the insecticides factory almost all the money invested by foreign collaborators was for employing consultants.

Fourteen industries are actively collaborating with the national laboratories by way of referring their problems including developmental programmes.

It is gratifying to find that the big establishments have started to train qualified scientists within the industry so that in future they become motivated towards research for solving the problems of the firms concerned. Ten firms out of twenty-five covered in the survey are primarily acquiring technical knowledge from foreign countries (two through their parent companies abroad).

## UNIVERSITY OF NEWCASTLE UPON TYNE

### MERZ COURT

By PROF. J. M. COULSON

Department of Chemical Engineering, University of Newcastle upon Tyne

THIS is the age of university expansion, and the University of Newcastle, in common with other universities, has steadily expanded its facilities and is not only the largest university centre in the north of England but is also noticeably a key centre of technological activities. Although sited within the city boundaries, it is fortunate in being adjacent to the exceptionally large Town Moor, covering one thousand acres, which gives it a very pleasing aspect on the northern side.

The latest and largest addition to the buildings is Merz Court, which was officially opened by the Prime Minister on May 7 and which provides accommodation for Chemical and Electrical Engineering, and also for the Department of Mathematics (Fig. 1). The north-eastern region has a long history as one of the key areas of engineering, including electrical engineering, and there are also very substantial chemical installations in the region. It is therefore appropriate that the new building should reflect the industrial achievements of the area by being named after the Merz family, who indeed played a notable part in the development of both electrical and chemical activities.

Dr. Theodore Merz came from Germany to enter the chemical industry, first in Glasgow and then in Newcastle. He is perhaps best known for his activity in the founding of the North Eastern Electricity Supply Company, which has since merged into the North Eastern Electricity Board. He has, in addition, a permanent place in the history of the University as one of those responsible for the foundation of the Durham College of Science which later became a part of the present University. His book on the *History of Thought in the Nineteenth Century* gives him a permanent place with the thinkers of his time. His son, Charles Merz, born in Gateshead, was trained as an electrical engineer, and later founded with William McLellan the internationally known company of Merz and McLellan, who have been associated particularly with electric power installations over a long period of

years. His second son, Norbert Merz, played an important part in the development of the north-eastern region and contributed in particular to the North Eastern Electricity Supply Co., Messrs. Reyrolle, Ltd., and Thermal Syndicate, Ltd.

Merz Court provides a usable floor area of 114,000 sq. ft. on a site area of only 47,000 sq. ft. This is broadly divided so that Chemical Engineering has 30,000 sq. ft., Electrical Engineering 50,000 sq. ft. and Mathematics 20,000 sq. ft., with the remainder for general purposes. To provide this large building on so small a site presented a major problem to the architects, not only because the Engineering Departments required large laboratories, many of which must be on the ground floor, but also because the soil required strengthening in order to support the heavy loads of such a building. The building is formed round a quadrilateral base with a central courtyard. In general there are six storeys, but some of the laboratories spread through two floors. The west side houses the Electrical Engineering Department on all floors, while on the east the Chemical Engineering Department occupies the lower three floors with Mathematics on the upper floors. The south side provides a group of lecture rooms available to all departments with a top floor again for the use of the Mathematics Department. The much shorter north side contains the workshops of the Engineering Departments, a students' common room, a seminar library for the building, and some small offices.

The construction of the building is of reinforced concrete and the east and west wings are each of 60 ft. in width, providing, in the case of Electrical Engineering, very large laboratories. The south side includes the largest lecture theatre, which will accommodate about two hundred students and which is well fitted for sound amplification and projection units. There are two preparation rooms which serve the other lecture rooms in this section. This south front is supported on a single span of 111 ft. over the entrance to the inner courtyard.

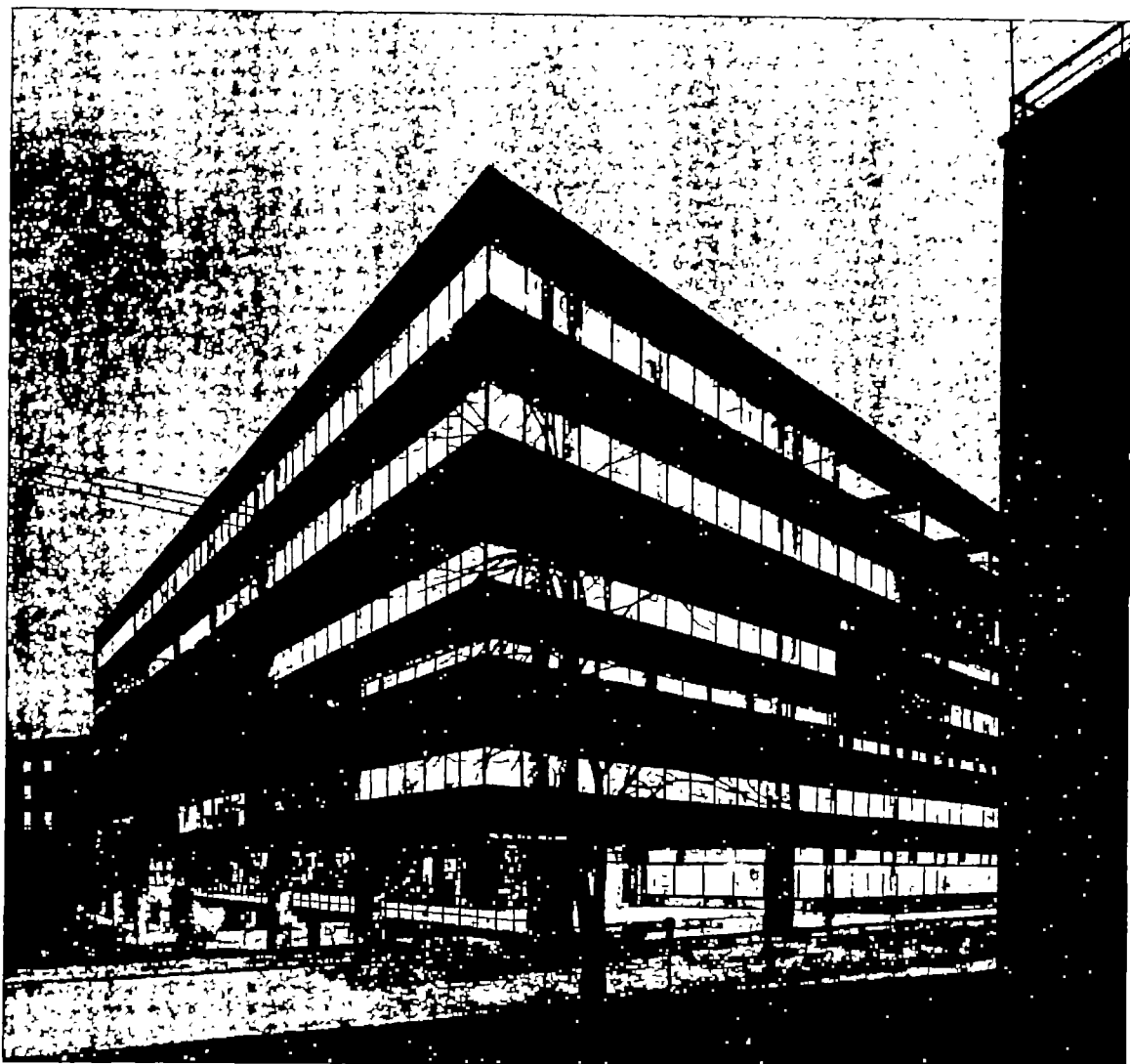


Fig. 1. Mers Court, University of Newcastle upon Tyne

Such an arrangement provides an open access on the ground-level and breaks up what would otherwise be a rather heavy front to this side of the building. The front is further relieved by an unusual bridge pathway which provides communication between the other engineering buildings and the main University Library.

The internal wall finishes are fair-faced concrete and fair-faced brick in the laboratories, and plastered concrete in the lecture and administrative rooms. Wood-block and cork-tile floors have been widely used except in some Chemical Engineering laboratories, where tiled floors, resistant to acid and solvents, have been laid. The door frames and much of the joinery is from the hard paduak wood and many of the flush doors are finished in the new polyurethane paint. The arrangement of the ceilings is interesting in that the use of softwood slats spaced very slightly apart and backed with fibre-glass gives sound insulation at an economical cost. The corridors, which have been kept to a minimum in the general design, have softwood open slatted ceilings which have been suspended where necessary. These slats can easily be removed to provide access to the surface ducts—a very important requirement in an engineering building.

The building is heated from an oil-fired installation in the basement, giving a total output of 34 million B.Th.U./h. This plant supplies most of the building with low-pressure hot water pumped through finned tube convectors.

Control is provided by fans operating from thermostats, such an arrangement giving fairly speedy heating-up when required. Special time-switches enable the heating load to be modified for night operation. The boiler plant also supplies steam for experimental purposes to the Chemical Engineering Department at a pressure of 100 lb./in.<sup>2</sup> and a separate electrode steam boiler provides steam at 500 lb./in.<sup>2</sup> to one laboratory.

#### Laboratory Supplies and Equipment

The supplies of electricity to both the Electrical and the Chemical Engineering Departments are extensive and complex. Two 750-kVA transformers mounted in the basement of the Electrical Department provide the main source of power; other equipment generating the various forms of supply is also mounted in the basement. The supplies to the five floors of the Electrical Department are taken through coded cables in large ducts with ample spare capacity. These cables feed either directly to central distribution boards or to small laboratory panels. Three 3-phase and neutral a.c. supplies are distributed from the basement. These are 415 V at 600 amp, 240 V at 200 amp, both of 50 c/s, and for servo work 60 V at 200 amp, and a further supply at 500 c/s. Six mercury arc rectifiers provide 120–0–120 V at 1,000 amp d.c., and in addition a large lead acid battery provides 120–0–120 V

d.c. There are also available two motor generator sets giving 100 amp at 240 V. In these laboratories, special panels designed in the Department are used. These panels, which may be regarded as engraved line diagrams, provide sufficient information for students to understand the arrangements for supply and when necessary modify connexions relatively easily. The various connexions throughout the Electrical Department enable pieces of equipment to be connected electrically from one laboratory to the other without much difficulty. This flexibility is regarded as a considerable asset.

In the Chemical Engineering Department the supplies of electric power are much simpler but are substantial in quantity. The general scheme is to arrange bus-bars either at one level or, in the case of the pilot plant laboratory, at two levels. Tappings from these bus-bars can readily be obtained at any desired position, but a number are fitted in for initial operation.

The Chemical Engineering Laboratories are also provided with compressed air, gas, water, and in many cases steam at 100 lb./in.<sup>2</sup>. There are no general facilities either for vacuum or for hot water, but these can be provided when required for individual pieces of equipment.

### Research and Teaching

Both the Engineering Departments have some special laboratories for research purposes, but in many cases, particularly in the Chemistry Section, the research and undergraduate teaching are combined so far as possible. Some special facilities in the Electrical Area include a screened room and a high-voltage laboratory. They are also provided with equipment for developing analogue computers and electronic circuitry. On the heavy current side, work is mainly on speed control of motors.

In Chemical Engineering the research activities are broadly related to the new field of electro-chemical engineering, for which there is a large special laboratory, to com-

bustion research, reactor design, and to the field of high-intensity heat transfer. Again, both Engineering Departments have their own workshops covering machine work, electronic work, instrument work and glass technology.

The top floor, mainly devoted to the Department of Mathematics, gives an added sense of lightness as a result of the fitting of a number of glass domes on the flat roof. In this section, the architects have successfully provided a little more perambulatory space, some of which is available on two terraces outside the normal rooms. The greatly enlarged premises for Mathematics are a source of considerable pleasure since this Department has managed to grow rapidly to one of those providing a significant contribution to the training in this key discipline. They have active research work in hand in functional analysis, group theory, mathematical logic, potential theory, continuum mechanics, fluid dynamics, relativity and mathematical statistics.

To the visitor and to those who work in it, there is no doubt that this building represents a step forward in the general style for the University. The large area of walls finished in white with rather light ceilings gives an air of space which is much appreciated. The wide variation in heights of room and the interesting development of the common-user lecture block facing south all help to excite the visitor and give the permanent dweller satisfaction. To have fitted three such different departments into the same block and woven them to form a united home is an undoubted achievement, and those who work there will be indebted to the architects, Messrs. Richard Sheppard, Robson and Partners. Each of the departments has a strong postgraduate research school, and Merz Court can be looked on as one of the major centres of new development in the north-east.

In the course of his tour round the building, the Prime Minister pointed out the general importance of modern technologies and the need for each part of the country to play its individual part in a successful community.

## OBITUARY

### Prof. John E. Driver

PROF. JOHN E. DRIVER, who died in Winchester on March 27, was the youngest son of Frederick and Mary Eleanor Driver, and was born in Montserrat in the West Indies on October 26, 1900. He left Montserrat at the age of ten and continued his education at West Buckland School in Devon and High Pavement School in Nottingham, from which he won a scholarship to what was then University College, Nottingham. He was remarkably advanced for his age, and as a student during the First World War, doing part-time training in the Officers Training Corps, read for an honours degree in chemistry under Prof. F. S. Kipping, and graduated at the early age of nineteen. The commanding officer of the Officers Training Corps, Major S. R. Trotman, was city analyst and part-time lecturer in applied chemistry, and Prof. Kipping also assisted the Corps as musketry instructor, so that there was close integration of his academic and military studies. This occurred in another form in his service during the Second World War.\*

Driver was appointed to the staff of University College, Nottingham, in 1920 and remained there until 1938 when he left to take up a similar post at Cambridge, which he held until the end of 1945, though absent on war service for much of the time. Although conditions for research at Nottingham in the pre-war years were difficult and formal teaching duties very heavy, he began research which continued throughout his career, making important contributions to such topics as the chemistry of crude drugs, dyes of the aurin type, the Fries re-arrangement, and

phenol aldehyde reactions, publishing mainly in the *Journal of the Chemical Society*. He was a lucid and inspiring teacher to pharmacy as well as chemistry students and was popular with staff and students alike. For some years he was an examiner of the Pharmaceutical Society of Great Britain. He showed a flair for authorship too, especially in the field of pharmaceutical chemistry and the chemistry of crude drugs. In collaboration with A. O. Bentley he published the *Textbook of Pharmaceutical Chemistry* in 1925, and was largely responsible for the revision of this book in the several new editions produced in the subsequent 40 years. He also published, with G. E. Trease, *The Chemistry of Crude Drugs*. His other books were *Bakery Science* and *Tables of Qualitative Analysis*, and he was checking the proofs of the seventh edition of the popular *Analysis* book at the time of his death. His career at Cambridge had scarcely begun when it was interrupted by the Second World War. His qualifications and experience led him naturally in the Services into the Chemical Warfare Branch of the Royal Engineers. His service in the Army during 1940-45 included a period of two years at the School of Chemical Warfare at Winterbourne Gunner as brigade major, as well as periods with the 21st Army Group and the War Office.

Driver became the editor of the *Journal of the Chemical Society* in 1946, filling this office very effectively until 1949 when he accepted the invitation to become professor of chemistry in the University of Hong Kong. He took up his appointment on April 11, 1949, and filled it with distinction until an intermittent but serious illness forced him to retire on March 11, 1960. The Faculty of Science

in the University of Hong Kong had been formed only just before the Second World War, though chemistry, physics and mathematics had been taught earlier within the Faculty of Arts. The newly created Faculty had its first meeting on July 4, 1939. Development was interrupted in December 1941 by the invasion of Hong Kong, when the University was forced to close. It did not re-open until 1946 and had to start again almost from scratch. In this vital phase of re-establishment and expansion he played an important part. He planned a new chemistry building, completed in 1953 and described in the *Proceedings of the Chemical Society* in 1958, which has proved one of the University's finest buildings. He was able also to resume his research activities. Perhaps his main contributions were nevertheless in the exercise of his flair for clear thinking, clear presentation of views, and as an administrator. He was elected dean of the Faculty of Science in November 1950 and held office continually until November 1959. His talents as an author were particularly helpful in his duties as chairman of the Board of the University of

Hong Kong Press. His valuable services were, however, by no means confined within the University. He acted several times as external examiner in the University of Malaya. He took also a great interest in extra-mural affairs. He served as a Justice of the Peace. He served also on the Hong Kong Government Pharmacy Board and Dangerous Goods Committee. He was a Fellow of St. John's College in the University and a member of the Council of St. John's Cathedral in Hong Kong. His notable contributions to all aspects of University life led the Senate to confer on him the title of emeritus professor on his retirement.

Despite poor health, retirement did not end his professional career entirely. He continued to revise his books, and the University of Hong Kong was very glad to have his continued service as its representative in the United Kingdom. He retired to Hampshire, where country life and the devoted care of his wife brought back a measure of health and the ability to sustain his professional and other interests.

J. MILLER

## NEWS and VIEWS

### Director-General of the Meteorological Office:

Sir Graham Sutton, F.R.S.

SIR GRAHAM SUTTON, who will be retiring from his position as director-general of the Meteorological Office on September 30, was appointed head of the Office in 1953 after unusually varied experience in Government Science: superintendent of research at the Chemical Defence Experimental Establishment, Porton; superintendent, Tank Armament Research; chief superintendent, Radar Research and Development Establishment, Malvern; and latterly as Bashforth professor of mathematical physics and dean of the Royal Military College of Science. His personal scientific reputation rested on his pioneer researches in diffusion and other problems of the Earth's atmospheric boundary layer, consolidated by his textbook *Micrometeorology*, which appeared first in 1953 and has become the standard text. Under his direction the Meteorological Office has undergone numerous changes towards meeting its primary task of providing a national meteorological service, its hitherto dispersed headquarters have been unified at its new and impressive scientific and administrative centre at Bracknell, Berkshire, and Weather Centres, open to the public, have appeared in a number of cities; but perhaps the large development of the research side of the Office, to the status of a directorate employing some sixty research scientists with high-speed computers and other modern facilities to match, will come to be regarded as his most far-reaching innovation. He leaves the Meteorological Office as a well-founded, up-to-date, scientific institution. Fortunately, in his new post as first chairman of the Natural Environment Research Council (*Nature*, 205, 748; 206, 873; 1965) his wide experience and proved ability will continue to be devoted to the benefit of science for some time to come.

Prof. B. J. Mason, professor of cloud physics in the Imperial College of Science and Technology (*Nature*, 188, 781; 1960), has been appointed to succeed Sir Graham Sutton as director-general of the Meteorological Office.

### Controller in the Ministry of Technology:

Dr. J. B. Adams, C.M.G., F.R.S.

THE Minister of Technology, the Right Hon. Frank Cousins, has appointed Dr. J. B. Adams, director of the Culham Laboratory of the U.K. Atomic Energy Authority, to be controller in the Ministry of Technology. In addition

to his duties with the Ministry, Dr. Adams will continue to direct the work of the Culham Laboratory.

John Bertram Adams, who is forty-five years of age, entered Siemens Research Laboratory from Eltham College. He was employed between 1940 and 1945 in the Telecommunications Research Establishment, Malvern, and afterwards in the Atomic Energy Research Establishment, Harwell. During 1953-61 he served with the European Organization for Nuclear Research (CERN) at Geneva, for the last fifteen months as director-general. As director of the Proton Synchrotron Division, he was responsible for the design and installation of the highly successful 28-GeV proton synchrotron which is the major equipment of the laboratory. In 1961 he rejoined the U.K. Atomic Energy Authority as director of the newly established Culham Laboratory. Dr. Adams was made a Companion of the Order of St. Michael and St. George in 1962. He was awarded the honorary degree of D.Sc. by the University of Geneva in 1960 and by the University of Birmingham in 1961. Dr. Adams was elected a Fellow of the Royal Society in 1963.

### Mathematics in the Imperial College of Science and Technology: Prof. G. E. H. Reuter

PROF. G. E. H. REUTER has been appointed professor of mathematics in the Imperial College of Science and Technology from October 1965. For the past six years he has been professor of pure mathematics at the University of Durham. Educated at Trinity College, Cambridge, he took up his first appointment, in the Scientific Civil Service, in 1941. In 1946 he moved to the University of Manchester where he was made senior lecturer in 1955. His research has been chiefly concerned with the analytic theory of Markov chains. The development of a population of any kind may be viewed as a random process and a Markov chain may be thought of as a far-reaching generalization of such a process. While Prof. Reuter has, for example, made a study of competition between insect populations, his most important work concerns the theory of general Markov chains. In the case of populations, one assumes known the birth and death rates at the possible population sizes and attempts to deduce various aspects of the population behaviour from these. To solve the analogous problems in general is perhaps the main aim of Markov chain theory. Prof. Reuter has shown that operator theory is a natural tool for tackling

many of these problems and that it may be applied to solve some of them under wide conditions. One might single out his work on the Kolmogorov equations and his work with Dr. D. G. Kendall (now professor of mathematical statistics at Cambridge) on ergodic theory and on 'pathological' Markov chains.

#### Pure Mathematics in the University of Durham

FOLLOWING the appointment of Prof. G. E. H. Reuter to a professorship in mathematics in the Imperial College of Science and Technology, two professorships have been established in pure mathematics in the University of Durham. Dr. T. J. Willmore and Dr. C. Hooley have been appointed to these chairs.

#### Prof. T. J. Willmore

Dr. Willmore was educated at Palmer's School, Grays, and King's College, London, where in 1939 he gained a B.Sc. degree with first-class honours in mathematics. He was appointed an assistant in the Mathematics Department at King's College, but the outbreak of the Second World War changed his plans and from 1939 until 1946 he was on the scientific staff of the Royal Air Force at Cardington. After the War, preferring a university career to the Scientific Civil Service, he resigned his appointment and in 1946 became a lecturer in mathematics at Durham. In 1954 he was appointed lecturer in the Department of Pure Mathematics at Liverpool, and became a senior lecturer in 1956 and a reader in 1962. Dr. Willmore's early research was on elasticity and relativity theory, but he soon became interested in differential geometry and most of his work has been in this field. He has published many papers on harmonic spaces, parallel distributions and derivations on manifolds, and has more recently become interested in global problems, particularly those related to curvature. He is the author of *An Introduction to Differential Geometry*, which is now a standard undergraduate and postgraduate text in Britain and the United States. He also collaborated with H. S. Ruse and A. G. Walker in the production of a monograph on harmonic spaces and with I. R. Porteous in an undergraduate text on topological geometry which is soon to be published. The training of mathematics teachers is a particular interest of Dr. Willmore's and he has for some time actively assisted the Institute of Education and the Extra-Mural Department in Liverpool, where he helped to organize and run courses designed to bring teachers up to date in modern mathematics. It is always a compliment to be appointed as a senior member of a university where one was formerly a junior, and Dr. Willmore's distinction as a geometer and a teacher, together with his energy and wide interests, will contribute much to his former university and its Mathematics Department. Dr. Willmore takes up his new appointment on October 1.

#### Dr. C. Hooley

Dr. Hooley was educated at Abbotsholme School, Derbyshire, and (after two years national service) at Corpus Christi College, Cambridge. He was a Fellow of the College from 1955 until 1958, and since then has been a lecturer in mathematics at the University of Bristol. Dr. Hooley's researches in the analytic theory of numbers have brought him an international reputation among experts in the field. The subject is one which has been deeply investigated during the past fifty years, and the frontier of the knowledge which is attainable by conventional methods is fairly well defined. But it is sometimes possible to make further advances by a combination of analytical and arithmetical reasoning, the latter being technically elementary but usually very difficult. In this way Dr. Hooley has succeeded in proving a number of results that had previously appeared to be out of reach. As an illustration, one may mention his proof (*Math. Zeitschrift*, 69, 259; 1958) that for 'almost all' those

numbers that are representable as  $x^2 + y^2$ , with positive integers  $x, y$ , there is only one such representation.

#### Medical Research Council: Appointment of Head of Division of Physiology and Pharmacology, National Institute for Medical Research

PROF. B. DELISLE BURNS has been appointed to the scientific staff of the Medical Research Council as head of the Division of Physiology and Pharmacology at the National Institute for Medical Research, in succession to Prof. W. S. Feldberg, who will be retiring from this post in November 1965. Prof. Burns, at present professor and chairman of the Department of Physiology, McGill University, Montreal, was a member of the Council's staff at the National Institute from 1946 until 1950. He has been particularly concerned with neurophysiological investigations of the brain, and it is intended that his work in this field should be continued when he takes up his appointment at Mill Hill next year.

#### Computers, Technologists and their Applications

IN a written answer in the House of Commons on June 24, the Minister of Technology, Mr. F. Cousins, gave the names of 17 research associations which actively encouraged the use of computers in their respective industries; of 18 research associations which had access to computers on their premises, at universities or at member firms; of 15 research associations using computers in their routine research (the names of some associations appear on all three lists). In another written answer on June 24, Mr. Cousins stated that of 4,064 non-industrial Civil Servants employed by his Department on June 1, 1965, 1,400 had university degrees or equivalent qualifications in scientific or technological subjects, and about another 1,400 had other scientific or technological qualifications. In a third written answer, Mr. Cousins stated that since the creation of his Department, besides the sponsored study by the Tavistock Institute of Human Relations of factors affecting the status of engineers, and work on behalf of the Committee on Manpower Resources for Science and Technology, action was in hand, in collaboration with other Departments, to promote the greater use of technological subjects in television and radio programmes, and to produce special booklets and films for wide distribution among young people.

#### Oceanographic Research

IN reply to questions regarding oceanographic research and the representation of the Ministry of Defence on the Natural Environment Research Council, in the House of Lords on June 22, the Minister of State for Education and Science, Lord Bowden, said that the Government was quite satisfied in this respect. The membership of the Council included Prof. M. J. Lighthill, Sir Edward Bullard, Prof. J. E. Harris and Dr. C. M. Yonge, all of whom had made important contributions to oceanography or related fields and had served on the National Oceanographic Council. The terms of reference of the Natural Environment Research Council were adequate for it to continue to advance the national effort in scientific oceanography, and this would be assisted by the fact that there would be one source of funds for basic oceanographic research. The Ministry of Defence would have an assessor on the Natural Environment Research Council, and would also be represented on the Council's Committee responsible for oceanography. The Minister was anxious to continue to co-operate, and the Royal Navy's representatives on the Committee were determined that their Service's interest should be pursued as strongly as when the Admiralty administered the National Institute of Oceanography. There must be close co-operation between the Navy and oceanographic research—on which the Navy spent £590,000 in 1964. On priority of oceanographic research, Lord Bowden said that priorities for various

research programmes were to be assessed by the new Research Council, and it was the Government's intention, in the light of the advice it received from the Research Council, to do what it could in oceanographic research. This was not a field in which inadequate resources need limit Britain's participation.

### Business Schools

In reply to questions in the House of Commons on June 24 regarding progress in establishing the new business schools, the Secretary of State for Education and Science, Mr. A. Croxall, stated that both schools had leased temporary premises which were being adapted and equipped, and staff were being recruited. The first full-time course at Manchester would begin next September. In London it was hoped to hold some part-time courses this autumn and full-time courses would start next February. The London School had appointed its director. He was satisfied that liaison between the Schools and the Ministry of Technology and the Government generally would be close and sufficient.

### Liberal Studies in a Technological Education

UNDER the title *Symposium on Liberal Studies* the British Association for Commercial and Industrial Education has collected a series of articles issued in the Association's *Journal* over the past 18 months (Pp. 40. London: British Association for Commercial and Industrial Education, 1964. 10s.). The president of the Association, Sir Willis Jackson, contributes a foreword, and there is an introduction by Sir Eric Ashby, in which he states his reason for regarding the liberal studies programme in the colleges of advanced technology as one of the initially important educational experiments of this century. There is also a select bibliography compiled by Mr. D. R. O. Thomas, characterized by breadth and vision, though, as always, there is room for two opinions as to the merits of including some particular books as against alternatives. In the first of the six articles, Prof. T. Lupton argues the claims of industrial administration to be a liberal undergraduate study. In the second, H. N. Sheldon describes the aims, content and methods of these studies, which are the responsibility of the general or liberal studies departments in colleges of advanced technology and assesses their value as an integral part of the training of undergraduate technologists. In the third, "Teaching Technology—a Liberal Approach", Mr. J. P. Moore describes the present aims and methods in teaching metallurgy at Bitterne College of Technology; Mr. D. J. Isaac's "Concepts and Methods in Science Teaching" examines the attitudes and assumptions of students and teachers at Brunel College and describes the action taken to remedy an unsatisfactory situation. Under the title "Humanities and Technology—a New Discipline", Mr. A. M. Duncan discusses the imperative need for a single system rather than two separate aspects of the same culture. Finally, Sir Lealie Rowan in an article, "Liberal Studies in Technology", examines the need for a change in attitude to training for management.

### British Admiral: A Super Oil Tanker

ON March 17, 1965, *British Admiral*, the first tanker of 100,000 tons deadweight to be built in Europe, was christened and launched by H.M. The Queen at Barrow-in-Furness, at the yard of Vickers-Armstrongs (Shipbuilders), Ltd. This was a great occasion, but there was also another soon to follow. In April 1965 the British Petroleum Tanker Company, the shipping subsidiary of the British Petroleum Company, celebrated its jubilee and to mark both events a recent issue of the *BP Magazine*, that colourful quarterly, is devoted entirely to sea-going oil-tankers and to the history of the British Petroleum Tanker Company in particular (15, Spring 1965. Pp. 32. British Petroleum Co., Ltd., London, E.C.2). "During these 50 years, the

British Petroleum Tanker Company has grown from modest beginnings to become the operator of one of the largest privately owned fleets in the world. The ships themselves have grown in size and complexity and the business of tanker operation, whether from the viewpoint of the man at sea or in the office, has developed to the point where it is at once both an art and a science." The articles in this publication, written by experts, aided by a series of excellent illustrations and diagrams, not only record the achievements of the British Petroleum Tanker Company over the years, but they are also a valuable insight into the evolution of oil-tankers in general; as such, they make commendably instructive reading for all interested in this vital aspect of international oil operations. The contributions are: "From Whaling Brig to Super Tanker", by M. Clémans and R. Ilian; "Those Who Go to Sea" (anon.); "A Tanker is Built", by Commander E. H. W. Platt; "Special Services", by Captain G. A. B. King. There is a special supplement in photogravure illustrating the actual launching of *British Admiral* and inside this folder are representations of some of the original plans used for its construction. From these and some explanatory notes it is learnt that, compared with the *Queen Elizabeth* (overall length 1,031 ft.), *British Admiral* is 917.5 ft.; breadth is 118 ft., and 128 ft., respectively; the tanker's depth to upper deck is 66 ft.; her service speed, loaded, about 15.5 knots. Other details of engines and general layout of this 'big ship', also of the commodore and chief engineer, whose responsibility she will be, complete what is a most worthy tribute to all concerned with her commission.

### The Quekett Microscopical Club

THIS year the Quekett Microscopical Club celebrates the centenary of its foundation on July 7, 1865. The title of the Club commemorates the name of John Thomas Quekett, professor of histology and conservator of the Hunterian Museum at the Royal College of Surgeons of England. A prominent microscopist in his day, a founder member of the Microscopical Society of London (now the Royal Microscopical Society), Fellow of the Royal Society and of the Linnean Society of London, and author of the *Practical Treatise on the Microscope*, Quekett died in 1861 at the early age of forty-six, greatly respected by all who knew him. There were at the time several suggestions current for commemorating his name in some permanent way. As events have proved, no more appropriate memorial to him could have come of this desire than the association of his name with this Club, which has met twice-monthly for the past hundred years, and to-day enjoys a world-wide membership. Appropriately, the Club's president in this centenary year is Prof. George J. Cunningham, who occupies the chair of pathology at the Royal College of Surgeons. The principal event to mark this occasion will be a two-day celebration meeting and exhibition of microscopy, which will be open to visitors. This will be held in the Central Hall, Westminster, S.W.1, during October 8-9. The theme of the meeting will be two-fold: first, to illustrate the founding and history of the Club; and secondly, to present a survey of present-day microscopy and microscopical equipment in science and industry, in addition to its recreational aspects. Demonstrations of films, slides and microprojection will also be staged throughout the two days.

### The Manchester Museum

THE report for the year ending July 31, 1964, recalls that for several years the policy of the Manchester Museum has been to concentrate on the more attractive display of its extensive collections (Pp. 20. Manchester: The Manchester Museum, The University, 1965). To this end, a new Japanese gallery has been arranged and the Cannon Aquarium opened to the public. The latter was removed from the Zoology Department of the University and



includes displays of tropical and cold freshwater fish, amphibians, snakes and lizards and a variety of invertebrates. Another feature of outstanding interest during the year was an exhibition of the Moon. A commercial firm in the town centre has given the use of a shop-window for the display of a small exhibit. This is changed each month and is already proving an attraction.

### The Coryndon Memorial Museum, Nairobi

DURING the year 1963-64 the authorities at the Coryndon Memorial Museum realized that with the importance now given to East Africa in matters concerning the conservation of wild-life, they were becoming the obvious centre of visual education and propaganda in this connexion (report for the period July 1, 1963, to June 30, 1964, of the Coryndon Memorial Museum, and the Coryndon Museum Centre for Prehistory and Palaeontology. Pp. 52. Nairobi: Coryndon Memorial Museum, 1964). Consequently, the Museum has featured to an ever-increasing extent as a notable tourist attraction. The Coryndon Museum Centre for Prehistory and Palaeontology, established in buildings situated within the Museum grounds, has now completed its second full year of operation. Under the honorary director of the Centre, Dr. L. S. B. Leakey, the results achieved have been extremely encouraging, and the necessary finance has been raised to construct a much-needed additional laboratory. Work has continued on extending the exhibition space in the Snake Park, and a large aviary has now been completed.

### Marble of the Cyclades Islands

THE report for 1964 of the Rugby School Natural History Society contains a report by J. C. Broome which won for him a Trevelyan scholarship (Pp. 24+1 plate. Rugby: Rugby School Natural History Society, 1965). He describes a visit to the Cyclades Islands in the Aegean Sea, which are part of an ancient massif, probably of Cambrian age. The rocks underwent considerable metamorphism and marble was formed from the limestone originally present. Orogenesis during Tertiary times established ridges which later became largely submerged, by movements still in progress, to form island chains. In the Cyclades there are two such chains, continuing the lines of Euboea and Attika, respectively. Paros and Naxos lie between them and are made up of older rocks projecting through the more recent. True calcitic marble is found on almost all the islands, and on many it has been quarried. On Tinos there is also a rock which is known commercially as a 'marble', but which is, in fact, an opicalcite.

From the beginning of the third millennium B.C. the islands were the home of the Cycladean branch of the Aegean Civilization. During the early bronze age the islanders produced various kinds of marble objects, the most important of which are small human figurines. The earliest specimens are flat, violin-shaped objects, scarcely recognizable as human, but the more advanced figures often show rounding of limbs and indications of facial features. The centre of the civilization was Paros, and most of the figures are in Parian marble. Marble was first quarried in the sixth century B.C. The most important of its early uses was statuary, which at this period was limited to two forms, both common on the Cyclades, the kouros, a nude male figure, and the koure, a draped female figure. Except on Naxos, where there was a flourishing school of sculpture using local marble, the islanders worked in Parian marble, which was held in high esteem throughout the world and rivalled only by a variety from Mount Pentelion, near Athens. The first use of marble in architecture was for roofing tiles. Later, in the Temple of Apollo at Delphi, the façade was of marble (Parian) and finally the whole building, as, for example, in the case of the Parthenon (Pentellic with

Parian roofing). The quarries passed to Imperial Rome in the first century B.C. and many Cycladean varieties were exported in large quantities to Rome for building materials. Parian marble was extensively used for making statues. During the Byzantine period the quarries were abandoned, but in the nineteenth century a move was made to reopen them. The only ones that are economically run to-day are those of Paros, Naxos and Tinos, which Broome discusses.

### Hyperbaric Oxygenation

THE collection of papers presented at a conference held by the New York Academy of Sciences in February 1964 provides a comprehensive and stimulating review of the present status of hyperbaric oxygenation (*Annals of the New York Academy of Sciences*, 117, Article 2: Hyperbaric Oxygenation. By S. Attar and 37 other authors. Pp. 647-890. New York: New York Academy of Sciences, 1965. 7 dollars). Treatment of patients under conditions of high-pressure air or oxygen is often considered to be a new science, so it comes as a surprise to read of experiments starting in 1662 and flourishing in the nineteenth and early twentieth centuries. The clinical aspects of treatment of patients suffering from heart complaints or gas poisoning are discussed at some length, as are the effects of high oxygen partial pressure on micro-organisms and toxins. It was encouraging to note that considerable emphasis was placed on the safety aspects that should be observed. One must always remember that when treating a patient in a large chamber, the medical staff are also subjected to the high-pressure conditions and must observe the correct decompression cycle when returning to normal pressure. For example, if they have been at a pressure of 3 atmospheres absolute for 170 minutes, the time taken to return to normal pressure (1 atmosphere absolute) must not be less than 98.8 minutes. The fire hazards associated with high partial pressures of oxygen are also worthy of note since many pieces of equipment normally used in operating theatres may burn with explosive force under these conditions. The appeal made by one contributor for standardization in terminology is especially worthy of notice. For example, chamber pressures are expressed in pounds per square inch gauge pressure, p.s.i. absolute, atmospheres, depth of water and mm of mercury. This provides an exercise in mental arithmetic, but makes comparisons of various conditions tedious.

### Nutrition Research in Hungary

RESEARCH on nutritional and allied problems is expanding in most countries. A small volume recently published—*Year-book of the Budapest Institute of Nutrition 1963*—clearly shows that Hungary is no exception (Pp. xiv+86. Budapest: Institute of Nutrition, 1964). This publication reflects in abbreviated form the varied activity of the Institute. Its staff published seventy communications during the year, and some of them are summarized here. As in some other nutritional research institutes, the coverage extends into fields of food chemistry, microbiology, and also into problems of food and cosmetics toxicology. Among investigations of interest is a series on the factors which influence the composition of human milk. It is shown, for example, that in Hungary the milk tends to contain less vitamin A and vitamin C (in the winter and spring) than in other countries, which is related to a lower intake of these vitamins. On the other hand, a higher allowance of vitamin C is recommended than in the British Medical Association proposals. The effect of fats on protein metabolism is reported. Lard and butter are shown to result in greater excretion of nitrogen than when margarine and sunflower seed oil are ingested; the latter being recommended for normal nutritional benefits and in post-operative diets. Paraffin is demonstrated to interfere with the utilization of carotene but not with its absorption from the intestinal



tract. Other matters reviewed here are a survey of radioactivity in foods, nutritional values of wheat and cabbage proteins, and the use of thin-layer chromatography for lipid examination. An interesting section contains a number of papers on the effects of high-frequency and infra-red cooking (evidently in increasing use in Hungary) on the physical properties of foods and their nutritional losses.

#### Quaternary Diatom Flora in West Sweden

*Diatom Floras in the Quaternary of the Göta River Valley (Western Sweden)*, by Dr. Urve Miller, describes the diatom succession in the silts in the Göta River from Glacial times onwards (*Sveriges Geologiska Undersökning. Series Cc, No. 44*. Pp. 67+6 plates. Stockholm, 1964. 30 kr.). The silts contain a few marine fossils, which prove that they were deposited in an estuary, but at most levels the vast majority of the diatoms are freshwater planktonic species, mainly no doubt carried down by the river, but in part perhaps living in fresh water floating on the top of the sea-water. The succession, which shows some well-distinguished phases, is interpreted as the result of both changing climate and changing freshwater supply. Carbon dating is used. The changes are recorded in the ways used for pollen analysis—a fairly large number is counted (here the number chosen was 2,000) and the percentages of the different species or larger groups are recorded. A good many rare species are noted and figured.

#### The Royal College of Surgeons of England

THE annual report of the Council of the Royal College of Surgeons of England for the year ending July 31, 1964, which includes the report of the Institute of Basic Medical Sciences, contains scientific reports from the Departments of Anatomy, Pathology, Physiology, Pharmacology, Dental Science and Biochemistry, Ophthalmology and Anaesthetics, and the research establishment at Buckton Browne Farm (Pp. 124. London: Royal College of Surgeons of England, 1965). The departmental reports include summaries of research progress, and lists of publications during the year are appended.

#### The Leopoldina German Academy of Sciences: Elections

THE Deutsche Akademie der Naturforscher Leopoldina, Halle, recently elected the following new members in the sections indicated: *Astronomy*, Prof. M. G. J. Minnaert (Utrecht). *Pharmacology*, Prof. F. Markwardt (Erfurt). *Pathology*, Prof. W. Doerr (Heidelberg). *Internal Medicine*, Prof. A. E. Sundermann (Erfurt). *Oto-, Rhino-, Laryngology*, Prof. Rosemarie Albrecht (Jena); Prof. H. Jakobi (Halle); Prof. H. Naumann (Berlin); Prof. H. F. Schuknecht (Boston). *Dermatology*, Prof. H. Kleime-Natrop (Dresden). *Stomatology*, Prof. R. Ritter (Heidelberg).

#### John F. Kennedy Memorial Fellowships at the Weizmann Institute of Science

UNDER the John F. Kennedy Memorial Fellowships Scheme, established by the Weizmann Institute of Science, Dr. Sidney Borowitz, chairman of the Department of Physics of New York University, New York City, and Dr. Henry Wallman, professor of applied electronics of Chalmers University, Goteborg, have been awarded one-year fellowships at the Weizmann Institute of Science for the academic year 1965-66, and Dr. John Blatt, professor of applied mathematics at the University of New South Wales, has been named a visiting professor at the Institute for a one-year period. Two scholarships at the Feinberg Graduate School of the Weizmann Institute were awarded to Y. Frishman, for research in nuclear physics, and to M. Lahav, for research in X-ray crystallography. Two additional scholarships will shortly be announced.

The John F. Kennedy Memorial Foundation, the funds of which have enabled the awards to be made, was established by the Weizmann Institute of Science in 1964; it is intended as a 5-million dollar endowment fund to advance scientific research. Half the fund has already been raised, in the sum of £6.5 million, including £1 million from the Israeli Government, £2 million from American contributors, £3 million from the Weizmann Institute's Endowment Fund, £800,000 from Israel donors. The first recipients were chosen by an international scientific committee consisting of Lord Rothschild (University of Cambridge), Prof. A. Lwoff (Pasteur Institute of the University of Paris), Dr. I. I. Rabi (Columbia University) and Dr. J. Robert Oppenheimer (recently retired director of the Institute for Advanced Study, Princeton).

#### University News:

##### Birmingham

THE following appointments have been announced: *Lectureships*, Mr. G. R. Isaak (Physics); Mr. Y. W. Lam (electronic and electrical engineering); Dr. D. R. Lloyd (chemistry); Dr. N. Crawford (physiological chemistry); Dr. D. G. Gwyn and Dr. D. B. Thomas (anatomy). *Research Fellowships*, Mr. M. M. Bibby (chemistry); Mr. G. S. Boulton (geology); Mr. D. P. S. Peacock (application of scientific techniques to archaeology); Mr. C. S. Repton (electronic and electrical engineering); Mr. J. R. Tittensor (chemistry); Dr. K. Burdett (medical biochemistry and pharmacology).

##### Keele

DR. R. C. MADDISON has been appointed lecturer in physics as from October 1.

##### Nottingham

THE following readers have been appointed: Dr. W. B. Hugo (pharmaceutics); Dr. H. McCallion (mechanical engineering); Dr. R. H. Osborne (geography); Mr. C. Snell (stress analysis); Dr. A. Wright (electrical engineering).

#### Announcements

DR. J. H. QUASTEL, professor of biochemistry at McGill University and director of the McGill-Montreal General Hospital Research Institute, has received the annual award of 500 dollars of the Canadian Society of Microbiologists, in recognition of his many years of outstanding research in microbial physiology and allied fields.

OPEN days will be held at the Mining Research Establishment of the National Coal Board, Isleworth, during July 20-21. Further information can be obtained from C. S. Makower, Mining Research Establishment, Worton Hall, Worton Road, Isleworth, Middlesex.

THE 451st meeting of the Biochemical Society will be held jointly with the Physiological Society in the University of Oxford during July 15-17. Further information can be obtained from the Biochemical Society, 20 Park Crescent, London, W.1.

THE second international conference on "Protozoology" will be held in the Imperial College of Science and Technology during July 29-August 5. Further information can be obtained from Dr. R. S. Bray, London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1.

AN international neurochemical conference entitled "Variation in the Chemical Composition of the Nervous System as Determined by Developmental and Genetic Factors", organized under the auspices of the Neurochemical Commission of the World Federation of Neurology, will be held in Oxford during July 25-30. Further information can be obtained from Dr. G. B. Ansell, Department of Experimental Neuropsychopharmacology, the Medical School, Birmingham 15.

## THE MATHEMATICAL ASSOCIATION ANNUAL CONFERENCE

FOR the first time Oxford was the venue of the Mathematical Association's annual conference, the largest ever, which was held during April. Members were accommodated at St. Hugh's (the College of this year's president, Dr. I. W. Busbridge), Somerville, Trinity and Wadham Colleges. They, with large numbers of non-resident members, attended the inaugural meeting in the Sheldonian Theatre and later lectures and meetings in the spacious new buildings of the Oxford College of Technology.

The Association was received on arrival by the Lord Mayor and Lady Mayores at a civic reception, and later by the Vice-Chancellor and Mrs. Wheare at a University reception at Rhodes House. The Association's annual dinner was held in St. Catherine's College. One afternoon was devoted to group visits and tours. Parties visited the University Engineering Laboratory, the *Atlas* computer at Harwell, the Hydraulics Research Establishment, the University Press, and various other places of special interest in and around Oxford. The exhibition of working school computers was a special feature of the main conference. These computers, which were demonstrated by senior pupils from the eight schools exhibiting (Abingdon, Bedford, Exeter, Marlborough, Oundle, Rugby, St. Albans and Sherborne) were, with one exception, made by the schools concerned and included digital and analogue machines, together with some smaller 'games' machines.

The various official reports at the annual business meeting indicated that the Association is in a flourishing condition with many worth-while projects in hand and a membership of now more than 5,000. The Treasurer, however, sounded a clear note of warning that, with increasing costs, the normal rate of increase of membership will be insufficient to provide the necessary finance for all the projects to be completed. He set the Association a target of 7,000 members by its centenary year, 1971. The Schools and Industry Committee has been working on the £3,000 given by the British Petroleum Company, and has good hopes that further assistance will be available to enable it to continue work beyond present horizons. The Association's Diplomas (Teaching and Technology) are now well established and the Teaching Diploma is keeping abreast of modern trends of teaching.

Following the arithmetic, mechanics and statistics reports, the Teaching Committee is now taking a more general look at mathematics as a whole. In particular, the teaching of mathematics between the ages of eleven and sixteen is to receive special attention: an attempt will be made to synthesize the various 'mathematical projects' and form some conclusions. (Meanwhile, the Association is itself publishing a number of papers on selected 'modern' topics, written by individual members: these papers are not intended to represent the Association's considered views in these matters.) The Teaching Committee will also review its primary school and technical college reports, with the view of rewriting them.

Mr. J. T. Combridge, reporting generally on "Current Affairs", spoke of the way in which the Association and the Institute of Mathematics and Its Applications are working closely together to the common good, each in its own field. He saw little danger that the advent of the Institute, which owed much to the Association for its inception and is now well established, would mean any diminution of the Mathematical Association.

The place of the computer in mathematical education is one topic in which the existence of a professional mathematical institution has helped the Association.

They have both worked through the Joint Mathematical Council in deciding how best the computer needs of schools can be met, not only for class instructional purposes, but for familiarization courses for teachers. The present choice would seem to lie between the central computer based on a university or college of advanced technology, and the travelling computer. There is no support for individual computers for schools.

For the International Congress of Mathematicians at Moscow in 1966, the three topics to be discussed by the International Committee for Mathematics, and their United Kingdom sponsors, are: (a) the programme of university training in mathematics for the future physicist (the Institute of Physics and the Physical Society); (b) the use of the axiomatic method in secondary teaching (the Mathematical Association); (c) the development of mathematical activity in school children; the place of the problem in their development (the Association of Teachers of Mathematics).

### Robbins and All That

Dr. I. W. Busbridge began her presidential address by directing the attention of the large audience in the Sheldonian Theatre to the building designed by a mathematician, Christopher Wren, and to Robert Streater's painted ceiling with its motif 'Truth descending upon the Arts and Sciences', and its mathematical significance.

In this age, if we are to achieve an educated nation, much "blood, toil, tears and sweat" lies ahead. Dr. Busbridge suggested that it was worth considering similar problems which must have faced Britain when the building of the Sheldonian was started in 1664. Galileo had died but twenty-two years before, the Royal Society had just been founded, Wren and Newton were young men, Halley a child, and, with the Commonwealth upheaval, the whole period was a ferment of ideas in many ways similar to our situation to-day. The Visitations at Oxford and Cambridge and their effect were paralleled to those of Robbins on the present university. It was of interest that the appointment in 1647 of John Wallis, for fifty years Savilian professor of geometry, was due to his predecessor being replaced by the Visitors for possessing Royalist sympathies. Both Wren and Halley must have been taught by him. The Visitors tried to impose new statutes on universities and colleges, but then, as now, Oxford demanded the right to take its own government into its own hands and to write its own statutes. Even then there were proposals for new universities, with London, York, Manchester and Durham all claiming consideration, and much outspoken criticism of the older universities. There were advocates for a university for every city, and suggestions for turning universities into scientific and technological institutes. With the Restoration came the reaction against science, and the dedication of the Sheldonian provided the popular preacher, Robert Smith, with an opportunity to damn the new philosophy.

In spite of this reaction, the golden age of British mathematics and mathematical physics blossomed: the time was ripe for Newton, whose *Principia* was initiated, and the printing paid for, by Edmund Halley, whose observatory is still to be seen in nearby New College Lane. This new learning found its way into the schools, and men such as Dr. Thomas Gale of St. Paul's School, later a Fellow and Secretary of the Royal Society, were firing the minds of boys like the young Halley. An important experiment in mathematical education was the foundation in 1673 of the Royal Mathematical School at Christ's Hospital to provide navigators for the Royal

Navy and merchant marine—an early technical section of a comprehensive school. The foundation had the benefit of advice and help from Sir Jonas Moore, inspector general of Ordnance, and one of the best practical mathematicians of the day, and the continuing interest of Newton, Flamsteed, Wren and Pepys, all governors of the school. Halley, who lived nearby, helped with the publication of the text-book on navigation. Unfortunately there was—just as now—a shortage of teachers of mathematics, and, in 100 years, no eminent mathematicians were produced by this School.

Before coming to the problems of the present day, Dr. Busbridge referred to a later 'Robbins Report'—the Royal Commission on the Universities of 1850. Although some steps had been taken before 1850 to establish degrees in science at Oxford and Cambridge and the setting up of laboratories at Balliol and Christ Church, the Commissioners made enlightened proposals regarding the provision of a School of Mathematics and Physical Sciences with two departments: (a) Pure and Applied Mathematics; (b) Mechanical Philosophy, Chemistry and Physiology, together with subordinate science. They also proposed that proficiency in mathematics might compensate for weakness in Latin in a new matriculation examination. The reactions at Oxford were violent, but reforms came steadily.

Turning to the Robbins Report itself, Dr. Busbridge considered the recommendations for a two- or three-subject degree to include mathematics, since "the great majority of undergraduate students of mathematics have neither the aim nor the ability to become mathematicians of the front rank, and, for them, somewhat less concentration would be appropriate, in order to make way for the study of some other suitable subject such as physics or chemistry". Dr. Busbridge, while completely in favour of two-subject courses involving mathematics provided such a course is well integrated, was concerned at the definition of 'front-rank' mathematicians. Assuming that such a mathematician is one capable of profiting from a postgraduate course in advanced mathematics, that is, those with first and top seconds in Finals, she disagreed profoundly with the suggestion that all the rest would be better doing two-subject degrees. It is well known that almost one-third of the students entering mathematics courses fall by the way. The proposals were: (a) more help and guidance to be given to first-year mathematics students—the year which even quite able students find difficult; (b) grants should be extended to allow mathematics honours failures to transfer to a double-subject honours course (this policy is understood to be now accepted in principle); (c) a lowering of the standard of the first-year course. However, she appreciated that each university must solve the problem for itself.

Dr. Busbridge was next concerned with the shortage of teachers of mathematics and, with the increased requirements of the universities and other colleges of further education, she feared a shortage in the schools for some time to come. The Oxford system of lectures to perhaps 150 undergraduates, with a wide range of abilities, made it desirable that there should be an adequate backing of tutorials; but this again created a staffing problem, though without two-way traffic at all levels, lectures cannot expect to be successful. The tutorial system is equally good for the "embryo Newton", the steady-going second-class man, and for the man at the bottom of the list. Dr. Busbridge did not advocate the abolition of the Oxford entrance and scholarship system. Her experience showed that among her most able students were those who on the results of the Advanced Level Examination for the General Certificate of Education would never have been selected.

The new school mathematics courses are bound to create problems over the next few years, and universities will have to design their first-year courses over that period so that all students arrive at the second year with a common basis of knowledge. This must be done without

boring "modernists" who have been stimulated in their schools or confusing the "traditionalists" by not laying a true foundation for the new work. The Association's Universities and Schools Committee is trying to compile a basic syllabus which would be common to all Advanced Level courses for the General Certificate of Education and without which a first-year university course would be unintelligible. This basic syllabus would also be the backbone of the one-subject Advanced Level course, thus facilitating the transfer from science courses to mathematics.

Dr. Busbridge appealed for more experimentation in schools. She did not expect them to go all the way with some of the new projects, but hoped teachers would endeavour, whenever appropriate, to make injections of modern mathematics into the traditional system. This would stimulate both teacher and pupil. Syllabuses were never intended to be adhered to rigidly. Every teacher of mathematics has two objects: (a) to give to every student the opportunity of developing his or her mathematical powers to the full; (b) to foster those of special ability for the sake of the country as a whole and for the advancement of knowledge. Dr. Busbridge criticized the Leicestershire form of comprehensive schooling (with its junior and senior high schools) as it is likely to affect the fulfilment of these objects. The years twelve to fifteen are the most important in the production of the mathematician, yet, under the Leicestershire scheme, experience is showing that few qualified mathematicians are opting to teach in the junior high schools. For Britain to survive we have to increase the supply of mathematicians, and yet many local authorities are turning to the Leicestershire plan as the solution to all their difficulties. In the true comprehensive school no such difficulty is created. She considered that the Mathematical Association should press for a dovetailing of time-tables between the junior and senior high schools under the Leicestershire plan so that at least the 'A' streams would be taught by fully qualified mathematicians. In the universities the process of levelling up or levelling down is also working. Whatever happens, this must be a levelling up, since Britain needs the best mathematicians that she can produce if the 'brain drain' is ever to be slowed and a reverse flow stimulated. Eminent research schools must be built up which will provide such intellectual and material attractions that many of the ablest young mathematicians and scientists will choose to study in them.

In conclusion, Dr. Busbridge, viewing all the difficulties ahead, took heart from the past surrounding her audience. Life under the Puritans must have been pretty grim, and yet it was the continuing effort of men with vision and faith that brought in the golden age of British mathematics. Now, as in 1885, we need the vision of Christopher Wren and Robert Streater when they chose 'Truth descending upon the Arts and Sciences' as the subject for their great painted ceiling.

#### The Mathematical Association, the Institute of Mathematics and the Joint Mathematical Council

The first discussion of the conference was on what the foregoing bodies do for the teacher of mathematics, and was opened by Prof. T. A. A. Broadbent on the Mathematical Association, and Prof. J. G. Semple on the Institute of Mathematics and Its Applications and the Joint Mathematical Council.

Prof. Broadbent challenged members as to whether they were accepting their individual responsibility to see that things which needed to be done were done. In his view, the Association to-day offered very good value to members for their subscriptions, with a first-class *Gazette*, frequent reports, ample branches, diplomas, etc.

The Association's primary purpose is the teaching of mathematics, but we must appreciate that the mathe-

matics is at least as important as the teaching. We must teach good mathematics in a good way.

He was sorry to note how few of the younger university dons and lecturers were members. This he felt to be a bad thing on a number of counts, but particularly for the inevitable widening which must result in the gap between the universities and the schools, a gap which only the Association is qualified to fill.

Prof. Broadbent hoped to see more young men and women playing their full part in the activities of the Association, and charged the older members to see that this was possible and encouraged. This is not a matter of what 'they' should do; it is a 'do-it-yourself' problem.

Prof. Semple, secretary of the Joint Mathematical Council, outlined the steps which have led in the past two or three years to the setting up of a Joint Mathematical Council of the United Kingdom, and of an Institute of Mathematics and Its Applications. He paid tribute to the part which the Mathematical Association, and in particular Mr. J. T. Combridge, had played in their inauguration.

In addition to setting up the Institute of Mathematics and dealing with international conference matters, the Joint Mathematical Council has been mainly involved with two projects which both affect the teacher: (a) the setting up, under the Organization for Economic Co-operation and Development, of a national committee, under the chairmanship of Dr. E. Kerr (Salford Royal College of Advanced Technology), to report on mathematics as a component of the training of engineers to first-degree level in Great Britain; (b) the formulation of plans for improving the standard of mathematical instruction in schools and increasing the supply of trained mathematicians.

Dr. Kerr's committee has worked out a 'core' syllabus in mathematics for engineers which contains a substantial section on numerical analysis and computer programming science. It has also prepared a suggested Advanced Level syllabus which would provide the kind of school mathematical training most useful to engineers.

Discussions are now proceeding with the appropriate institute on how time for this core syllabus can be found from that at present allocated to other engineering subjects, and how mathematics departments generally are to equip themselves to teach in the spirit envisaged in the report from the Organization for Economic Co-operation and Development.

The Joint Mathematical Council's most difficult project has been that of the present complex of problems relating to mathematical instruction in general, and particularly in schools, both primary and secondary. The Council's approach to this problem has been taken under three main heads: (a) the possible danger of the threatened proliferation of General Certificate of Education syllabuses, and the possibility or desirability of some degree of control, for example, by means of agreed minimal core syllabuses at the various levels; (b) the provision of a regular and effective information service for all teachers of mathematics; (c) the expansion, both for the present emergency and on a permanent basis, of facilities for the in-service training of teachers of mathematics in schools.

To try to reach a decision on (a), the Joint Mathematical Council has charged all its constituent member associations, covering the university-level to the primary school, to examine this problem independently and to report back to the Education Committee of the Council on which selected Her Majesty's Inspectors of Schools are attending members.

The foregoing items (b) and (c) have not posed the initial problems that (a) has done and is likely to do. Recommendations for the in-service training of teachers and for the setting up of an information service for teachers have already been welcomed by the Minister of State for Education and are now under his consideration. These proposals include the setting up for the development

of in-service training of teachers of: (a) local mathematics centres (each located in a school and covering a considerable group of primary and secondary schools in a local education authority neighbourhood); (b) regional advisory units (based on institutes of education); (c) a national committee. In addition, it is proposed that a national information centre should be established, located possibly in one of the larger institutes of education.

The subsequent discussion showed that the Association was very conscious of its failure to make an impact in the primary field and that the fault lay in the first instance with the members, who had failed to accept their responsibility as mathematicians to help to bridge the gap. Courses and lectures were arranged, but secondary and university teachers were all too conspicuous by their absence.

### Mechanics Report and Other Discussions

The second and third discussions of the conference were on the Association's recently issued second reports on the teaching of mechanics and arithmetic in schools.

The Mechanics Report was described by the president as one of the Association's outstanding reports and a great addition to its literature.

Mr. J. T. Combridge, chairman of the Report Sub-Committee, outlined some of the thoughts which had guided its preparation. While applied mathematics is tending to disappear from the examination syllabuses, there is still plenty of applied mathematics which needs to be taught. It is a suitable subject for all secondary pupils whatever the age or ability, and it should provide the opportunity for co-operation with teachers of science subjects. It is most certainly not a dead subject: it is indeed a live and developing subject and teachers should endeavour to make it a live subject in keeping with modern times, whether by experiment or other means. The report endeavours to lead.

Mr. S. L. Parsonson (Harrow School) described the report as a spirited plea for the retention of a subject which surely must be needed in times when the demand for technologists, applied mathematicians, and scientists capable of doing applied research is so great. He blamed the examination system of the past twenty or thirty years for the subject's present disrepute. It has removed the subject from the real world into that of unreal problems, and any questions set involving a test of the principles of mechanics soon showed the students have a serious lack of understanding of the basic principles.

The only major criticisms of the report from the floor emphasized that frames of reference were not sufficiently stressed and that insufficient attention was directed to the needs of the teachers at the Certificate of Secondary Education level who need more guidance than their more highly mathematically qualified colleagues.

The problem of the mathematical model and the difficulty of setting examination questions which are both sensible and soluble at the appropriate level was one to which no ready answer was given. All were agreed on the early introduction of the use of vectors.

The Arithmetic Report (a completely new report to replace the previous 1931 report) was described by Mr. C. G. Nobbs, a member of the Report Sub-Committee, as one based on the concept that computation, seen as a mechanical process, is being left increasingly to the machine, and that the time thus saved allows a more informal approach leading to better understanding.

Mr. Ellerby (St. Dunstan's, Catford), speaking as a junior school master, felt that the report should have given more guidance, though this view was not supported from the floor. Many felt that teachers must be prepared to consider alternative methods which may seem equally desirable, weigh them and make up their own minds.

Some speakers would have liked to see statistics, as a branch of arithmetic, receiving even greater emphasis in the report.

The final discussion of the conference was on school mathematical societies. The main speakers were Mr. D. Hobbs (Exeter School) and Mr. A. R. Tammadge (Abingdon). Mr. Hobbs outlined the activities of an essentially practical society which thrived on the making of mathematical machines and models of all types, working computers being demonstrated. Mr. Tammadge spoke of the more culturally inclined senior society the *modus vivendi* of which was based socially and intellectually on that of the university society.

### General Lectures

Dr. J. F. Scott, deputizing for Prof. McKie, gave the Association a most absorbing account of the early history of the Royal Society up to the early eighteenth century.

Prof. M. F. Atiyah, Savilian professor of geometry in the University of Oxford, speaking on "Linear and Non-Linear Mathematics", took members to some of the frontiers of mathematics to-day, indicating the unity and simplicity of the subject. He showed the significance of linear methods in various parts of mathematics and the advantages in translating certain kinds of non-linear problems into linear form: in this, one does not restrict oneself to finite dimensional linear problems but allows infinite dimensions.

Dr. K. Austwick, Department of Education, University of Sheffield, in an illustrated lecture entitled "Automation in the Classroom", gave a balanced idea of the way in which teaching machines can be used as an aid in the teaching of mathematics. The present machines, which are relatively unsophisticated, are mainly effective in the teaching of motor skills and are more useful for revision, remedial and self-tutoring work with students capable of constructing responses. Conventional teaching

cannot fail to benefit. In this respect his concluding remark was very pertinent: "Any human teacher who can be replaced by a machine ought to be".

Mr. J. H. Durran (Winchester College), speaking under the title "Do-it-yourself  $\chi^2$ ", delightfully demonstrated how the testing of goodness of fit in statistical work can be approached in an intuitive and experienced way.

The final lecture and film of the conference was "I Do and I Understand—a New Look at Primary School Mathematics", by Mr. J. W. G. Boucher, a former primary school teacher, and now a Fellow of the Nuffield Research Foundation. The attendance and interest shown by members at this final lecture showed that there is an increasing awareness among mathematicians that, using as practical an approach as possible with young children, mathematics can become the favourite subject for all children.

### Elections and Future Conferences

At the annual general meeting, Mrs. E. M. Williams was elected president for 1965–66. The following honorary officers were re-elected. *Treasurer*, Mr. R. E. Green; *Secretaries*, Mr. F. W. Kellaway and Miss R. K. Tobias; *Editor*, Dr. E. A. Maxwell; *Librarian*, Prof. R. L. Goodstein; *Assistant Secretaries*, Mr. K. J. Backhouse, Instructor Captain R. G. Cross, Mr. B. J. F. Dorrington, Miss E. M. Holman, Dr. E. Kerr and Mr. C. Steele; *Assistant Treasurer*, Mr. N. Q. Dodds.

The 1966 meeting will be held in the University of North Staffordshire during April 13–16, 1966. The centenary meeting (1971) is being planned for University College, London, where the inaugural meeting of the Association for the Improvement of Geometrical Teaching (now the Mathematical Association) was held in 1871.

R. G. CROSS

## SIXTY THOUSAND PERIODICALS

THE *World List of Scientific Periodicals*\* was initiated late in 1920, when Sir Sidney F. Harmer, director of the Natural History departments of the British Museum, wrote to Sir Peter Chalmers Mitchell, secretary of the Zoological Society of London, asking him to direct the attention of the Conjoint Board of Scientific Studies to the problems of what is now referred to as information retrieval. The multiplicity of periodicals, issued in various languages throughout the world, was already confusing. No single library held more than a fraction of the total number, and the Board was invited to consider the preparation of a list of scientific periodicals with an indication of the libraries where they were kept.

The Board adopted the suggestion, and work began late in 1921, under the guidance of the World List Association, incorporated as a limited company. The first edition concerned the years 1900–21; it was issued in two volumes of which the first, published in 1925, listed 24,128 titles, while the second volume, published in 1927, gave standard abbreviations and library locations. The second edition was issued in one volume in 1934, and covered the years 1900–33; the third edition, covering 1900–50, was issued in 1952 and reprinted in 1958. The present edition covers the years 1900–60 so that, like its predecessors, it attempts to include every periodical which survived into the present century. About a quarter of the entries are titles published since 1950 and the total number is now more than 60,000, and the *List* is now issued in three volumes. In spite of the inconvenience in handling

which is inevitable in a multi-volume work of reference, this edition is even more generally useful, and readily consulted, than its predecessors. A new type-face has been employed, words used in the alphabetical arrangement are in bold type, and the number of cross-references has been increased. The transliteration of Cyrillic and Greek alphabets is in accordance with the recommendation of the Royal Society, modified and published as British Standard 2979: 1958.

The abbreviation of titles of scientific periodicals according to a carefully considered system, which has been a feature of the *World List* since its inception, has been instrumental in effecting a degree of uniformity in bibliographic usage throughout the scientific world. The abbreviations in this edition follow a forthcoming British Standard, not greatly different from the system followed in previous editions.

This is to be the last edition of the *World List* compiled and produced in this way, by an Association which has no permanent staff and by editors whose main occupation is elsewhere. The first three editions were prepared under the supervision of the late Mr. W. A. Smith, of the British Museum. After his death, before work on the fourth edition was begun, Mr. Peter Brown, also of the British Museum, undertook the editorship with Mr. G. B. Stratton, of the Zoological Society, who has been associated with the *World List* from its earliest days. The Association has had four successive chairmen: Sir Peter Chalmers Mitchell, Dr. A. S. Neave, Dr. E. J. Holmyard, and the present chairman, Dr. L. Harrison Matthews. In future, it is proposed to continue the *List* in the form of an annual cumulation of the material published quarterly by *Bucop*, the British Union Catalogue of

\* *World List of Scientific Periodicals Published in the Years 1900–1960* Edited by Peter Brown and George Burder Stratton. Vol. 1: A–H. Pp. xxv+1–532. Vol. 2: I–P. Pp. xx+533–1196. Vol. 3: Q–Z and Periodic International Congresses. Pp. 1187–1824. (London: Butterworths, 1963, 1964 and 1965.) 500s. per set of three volumes.

Periodicals, published by the National Central Library. It is hoped that it will be possible to prepare 5- and 10-year compilations, offering to the user the convenience of the present compilation and incorporating the name *World List* in the title. *Bucop* employs a computerized system and the National Central Library commands resources commensurate with the immense amount of material which it will be necessary to handle.

The possibility of providing an index, to assist (for example) in tracing a periodical the title of which is incompletely known, is being borne in mind by the compilers of the computerized catalogue. It could hardly be expected within the framework of the *World List* as it is to-day. This edition is not absolutely free of errors, but it is clear that it has been most meticulously

prepared. It is obviously impossible to guarantee the inclusion of every scientific periodical in a list compiled from information volunteered by contributing libraries the stocks of which may not include every publication that could qualify. However, the number of libraries contributing to the present edition has been increased and, by courtesy of the Director, the compilers have had access to a number of titles held in the National Lending Library for Science and Technology, which was not officially opened until 1962.

Many new journals have been started since 1960, but nevertheless these three volumes provide an invaluable guide to all but a small fraction of the periodical literature a scientist may wish to consult, and to its whereabouts in British libraries.

## FIELD STUDIES COUNCIL

ACCORDING to the latest annual report of the Field Studies Council\*, the year 1963-64 saw a continuance of the growth in the number of students attending the Council's centres. The total number of student-weeks reached 11,617 (an increase of 12 per cent)—mainly as a result of additional accommodation at Slapton Ley and of a full season at Orilston, but also because of larger bookings at other centres. This was divided between subjects as follows:

Biological subjects	5,979
Countryside and natural history	232
Geographical and geological	4,686
Art	245
Archaeology	219
Other courses	196

The number of individual attendances as distinct from the number of student weeks was 11,629, divided as follows:

Sixth forms	7,736
Other forms	192
Training colleges and teacher courses	1,569
Universities	1,160
Technical colleges	145
Amateurs	827

Despite the extra numbers which the centres have been able to take, many hundreds of would-be students had to be turned away. It is the Council's aim and intention to open further centres when the necessary finance becomes available, and the New Centres Working Party is con-

tinuing its search for suitable properties in areas where centres might be established. The Council was greatly heartened in the early part of the year to learn that the Worshipful Company of Drapers was proposing to make a grant of £25,000 to it to mark the occasion of the sixtieth anniversary of the granting of the Company's first charter. Payment is to be made by instalments over a period of not more than five years; a condition of the grant is that it shall be used for purchasing and converting a property situated in North Wales to a field studies centre. Search immediately began for a suitable building. It was decided that the property most suitable to the Council's requirements was Rhyd-y-Creua, a house situated in the Conway Valley, one mile from Betws-y-Coed. Rhyd-y-Creua is unlikely to be ready to accommodate students before the 1966 season commences.

Following the recommendation of the Scientific Advisory Committee, Preston Montford and Slapton Ley have been inspected by the Department of Education and Science, the reports of which are now being awaited. Preston Montford was also visited by two scientists from the Sub-committee and as a result of their report and suggestions a new experimental biology course was inaugurated which, after one season, is considered to be very successful.

A small working party has been set up, the brief of which is to consider the educational aims and practice of the Field Studies Council in the light of present and future needs and to make recommendations to the Council.

\* Field Studies Council. Annual Report, 1963-1964. [Pp. 39+8] photographs. (London: Field Studies Council, 1965.)

## PHILOSOPHIES OF PIERRE TEILHARD DE CHARDIN

I FIRST met Pierre Teilhard de Chardin as a young man, when we were assisting in prehistoric investigations in north Spain in 1912 or thereabouts. I also had occasion to see him from time to time until his death. He had a charming personality and was an excellent research worker in the fields of palaeontology and geology, and did much administrative work while living in China. He read deeply in the realm of philosophy, and was accepted wholeheartedly by others practising in that discipline. Above all, he was a profound Christian—a Roman Catholic and indeed a Jesuit. Not many men have reached the front rank in all these three disciplines, and therefore what he has to say is worthy of great consideration.

Teilhard was a firm believer in evolution—indeed, he was to some extent persecuted by his own hierarchy for

this belief—but he did not confine evolution to living organisms; for him everything from the atom to reflective man has been influenced by evolution. He made no distinction between man and the animals, nor indeed between animate and inanimate objects. Matter had 'pre-life', an imperceptible particle of life, and was therefore also influenced by evolution. At present the effects of evolution tend to divergence, but in the case of reflective man must ultimately converge towards a unity which he called omega. For Teilhard this omega was equated with the incarnation, birth, life, death and resurrection of Jesus, the Christ.

Such a bald series of statements is, of course, grossly inadequate. It takes many hours of hard study properly to appreciate the width and depth of Teilhard's thought. It is a great pity that the older generation of Jesuits in



the 'twenties failed to recognize what a great man he was. They seldom allowed him to come to Paris, and he mostly lived and worked outside his native France. He died in 1955 in the United States. Only after his death was it possible for his great books to be properly produced. The Abbé Breuil once told me that what he was able to publish during his lifetime was only because he chanced to be a friend of the then Pope. Several times friends suggested that he should cease to be a Jesuit. But Père Teilhard was loyal both to his Church and to his Order.

*Pierre Teilhard de Chardin—Some Aspects of his Thought\** is an admirable introduction to certain aspects

\* Proceedings of the University of Newcastle upon Tyne Philosophical Society, Vol. 1, No. 8: *Pierre Teilhard de Chardin—Some Aspects of his Thought*, P. G. Fothergill, Pp. 24–84. (Newcastle upon Tyne: University of Newcastle upon Tyne Philosophical Society.)

of his philosophies. It should be read before attempting to tackle the great life by Claude Cuénot, or indeed the two major works of Teilhard himself, namely, *The Phenomenon of Man* and *Le Milieu Divin* (the French title has been retained in the English translation). Not everyone after careful study will entirely agree with Teilhard's thought. There will always be those who separate entirely the soul of man from the rest of creation and treat it like the mistletoe on an apple tree, which for a time is bound to the tree yet is really totally distinct. But Teilhard's philosophy is worthy of very careful thought, and anyone interested cannot do better than to read carefully Fothergill's *Pierre Teilhard de Chardin: Some Aspects of his Thought*.

M. C. BURKITT

## ONCOLYSIS WITH LASER ENERGY COMBINED WITH CHEMOTHERAPY

By JOHN PETER MINTÓN

Surgery Branch

AND

PROF. GEORGE H. WEISS and PROF. MARVIN ZELEN

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RECENT experiments have shown that pulsed laser energy can be used for the rapid and precise destruction of malignant tumour implants in animal systems<sup>1</sup>. It has been shown quantitatively that the probability of destroying a tumour implant in a mouse depends on the ratio of laser energy to tumour size. The destruction probability increases with this ratio in a manner which can be made mathematically precise<sup>2</sup>.

It is the purpose of this article to report on experiments where chemotherapy has significantly increased the destructive effect of laser energy on the tumour system. Other investigators<sup>3,4</sup> have also attempted to find a way for potentiating the destructive effects of the laser energy on tumour systems. These investigations involved painting the surface of a tumour with a dye or systemically injecting various pigmented solutions into the animal. The results by these methods have been relatively unsuccessful.

The results of experiments comparing the effect of laser energy alone with the combination of laser energy and a course of cyclophosphamide on tumour-bearing mice are summarized here. The results show that permanent tumour destruction by laser energy is significantly increased in mice receiving cyclophosphamide as compared with mice not receiving this course of chemotherapy.

The experiment consisted of injecting 115 six-week-old CDF<sub>1</sub> female mice with 0.04 c.c. of a 1:10 dilution of Cloudman S91 melanoma<sup>5</sup>. At the end of two weeks of tumour growth, the animals were arranged in order of size of tumour which ranged from 1 mm to 10 mm in diameter. The animals were paired according to size such that animals in a pair had tumours of comparable size. Among the 57 pairs, 11 pairs were designated as 'control pairs'; the remaining 46 were termed 'treatment pairs'. The control pairs were assigned to every fifth pair in order of size of tumour. One animal in each of the control and treatment pairs then received an 11-day course of cyclophosphamide chemotherapy injected subcutaneously at a daily dosage of 25 mg/kg/day. At the end of the fifth or sixth day of chemotherapy, all the animals in the treatment pairs were exposed to laser energy. Animals in the same pair received the same amount of energy from a ruby laser (6889 Laser Unit—Maser Optics, Boston, Mass.). The ruby rod was 1.6 cm in diameter and 17.7 cm in length and utilized a Fabry-Perot etalon dielectric coating for internal reflexion. It was stimulated

by two 7.5 kW xenon flash lamps. The laser energy was delivered in several pulses, each of which was 48–56 joules. Energy determinations were obtained by direct calorimetry at varying intervals between the series of tumour exposures. Each laser pulse was delivered through a biconvex lens with a 12.5 cm focal length.

In order to destroy a tumour, it is necessary that the entire tumour be exposed to laser energy. Therefore, large tumours (greater than 5 mm in diameter) received multiple pulses. Animals were observed for five weeks after exposure to the laser energy.

There were 42 treatment pairs in which results could be evaluated; the death of two mice invalidated results from two other pairs. These results are summarized in Table 1.

The only experimental results which can be used for evaluating the comparison between the two treatments are those pairs in which the tumour on one mouse was destroyed, while the other mouse's tumour recurred.

There were 15 such pairs. Of these, 14 showed tumour destruction by the combination of laser and chemotherapy with tumour recurrence on the paired mouse which received laser only. Clearly this is a highly significant result.

The effect of chemotherapy before irradiation by laser energy was to decrease the growth rate of the tumour. Consequently at the time of laser radiation, the animals receiving chemotherapy had tumours which were (on the average) slightly smaller than those receiving no chemotherapy. The average (geometric) diameters were 8.27 mm and 7.92 mm for the non-chemotherapy and chemotherapy animals respectively.

In order to determine if the slight difference in tumour size could possibly account for the potentiation observed with the chemotherapy, the animals were paired a second time (after being exposed to the laser energy). The animals in the same pair received the same amount of laser energy, but this time the (geometric) diameters of the tumour at the time of laser exposure were matched. This classification yielded pairs, in which the average

Table 1 RESULTS OF TREATMENT PAIRING  
(Pairing done before chemotherapy)

	Laser plus chemotherapy	
	Tumour destroyed	Tumour recurred
Laser only	<div> <div>Tumour destroyed</div> <div>Tumour recurred</div> </div>	<div> <div>1</div> <div>26</div> <div>27</div> </div>
	15	43



diameter of the non-chemotherapy and chemotherapy animals was 7.97 mm and 8.01 mm respectively. The experimental results for this second pairing are summarized in Table 2. The number of pairs for which the

Table 2. RESULTS OF SECOND PAIRING (Pairing done the fifth or sixth day of chemotherapy just prior to laser exposure)				
Laser only	{	Laser plus chemotherapy		
		Tumour destroyed	Tumour recurred	
		1 9 10	1 20 21	2 29 31

outcome for both animals was different is 10. Of these, 9 were favourable to the combination therapy. Again these results are highly significant ( $P=0.026$ ).

An incidental observation was that tumours which recurred after laser radiation appeared to grow at a much faster rate than before being irradiated. In contrast, recurrent tumours after combination chemotherapy and irradiation had a reduced growth rate (in comparison to normal tumour growth rate).

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<sup>5</sup> Snell, G. D., *J. Nat. Cancer Inst.*, **13**, 1511 (1953).

## MAGNETIC AND SPECTRAL EVIDENCE FOR TRINUCLEAR CLUSTERS IN MOLYBDENUM CHLORIDES

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IN a recent communication<sup>1</sup> we directed attention to the fact that compounds involving polynuclear metal clusters are not necessarily diamagnetic, and that paramagnetism can often be of diagnostic value for detecting the presence of metal-metal bonding. If a core is constituted of an odd number of metal atoms with uneven electron configuration, the cluster is likely to be weakly paramagnetic and to obey Curie's Law. Thus, the low magnetic moment of rhenium (IV) chloride,  $\mu_{\text{eff}} = 1.0$  Bohr Magnetons (B.M.) is independent of temperature and is consistent with a trinuclear species  $\text{Re}_3\text{Cl}_{12}$  with a single unpaired electron distributed over the  $\text{Re}_3$ -core<sup>1</sup>. On the other hand, if the core contains an even number of metal atoms, diamagnetism is generally observed (for example,  $[\text{Mo}_4\text{Cl}_{14}]^{4+}$ ,  $[\text{W}_4\text{Cl}_{14}]^{4+}$ ,  $[\text{Ta}_4\text{Cl}_{14}]^{3+}$ ) (refs. 2 and 3), although paramagnetic species may also be found (for example,  $\text{Mo}_4\text{Cl}_{14}$ ,  $[\text{Cr}_4\text{Cl}_{14}]^{3+}$ ,  $[(\pi\text{-cpd})_4\text{Ti}_4\text{Cl}_{14}]$ , refs. 4-6).

Table 1. MAGNETIC PROPERTIES OF MOLYBDENUM (III) CHLORIDE

Temp. (°K)	$\chi_M \times 10^3$	$\mu$ (B.M.) = $2.84 [ \chi_M(T+100) ]^{1/2}$
300	309	1.00
281	320	0.99
256	335	0.98
231	360	0.98
209	377	0.97
158	466	0.99
141	495	0.98
115	546	0.97
91	643	1.00

An early investigation<sup>7</sup> of the magnetic properties of molybdenum (III) chloride at three temperatures showed that the magnetic moment was about 0.8 B.M. This value is anomalously low for molybdenum (III) compounds, which usually show magnetic moments of the order of 3-8.3 B.M.<sup>8</sup>. We have now investigated in detail the variation of the magnetic susceptibility of molybdenum (III) chloride with temperature and find our results agree well with those of Klemm and Steinberg<sup>7</sup>. Extrapolation to infinite susceptibility of a plot of  $\chi_M^{-1}$  against  $T$  (Curie-Weiss plot) shows the presence of an antiferromagnetic interaction with a  $\theta$ -value of 100°. With the inclusion of this term the magnetic moment of molybdenum (III) chloride is 1.0 B.M. and independent of temperature. This suggests that the compound is also a trimer  $\text{Mo}_3\text{Cl}_9$  with the ground state configuration  $(a_1')^2(a_1'')^2(e')^2(e'')^1$  isoelectronic with that of  $\text{Re}_3\text{Cl}_{12}$  (ref. 1). The deviation from a true Curie law is not unexpected if the compound has a structure of the same

type as that found for rhenium (III) chloride, the weak antiferromagnetic interactions occurring via bridging chlorine atoms joining the adjacent trimer units<sup>9</sup>. The experimental results are presented in Table 1.

The diffuse reflectance spectrum of molybdenum(III) chloride is not unlike those of the  $\text{Re}$  (III) clusters examined by Cotton *et al.*<sup>9</sup> with well-defined absorption peaks at 530 m $\mu$  and 760 m $\mu$ , as shown in Table 2.

Table 2. SPECTRA OF MOLYBDENUM CHLORIDE CLUSTERS

Compound	Electron configuration	Positions of absorption maxima, cm <sup>-1</sup> ( $\epsilon_{\text{max}}$ )	Solvent
$\text{Mo}_3\text{Cl}_9^{3+}$	$d^3$	14,100 (160), 21,500 (1,550)	Acetonitrile
$\text{Mo}_3\text{Cl}_9$	$d^3$	13,150; 19,100	Reflectance
$\text{Re}_3\text{Cl}_{12}$	$d^1$	13,300 (410); 19,400 (1,610)	Acetone <sup>9</sup>

Our evidence for the trinuclear nature of molybdenum (III) chloride is at variance with unpublished data quoted by Schafer and Schnering<sup>10</sup>, who suggest that the compound contains  $\text{Mo}_4$ -clusters and is diamagnetic ( $\chi_M = 19 \times 10^{-4}$  o.g.s., B.M.U.). The trichlorides of several transition metals, notably those of titanium<sup>11</sup>, rhenium<sup>12</sup> and ruthenium<sup>13</sup>, are known to exhibit polymorphism; possibly Schafer prepared a different polymorph. The material used in our investigations was prepared by thermal decomposition of molybdenum (V) chloride in nitrogen, the temperature being kept as low as possible to avoid further decomposition to molybdenum (II) chloride. Preliminary attempts to ascertain, by X-ray powder methods, whether  $\text{MoCl}_3$  and  $\text{ReCl}_3$  are isostructural are inconclusive, although the diffraction patterns of the two compounds are similar.

Mr. D. F. Stewart and Dr. T. A. O'Donnell, of this Department, have recently succeeded in isolating a chloro-cation of molybdenum (IV),  $[\text{MoCl}_4]^+$ . The magnetic moment of this species lies between 1.6 and 1.7 B.M. and the Curie law is obeyed. The electronic spectrum of solutions of the cation in acetonitrile closely resembles those of other  $d^3$  and  $d^4$  trimers, which suggests that this cation should be formulated as  $[\text{Mo}_3\text{Cl}_9]^{3+}$ . Reference to the M.O. diagram<sup>1</sup> shows that the expected ground state electronic configuration of  $(a_1')^2(a_1'')^2(e')^2$  should be associated with two unpaired electrons per  $[\text{Mo}_3\text{Cl}_9]^{3+}$  trimer with a corresponding magnetic moment of 1.64 B.M., that is, 2/3 of an electron per molybdenum (IV) atom. Preliminary magnetic measurements on molybdenum (IV) chloride, tungsten (V) chloride and tungsten (V) bromide suggest that these compounds also involve trinuclear clusters, the latter compounds existing as  $[\text{W}_3\text{Cl}_{12}]^{3+}$  and  $[\text{W}_3\text{Br}_{12}]^{3+}$  cations.

Table 3. COMPLEXITIES OF MOLYBDENUM CHLORIDE CLUSTERS

Oxidation state of molybdenum n in Mo <sub>n</sub> -cluster	+1	+2	+3	+4	+5	+6
	unknown	6	3	3	2	unknown

Table 4. POSSIBLE CLUSTERS FOR MOLYBDENUM CHLORIDES

Molybdenum Oxidation state	Mo <sub>2</sub> Cl <sub>12</sub> type	Re <sub>2</sub> Cl <sub>12</sub> type	Mo <sub>3</sub> Cl <sub>15</sub> type
Mo (VI)	[Mo <sub>2</sub> Cl <sub>12</sub> ] <sup>2+</sup>	[Mo <sub>2</sub> Cl <sub>12</sub> ] <sup>2+</sup>	[Mo <sub>3</sub> Cl <sub>15</sub> ] <sup>3+</sup>
Mo (V)	[Mo <sub>2</sub> Cl <sub>12</sub> ] <sup>+</sup>	[Mo <sub>2</sub> Cl <sub>12</sub> ] <sup>+</sup>	[Mo <sub>3</sub> Cl <sub>15</sub> ] <sup>2+</sup>
Mo (IV)	[Mo <sub>2</sub> Cl <sub>12</sub> ] <sup>0</sup>	[Mo <sub>2</sub> Cl <sub>12</sub> ] <sup>0</sup> [Mo <sub>3</sub> Cl <sub>15</sub> ] <sup>+</sup> [Mo <sub>3</sub> Cl <sub>15</sub> ] <sup>0</sup>	[Mo <sub>3</sub> Cl <sub>15</sub> ] <sup>+</sup>
Mo (III)	[Mo <sub>2</sub> Cl <sub>12</sub> ] <sup>-</sup>		[Mo <sub>3</sub> Cl <sub>15</sub> ] <sup>0</sup>
Mo (II)	[Mo <sub>2</sub> Cl <sub>12</sub> ] <sup>-</sup>	[Mo <sub>2</sub> Cl <sub>12</sub> ] <sup>-</sup>	[Mo <sub>3</sub> Cl <sub>15</sub> ] <sup>-</sup>
Mo (I)	[Mo <sub>2</sub> Cl <sub>12</sub> ] <sup>-</sup>	[Mo <sub>2</sub> Cl <sub>12</sub> ] <sup>-</sup>	[Mo <sub>3</sub> Cl <sub>15</sub> ] <sup>-</sup>

The pronounced tendency of molybdenum to form metal-metal bonds in the +2 oxidation state is well known. We have also obtained evidence, to be presented later, that metal-metal interaction occurs in dimeric molybdenum (V) chloride. The results presented here demonstrate that this tendency is not restricted to these oxidation states but is common to all the established oxidation states of molybdenum other than +6.

An interesting progression in the complexity, *n*, of halide clusters with varying oxidation state of the metal atom emerges from these investigations (Table 3). The inverse relationship can be rationalized in terms of the suggestion of Cotton and Haas<sup>1</sup> that the preferred structure is one which will minimize the electrostatic charge carried by the cluster. Table 4 summarizes the possible

clusters of molybdenum chlorides in all six oxidation states on the basis that they adopt one of the established Mo<sub>2</sub>Cl<sub>12</sub>, [Re<sub>2</sub>Cl<sub>12</sub>]<sup>2+</sup> (or Re<sub>2</sub>Cl<sub>9</sub>), or [Mo<sub>3</sub>Cl<sub>15</sub>]<sup>4+</sup> structures.

The clusters known to exist (enclosed by full lines in Table 4) conform both to the foregoing electrostatic requirement and that of filling only those metal-metal molecular orbitals which are bonding.

We assume here that molybdenum (III) chloride, like rhenium (III) chloride, achieves the prototype structure (that is, [Re<sub>2</sub>Cl<sub>12</sub>]<sup>2+</sup>) via inter-cluster chlorine bridging of adjoining trimers. On the other hand, the cation [Mo<sub>3</sub>Cl<sub>15</sub>]<sup>2+</sup> derived from trimeric molybdenum (IV) chloride can presumably solvate in donor solvents like acetonitrile.

The implications of Table 4 should be of general validity and not necessarily restricted to the chemistry of molybdenum.

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## USE OF COMBINATION BANDS IN THE VIBRATIONAL SPECTROSCOPY OF METAL CARBONYLS

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THE use of infra-red spectroscopy in inorganic chemistry has now become widespread. It is a particularly valuable method of studying transition metal carbonyl compounds because absorptions around 2,000 cm<sup>-1</sup> can be specifically associated with C—O stretching vibrations.

Metal-carbon stretching and MCO angle deformation modes occur<sup>1-4</sup> over a relatively wide range, 350–700 cm<sup>-1</sup>. The remaining vibrational modes for mononuclear carbonyls, MCM angle bending, probably occur below 250 cm<sup>-1</sup>.

Measurements of the infra-red spectra of carbonyl compounds in the C—O stretching region, 1,700–2,200 cm<sup>-1</sup>, have been used to provide information on molecular stereochemistry and to study variations of bonding in series of compounds. In all but monocarbonyl compounds there are several C—O stretching vibrations, and in order to make a study of bonding it is necessary to know as much as possible about all of them. Many carbonyl compounds are highly symmetrical and consequently only a few of the fundamentals are infra-red active, and even when several are observed their correct assignment may prove difficult. Raman spectra yield more frequencies and polarization data which afford additional means of assignment. Unfortunately the Raman spectra of only

a few carbonyl derivatives are available because the compounds are often coloured and absorb, rather than scatter, the incident radiation, or are decomposed by it. Limited solubility also restricts the use of Raman methods in many cases. Stammreich *et al.*<sup>1</sup> have overcome some of these difficulties by using sources of long-wave-length radiation and their results have greatly extended our understanding of carbonyl compounds.

As an alternative to Raman spectroscopy for determining 'infra-red-forbidden' fundamentals the infra-red combination frequencies can be measured. We have found that, for a large number of carbonyl derivatives, binary combinations of C—O stretching fundamentals occur near 4,000 cm<sup>-1</sup> with extinction coefficients of about 5–30. Such bands have been reported by Jones for the Group VI hexacarbonyls<sup>2</sup> and for nickel carbonyl<sup>3</sup>, and by Jones<sup>4</sup> and Edgell<sup>5</sup> for iron pentacarbonyl, but have not previously been studied in detail.

The commonest type of infra-red active binary combination is that of an 'allowed' with a 'forbidden' fundamental. Direct overtones are less commonly permitted. Thus, by subtraction of the known active fundamental from the combination frequency, a value for the inactive fundamental may be obtained. Our experience is that the error in frequencies so calculated is not likely to exceed 20 cm<sup>-1</sup>,

and is almost invariably such that the calculated frequency is low. Higher-frequency inactive fundamentals are obtained more accurately than lower ones; we think that this results from a partial cancelling of effects due to anharmonicity and Fermi resonances.

These principles may be illustrated by reference to chromium hexacarbonyl. The molecule has  $O_h$  symmetry and there are three fundamental C—O stretching modes, belonging to the representations  $a_{1g}$ ,  $e_g$  and  $t_{1g}$ . Only the  $t_{1g}$  mode is infra-red active and occurs at 1985  $\text{cm}^{-1}$ . Two binary combinations are allowed ( $a_{1g} + t_{1g}$ ), symmetry  $T_{1g}$ , and ( $e_g + t_{1g}$ ) which spans  $T_{1g} + T_{2g}$ . (Lower case is used for fundamentals, for example,  $a_{1g}$ . Capitals are used for symmetries of combination terms.) They are observed at 4,082  $\text{cm}^{-1}$  and 3,985  $\text{cm}^{-1}$ ; subtraction of 1,985  $\text{cm}^{-1}$  yields 2,097  $\text{cm}^{-1}$  and 2,000  $\text{cm}^{-1}$  for the forbidden fundamentals. These figures may be compared with the values 2,100  $\text{cm}^{-1}$  and 2,020  $\text{cm}^{-1}$  recorded in the Raman spectrum and assigned, respectively, to  $a_{1g}$  and  $e_g$  fundamentals. It is probable that the greater accuracy of the  $a_{1g}$  frequency is due to the effects of (1) anharmonicity, which tends to lower both  $a_{1g}$  and  $e_g$  values, and (2) Fermi resonance between the  $T_{1g}$  components of the combination terms which would also lower the calculated  $e_g$  frequency, but would raise the  $a_{1g}$  value in opposition to the anharmonicity effect.

Interpretation of the infra-red spectra of manganese and rhenium carbonyl derivatives presents an interesting problem. The parent compounds are binuclear molecules,  $M_2(\text{CO})_{10}$ , and, in addition to the usual vibrations, the metal-metal stretching mode must be considered. A knowledge of the fundamental frequencies and force constants of metal-metal bonds would be of considerable value. We have measured the spectra of many manganese carbonyl derivatives but discuss here only the parent compounds  $\text{Mn}_2(\text{CO})_{10}$  and  $\text{Re}_2(\text{CO})_{10}$ . The C—O stretching fundamentals and binary combinations are shown in Tables 1 and 2. The Raman spectrum of the colourless  $\text{Re}_2(\text{CO})_{10}$  has also been recorded (Table 3) and enables us to check how correct are the assignments made from binary combinations in the infra-red. The spectra were all measured in carbon tetrachloride solution; they are slightly solvent-dependent, but we shall discuss these variations in later publications. The Raman spectrum of  $\text{Re}_2(\text{CO})_{10}$  was also measured in cyclohexane, in order to observe some of the lower frequency bands masked by carbon tetrachloride lines. Infra-red fundamentals are accurate to  $\pm 0.5 \text{ cm}^{-1}$  combination bands and Raman lines to  $\pm 2 \text{ cm}^{-1}$ .

Manganese carbonyl has a solid-state structure\* in which two CO groups and the metal atoms are co-linear, the remaining CO groups being arranged in two staggered squares as shown in Fig. 1. The metal atoms are displaced outwards from the centres of these squares. The shape is a very good approximation to  $D_{4h}$  symmetry, although the site symmetry is only  $C_2$ . We assume that the  $D_{4h}$

Table 2. SPECTRUM AND ASSIGNMENTS OF $\text{Re}_2(\text{CO})_{10}$			
Observed frequency ( $\text{cm}^{-1}$ )	$\epsilon$	Assignment	Calculated frequency
1,974	6,500	$b_1$	—
2,018	35,600	$e_1^*$	—
2,074	8,500	$b_1^*$	—
—	—	$a_1 + b_1$	3,908
3,947	14.1	$a_1 + e_1$	3,947
3,981	Shoulder	—	—
3,999	14.9	$\{a_1 + e_1, a_1 + b_1^*\}$	4,003
4,037	17.7	$\{e_1 + e_1, e_1 + b_1^*\}$	4,008
4,053	12.5	$\{2a_1, a_1 + b_1^*\}$	4,040
4,077	0.1	—	4,054
4,099	5.7	$a_1^* + b_1$	4,053
4,123	Shoulder	—	—
4,138	32.2	$a_1^* + e_1$	4,100
4,198	4.0	$a_1^* + b_1^*$	4,130

Calculated frequencies were obtained from Raman fundamentals, except for the  $a_1$  combinations.

Table 3. RAMAN SPECTRUM OF  $\text{Re}_2(\text{CO})_{10}$

Frequency ( $\text{cm}^{-1}$ )	Character	Assignment
123	s pol	$a_1$ $\alpha(\text{Re-Re})$
225	vw	$\beta(\text{OReO})$
251	vw	
386	s pol	$a_1$
432	ms dep	$\alpha(\text{Re-O})$
454	ms pol	
535	w	$\delta(\text{ReOCO})$
1,967	vw	
1,989	s pol	$\alpha(\text{CO})$
2,027	s dep	
2,080	w	
2,126	s pol	

vw, very weak; w, weak; ms, medium strong; s, strong; pol, polarized; dep, depolarized.

Fundamental:  $a_1^*$   $e_1$   $b_1^*$   $b_2$   $a_1$   $e_1$   $e_2$   
Frequency ( $\text{cm}^{-1}$ ): 2,126 1,989 2,074 1,974 2,013 2,037 1,934

arrangement is preserved in solution and that rhenium carbonyl is similar. The possibility of the squares being in an eclipsed configuration ( $D_{4h}$  symmetry) cannot, however, be excluded on spectroscopic grounds.

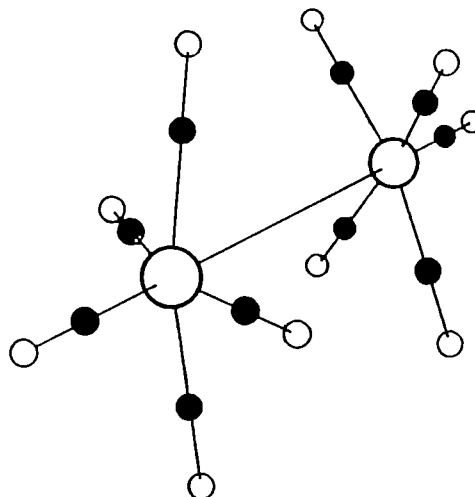


Fig. 1. The structure of manganese carbonyl

The  $D_{4h}$  arrangement gives rise to seven C—O stretching modes belonging to species  $a_1$  (two),  $b_1$  (two),  $e_1$ ,  $e_2$  and  $e_3$ . Their forms are shown in Fig. 2. Only the two  $b_1$  and the  $e_1$  vibrations are 'infra-red allowed' and just three strong bands are found in the 2,000  $\text{cm}^{-1}$  region, which have been assigned by Flitcroft, Huggins and Kaesz\* as shown in Tables 1 and 2. The four remaining fundamentals,  $a_1$  (two),  $e_2$  and  $e_3$ , are Raman active. It may be noted that mutual exclusion of infra-red and Raman lines occurs here, although these molecules are not centrosymmetric.

Table 1. SPECTRUM AND ASSIGNMENTS OF  $\text{Mn}_2(\text{CO})_{10}$

Observed frequency ( $\text{cm}^{-1}$ )	$\epsilon$	Assignment	Calculated frequency
1,980	4,590	$b_1$	—
2,014	25,500	$e_1^*$	—
2,046	12,100	$b_1^*$	—
3,906	<0.1	—	—
3,961	• 10.5	$\{a_1 + b_1, a_1 + e_1, a_1 + b_1^*\}$	3,956
3,990	10.8	$\{e_1 + e_1, e_1 + b_1^*\}$	3,981
4,023	12.5	$\{2a_1, a_1 + b_1^*\}$	3,985
4,035	5.9	$\{e_1 + e_1, 2e_1\}$	3,990
4,061	<0.1	—	3,993
4,096	2.9	$a_1^* + b_1$	4,023
4,109	<0.1	—	4,032
4,125	24.3	$a_1^* + e_1$	4,035
4,163	7.3	$a_1^* + b_1^*$	4,036

Fundamental:  $a_1^*$   $e_1$   $b_1^*$   $b_2$   $a_1$   $e_1$   $e_2$   
Frequency ( $\text{cm}^{-1}$ ): 2,116 1,976 2,046 1,980 2,014 2,018 1,947

\* The asterisk is used to distinguish the higher of two fundamentals in the same symmetry class.

The twelve 'infra-red-allowed' binary combinations of these fundamentals are as follows:

- $(a_1 + b_2) (B_2)$ . Four separate bands.
- $(a_1 + e_1) (E_1)$ . Two separate bands.
- $(e_2 + b_2) (E_1)$ . Two separate bands.
- $(e_2 + e_1) (B_1 + B_2 + E_2)$
- $(e_2 + e_1) (E_1 + E_2)$
- $(2e_2) (A_1 + B_1 + B_2)$
- $(e_2 + e_2) (E_1 + E_2)$

Seven principal peaks are observed in the 4,000  $\text{cm}^{-1}$  region for both manganese and rhenium carbonyls. The analysis shows that there is considerable overlap of the twelve combinations. Assignment of the spectra has been carried out using: (1) intensity relationships; (2) comparison with related compounds such as  $\text{Mn}(\text{CO})_5\text{Br}$  and  $[\text{Et}_3\text{PMn}(\text{CO})_4]_2$ , the latter compound having the same geometrical structure<sup>6</sup> as manganese carbonyl with the two axial CO groups replaced by  $\text{Et}_3\text{P}$ ; (3) a method of elimination based on internal consistency. Twenty-four possible assignments were tested and only two appeared to be satisfactory. The Raman spectrum of rhenium carbonyl gives complete confirmation of the assignment of the two  $a_1$  modes. The other strong Raman line, at 2,027  $\text{cm}^{-1}$ , must be either  $e_2$  or  $e_1$ , but on the evidence available it is impossible to distinguish between them. However, the form of the equations relating frequencies to a Cotton-Kraihanzel force field<sup>7</sup> indicates that the frequency of the  $e_2$  vibration should be much less than that of the  $e_1$ . For this reason we favour the assignments given in Tables 1-3. We cannot at present explain the weak Raman line at 2,090  $\text{cm}^{-1}$ .

In addition to  $\text{Mn}_2(\text{CO})_{10}$ ,  $\text{Co}_2(\text{CO})_{10}$  and  $\text{Re}_2(\text{CO})_{10}$ , Flitcroft *et al.* have discussed the infra-red active fundamentals of the mixed carbonyl,  $(\text{CO})_4\text{Mn}_2\text{Re}(\text{CO})_6$ . This compound probably has  $O_h$  symmetry and may be expected to show more bands in the infra-red spectrum than the others. In  $O_h$  symmetry  $a_1$  and  $e_g$  modes are infra-red active, and by considering the correlation between representations of groups  $O_h$  and  $D_{4h}$  and by assuming that the general form of the normal modes is similar in homo- and hetero-nuclear carbonyls, it would be anticipated that the three fundamentals corresponding to  $a_1$  and  $e_g$  in  $D_{4h}$  symmetry should become infra-red active. The  $b_2$  and  $e_1$  modes should not change their appearance. The spectrum of  $(\text{CO})_4\text{Mn}_2\text{Re}(\text{CO})_6$  shows three strong bands similar to the other compounds, together with six weaker bands, given as 1945 ( $J$ ), 1992 ( $H$ ),

1998 ( $O$ ), 2031 ( $G$ ), 2044 ( $F$ ), and 2124  $\text{cm}^{-1}$  ( $E$ ). The same letters are used for identification by us.

Flitcroft *et al.* suggest, very tentatively, that band  $E$  may be a binary combination of the  $a_1$  C—O stretching fundamental at 2,054  $\text{cm}^{-1}$  with the Mn-Re stretching vibration. However, we have the following reasons for believing that this band is, in fact, an  $a_1$  fundamental of  $(\text{CO})_4\text{Mn}_2\text{Re}(\text{CO})_6$ , which is infra-red active in  $O_h$  symmetry:

(i) As we have shown, both  $\text{Mn}_2(\text{CO})_{10}$  and  $\text{Re}_2(\text{CO})_{10}$  have infra-red inactive  $a_1$  fundamentals at about 2,120  $\text{cm}^{-1}$ .

(ii) A combination of  $[a_1(2054) + a_1(M'-M)]$  in a molecule of  $O_h$  symmetry corresponds to a combination  $[b_2 + a_1(M_1-M)]$  in a  $D_{4h}$  system which would be permitted in the infra-red. No such frequency can be found.

(iii) The assignment of Flitcroft *et al.* yields a Re-Mn stretching frequency of 70  $\text{cm}^{-1}$ . However, we attribute the strong polarized Raman line in  $\text{Re}_2(\text{CO})_{10}$  at 128  $\text{cm}^{-1}$  to Re-Re stretching, assuming an effective rhenium mass equal to the atomic weight, this corresponds to a force constant of 0.9  $\text{md}/\text{A}$ . With the same force constant, we calculate the metal-metal stretching frequencies in the related compounds as follows: Mn-Re 190  $\text{cm}^{-1}$ , Mn-Mn 236  $\text{cm}^{-1}$ , Re-Re 176  $\text{cm}^{-1}$ .

(iv) The weakness of the band  $E$  in the infra-red is consistent with the small dipole change expected for an  $a_1$  vibration of this sort.

Discussion of the other weak bands reported by Flitcroft *et al.* in all the compounds which they investigated cannot be included here in any detail, but it seems to us probable that band  $O$ , which occurs in all their spectra, is due to the  $e_1$  vibration of molecules with a  $^{13}\text{CO}$  group in a radial position. In  $(\text{CO})_4\text{Mn}_2\text{Re}(\text{CO})_6$ , assignment of band  $H$  to the expected  $a_1$  vibration is in good agreement with the lower  $a_1$  frequencies which we find and band  $J$  may well be the  $e_g$  mode corresponding to  $e_2$  in the homonuclear carbonyls. However, it is at least equally likely that band  $J$  is a  $^{13}\text{CO}$  satellite of the frequency at 1,978  $\text{cm}^{-1}$ , as suggested by Flitcroft *et al.*

There are several features of the assigned fundamentals of manganese and rhenium carbonyls which are worthy of mention. The fundamentals,  $a_1$ \*, have very high values. Qualitatively there seem to be two reasons for this, which reinforce each other. Coupling between different CO groups on one metal atom generally has the effect of raising the frequencies of those vibrations in which the C—O groups stretch in phase. This effect raises both  $a_1$ \* and  $b_2$ \* frequencies. It may be seen from Fig. 2 that in both these modes the five C—O groups on one metal atom

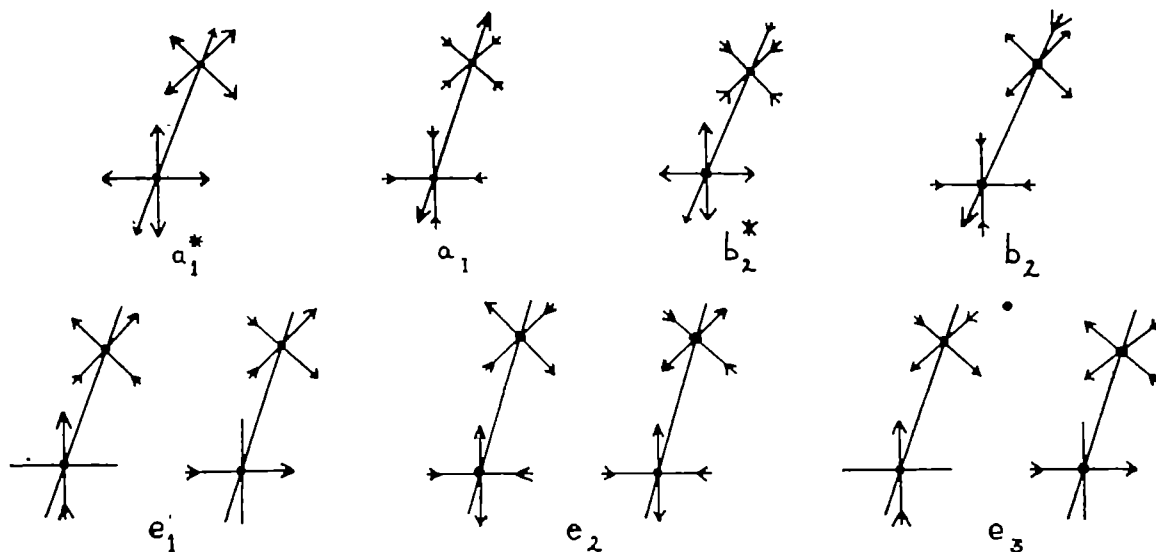


Fig. 2. The forms of the C—O stretching vibrations for a molecule,  $\text{M}_2(\text{CO})_{10}$ , of  $D_{4h}$  symmetry

oscillate with the same phase. Both valence bond theory and molecular orbital theory, however, predict that coupling between O—O groups on adjacent metal atoms would result in a lowering of the in-phase frequencies. In terms of potential energy functions this statement means that the relevant interaction force constants should be negative. Thus it would be predicted that the  $a_1^*$  frequency should be less than  $b_1^*$ . It appears to us that the  $a_1^*$  value is abnormally high because of steric repulsions between the radial OO groups on different metal atoms; these repulsions are most effective when neighbouring OO groups on the two metal atoms stretch in phase, which occurs to the maximum extent in the  $a_1^*$  mode and to the minimum extent in the  $b_1^*$  mode. This explanation can also account for the  $a_1$  frequency being so much higher than the  $a_2$ . These ideas are only qualitative, but we intend to make force constant calculations based on the model suggested by Kraihanzel and Cotton<sup>8</sup>. This model has been used by several workers<sup>9-11</sup>, but has never been fully tested owing to insufficient data.

From these examples, it will be apparent that the utility of measurements in the near infra-red region is of

considerable importance in both the assignment of infra-red active modes and in elucidation of infra-red inactive modes, particularly for complexes where Raman data are difficult to obtain.

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## SEPARATION OF LIQUID MIXTURES BY NON-FOAMING BUBBLE FRACTIONATION

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OVER the past few years there has been increased interest in the technique of partially separating (concentrating) the components of a liquid mixture by means of foam fractionation. Studies reported include those of our own group<sup>1-4</sup>, as well as those of a number of other investigators<sup>5</sup> including one investigation which appeared recently in *Nature*<sup>6</sup>.

Foam fractionation is based on the preferential adsorption of a solute at the interface of bubbles which are usually formed by deliberately bubbling a gas through a liquid mixture. These bubbles rise, forming a foam which carries the solute off overhead, thus effecting a partial separation of components. Unfortunately, by its very nature, use of the technique is limited to systems which will in fact foam. Accordingly, the present investigation was initiated to examine the feasibility of using a 'foamless foam column'; that is, just a vertically elongated pool of liquid. So far as we are aware, this has been a virtually neglected area of study.

The simple device used is shown in Fig. 1a. It is called a 'bubble column' in contradistinction to a foam column. In the work recorded here it was a vertical glass tube filled to a convenient height with a very dilute aqueous solution of a surface-active agent, namely, technical monobutyl diphenyl sodium monosulphonate. Nitrogen gas (which was first thoroughly humidified to eliminate subsequent evaporation) was bubbled up through the liquid in the column. The objective was to see whether a significant concentration gradient would occur in the column despite the fact the solution was too dilute to foam.

After bubbling for several hours at room temperature, samples were withdrawn from the top and bottom of the column and analysed for solute spectrophotometrically by the standard methylene blue test<sup>7</sup>. A number of runs were conducted. For initial charge concentrations of several parts per million, the concentration at the top was as much as quadruple that at the bottom. Such marked separation shows that, even at concentrations below the foaming threshold, the rising bubbles were quite effective in adsorbing solute at their surfaces and carrying

it to the top of the column. (Bubble fractionation is based on surface adsorption of non-volatile solute and should not be confused with the familiar but quite different operation of desorption which involves diffusion of volatile solute into the bubble interior.) This transport establishes the concentration gradient within the column.

Of course, the operation has its internal limitations. The rising bubbles drag lean liquid from the bottom up to the top. Rich liquid from the top then flows down to the bottom as replacement. The mixing action of this circulation limits the separation attainable.

With bubble sizes in the narrow range of 0.36–0.38 cm diameter, varying the frequency from 120 to 250 bubbles per min was of comparatively little effect. This accords with the aforementioned limitation. Increasing the bubble

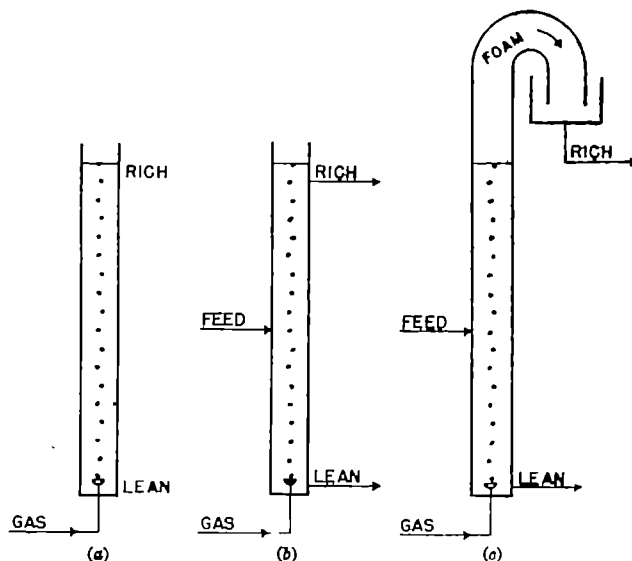


Fig. 1. Bubble fractionation apparatus (not to scale). a, Batchwise operation as used here; b, projected flow operation; c, projected application to low-threshold foam fractionation.

frequency increases the undesirable mixing as well as the desirable adsorbing surface.

Fig. 2 and Fig. 3 show the effects of several independent variables on the separation ratio (which is defined as the ratio of the concentration at the top of the column to that at the bottom of the column). The degree of separation increased with the height of the liquid column and with the charge concentration, but decreased with the column diameter. This last would seem to result from a wall effect which is diminished at larger diameters, thus allowing the aforementioned undesirable circulation to proceed with less hindrance.

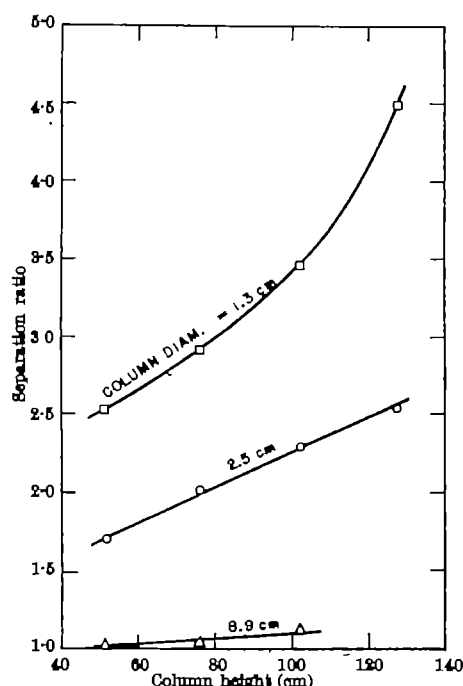


Fig. 2. Effect of column dimensions on the degree of bubble fractionation for a charge concentration of 3.1 parts per million

The experiments performed here were in batch. However, with sufficient hold-up (liquid residence time) it should be possible to operate continuously as shown in

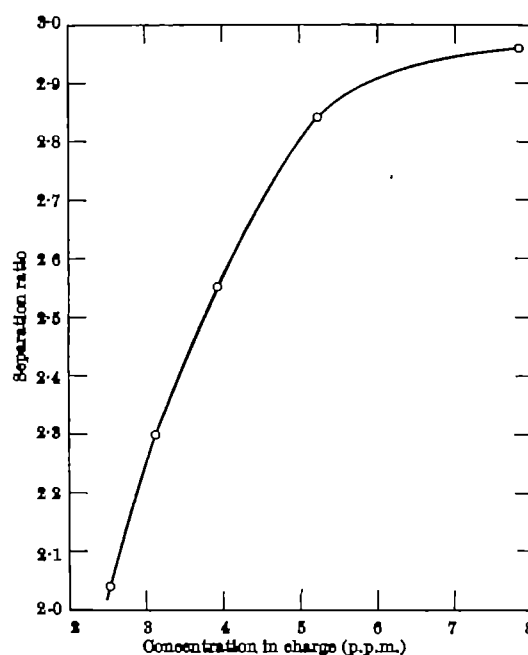


Fig. 3. Effect of charge concentration on the degree of bubble fractionation in a column of 2.5 cm diameter and 102 cm height

Fig. 1b. Finally, for a solution the concentration of which is only slightly below the foaming threshold, it should be possible to enrich the top of the liquid sufficiently by bubble fractionation so that foam fractionation could be used above it. This is illustrated in Fig. 1c.

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## LOWER OLIGOCENE BIRD-TRACKS FROM NORTHERN SPAIN

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IN the course of a preliminary sedimentological investigation of a Lower Oligocene formation outcropping in the provinces of Navarra and Zaragoza, Spain, one of us (de R.) discovered bird-tracks. Some time afterwards his co-authors collected more material from the same locality and, by proceeding along the strike, sampled scores of other bird-tracks and natural casts. The collected material, now kept in the Rijksmuseum van Geologie en Mineralogie, Leiden, derives from an outcrop area some 40 km long, starting from the westerly wedge-out of the fossiliferous formation, via the Liedena area where bird-tracks had been first observed by Mangin<sup>1</sup>, to the east (Bailo-Salinas de Jaca area), where it is increasingly better developed, but where tracks are apparently lacking.

The lowermost part of the continuous Oligocene section discussed here contains in the Liedena area lagoonal gypsiferous deposits, which are a few tens of metres thick. They are overlain by Mangin's 'grès à ripplemarks' and together with the latter form a complex almost 100 m thick.

With respect to the mode of deposition, two types of sandstone can be distinguished in this sequence, namely, graded sandstones with bases channelling into the beds below and others the deposition of which evidently did not alter the original upper surface features of the underlying bed. As regards sole markings, flute casts are restricted to the first type, casts of bird-tracks and lower surface ripple-marks to the second type.

These sediments form the base of the post-orogenic fill of the Ebro basin and should not be considered a 'flysch', as Mangin contends, in spite of their rhythmic aspect and local grading<sup>2</sup>. In slightly more detail, this type of sequence is as follows: The basal part of the Oligocene in the region with bird-tracks outlined here generally consists of red and grey to greenish calcareous shales with thin intercalations of siltstone. Both the shales and siltstones contain a certain amount of gypsum. This sequence grades upwards into a much more arenaceous succession with scores of beds with bird-tracks (Fig. 1). Generally, it consists of alternating rather fine to medium-grained sandstones, siltstones and calcareous to silty shales. There would appear to be a predominance of non-graded beds, particularly as regards the thinly bedded fine-grained units. Sandstones containing irregular silty to pelitic trends are not infrequent. Several thin beds show evidence of slumping. Many of the sandstones and siltstones display ripple-marks on their lower or upper surfaces. There are some apparently symmetrical ripple-marks, which are generally thought to be due to wave action.

The best examples of grading (ref. 2, p. 195) were found in the thickest sandstone beds (thickness about 1 m or more). On the sole of the graded units flute casts and groove casts are occasionally present. Mudflakes were observed near the erosional base of several of these beds.

Towards the top of the arenaceous succession the sequence becomes generally finer-grained and consists predominantly of finely grained thin-bedded sandstones and siltstones alternating with calcareous pelites. The lower surfaces of the arenaceous beds are here particularly rich in very delicate sole markings connected with bioturbation, the presence of pseudomorphs after salt crystals, striations, etc. It was interesting to find coprolites of the same nature as those described from the Rotliegendes by Reineck<sup>3</sup> as *Planolites rugulosus* n.sp., stemming from a similar environment. Bird-tracks, both on sandstones or siltstones (occasionally on ripple-marked surfaces or

associated with salt pseudomorphs) and on shales (evidenced as natural casts on the sole of overlying arenaceous beds), occur in the entire arenaceous succession, although more frequently in its lower part.

Finally, there are mud cracks and also, not infrequently, rain imprints mainly on shaly, silty beds throughout the sequence.

Eastwards, the whole arenaceous succession thickens and red colours become predominant. Burrows, presumably made by crabs, and at places passing from a sandstone through a thick shale bed into the underlying sandstone, finally occur in such abundance that the observed concurrent lack of bird-tracks may be confidently attributed to their being obliterated by intensive burrowing activities. As to the environment of deposition of the sediments discussed here, our observations do not seem to tally with opinions often expressed regarding depth of formation. Thus, the individual graded units encountered in the various sections traversed are often quite similar to deep-water deposits in other formations, at least in outward appearance. However, there can be little doubt that the sequence described here was deposited in an environment with shallow or negligible aqueous cover, in a coastal belt with temporary and local evaporitic conditions. In such an environment, minor streamfloods originating in a hinterland with little topographic relief after cloud bursts may be the depositing agent of the generally rather finely grained graded beds encountered.

It finally remains to consider the extraordinary, and often beautiful, preservation of a truly amazing abundance of bird-tracks in an area of the order indicated. It is hard to see how tracks abounding in all directions so repeatedly could be preserved at all with such regularity without invoking aeolian action. Only thus can we envisage the much-repeated mechanism of quick burial and most successful preservation of the tracks after their imprint in exposed wet arenaceous to clayey sediments, first with wind-blown salt and sand derived from drying flats and only later by more sediment transported by water.



Fig. 1



However, observations on the subject are, we believe, still largely lacking and the matter would require some considerable further study.

In this article no attempt will be made to describe the fossil tracks in any detail. Again, there seems, at this stage, little point in figuring all the various types of tracks discovered. It may be stated that the tracks appear to have been made largely by semi-aquatic birds on slightly emergent mud and sand or salt flats, probably subject not only to repeated inundations but also to repeated drying with associated aeolian transport. Most of the tracks were apparently made by a limited number of species which flocked together in a favoured environment and did so for a very long time. The various fossil-track assemblages, when fully studied, may well give us an interesting insight into the animal life of the period concerned in a fairly wide region in northern Spain.

Fig. 1, showing one of the largest slabs of siltstone collected, is given here as an example of the more interesting assemblages (imprints) discovered. As in most instances observed by us, the majority of tracks were evidently made by small wading birds, and some of these, at least, show affinity to tracks made by sand-pipers, snipe-like birds, and other small birds, or by gulls. More spectacular are the large tracks, often of astounding

clarity of impression, of a large web-footed bird. A preliminary investigation suggests that we may well have to look among the heron types of wading birds, more particularly the stork family (Ciconiidae), for the nearest relatives of these web-footed birds. It is not surprising that the tracks should have a 'modern' appearance, for most of the bird families now living had already evolved by Upper Eocene time.

So far, bird-tracks have been but rarely reported from northern Spain<sup>1,4</sup> and those reported by Hernandez<sup>4</sup>, although likewise of an Oligocene age, are quite unlike any discovered by us. Recent literature on the subject of bird-tracks (compare Abel<sup>5</sup>) includes interesting finds in Poland, the Rumanian Carpathians<sup>6</sup>, and the Uinta Basin (Eocene), Utah, United States<sup>7</sup>.

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## ENVIRONMENTAL DETERMINATION OF INSULAR VARIATION IN BIRD SPECIES ABUNDANCE IN THE GULF OF GUINEA

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FIGURE 1 illustrates for the avifaunas of the four islands in the Gulf of Guinea off West-Equatorial Africa the well-known species-area and species-isolation curves. The first is positive, the latter is negative, and the species numbers vary positively when plotted against maximum elevation of the islands. The data are from Amadon<sup>1</sup> and indicate that bird species numbers increase with area (slope =  $z = 0.489^*$ ) less rapidly than with decreased distance from the mainland ( $z = -0.866$ ). The question arises: do bird species numbers ( $Y$ ) vary among the islands in a way that  $Y$  depends on several factors (such as area, elevation and isolation) for determination of its value? Here it can be assumed that insular area and elevation are positive indices of ecological diversity and the likelihood of newly arrived dispersers finding vacant or available ecological niches, and that isolation, measured as each island's distance from the African mainland, makes negative the chances for dispersers to reach given islands<sup>2,3</sup>.

Multiple regression analysis provides one test for the problem, and among others two models or estimating equations are convenient and standard:

$$\hat{Y} = a_{Y.12} + b_{Y.1}X_1 + b_{Y.2}X_2 \quad (\text{Model I}) \quad (1)$$

and

$$\log \hat{Y} = \log a_{Y.12} + b_{Y.1} \log X_1 + b_{Y.2} \log X_2 \quad (\text{Model II}) \quad (2)$$

Here,  $b$  is a pure number, being the ratio of increase in  $Y$  (dependent variable) with that of  $X$  (independent variable), independent of other  $X$ s. The intercept value or constant ( $a$ ) is the value of  $Y$  or  $\log Y$  when the  $X$ s or  $\log X$ s are zero. The two models derive respectively from the linear and non-linear equations:

$$y = bx, \quad (I) \quad (3)$$

where  $x = x^z$  with  $z = 1$ , and

$$y = bx^z \quad (II) \quad (4)$$

where  $z \neq 1$ .

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For multiple regression analysis involving four variables (1 dependent, 3 independent), we have found with our computer programme (University of Texas: Tennessee Valley Authority Multiple Regression Comprehensive Analysis) that a minimum of 5 sample items is necessary for consistent calculation of the standard deviations ( $s_y$ ) (ref. 4) of the partial regression coefficients. For a fifth sample we selected the highlands of Mt. Cameroon (13,350 ft.) on the mainland coast of the Gulf. Searle<sup>5</sup> records 67 bird species for the montane forest (lower limit: 5,000 ft.) of this volcano, which is about 22 miles from Fernando Po in the Gulf. If from standard maps we estimate the radius ( $r$ ) of the volcano at 5,000 ft. as 7 miles, and if we assume a right cone with a height ( $h$ ) of 8,350 ft. for the volcano's 'cap' above 5,000 ft., then the new sample's area or curved surface area ( $csa$ ) is 169 sq. miles by the formula  $csa = \pi r \sqrt{r^2 + h^2}$ . Although isolation for

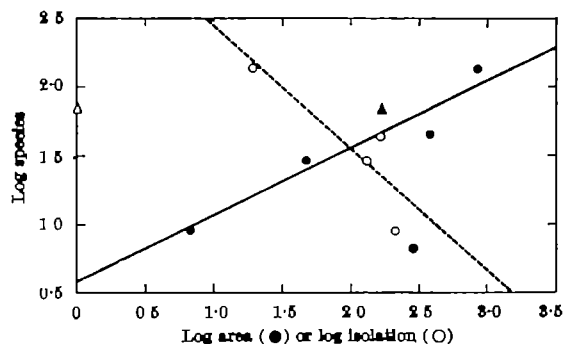


Fig. 1. Bird species-area and species-isolation curves for the four islands in the Gulf of Guinea. To show by closed (●) and open (○) circles their respective positive and negative trends. Closed and open triangles designate respective values for the montane sample of Mt. Cameroon, but these values are not utilized in the calculation matrices for the regression lines. Raw data ( $Y$ , species;  $X_1$ , area;  $X_2$ , elevation,  $X_3$ , isolation) for each island or sample. Annobon (9 bird spp., 6.8 sq. miles, 1,969 ft., 211.2 miles); Principe (20, 48.7, 3,110, 136.7); São Tomé (44, 536.1, 6,640, 174.0); Mt. Cameroon (67, 169, 8,350, 1); and Fernando Po (133, 852.6, 9,350, 19.9)

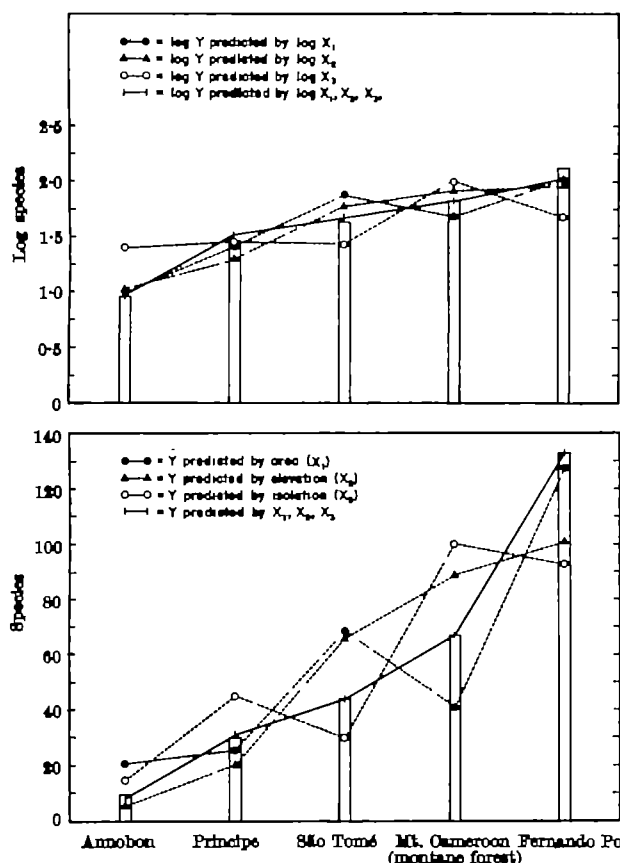


Fig. 2. Predictions by single and multiple regression analyses of the variation in bird species numbers in the Gulf of Guinea. Lower plot, predictions by Model I; upper plot, by Model II. Open bars designate vertically reported numbers of bird species. To show that knowledge of area, elevation, and isolation gives near-perfect prediction of the insular variation in bird species numbers. Standard errors of the estimate for  $\hat{Y}$  and  $\log \hat{Y}$  predicted by the three variables (equations (3) and (4)) are 1.6 species and 0.068 log species.

this continental sample should be zero or negligible, it can be assigned a token value of 1 mile. This avoids for Model II the problem of working with the common logarithm of zero, or minus infinity. That Amadon<sup>4</sup>, Searle<sup>5</sup>, Moreau<sup>6</sup>, and others have discussed the strong geological and avifaunal affinities between this volcano and its insular relatives nearby in the Gulf is considered justification for our use of this additional sample.

Table 1 summarizes computer results of an examination by the models of the regression of  $Y$  on the  $X$ s and of  $\log Y$  on the  $\log X$ s, and we note that a secondary aim of this report is to check the feasibility and appropriateness of a multiple regression investigation of a biological system with few sample items. Goodness of fit for regression of  $Y$  or  $\log Y$  on the  $X$ s or  $\log X$ s is estimated by the size of  $R^2$  or the coefficient of multiple determination ( $= \Sigma \hat{y}^2 / \Sigma y^2$ ) (ref. 4). All single and partial regression coefficients are tested by Student's  $t$ -distribution ( $t_0 = b/s_b$ ), and may be considered insignificant unless designated by an asterisk\* (significant:  $0.05 > P > 0.01$ ) or asterisks\*\* (highly significant:  $P < 0.01$ ).

Preston<sup>7</sup> and MacArthur and Wilson<sup>8</sup> have suggested the respective indexing of isolation by the reciprocal and square of the distance, and Hamilton *et al.*<sup>9</sup> have used the linear (that is, arithmetic or untransformed) value of distance. For  $Y$ , but not  $\log Y$ , we have tested the reciprocal and square transformation ( $X_3$ ,  $X_7$ ) of the isolation or distance measurement ( $X_1$ ,  $X_2$ ), along with the squares ( $X_4$ ,  $X_5$ ) or area ( $X_1$ ) and elevation ( $X_2$ ). The  $X$ s are used in single and multiple regression for two and three independent variables with 'overlapping' not allowed. For example, for predictions with area, elevation,

and isolation, only one measurement or transformation of each variable ( $[X_1$  or  $X_4] \cdot [X_2$  or  $X_5] \cdot [X_3$  or  $X_6$  or  $X_7]$ ) is used in any multiple regression.

The results summarized in Table 1 (cf. values for  $R^2$ ) are good in most cases, but the better predictions are by the linear measurements of  $X_1$ ,  $X_2$ , and  $X_3$ , or their logarithmic counterparts. For isolation, however, the square of the distance is clearly superior to the reciprocal in predicting  $Y$ —a point of some value in speculations on the shape of the dispersal curve for bird species crossing water-gaps (cf. Hamilton and Rubimoff<sup>9</sup>).

Either by Model I or II it is obvious (Fig. 2) that an almost perfect prediction ( $R^2 = 0.99$ – $1.00$ ) of Amadon's and Searle's data for variation in bird species numbers in the Gulf of Guinea can be obtained by use of three measurements of their insular or sample environment: area, elevation, and isolation. For predictions noted in Fig. 2, the multiple regression equations for 3  $X$ s are:

$$\hat{Y} = 78.180 + 0.110 * (\text{area}) - 0.004 (\text{elevation}) - 0.302 * (\text{isolation}) \quad (\text{I}) \quad (5)$$

and

$$\log \hat{Y} = 9.712 + 1.225 (\log \text{area}) - 2.702 (\log \text{elevation}) - 0.391 (\log \text{isolation}) \quad (\text{II}) \quad (6)$$

In equation (5)  $\Sigma y^2$  is 9077.2, and the sum of squares attributable to regression ( $\Sigma \hat{y}^2$ ) is 9074.7. All parts of equation (6) are insignificant, and its intercept value ( $a$ ) is extraordinarily high, being approximately  $10^{10}$  bird species when the  $\log X$ s are projected to zero. This is of mathematical rather than of biological significance, and stems from the inclusion in the equation of  $\log$  elevation, with which  $\log Y$  in this instance varies negatively, and thus increases (1) as elevation approaches zero (see (3) below).

The better multiple regressions with two independent variables occur by the following equations:

$$\hat{Y} = 54.150 + 0.094 ** (\text{area}) - 0.231 * (\text{isolation}) \quad (\text{I}) \quad (7)$$

and

$$\log \hat{Y} = 0.880 + 0.444 * (\log \text{area}) - 0.117 (\log \text{isolation}) \quad (\text{II}) \quad (8)$$

Standard errors of the estimate ( $s_y$ ) for  $\hat{Y}$  and  $\log \hat{Y}$  predicted by the two equations are respectively 6.1 species and 0.134 log species. With elevation ( $X_2$ ) withdrawn from the calculation matrices, the models account here

Table 1. INSULAR VARIATION IN NUMBER OF BIRD SPECIES IN THE GULF OF GUINEA

Coefficients of determination ( $r^2$ ) and multiple determination ( $R^2$ ) for regression on three environmental factors and their transformations

Independent variables		Models†		Variables	I	II
		I	II			
Area	$X_1$	0.839*	0.803*	$X_1, X_2$	0.849	—
Area <sup>2</sup>	$X_4$	0.825*	—	$X_4, X_2$	0.907*	—
Elevation	$X_2$	0.773*	0.807*	$X_2, X_3$	0.975*	—
Elevation <sup>2</sup>	$X_5$	0.847*	—	$X_5, X_3$	0.439	—
Isolation	$X_3$	0.646	0.358	$X_3, X_1$	0.924*	—
1/Isolation	$X_7$	0.012	—	$X_7, X_1$	0.859	—
Isolation <sup>2</sup>	$X_6$	0.653	—	$X_6, X_1, X_2$	1.000**	0.990
				$X_6, X_2, X_3$	0.968	—
	$X_1, X_2$	0.907*	0.918*	$X_1, X_2, X_3$	0.981	—
	$X_1, X_3$	0.930*	—	$X_1, X_2, X_3$	0.978	—
	$X_2, X_3$	0.991**	0.964*	$X_1, X_2, X_3$	0.982	—
	$X_1, X_4$	0.936*	—	$X_1, X_2, X_3$	0.980	—
	$X_1, X_5$	0.990**	—	$X_1, X_2, X_3$	0.970	—
	$X_2, X_4$	0.890	—	$X_2, X_3, X_6$	0.906	—
	$X_2, X_5$	0.958*	—	$X_2, X_3, X_6$	0.948	—
	$X_3, X_4$	0.846	—	$X_3, X_2, X_6$	0.990	—
	$X_3, X_5$	0.799	—	$X_3, X_2, X_6$	0.977	—
	$X_6, X_7$	0.991**	—	$X_6, X_2, X_3$	0.978	—

† Under Models I and II are listed the respective values for  $R^2$  ( $= r^2$  for single variables) for the arithmetic-to-arithmetic and logarithmic-logarithmic regressions.  $R^2$  or  $r^2 = \Sigma \hat{y}^2 / \Sigma y^2$  = fraction of variation accounted for by regression of the dependent variable on the independent variable(s). Degrees of freedom with  $n = 5$ : for 1  $X$ , 3; for 2  $X$ s, 2; for 3  $X$ s, 1. Note: no attempt is made here to evaluate relative goodness of fit between the models, or between the various variable combinations where each set has a high  $R^2$  value.

Table 2. INSULAR VARIATION IN NUMBER OF BIRD SPECIES IN THE GULF OF GUINEA

Variation and its sources†	Analysis and partitioning of the variance Degrees of freedom	Sum of squares	Mean squares
Bird species sample Nos.	4	$\Sigma y^2 = 9,077.2$	
Environmental variation	2	$\Sigma y^2_{12} = 9,002.1$	4,501.1**
Unexplained variation	2	$\Sigma y^2_{12} = 75.1$	37.5
		$F = 4,501.1/37.5 = 108; P = < 0.01$	
Area and isolation	2	$\Sigma y^2_{12} = 9,002.1$	
Area alone	1	$\Sigma y^2_{12} = 7,622.7$	
Isolation minus area	1	1,379.4	1,379.4**
Area and isolation	2	$\Sigma y^2_{12} = 9,002.1$	
Isolation alone	1	$\Sigma y^2_{12} = 5,878.2$	
Area minus isolation	1	3,123.9	3,123.9**
Unexplained variation (error)	2	75.1	37.5

Interpretation of analysis: (i) by test of null hypothesis, no significant variation in bird species numbers is unaccounted for by variation in area and isolation; (ii) variations in area and isolation contribute independently of one another to separate, significant components of bird species variance.

† Variation in bird species numbers ( $Y$ ) =  $\Sigma Y^2 - (\Sigma Y)^2/n = \Sigma y^2$ ; environmental variation =  $\Sigma y^2_{12}$  = sums of squares accounted for by regression of  $Y$  on  $X_1$  (area) and  $X_2$  (isolation).  
 $F$  = Fisher's<sup>12</sup> and Snedecor's<sup>11</sup> variance ratio.

respectively for 99 and 95 per cent of the variation in  $Y$  and log  $Y$ . Note also the marked decrease in the intercept value of equation (8) (cf. equation (6)). By comparing values for  $R^2$  in Table 1 for predictions by the three possible pairings of the  $X$ s or log  $X$ s, one readily sees that area and isolation are the more important contributors to bird species' variance by either Model I or Model II. A 'quick and dirty' test of significance for  $R^2$  values in Table 1 is by evaluating their square roots ( $= r$  or  $R$  = coefficient of correlation or multiple correlation) against R. A. Fisher's significance table for correlation coefficients<sup>13</sup>. Thus with 3, 2 or 1 degree(s) of freedom, any  $r^2$  or  $R^2$  value in Table 1 must be 0.771, 0.903 or 0.994 to attain at least a 0.05\* level of significance.

Analyses and partitioning of the variance<sup>4</sup> of  $Y$  and log  $Y$  support the conclusion from the comparison of  $R^2$  data (Table 1) that area and isolation are the important 'pushers' of bird species' numbers. For equation (7) (Model I), Table 2 presents such an examination of the variance, and shows by results of  $F$ -tests: (i) that no significant variation in  $Y$  remains unaccounted for by regression on area and isolation ( $\Sigma y^2 = 9,077.2$ ;  $\Sigma y^2_{12} = 9,002.1$ ); (ii) that each variable accounts separately for a significant component of  $Y$  variance. As the small sample size ( $n = 5$ ) suggests a probable departure of  $r$  from normality, we have tested the correlation and multiple correlation values by R. A. Fisher's normalizing transformation of  $r$  to  $z_r$ , where  $z_r = 0.5 [\log_e (1 + r) - \log_e (1 - r)]$ . In an important instance, a value of 0.998 for  $R_{12}$  (cf. Table 2 where  $R^2_{12}$  is 0.991) is significant\*\* both by  $t$ -test and by  $z_r$ -test, with  $z_r$  being 2.394 as determined (see ref. 9, 280) by the difference between 0.5 log<sub>e</sub> for mean square of  $\Sigma y^2_{12}$  and such for  $\Sigma y^2_{12}$ .

**Concluding discussion.** (1) Multiple regression analysis reveals that insular area, isolation, and elevation may be respectively considered as major, minor, and trivial contributors to variance for bird species numbers of isolates and a sample in the Gulf of Guinea. Together the variables give near-perfect prediction (Fig. 2) of Amadon's<sup>1</sup> and Searle's<sup>8</sup> data. That coefficients of multiple determination ( $R^2$ ) are greater than those of single determination ( $r^2$ ) (Table 1) suggests strongly that insular variation in bird species abundance is determined by several factors—a principle rather obvious, but rarely demonstrated. In the Gulf of Guinea, then it appears that two factors 'working' in opposition to one another interact to 'fix' insular avifaunal size: one, area presumably reflecting environmental opportunity or ecological diversity; and the other, isolation.

(2) Recently, interest has been expressed in the value of  $s$  or the slope of the regression line ( $b$  by our Model II) for log species regressed on log area<sup>1,7,8</sup>. In the present work,

the first-order, species-area curve ( $z_{y1}$ ) is 0.50\* for the five sample items. When 'corrected' in multiple regression,  $z_{y1.12}$  becomes 1.22\*, and when 'recorrected' by removal of log  $X_2$  (trivial contributor to variance of log  $Y$ ) the value (now  $z_{y1.2}$ ) becomes 0.44\*. This is larger than such exponential values reported for the three other, more isolated archipelagos, the avifaunas of which have been analysed correspondingly (West Indies, 0.24\*\*, East Indies, 0.28\*\*, and East-Central Pacific, 0.30\*\*) (ref. 2).

Preston in his canonical theory<sup>7</sup> for species abundance considers  $z$  to become smaller as oceanic isolates become continental samples. MacArthur and Wilson, in their equilibrium theory<sup>8</sup>, proceed from population-turnover theory to statistical aspects of species numbers distribution and conclude that  $z$  becomes larger as islands or sets of islands become more isolated. In this instance the different theories of the workers cited predict the same (i) response, namely, that  $z$  becomes smaller with decreased isolation. Although the small sample of 5 prevents our conclusion from being taken seriously, the bird species-area curve for the Gulf of Guinea is the opposite of what would be expected by either the canonical or equilibrium theory. That is, it is larger, not smaller, for a set of islands next to a continent, the equatorial avifauna of which probably has a relative ancient history. It should be noted, turning the discussion coin to its other side, that the findings of Hamilton and co-workers<sup>3</sup> for 'corrected' species-area curves for the two Pacific and West Indian avifaunas offer support for both theories. The values listed here at once average 0.273 (Preston's theoretical  $z$  value: 0.270) and show an increase in value (0.24 → 0.28 → 0.30) when listed in increasing order of their archipelago's oceanic isolation from continental or zoogeographic source regions (MacArthur and Wilson's conclusion).

(3) Finally, the negative partial regression coefficient for species-elevation variation by both models (equations (3) and (4)) hints at the minimum of decrease in bird species numbers when such variation is independent of area and isolation. This is a non-obvious finding, for the regression of  $Y$  on  $X_3$  only is positive ( $b_{y3} = 0.013^*$ ). Since the contribution of elevation ( $X_3$ ) to  $\Sigma y^2$  is trivial and insignificant, the finding may either be an insignificant variability due to the small value of  $n$  or be a result of correlation or confounding between the  $X$ s themselves (but  $r_{12}$  or  $r_{23}$  is each less than 0.80; see ref. 4); or the finding may be an indication of a reality which would occur if  $n$  were larger! Having noted these alternatives, we propose to consider the latter, improbable (admittedly) possibility for heuristic purposes only:

Imagine a circular, sea-level island. To increase in elevation without increasing in surface area, a reduction in sea-level circumference must be attendant to the rise of an elevation peak. The problem resolves to the question of how a shift in space from the lowlands to the highlands—without insular change in total surface area or isolation—might result in fewer species?

Writing in the vein of theorists and systems-makers, we offer three of various possible answers to the questions posed: (i) Bird species may immigrate into new areas less rapidly than they become extinct by the loss of equal area. (ii) Bird species restricted or adapted to highland zones may have a higher extinction rate than their lowland counterparts. (iii) Suggested by R. H. MacArthur and possibly related to (ii). Insular bird species adapted to montane habitats may have a lower immigration rate than lowland species as a result of the greater distances necessary for their inter-island or continent-to-island dispersal. Since there is less space for species in the highlands than in the lowlands, suggestion (ii) finds support in—indeed, it may be another manifestation of—the observation<sup>14</sup> that species of small islands or continental isolates become extinct more rapidly than species in the larger situations.

Making an analogy with decrease in number of species per unit area on passing from tropical to temperate to

arctic latitudes, we thus predict that when bird species-sample area studies are carried out at various elevations within these and perhaps other islands the species-elevation curve for intra-island situations will in the main be negative.

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## GEOLOGY OF RÉUNION ISLAND, INDIAN OCEAN

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IN recent years petrologists have devoted considerable attention to the volcanic islands of the oceans, and the Hawaiian archipelago, in particular, has been accepted as a classic area for the study of basalts<sup>1-3</sup>. From a review of the literature on oceanic basalt islands it became apparent that Réunion in the Indian Ocean is one of the few other oceanic islands offering a volcanic sequence comparable in thickness and variety to those of the larger Hawaiian islands, and that it would be profitable to make a detailed study of its petrology. The following account presents the preliminary results of this work, based on two periods of field-work in July and August in 1963 and 1964.

Réunion is situated at 55° 30' E. and 21° S. on the southern extremity of the Mascarene ridge in the western Indian Ocean. The island is ovoid in plan with a long axis directed N.W.-S.E. and an area of 2,512 km<sup>2</sup>. It emerges from depths in excess of 4,000 m to culminate in two volcanic summits: Piton de la Fournaise, in the south-east, is an active basalt cone reaching 2,631 m above sea-level, while Piton des Neiges in the north-west, despite its long extinction and severe erosion, still attains a height of 3,069 m. The diameter of the whole volcanic complex at the -4,000-m contour is c. 190 km and the average gradient from this depth is 4.5°.

Piton de la Fournaise has been described in considerable detail by a number of investigators, among whom Lacroix<sup>4,5</sup> is outstanding. But the older massif of Piton des Neiges has received much less attention<sup>6,7</sup>. Piton des Neiges is apparently unique in its combination of extreme dissection and variety of rock types. Not only are the youngest lavas and ashes largely preserved but, as a result of amphitheatre-headed valley erosion on a prodigious scale, relatively ancient suites of effusive and intrusive rock, ranging from ultrabasic to acid in composition, are displayed for examination deep within the core of the original volcano. The lavas fall naturally into two groups generally separated by a well-defined erosional discontinuity; the younger Differentiated Series consists mainly of hawaiite, mugearite and feldsparphyric basalt flows with thick intercalated pyroclastic deposits, and the older Oceanite Series consists mainly of olivine-rich basalt flows, which tend to be thinner, to have lower dips and to be associated with much less interbedded pyroclastic material than the lavas of the Differentiated Series. Ultrabasic inclusions occur sporadically among the flows of the Oceanite Series.

The lowest structural levels of the volcano are encountered in the floors of the three great erosional cirques

which surround the summit of Piton des Neiges. Here, the lavas of the Oceanite Series are underlain by a massive accumulation of poorly bedded agglomeratic material, at least 1,000 m thick, composed entirely of fragments of olivine-rich basalts, identical in type to the overlying lavas. The total thickness and lateral extent of this Cirque Agglomerate is not known, because only the top part of the pile is seen and it is not clear whether it accumulated within a caldera or over the flanks of the volcano during its earlier history.

Deep erosion to the north-east of the summit in the Salazie cirque has revealed zones of intense mylonitization in the Oceanite Series. The mylonitization appears to be associated with the occurrence of low-angled thrust-planes dipping towards the volcano centre. At one locality a slice of layered gabbro, tens of metres thick, occurs among the crushed lavas and has itself been partially mylonitized. The Oceanite Series and the Cirque Agglomerate are dissected by dykes, sills and inclined sheets, which become concentrated towards the core of the volcano into intense 'sheet-swarms' where the country rock makes up only a very small percentage of the total outcrop. The earliest members of this hypabyssal suite are ultrabasic sheets, some of which have been observed to cut the layered gabbro. These were succeeded by innumerable basic and intermediate intrusions including at least one strongly differentiated sill. The largest intrusions are massive undulatory sheets of syenite, up to 100 m thick and containing numerous narrow veins of quartz-sandstone-calcite pegmatite. The syenites are among the latest intrusions cutting the Oceanite Series and the Cirque Agglomerate, but are themselves cut by occasional thin basic dykes. No clear pattern of intrusion is discernible among the minor intrusives exposed in the floors and walls of the cirques, but a radial swarm of olivine basalt and oceanite dykes can be recognized within the Oceanite Series on the flanks of the volcano.

At some time before the extrusion of the Differentiated Series and probably before deep dissection of the primitive olivine basalt shield, zeolites were deposited in the vesicles of the lavas forming the central part of the volcano. The zeolitization affects the Cirque Agglomerate wherever it is seen, the inner and lower parts of the Oceanite Series and the great majority of the basic and ultrabasic intrusions. The later intrusions, including the syenites, are not zeolitized and clearly represent a later event probably synchronous with the eruption of the Differentiated Series.

On the northern and western flanks of the mountain the lavas and fragmental rocks of the Differentiated Series were laid down in an apparently conformable sequence with no obvious erosional breaks, and the strata thin very rapidly away from the volcano centre. The surface of the youngest material in these areas has been little modified by erosion since its formation; on the higher ground this surface is composed of moderately consolidated ash a few metres thick. Around the western flank of the volcano the lower slopes are built of apparently youthful, blocky flows of feldsparphyric basalt.

On the eastern flanks of Piton des Neiges the situation is much more complex. This is the side of the volcano which has received most of the precipitation from the east and south-east trade-winds, and here radial stream erosion has been extremely vigorous. During the later evolution of the volcano (at least) it would seem that no sooner was a deep ravine trenched into the mountain-side than it was occupied and obliterated by new flows from the summit crater. Between periods of extrusion new stream valleys were developed on either side of the flows thus channelled by earlier valleys and these, in turn, were filled by lava. The repetitive pattern of events, whereby valleys created by water during periods of comparative quiescence were destroyed by lava during subsequent volcanic activity, has been recognized by Stearns<sup>8</sup> in his work on the Hawaiian volcanoes. The consequences of this erosional bias to the windward or eastern side of Piton des Neiges included faster valley-head migration towards the summit crater with consequent concentration of flows on this side of the mountain, and the development of relatively thick flows as a result of restriction to steep-sided ravines. In other words, in spite of inequality of erosional rates around the mountain, an approximately symmetrical shield profile was maintained.

There is one clearly demonstrable intraformational erosional break within the Differentiated Series on the eastern side and others are strongly suspected. The youngest flows emitted from the summit cone were directed along valleys trenched into earlier members of the Differentiated Series. These distinctive young flows are of intermediate composition and carry phenocrysts of plagioclase, olivine, augite and magnetite. Like the feldsparphyric basalt flows near the west coast, the surface features have been little changed by subsequent erosion. The latest flows and pyroclasts of Piton des Neiges are believed to have been erupted from a relatively steep-sided (15–20°) summit cone the top of which lay approximately 300 m higher than the present summit. Massive and apparently horizontal flows, not accessible but thought to belong to the Differentiated Series, are exposed on the steep eastern side of the summit massif about 1,000 m below the summit itself, and are inferred to be caldera-filling flows. Thus, although there is no evidence whatever of caldera fracturing during the final stages of activity, it seems probable that such structures did exist at least once, during the earlier development of the Differentiated Series.

The most spectacular flows of the Differentiated Series are those forming the south wall of the Salazie cirque; these are very thick (up to 100 m), nearly horizontal units, exhibiting prominent columnar jointing. The accessible members of this group are seen to contain a very high proportion of basalt fragments representing earlier eruptions, in a glassy and flow-banded matrix containing abundant phenocrysts of anorthoclase and sanidine. The evidence suggests that these flows are 'nuée ardente' deposits formed during eruption of magma which was probably similar to that which produced the intrusive sheets of quartz syenite elsewhere, although the high content of included basalt chips precludes an accurate assessment of its composition.

The three great cirques of Piton des Neiges are erosional features which owe their distinctive form to the contrast between the lavas of the Differentiated Series and the

underlying rocks. The Oceanite Series is composed principally of thin vesicular flows separated by scoriaceous horizons and it is therefore relatively susceptible to erosion. This applies even more to the zeolitized parts of the Oceanite Series and to the Cirque Agglomerate. Thus, the older agglomerates and lavas, within a 7-km radius of Piton des Neiges's summit, have relatively little coherence and crumble readily. The overlying Differentiated Series, consisting mainly of tough massive flows of basalt and intermediate rocks, forms a relatively competent cover. Wherever a consequent stream has been initiated on this cover and eroded a gorge sufficiently deep to expose the older series, the process of undercutting, valley widening and deepening, and headward erosion is accelerated and reaches its extreme development when the valley heads penetrate the rotten core of the volcano comprising the zeolitized lavas and pyroclasts. Lateral undercutting and land-sliding become the principal erosional processes and the resulting avalanche deposits spread out as loosely consolidated sheets (shown as 'drift' in Fig. 1) over the valley floors where they are rapidly dissected into a 'bad-land' topography by stream run-off. Cilaos and Salazie, the principal towns within the cirques, are both built upon erosional remnants of great debris deposits of this type. The surfaces of such deposits may weather to form lateritic soils before becoming submerged by further avalanche flows, and hence a crudely bedded appearance results in some sections. Previous writers have not clearly distinguished the crumbling zeolitized lavas and agglomerate (both riddled by minor intrusions) from the superficial avalanche debris and fluvial sediments and have simply described all fragmental material forming the rugged floors of the cirques as 'basaltic detritus'. The development of only three large cirques (with possibly a fourth to the east, now filled with the youngest lavas), rather than a number of amphitheatre-headed valleys, seems to indicate that the first few valleys to penetrate the core proceeded to dominate the situation by relatively rapid lateral extension and 'capture' of the run-off previously feeding the neighbouring valleys.

A number of ancient terraces, composed of fluvial sands and pebble-beds, occur in the river gorges draining out from the cirques. Some of these, through which the streams have trenched deep ravines, are more than 100 m thick. At present, deposition of this sort of material is occurring only in the deltaic fans at the seaward ends of the gorges. It is probable that the older deposits were accumulated in large temporary lakes formed by the ponding up of the rivers behind dams created by massive land-sliding from unstable mountain-sides.

The eruptive histories of Piton des Neiges and Piton de la Fournaise have undoubtedly had considerable overlap, and Réunion must have passed through a stage when it consisted of a relatively steep, explosively active, cone to the north-west, emitting a variegated sequence of products, and a basalt shield volcano to the south-west, producing a rather uniform series of lavas. These basalts, olivine basalts and oceanites are strikingly similar to the older series of Piton des Neiges. In spite of being a highly productive volcano, Piton de la Fournaise is also deeply incised by river gorges. The drainage pattern is partly radial and in part directed by a series of caldera ring-fractures. The latter are closed (convex) towards the west but are open towards the east. It is probable that they have developed as a combination of vertical caldera-collapse (as at Kilauea and other Hawaiian volcanoes) and slumping towards the steep, unsupported, eastern side of the volcano. The modern cone has three intersecting crater-pits at the summit, of which the most easterly is the focus of contemporary activity. The cone is itself enclosed on its southern, western and northern sides by a caldera wall 100–300 m high. Most historic eruptions have occurred within the bounds of this caldera, the flows being predominantly directed towards the east. Prehistoric and historic flows have spilled into some of the

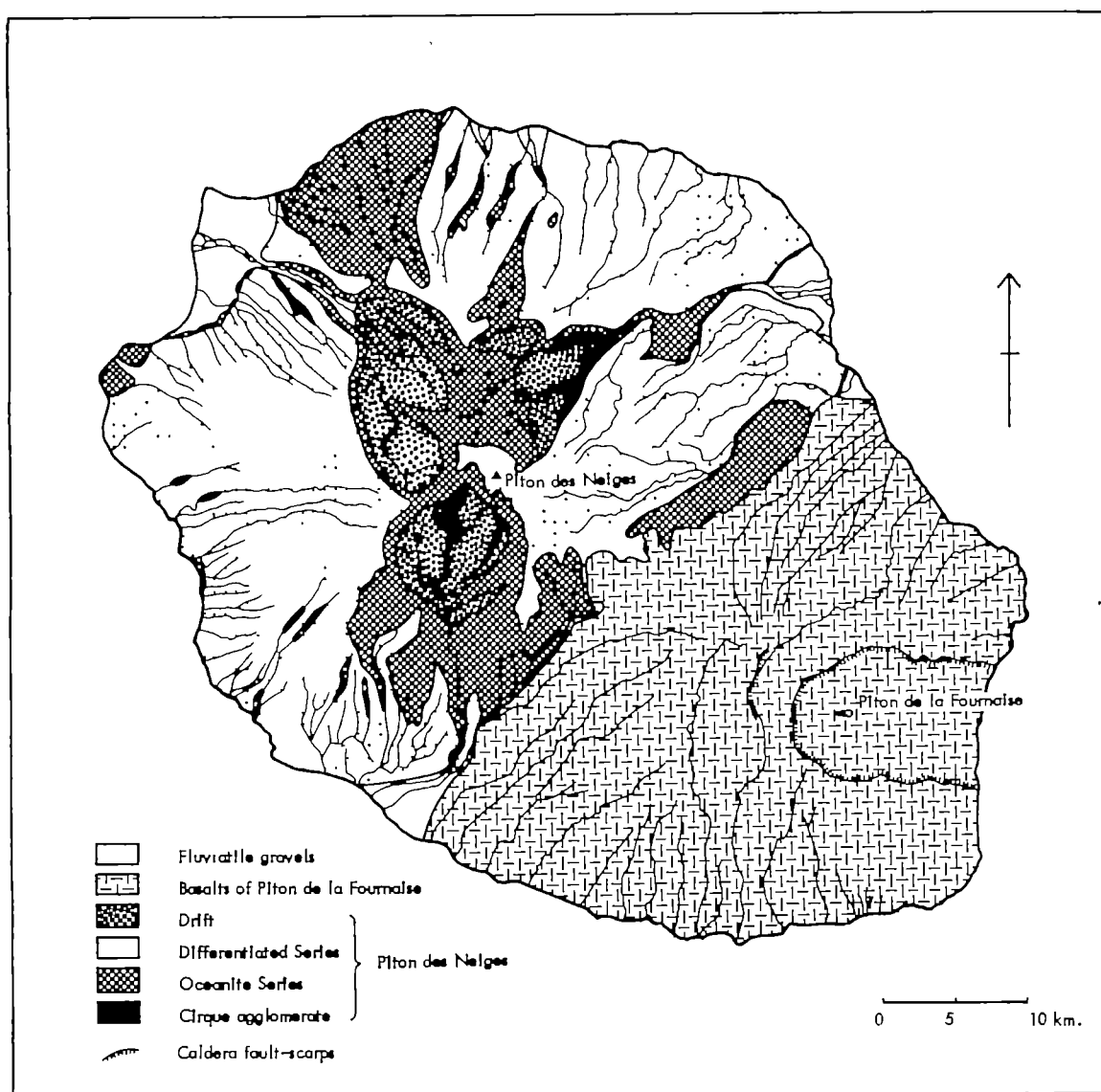


Fig. 1. Simplified geological map of Réunion

great river gorges on the mountain and conspicuous unconformities result. A former deep gorge, excavated by a river flowing north-east at the confluence of the dip slopes of the two volcanoes, has been largely obliterated by prehistoric flows spreading northwards from the summit region of Piton de la Fournaise. These erosional unconformities are, however, not marked by any significant change in lava type. The interpretation of a series of massive, horizontal flows as an ancient caldera-filling suite to the north-west of the modern eruptive centre, consideration of the existing caldera features, and the geology of the island as a whole, lead to the suggestion that there has been a steady overall shift in the centre of activity south-eastwards from Piton des Neiges.

A few small dykes are the only intrusions visible in the caldera walls and gorge sections on Piton de la Fournaise, and there is no suggestion of any zeolitization among the rocks of this volcano. Lacroix<sup>4</sup> has described the occurrence of ultrabasic nodules from Piton de la Fournaise; inclusions are absent from most flows, but locally they are

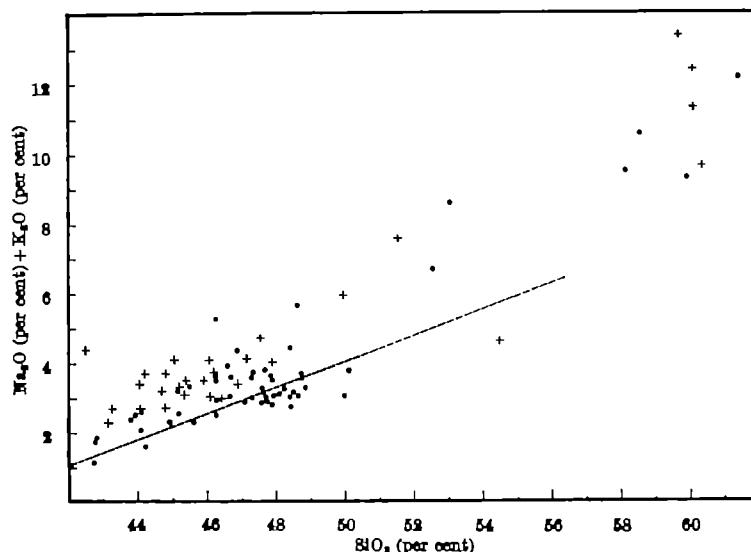


Fig. 2. Alkali-silica plot for lavas from Réunion and Mauritius. Solid circles, Oceanite Series of Piton des Neiges and basalts of Piton de la Fournaise; open circles, differentiated Series of Piton des Neiges; crosses, volcanic rocks of Mauritius. Analyses from Boudière<sup>5</sup>, Lacroix<sup>4</sup> and Walker and Nicolayson<sup>6</sup>. Dashed line indicates the boundary between Hawaiian tholeiitic and alkalic series<sup>7</sup>

very abundant. They are mainly dunite, occasionally grading into pyroxenite and very rarely carrying plagioclase.

The division of the lava sequences of many Hawaiian volcanoes into two main groups<sup>1</sup> can be generally matched in Réunion. The Differentiated Series of Piton des Neiges is clearly equivalent to the alkalic suite of lavas which typically forms the upper part of mature Hawaiian volcanoes, and similar rocks build the exposed part of the other Mascarene volcanoes, namely Mauritius<sup>2</sup> and Rodriguez<sup>3</sup>. The Oceanite Series of Piton des Neiges, and the lavas of Piton de la Fournaise, represent the 'primitive' basaltic suite, which in Hawaiian volcanoes is of tholeiitic type. The chemical affinities of the Réunion 'primitive' lavas are less clearly established; using the chemical data quoted by Lacroix<sup>4</sup> it can be shown that the majority of the olivine basalts contain significant amounts of normative hypersthene, which is generally a tholeiitic

characteristic, but an alkali-silica diagram (Fig. 2) indicates a composition intermediate between the tholeiitic and alkalic suites as defined by this method for the Hawaiian lavas<sup>5</sup>. The mineralogical evidence, particularly the nature of the groundmass pyroxenes, requires detailed investigation, but preliminary work indicates that Ca-poor pyroxenes are absent.

<sup>1</sup> Tilley, C. B., *Quart. J. Geol. Soc. London*, **196**, 87 (1960).

<sup>2</sup> Yoder, H. S., and Tilley, C. B., *J. Petrol.*, **3**, 843 (1962).

<sup>3</sup> Macdonald, G. A., and Katsura, T., *J. Petrol.*, **3**, 89 (1964).

<sup>4</sup> Lacroix, A., *Le Volcan Actif de L'île de la Réunion et ses produits* (Gauthier-Villars, Paris, 1936).

<sup>5</sup> Lacroix, A., *Le Volcan Actif de L'île de la Réunion (supplément) et celui de la Grand-Croix* (Gauthier-Villars, Paris, 1938).

<sup>6</sup> Lacroix, A., *Minéralogie de Madagascar*, **2**, *Lithologie* (Paris, 1923).

<sup>7</sup> Bumbrey, P., *Etude géologique de L'île de la Réunion* (Service Géologique, Tananarive, 1960).

<sup>8</sup> Stearns, H. T., *Hawaii Div. Hydros. Bull.*, **3** (1946).

<sup>9</sup> Walker, F., and Nicolayson, L. O., *Colonial Geol. Surv. (Min. Res. Div.)*, **4**, 3 (1964).

<sup>10</sup> McDougall, I., Upton, B. G. J., and Wadsworth, W. J., 1965 (in the press).

## CALEDONIAN OROGENY OF THE CENTRAL IRISH SEA REGION

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SINCE the publication<sup>1</sup> of a suggested scheme of correlation of Caledonian structural and igneous events in Britain, further research in North Wales, both by myself and others, now allows a fuller comparison of its history with that of S.E. Ireland to give a detailed picture of the Caledonian orogeny of the Central Irish Sea region. As was pointed out previously, a consideration of this orogeny should include not only the strong initial compressions which led to widespread folding and cleavage at the end of the Silurian period but also the succeeding phases of high-angle faulting and igneous intrusion continuing long into Devonian times (ref. 1, p. 796). The initial correlation of the Caledonian events (so defined) of these two areas on either flank of the Irish Sea was based entirely on my own work<sup>2,3</sup>. The subsequent recognition by Helm, Roberts and Simpson<sup>4</sup> that the fold-phases and cleavage observed by Simpson in the Isle of Man<sup>5</sup> are also present in North Wales allows, however, a closer correlation of the earlier phases of Caledonian history.

It is clear that the  $F_2$  fold system (and its associated cleavage) described by those authors is indicative of a stress-system corresponding to that which produced low-angle thrusting in S.E. Ireland; and that these localized structural features represent the second compressional phase of the orogeny, following the more widespread folding and cleavage of the first compression ( $F_1$ ). The  $F_2$  folds and cleavage of North Wales must then represent the second cross-phase, since the earliest Caledonian intrusions of northern Lleyn (the early granodiorite suite, intruded during the much weaker third compression) post-date all the more severe deformation of the country rocks. The  $F_2$  folds and cleavage are, like those of the  $F_1$  phase, closely localized, at least in the definite examples quoted by Helm, Roberts and Simpson<sup>4</sup>. Folds and cleavage of this generation in south-eastern Lleyn have since been described by me<sup>6</sup>, but no definite examples of  $F_2$  folds or cleavage have yet been observed in the areas of the peninsula that have been examined.

Further investigations by me to cover a more extensive part of the Lleyn peninsula<sup>7</sup> have also added further information on the later Caledonian history of North Wales. It has now become apparent that a group of

E.N.E. sinistral wrenches which have displaced the early granodiorite intrusions<sup>8</sup> can be separated from the north-easterly dextral wrenches such as the Nefyn fault (ref. 3, pl. 13) despite the approximate parallelism of these two sets of fractures. The Nefyn fault is very accurately dated by the penetration of aplites of the Bodelias granodiorite up minor fractures associated with this fault, which has clearly displaced the main intrusion (ref. 3, p. 174). Moreover, the direction of compression indicated by microgranodiorite dykes of Pennant type, associated with and of the same magma type as these early granodiorites, is correct for the formation of dextral wrenches with the direction of the Nefyn fault. Hence it appears that the north-easterly dextral faults pre-date the E.N.E. sinistral displacements, which apparently formed in the later part of the third cross-phase when a new generation of magma was emplaced to form the Pistyll microgranodiorite<sup>9</sup> with a form indicative of the north-easterly compression required for the formation of E.N.E. sinistral wrench faults.

In addition to some early N.N.W. faults which formed at the same time as the intrusion of the early granodiorite suite (ref. 3, p. 173), there are also later N.W.-N.N.W. fractures in the Lleyn peninsula, which clearly chop across the E.N.E. sinistral wrenches (ref. 7, p. 11) and include faults of sinistral wrench type as well as dip-slip faults and others which contain components of both types of movement. While some of the dip-slip faults are closer to N.W. in direction and somewhat sinuous in their outcrop, other faults with an important dip-slip component share the N.N.W. direction and straightness of those fractures which are largely or entirely sinistral wrenches. Hence it appears that a series of N.N.W. sinistral faults was formed first, and later with a slight change of the stress field some of these became modified or very largely overprinted by dip-slip movements, while some new, generally shorter, dip-slip faults also formed, closer to N.W. in direction. Both phases of movement can be recognized prior to the intrusion of felsite dykes<sup>8</sup> and hence both belong to the fourth compressional phase as defined by the events of S.E. Ireland, for as has previously been pointed out<sup>1</sup> the felsites (and some of the later events) of North Wales can be closely fixed in that scale. The pre-felsite W.N.W. faults across the southern end of the



Pistyll microgranodiorite, which were included in Table 1 of ref. 1, are now known to represent an uncommon fault direction in Lleyn. The reason for their formation in that locality, while N.N.W. sinistral wrenches were forming in most other parts of the peninsula, appears to have been due to a local modification of the vertical component of the stress field by the recently emplaced Pistyll intrusion.

Table 1. CORRELATION OF THE MID-PALAEZOIC STRUCTURAL AND IGNEOUS EVENTS OF SOUTH-EAST IRELAND AND NORTH WALES, SHOWING DIRECTIONS OF MAXIMUM HORIZONTAL COMPRESSION

Structural phases	Arklow area (S.E. Ireland) —Trenlett 1959	North Wales —various sources
	Stress direction Recorded events	Stress direction Recorded events
FIFTH CROSS-PHASE		
FIFTH COMPRESSIONAL PHASE		
FOURTH CROSS-PHASE		
FOURTH COMPRESSIONAL PHASE		
THIRD CROSS-PHASE		
THIRD COMPRESSIONAL PHASE		
SECOND CROSS-PHASE		
SECOND COMPRESSIONAL PHASE		
FIRST CROSS-PHASE		
FIRST COMPRESSIONAL PHASE		

The age-relationships of some of the later intrusions of North Wales have been discussed previously (ref. 7, p. 223), and it was seen that, contrary to the sequence suggested by Greenly<sup>8</sup>, their order of emplacement is: (1) ultramafic suite; (2) felsites and granophyres; (3) dolerites. While some of the ultramafic intrusions of Anglesey appear to have a sill-like form, there is no doubt that many are essentially dyke-like (possibly with locally concordant contacts) and have an outcrop parallel to that of the country rocks only because that was the orientation of the maximum horizontal compression during their emplacement. The ultramafic rock of Pen-y-rhiwian on the mainland appears to have been intruded as a dyke up one of the E.N.E. wrench faults<sup>7</sup>. This relationship is the only item of evidence on the earliest possible age for this suite, so it is possible that the ultramafic intrusions were emplaced during the third cross-phase (prior to the extensive N.N.W. and N.W. faulting of this region), but intrusion in the early part of the fourth cross-phase appears to me more probable.

The position in the time-sequence of the felsites and granophyres of North Wales is accurately fixed not only relative to the felsites of S.E. Ireland and the preceding N.W.-N.N.W. faults of the Lleyn peninsula (see above) but also in relation to the succeeding igneous events of the fifth compressional phase<sup>8</sup>. Though some of the intru-

sions have a circular outcrop and some follow pre-existent faults<sup>8,9</sup>, many develop a N.N.E. elongation<sup>8,10</sup> indicating the direction of maximum horizontal compression (albeit a weak one) during their emplacement.

The discovery of more rocks of Garnfor type at Garn Bentryrh<sup>8</sup> suggests that the W.N.W. orientation of the intrusions of these rocks and the dolerites at Garnfor may represent a local variation of a general north-westerly stress orientation rather than an earlier part of the fifth compressional phase as was previously postulated<sup>1</sup>. The events of the fifth cross-phase have previously been described<sup>1,8</sup>, consisting essentially of the intrusion of the Yr Eifl microgranite and dip-slip movements along north-easterly faults, but, whereas these faults in the Nefyn area were once thought to have originated at this time<sup>8</sup>, it is now apparent from studies over a wider area that they already existed as part of the E.N.E. sinistral wrench system prior to the intrusions of felsites, Garnfor hybrids and Yr Eifl microgranite. The small post-intrusive movements affecting these rocks represent only reactivation of already existent faults. Some small E.N.E. sinistral displacements of the margins of felsite intrusions are perhaps more likely to have occurred during this phase than at a later stage of the fourth cross-phase.

It will be noted that the Caledonian history of North Wales is now known in more detail than at the time of my previous publication on correlations of stress-chronology<sup>7</sup>. Nevertheless, as is indicated by a comparison of this new history with the earlier version, further research of North Wales has tended to amplify rather than modify the history previously advocated. Any modifications are minor and it is clear that the history deduced earlier was essentially correct.

A new comparison of the Caledonian history of North Wales with that of S.E. Ireland on the basis of the stress chronology of the two areas is given in Table 1. It will be seen from this that the filling in of the gaps in the Caledonian history of North Wales has tended to increase the similarity of its structural sequence to that of S.E. Ireland. The new information has not required the change of position of any of the correlated events, and supports the previous tentative correlation. Moreover, the further results of research in North Wales have not required the addition of any more compressional or cross-phases, and it begins to appear as if the five cycles previously detected in the Caledonian earth-movements of the region constitute the total number (apart from the Middle Devonian movements which must also be included in this orogeny—ref. 1, p. 799). The ubiquitous severe cleavage of the first compressional phase in S.E. Ireland, and the widespread development of thrusts and associated cleavage during the second compression, indicate that these main phases of the orogeny were much more severe in the western parts of this region, while the important faulting of the third compression indicates a continuation of this state of affairs, but in the later phases there is no general rule on which area had more severe stresses applied to it. Nevertheless, though some of the compressions were stronger in one area than the other and many of the structural episodes are represented by different events on either flank of the Irish Sea, it is apparent that when the events are represented by the direction of maximum horizontal compression there is a very close similarity of the structural history of the two areas which suggests an overall control of Caledonian orogenic processes over the whole region.

<sup>1</sup> Trenlett, W. B., *J. Geol.*, 71, 793 (1963).

<sup>2</sup> Trenlett, W. B., *Quart. J. Geol. Soc. Lond.*, 115, 17 (1959).

<sup>3</sup> Trenlett, W. B., *Lie. and Manch. Geol. J.*, 8, 157 (1963).

<sup>4</sup> Helm, D. G., Roberts, B., and Simpson, A., *Nature*, 200, 1060 (1963).

<sup>5</sup> Simpson, A., *Quart. J. Geol. Soc. Lond.*, 119, 257 (1963).

<sup>6</sup> Trenlett, W. B., *Geol. J.* (in the press).

<sup>7</sup> Trenlett, W. B., *Geol. J.*, 4, 207 (1964).

<sup>8</sup> Greenly, H., *The Geology of Anglesey* (Mem. Geol. Surv. G.B., 1919).

## SALT WATER IN THE BOTTOM LAYERS OF TWO NORWEGIAN LAKES

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**D**URING and after the last glacial period large areas of Norway were flooded by the sea, and the marine limit was in most places situated far above the present sea-level. Later, the sea retreated, and the lake basins in areas that had been flooded were left filled with sea-water. In deep basins in sheltered localities salt water can, because of its density, remain in the deepest layer of the lake for a long time. Consequently, trapped sea-water can still be found in the bottom layer of certain deep lakes situated below the marine limit.

In 1952 it was discovered<sup>1</sup> that lake Rørholtfjord in Telemark county, southern Norway (Fig. 1), situated 60 m above sea-level, has a layer of salt water extending from a depth of 134 m to the bottom (147 m).

(Fig. 1), were found to be meromictic, with salt water in the bottom layer.

The bedrock of the catchment area of these lakes (Fig. 2) is built up of gneisses and granite gneisses. The topography is irregular and most of the area is covered by only a thin layer of moraine material. Between Birkelandsvatn and Tronstadvatn there is a small moraine, and there are remains of moraine material in some places along their shores. In the catchment area there is a good deal of marshland and the soil is peaty. Most of the area is covered by coniferous forest, particularly pine (*Picea abies* and *Pinus silvestris*). With regard to the geological origin of the lakes, they are eroded along a fault-line, and their shores are very steep. Consequently the lakes are protected from the wind by the surrounding hills and the locality has a rather sheltered character.

Tronstadvatn is situated about 13 km from the sea and Birkelandsvatn about 22 km. The lakes were sounded using a 'Simrad' echo sounder (Simonsen Radio A/S, Ensjeveien 18, Oslo). The morphological details for the lakes are given in Table 1.

Tronstadvatn and Birkelandsvatn have catchment areas of about 53 km<sup>2</sup> and 22 km<sup>2</sup> respectively. According to data from the Norwegian Watercourse and Electricity Board<sup>2</sup> the mean drainage in this area is about 40 l./sec./km<sup>2</sup>, which gives 183,000 m<sup>3</sup> and 78,000 m<sup>3</sup> of drainage water per 24 h for Tronstadvatn and Birkelandsvatn respectively. The 'theoretical renewal time' of the whole

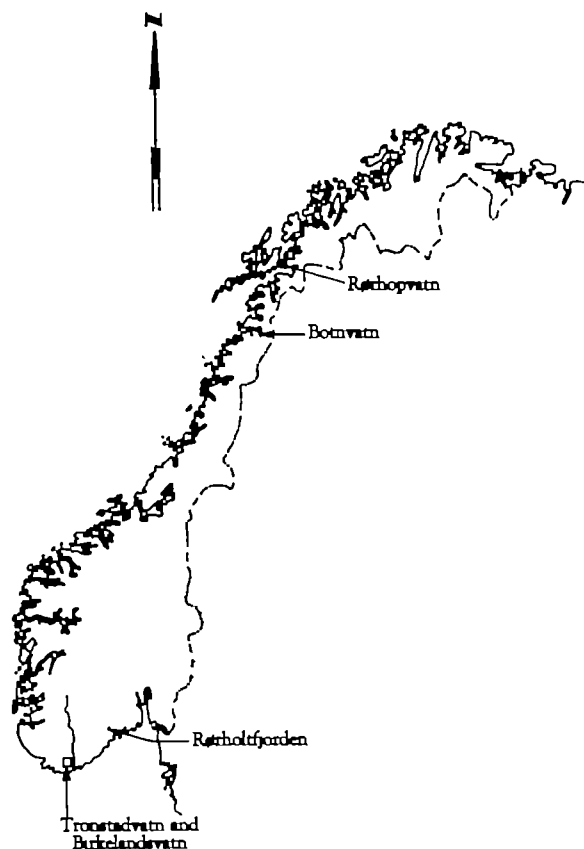


Fig. 1. Norwegian lakes with trapped sea-water

Since then, Strøm has also described<sup>3</sup> two similar lakes in northern Norway (Fig. 1). Botnvatn, situated 12 m above sea-level in the Salten district, is saline from a depth of 102 m to the bottom (113 m). Similarly, Rørholtfjord, 3 m above sea-level in the Lofoten district, has salt water from 44 m to the bottom (92 m). Strøm maintains that there is no doubt that the salt water in the bottom of these lakes is the remains of previous sea-water.

In 1963, during a limnological investigation of several lakes in the Kristiansand area in the south-western part of Norway, two lakes, Tronstadvatn and Birkelandsvatn

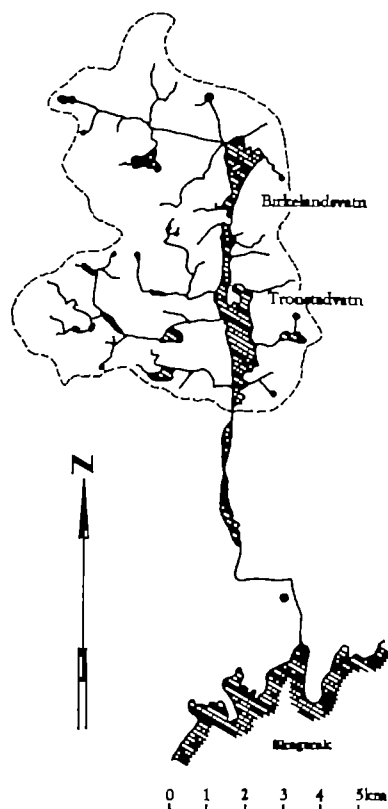


Fig. 2. Tronstadvatn and Birkelandsvatn, the catchment area.

Table 1. TRONSTADVATN AND BIRKELANDSVATN  
Morphological details

	Tronstadvatn	Birkelandsvatn
Altitude above sea-level	41	43
Maximum depth, m	98	69
Average depth, m	37	23
Surface area, km <sup>2</sup>	2.0	0.8
Volume, million m <sup>3</sup>	76.5	16.7
Maximum length, km	4.9	3.0
Maximum breadth, km	0.8	0.9
Cryptodepression, m	57	26

Table 2. FIGURES FOR COLOUR (mg Pt/L) AND COD (ml N/100 KMnO<sub>4</sub>/L) IN SOME LAKES IN THE KRISTIANSTAD AREA

Locality	Colour, mg Pt/L	ml N/100 KMnO <sub>4</sub> /L
Rossevatn	13	25.0
Tronstadvatn	15	27.5
Hammervatn	18	31.3
Reivvatn	21	33.8
Birkelandsvatn	23	36.5
Bjelklandsvatn	25	42.5
Sukkevatn	29	46.3
Storvatn	45	56.3

water mass is thus about 14 months for Tronstadvatn and 7 months for Birkelandsvatn.

Down to about 78 m in Tronstadvatn and 33 m in Birkelandsvatn the lakes are dimictic, and every spring and autumn the water turns over; during winter and summer a thermal stratification is established. During the summer stratification period the thermocline is situated at a depth of 7-8 m in both lakes. From 78 m in Tronstadvatn and 33 m in Birkelandsvatn the temperature increases with increasing depth (Fig. 3) and here the temperature conditions are constant during the year.

The pH and 'hardness' of the fresh water in Tronstadvatn and Birkelandsvatn are low, as in most of the other lakes in south Norwegian bedrocks. The lakes receive a load of allochthonous organic material from the catchment area, but, compared with other lakes in the area, the amount of humus present in the water is relatively small (Table 2). This is because the biological decomposition of such material is very effective in these comparatively large and deep lakes.

In the aerated water masses the oxygen saturation value lies between 80 and 90 per cent, but in the chemocline layer the value decreases from about 80 per cent to 0.

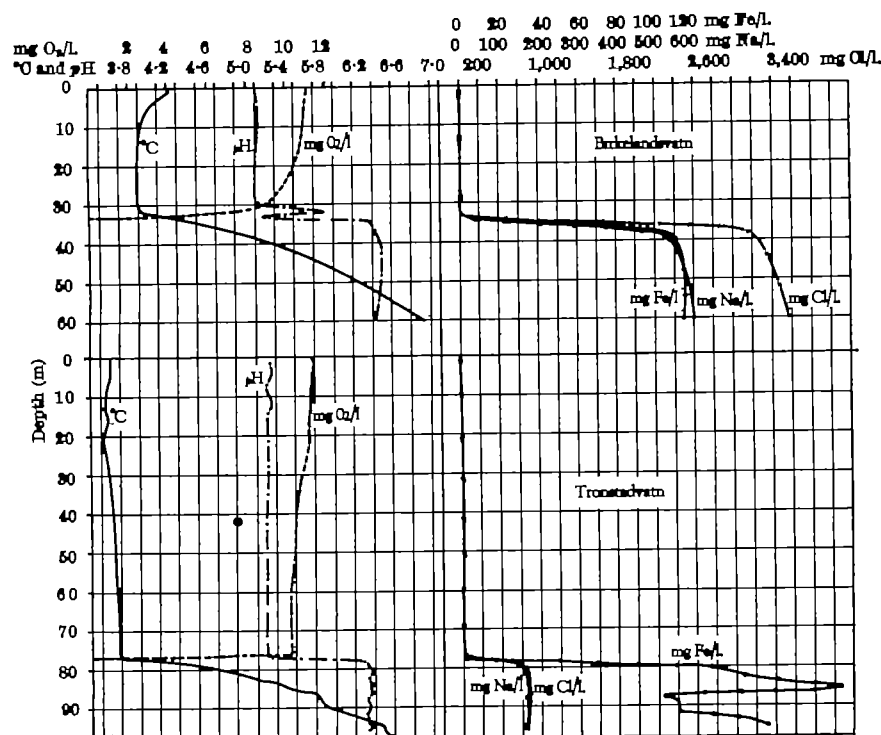


Fig. 3. Some chemical components in Tronstadvatn and Birkelandsvatn

The water has low concentrations of iron and manganese ions. These ions are probably associated with humic material carried to the lakes with the incoming drainage water. As is usual in surface waters in this region, the fresh water of the two lakes has a low content of chloride and sulphate. The concentration of these ions increases with depth in the fresh-water layer and the same applies to sodium and potassium.

Tronstadvatn and Birkelandsvatn are both meromictic lakes, and they have high concentrations of salts, methane and carbon dioxide in the deep water. The stagnant water in Tronstadvatn is situated between 78 m and the bottom (98 m), and has a volume of  $2.5 \times 10^6$  m<sup>3</sup>. In Birkelandsvatn the stagnant water extends from 33 m to the bottom (69 m) and the volume is  $2 \times 10^6$  m<sup>3</sup>.

The qualitative and quantitative composition of the salt water is somewhat different for the two lakes, as can be seen from Table 3.

Table 3. CHEMICAL COMPOSITION OF THE STAGNANT WATER IN TRONSTADVATN AND BIRKELANDSVATN AND OF SEA-WATER SALINITY (35 parts per thousand)

Parameter	Tronstadvatn	Birkelandsvatn	Sea-water (35‰)	B/T
$\kappa_s \times 10^6$	2,500	8,800	c. 48,000	3.5
Cl, mg/L	670	3,350	19,800	5.0
Br, mg/L	4	*	66	0.06
I, mg/L	165	600	11,100	3.7
Na, mg/L	5.2	10.3	290	2.0
K, mg/L	140	840	420	6.0
Ca, mg/L	102	300	1,330	3.0
Mg, mg/L	150	115	0.001-0.05	0.75
Fe, mg/L	10	1.2	0.001-0.01	0.12
Mn, mg/L				

\* Not analysed.

The figures in the last column (B/T) indicate the ratio between the concentrations of the different components in Birkelandsvatn and Tronstadvatn.

Table 4. RATIOS BETWEEN THE CONCENTRATION OF SOME COMPONENTS IN TRONSTADVATN, BIRKELANDSVATN AND SEA-WATER (35 parts per thousand)

Locality	Cl/Br	Na/K	Cl/Na	Br/Na
Tronstadvatn	165	23	4	1/41
Birkelandsvatn	60	60	5.6	
Sea-water	300	30	1.8	1/168

Table 3 shows that most of the components are present in greater concentrations in Birkelandsvatn than in Tronstadvatn except for iron and manganese, the concentrations of which are greater in Tronstadvatn. The concentration ratios between various components are shown in Table 4.

Table 4 shows that the ratio between chloride and bromide is only half as high in Tronstadvatn as in sea-water. The relationship between sodium and potassium is almost the same in Tronstadvatn as in sea-water, but only half that in Birkelandsvatn. There is no apparent correlation between chloride and sodium or bromide and sodium. It is not, therefore, possible on this basis to say whether the stagnant water in Tronstadvatn and Birkelandsvatn is the remains of previous sea-water.

Comparing the ratios of chloride and sodium concentrations in the fresh water and the stagnant water we find that these ratios increase with depth, as Table 5 shows.

In the fresh water in Tronstadvatn the chloride/sodium ratio is the same as in sea-water, but this is not so for the stagnant water. In both the fresh and the stagnant water the chloride/sodium ratio is greatest in Birkelandsvatn.

Table 5. RELATIONSHIP BETWEEN CHLORIDE AND SODIUM IN TRONSTADVATN AND BIRKELANDSVATN

	Depth (m)										
Locality	1	20	32	33	34	50	70	77	78	79	80
Tronstadvatn	1.7					1.8	1.8	2.1	4.2	4.1	4.1
Birkelandsvatn	2.2	2.8	3.2	4.6	5.5	5.6					

The origin of the salt water in Tronstadvatn and Birkelandsvatn is of considerable interest, especially as it can throw some light on the location of the marine limit in south-west Norway. Danielsen<sup>4</sup> maintained that the marine limit in the Kristiansand area is situated about 40–50 m above the present sea-level. On the other hand, Andersen<sup>5</sup>, in a comprehensive investigation of the quaternary geology of south-west Norway, states that the marine limit in the Kristiansand area is situated between 16 and 28 m above the present sea-level.

The geology of the area in which these lakes are situated is such as to preclude the idea that the salt water gained its mineral contents from the bedrock. It seems very likely, therefore, that the salt water in the bottom of the two lakes is of marine origin. This constitutes powerful evidence that the marine limit in this region was situated at least 40 m above the present sea-level.

The fact that the concentration ratios of the ions in the salt water of the lakes are different from those of sea-water does not necessarily argue against the sea-water theory. It is quite likely that the slow addition of organic and inorganic allochthonous material from the catchment area over thousands of years could have altered the composition of the salt water in the period that has elapsed since the lakes became isolated from the sea. Furthermore, dilution and selective adsorption of the various salts could have occurred in such a way that their ratios to each other are now quite different from what they are in sea-water.

It is, then, reasonable to suppose that the salt water in the bottom of Tronstadvatn and Birkelandsvatn has originated from sea-water left behind after the last glacial period. This is considered to be evidence for placing the quaternary marine limit in this area at a level at least 40 m above the present sea-level.

<sup>1</sup> Strøm, K., *Nature*, 180, 982 (1957).

<sup>2</sup> Strøm, K., *New Scientist*, 13, 284 (1961).

<sup>3</sup> Norges vassdrags- og elektrisitetsvesen, *Hydrologiske Undersøkelser i Norge* (1958).

<sup>4</sup> Danielsen, D., *Norg. Geol. Unders.*, 55 (1910).

<sup>5</sup> Andersen, B. G., *Norg. Geol. Unders.*, 210 (1960).

## ACCELERATED DEFORMATION OF ROCK SALT AT ELEVATED TEMPERATURE

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CHEMICAL reprocessing of power reactor fuel produces a chemically complex and hazardous effluent which cannot be handled by conventional waste-disposal methods. Special methods of disposal are required to contain these wastes for centuries, thus preventing the escape of fission products to the environment. The most promising method for disposal of high-level, heat-generating, power reactor wastes is the conversion of liquid wastes to solids, followed by the ultimate disposal of the solids in a salt mine<sup>1–3</sup>.

In order to be able to design a radioactive waste disposal facility in a salt mine, it is necessary to be able to predict the effects on mine stability of both supporting-pillar stresses (produced by the superincumbent Earth strata), and elevated temperatures (produced by radioactive decay of the fission products in the waste).

Recent work by Serata<sup>4</sup> and Obert<sup>5</sup> at ambient temperatures has shown that flow in rock-salt mines may be approximated by testing scale-model specimens uniaxially. It is generally known that elevation of the temperature increases the creep rates of stressed rock-salt specimens, but no data or experience are available which can be directly applied to mine conditions.

To simulate pillar, roof and floor conditions which would exist in mined cavities in rock salt, sample specimens are fabricated to represent scale models of salt pillars and their surrounding rooms. The test specimens used in the work recorded here are cylindrical in shape, with a portion of the centre ground out to form the pillar and surrounding rooms (Fig. 1). By 'epoxying' steel rings around the ends of the samples, effective confining pressure is applied to the roof and floor portions of the models when they are loaded. Constant uniaxial loads are applied to the models by hydraulic compression testers having capacities up to 300,000 lb. (1 lb. = 0.453 kg). Cavity closure is measured by mounting two dial gauges 180° apart on the rings. All tests were conducted in a controlled-temperature room equipped with automatic dehumidifiers to prevent condensation on the samples during extremely damp periods. Elevated temperature tests were performed with the specimens inside a cylindrical heating jacket and with barrier heaters on top and bottom between the specimen and the platens.

All model pillars were fabricated from 6-in.-diam. (1 in. = 2.54 cm) cores of rock salt taken in the mine of the Carey Salt Co., Lyons, Kansas. The pillars measure 4 in. in diameter and 1 in. in height. Tests were conducted at temperatures of 22.5° C, 60° C and 100° C for axial loads of 2,000, 4,000, 6,000, 8,000 and 10,000 lb./in.<sup>2</sup> (1 lb./in.<sup>2</sup> = 0.0703 kg/cm<sup>2</sup>). Durations of the tests varied from a few hours to several thousand hours. For certain temperature and load combinations, duplicate or triplicate tests were run in order to obtain an approximate measure of variation from sample to sample. Eventually, this will be done for most of the combinations. By the middle of February 1965, about 25 pillar models had been tested in this Laboratory.

From these tests it was observed that the deformation of the pillars increased markedly with increasing loads. However, even more significant was the greatly accelerated creep rates of the salt at elevated temperatures. The

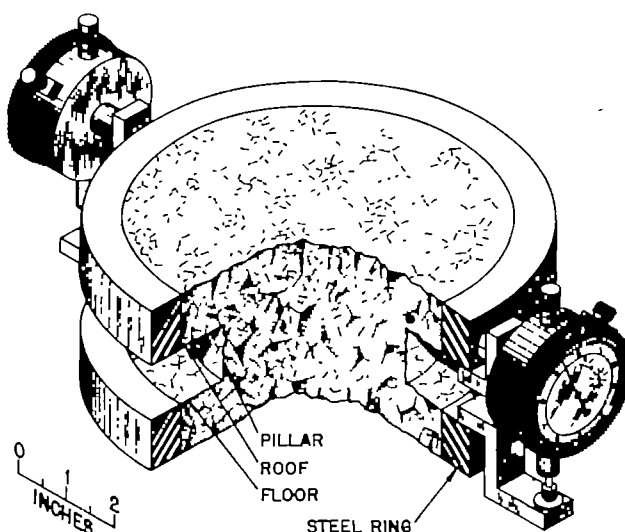


Fig. 1. Model of salt pillar

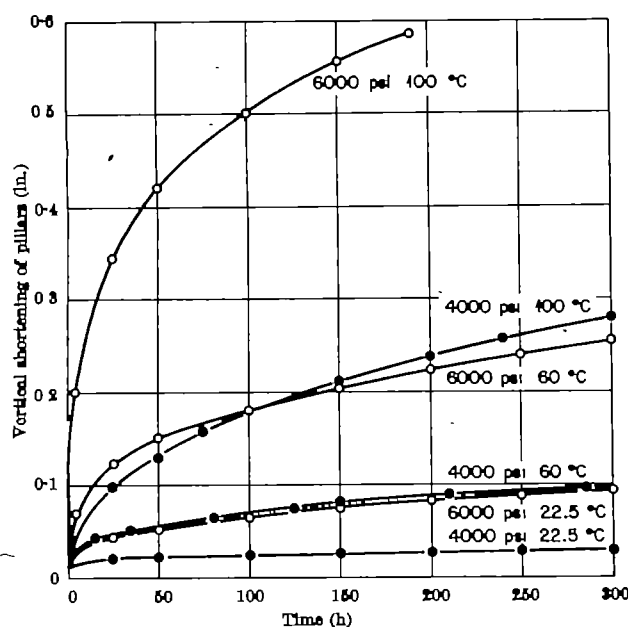


Fig. 2. Deformation of model rock salt pillars

cavity closures with time for models axially loaded to 4,000 lb./in.<sup>2</sup> and 6,000 lb./in.<sup>2</sup> at temperatures of 22.5° C, 60° C and 100° C are shown in Fig. 2. These curves, along with those at higher and lower stresses, all show general similarities. For each curve, there is initially a high creep rate that decreases with time. Tests of longer duration show that creep rates continue to decline even after 5,000 h. This is in agreement with measurements that we have made in salt mines which show that closure rates are still decreasing with time in openings up to 12 years. At room temperature (22.5° C) the percentage cavity closure (inches shortening of the pillar) for the 4,000-lb./in.<sup>2</sup> sample was about 3 per cent after 300 h and for the 6,000-lb./in.<sup>2</sup> model approximately 10 per cent, while for the 4,000- and 6,000-lb./in.<sup>2</sup> samples heated to 60° C the closure for the same period of time was about 10 and 25 per cent, respectively. When the temperature is increased to 100° C, the increase in the rates of cavity closure is even more pronounced. It is also of interest that the deformational behaviour of the salt pillar loaded to 4,000 lb./in.<sup>2</sup> at a temperature of 60° C is approximately the same as the behaviour of the sample loaded

to 6,000 lb./in.<sup>2</sup> at room temperature. Also, the creep curve for the 4,000-lb./in.<sup>2</sup> model at 100° C shows a close correlation with the behaviour of the 6,000-lb./in.<sup>2</sup> sample at 60° C. This strongly suggests that salt pillars subjected to elevated temperatures will behave like pillars under higher stresses.

We were encouraged to extend the pillar-model tests to elevated temperatures after we had analysed the results of a series of seven tests at ambient temperature which were performed for us by the Applied Physics Laboratory of the Bureau of Mines<sup>1</sup>. To these data we fitted an empirical equation, extrapolation of which produced predicted creep rates which were in reasonable agreement with vertical and horizontal creep-closure rates which we have been measuring in the Kansas mines since 1959 (ref. 6). The following empirical equations have been fitted to the results of the Oak Ridge National Laboratory pillar-model tests for times from 10 h on. The equation would require modification to fit the data from zero time.

$$\dot{\epsilon} = 3.2 \times 10^{-36} T^{15.5} \sigma^{2.5} t^{-0.55}$$

$$\epsilon = 9.2 \times 10^{-36} T^{15.5} \sigma^{2.5} t^{0.55} + A$$

where  $\dot{\epsilon}$  = strain rate (vertical convergence,  $\mu$  in. in.<sup>-1</sup> h.<sup>-1</sup>);  $\epsilon$  = cumulative deformation ( $\mu$  in. in.<sup>-1</sup>);  $T$  = absolute temperature (°K);  $\sigma$  = average pillar stress (lb./in.<sup>2</sup>);  $t$  = time (h); and  $A$  = total deformation at 10 h.

At ambient temperature these equations give approximately the same results as those derived from the Applied Physics Laboratory tests. Although the data are still too sparse to attempt a statistical analysis, the results appear to corroborate a previously developed hypothesis<sup>7</sup> that the effect of elevating the temperature is essentially the same as that of increasing the average pillar stress, and that the relationship between creep rate and axial stress follows the same power law regardless of the temperature.

This work was supported by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.

<sup>1</sup> Committee on Waste Disposal, Division of Earth Sciences, *Disposal of Radioactive Wastes on Land* (Nat. Acad. Sci.-Nat. Res. Council, Publ. 519, 1967).

<sup>2</sup> Bradshaw, R. L., et al., *Treatment and Storage of High-Level Radioactive Wastes*, 153 (Intern. Atomic Energy Agency, Vienna, 1963).

<sup>3</sup> Bradshaw, R. L., et al., *Trans. Amer. Nuclear Soc.*, 7 (2), 390 (1964).

<sup>4</sup> Sorala, E., in *Proc. Sixth Symp. Rock Mechanics*, 260 (Univ. Missouri at Rolla, 1964).

<sup>5</sup> Obert, L., in *Proc. Sixth Symp. Rock Mechanics*, 530 (Univ. Missouri at Rolla, 1964).

<sup>6</sup> Bradshaw, R. L., Boegly, jun., W. J., and Simpson, F. M., in *Proc. Sixth Symp. Rock Mechanics*, 501 (Univ. Missouri at Rolla, 1964).

<sup>7</sup> Bradshaw, R. L., in *Waste Treatment and Disposal, Quarterly Progress Report, May-October 1963*, ORNL-TM-757, ed. by Blanco, R. R., and Parker, F. L., 123 (1964).

## LOGIC OF COMPUTER-BASED INTRINSIC CLASSIFICATIONS

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WE believe that the rationale of the numerical techniques for the classification of elements into sets has been widely misunderstood. This misunderstanding has given rise, on the one hand, to exaggerated respect for the 'absolute' nature of the classifications so produced and, on the other, to unmerited summary dismissals of many such systems (see, for example, the strictures of Seal<sup>1</sup> on the work of Sokal and Sneath<sup>2</sup>). The intention of this article is to place these methods in their logical context, to reveal those areas in which empirical tests are appropriate, and to announce computer programmes capable of carrying out such tests on a more extensive scale than his hitherto been possible.

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### The Statistical Problem

From a statistical point of view any classificatory system involves three terms: the individuals that are to be classified, the attributes by which these individuals are described, and the uses to which the classification is to be put. Any one of these three may in principle be either a finite or an infinite set; if a set contains more elements than can conveniently be individually considered, we shall regard it as infinite. The uses will therefore only be treated as a finite set if they are all known, and in this case an extrinsic classification (that is, a classification with respect to one or more external criteria, such as that suggested by Macnaughton-Smith<sup>3</sup>) is required; we are not here

concerned with this case. The attribute-set, though always in principle infinite, is in practice always finite since no use can be made of information we do not possess. In intrinsic classifications, therefore, there are only two basic situations according to whether the individual-set is finite or infinite. Of the eight possible situations (finite or infinite sets of individuals, attributes or uses), only the case with all three terms finite is strictly non-probabilistic, because completely determinate, and this case is unlikely to arise in practice. However, if the user constates the only infinite set, it will be difficult to interpret the assigning of a probability or a significance-level to any fusion or separation of individuals or groups; it will normally only be possible to assess a result as 'informative' or 'profitable'. It is in this wider sense that Williams and Dale<sup>4</sup> have used the term 'non-probabilistic'.

The distinction between the two basic situations (probabilistic, or non-probabilistic in the wider sense) may to the user appear artificial or unimportant; but they represent two quite distinct statistical problems which we shall deal with in turn.

(1) *The case of the finite population.* The classic example of this case is that in which the elements are taxa the characteristics of which have been extracted from the relevant literature. A more troublesome case is that in which the characteristics have been obtained from an examination of representative specimens (for example, type-specimens in the nomenclatural sense). Here it is essential for the user to decide whether these are to be regarded as specimens (that is, individual samples from a population and thus subject to error) or as types (that is, convenient summaries of information which define taxa, as distinct from merely illustrating them).

The practical test in border-line cases is primarily whether the user does or does not wish to question the distinctness of the elements. If he does not, then these elements are members of a finite population: they are as completely defined as the user wishes, they are postulated as different, and the null hypothesis that they are samples from a larger population is highly artificial. This situation is non-probabilistic in the wider sense defined here, and it remains to explore the status of the information then produced by 'cluster-seeking' or 'similarity' methods. Consider, as an analogy, a family of ten children; to their mother they are unique and precisely known entities. She may nevertheless find it useful to group them in order to convey information economically: into, for example, two musical and eight unmusical, or into six boys and four girls. The musical classification would only be useful in a very specific context; but the sex classification would involve differences on many other attributes, and would serve a variety of purposes. Such a classification is, in fact, predictive; the precise purpose for which it is to be used may not be known at the time that it is made, and its function is simply to provide groupings which it is hoped will involve attributes additional to those immediately under consideration.

Two important conclusions follow: (a) Classifications so produced can never be true or false, or even probable or improbable; they can only be profitable or unprofitable. To define an optimum method we should have to formalize the situation sufficiently to estimate, and thence to maximize, the expected profitability. The purpose of such methods is not to displace the intuitive taxonomist, but to suggest to him potentially fruitful lines of investigation. (b) Such classifications are not unique. The ten children might equally well have been divided into three infants, five at school, and two out at work; and this grouping would have been more profitable than the sex-grouping for some purposes, less so for others. The classification must be tested empirically by the observation of further appropriate attributes.

If, however, the user does not choose to regard his taxa as inviolate, an alternative strategy is available; he may postulate that the differences between two similar taxa

might have arisen as a result of sampling errors, and he may then elect to analyse his finite group as if it were a sample from a hypothetical infinite population. This is in effect the strategy suggested by Goodall<sup>5</sup>. So far as the inter-relationships between elements are concerned, the group under investigation is regarded as a model of an infinite population; each pair of taxa can then be associated with a probability that the resemblance of the two elements has arisen by chance. This system has the interesting property that the degree of similarity between two individuals is related to the homogeneity of the group as a whole. (This concept is familiar to taxonomists who are well aware that the orders of differences which separate tribes in a homogeneous family such as the Labiatae would barely suffice to distinguish genera in the more diverse Ranunculaceae.) Goodall (personal communication) has under development a corresponding probabilistic cluster-analysis system; but even when this is available the computation involved is likely to preclude the use of the method in any but critical cases. For the 'primary survey' of taxonomy—the delimitation of groups likely to be worthy of further study—the user may normally be expected to use empirical coefficients in the predictive sense outlined here.

(2) *The case of the sample.* In ecology (in classification of vegetation) the population under investigation is usually a set of quadrats; in microbiology it may be a set of isolates. In such cases the population is manifestly a sample from a larger population, and is subject to bias and error in the sampling. Three strategies are now available:

(a) The user may, providing he does so overtly, choose to process the data as if it were a finite population. It then follows that, whereas with a truly finite population the results can be at worst unprofitable, they can now in addition be misleading. This strategy therefore imposes on the user a more stringent test of profitability; in ecology a habitat survey will provide such a test, as will the investigation of the behaviour of further attributes in taxonomy.

(b) The original classification may be applied to a fresh sample by means of an allocation rule, and the resulting sub-division appropriately tested for significantly high information-content; this strategy has received less attention than it deserves. Alternatively, but less satisfactorily, a second sample may be classified independently and the results compared; no precise test of the validity of such a comparison has been suggested, but groupings recognizable as common to the two analyses are unlikely to be due to sampling errors, though they may be due to bias.

(c) The user may choose to work in a genuinely probabilistic situation such as is provided by the Goodall coefficient. He has more commonly wished to take advantage of the kind of 'similarity' defined by the empirical systems, and to assign probabilities to the relationships so obtained. The difficulties of this approach have been briefly discussed by Williams and Dale<sup>4</sup>; the problem is notoriously intractable and, although it has given rise to a formidable literature, only in the case of the rarely used information statistic can there be said to be a satisfactory solution. Taxonomic writing has sometimes seemed to us to imply that this particular type of strategy is the normal one, and that its state of development is substantially satisfactory; such a view cannot but arouse misgivings.

### New Computer Programmes

We are not here concerned with monothetic classifications, which use a divisive strategy, but with the polythetic agglomerative ('similarity') methods which have been widely used for taxonomic purposes. In those cases in which empirical classifications can be justified, it is, as we have already implied here, to be expected that

different types of problem will be best served by different systems; the distinction frequently made in the literature between 'size coefficients' and 'shape coefficients' is a familiar example. Nevertheless, few comparative studies have been made of different systems on the same set of data. This is largely because any one worker has usually had access to a very limited range of alternatives; and we have therefore written, for the Control Data 3600, a flexible programme to facilitate such investigations. The specification, in outline, is as follows:

**Data (i) type:** qualitative or quantitative. Since the programme is intended for methodological study, provision has not been made for missing or inapplicable attributes. Automatic transposition is available for 'inverse' classifications of attributes\*.

**Data (ii) dimensions:** fast versions of the present programmes will accommodate 100 individuals specified by 50 attributes. Alternative versions, using magnetic tape as an intermediate store, will accommodate populations of very considerably increased size.

**Sorting strategies.** Both nearest-neighbour and group sorting are provided. The group sorting is of the type in which a group is replaced on formation by the co-ordinates of its centroid (or, in the case of the information statistic, by a definition of the whole group).

**Coefficients.** Four basic coefficients are provided:

(i) **Squared Euclidean distance.** Provision is also made for initial standardization of attributes to zero mean and unit variance, or for the reading in of a vector of weighting coefficients.

(ii) **'Non-metric coefficient'.** In the conventional notation of a  $2 \times 2$  table, this is  $(b + c)/(2a + b + c)$ : the quantitative counterpart between two vectors ( $x_1$ ) and ( $x_2$ ) is  $(\sum |x_{1j} - x_{2j}|)/\sum (x_{1j} + x_{2j})$ . It is open to mathematical objections, but has so commonly been used in ecology that it is important for comparative purposes.

(iii) **Correlation coefficient.**

(iv) **Information statistic.** If a group of  $n$  elements is defined by  $p$  attributes, and if the  $j$ th attribute is possessed by  $a_j$  elements, the loss of information on formation of the group from its elements may be defined by:

$$I = pn \log n - \sum_{j=1}^p [a_j \log a_j + (n - a_j) \log (n - a_j)]$$

The coefficient used for discrimination is the increase in  $I$  on fusion, which is to be minimum. It is available in this form only for qualitative data, and cannot meaning-

fully be used with nearest-neighbour sorting since it is then a constant multiple of Euclidean distance.

To allow as much space as possible for data, three separate programmes are provided: **QUALNEAB** (qualitative data, nearest-neighbour sort), **CENTROID** (qualitative or quantitative data, centroid sort) and **NUMNEAB** (quantitative data, nearest-neighbour sort).

Dr. J. M. Lambert has made a preliminary examination of a set of comparative runs of all methods on two qualitatively specified ecological populations. The results already suggest that, as is to be expected on mathematical grounds, centroid sorting is always more powerful than nearest-neighbour sorting; they also give grounds for the tentative suggestion that the information statistic is the most informative of the coefficients. A full account of these analyses will in due course be submitted for publication in an ecological journal. Meanwhile, users with other types of data may wish to undertake similar comparative studies, and specifications of the programmes can be obtained from Dr. G. N. Lance.

### Problem of Mixed Data

A striking characteristic of much genuine taxonomic data is the high proportion of multi-state attributes. These cannot in all cases be realistically dichotomized; nor can the separate states be regarded as qualitative attributes, since the states are not normally independent and spurious negative associations immediately arise. Taxonomists cannot be expected to use numerical methods until these are capable of processing their data without distortions imposed solely for the convenience of the method. The Goodall coefficient will accommodate mixed data, but a simpler method is desirable for everyday use. We have therefore developed a cruder empirical system based on the 'non-metric coefficient', which will accommodate qualitative, quantitative and multi-state attributes; it has been assembled into a programme (**MULTIST**) which uses centroid sorting. We regard this programme as still experimental, but it has given promising results on small-scale problems and is available for more extensive tests.

We thank Mr. P. Macnaughton-Smith, of the Home Office Research Unit, for his advice.

\* See H. J. Macnaughton-Smith, *Statistical Analysis for Biologists* (Methuen, London, 1964).

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\* Williams, W. T., and Dale, M. B., *Adv. Bot. Res.*, 2 (in the press).

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## NUCLEOLAR AND CYTOPLASMIC RIBONUCLEIC ACID INHIBITION BY EXCESS THYMIDINE

By DR. FREDERICK H. KASTEN, FREDY F. STRASSER and MARIAN TURNER

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AT low concentrations, actinomycin D inhibits DNA-dependent RNA synthesis without affecting DNA replication<sup>1</sup>. At the cytological level, this action manifests itself in the nucleolus, resulting in an inhibition of nucleolar RNA synthesis with failure to form ribosomal RNA (ref. 2) and a peculiar sorting out of the fine-structural components of the nucleolus<sup>3-5</sup>. During the course of an investigation of nucleoprotein synthetic patterns in synchronized mammalian cells, we obtained evidence for a similar selective inhibition of nucleolar RNA synthesis resulting from treatment with relatively high concentrations of thymidine.

Excess thymidine was first reported to induce partial synchrony by Xerxes<sup>6</sup>. An improved double-blocking technique has since been shown to induce synchrony in cultures of heteroploid human kidney cells<sup>7</sup>, HeLa cells<sup>8</sup> and diploid Chinese hamster ovary cells<sup>9</sup>. By adjusting the two excess thymidine exposures to coincide properly with the time parameters of the mitotic cycle, such cells then enter the S phase in a synchronized burst following transfer to normal medium. This conclusion is supported by our observations on CMP cells using Coulter electronic cell counts, mitotic counts, phase-contrast time-lapse cinematography film records, and tritiated thymidine



autoradiography<sup>10</sup>. Observations on thymidine inhibition of nucleolar RNA synthesis and cytoplasmic RNA are described in detail in this article.

A newly developed epithelial line of human adenocarcinoma cells was used. This rapidly growing line is designated as the CMP line (the growth characteristics, cytogenetics, and ultrastructure of CMP cells will be described in another publication from this laboratory) and is now in its 55th passage. All procedures to be described were carried out in a 37° C warm room in order to minimize temperature fluctuations and improve cell synchronization. Cells were grown in Eagle's medium plus 10 per cent foetal bovine serum, plus penicillin (100 units) and streptomycin at 50γ/ml. Trypsinized cultures were seeded on to coverslips in Yerganian tubes at a density of 20,000 cells per ml. and grown for 3 days. While in the logarithmic growth phase cultures were blocked for 24 h with 2.5 mM of thymidine. The blockage was broken by washing cells 3 times in pre-warmed Gey's balanced salt solution and incubating in normal medium for 15 h. A second thymidine blockage was imposed for 24 h.

At the end of the second thymidine treatment, tritiated uridine was added for 1 h to the thymidine-rich medium to give 20 μc./ml. (Schwarz lot 6501, 4.42 c./mM). Randomly growing cells were likewise exposed to labelled uridine for 1 h. Cells were fixed in Carnoy's acetic alcohol (1:3), brought to water, treated with 5 per cent cold trichloroacetic acid for 15 min to remove labelled RNA precursors, washed in water, dried, and covered with AR 10 stripping film. Duplicate sets of slides were stored at 4° C for 4–8 days. Preparations were developed in Kodak D19 and stained with haematoxylin and eosin. A third set of slides from the same experiment, but unlabelled, was fixed in 95 per cent methanol and stained by May–Grünwald–Giemsa for mitotic counts.

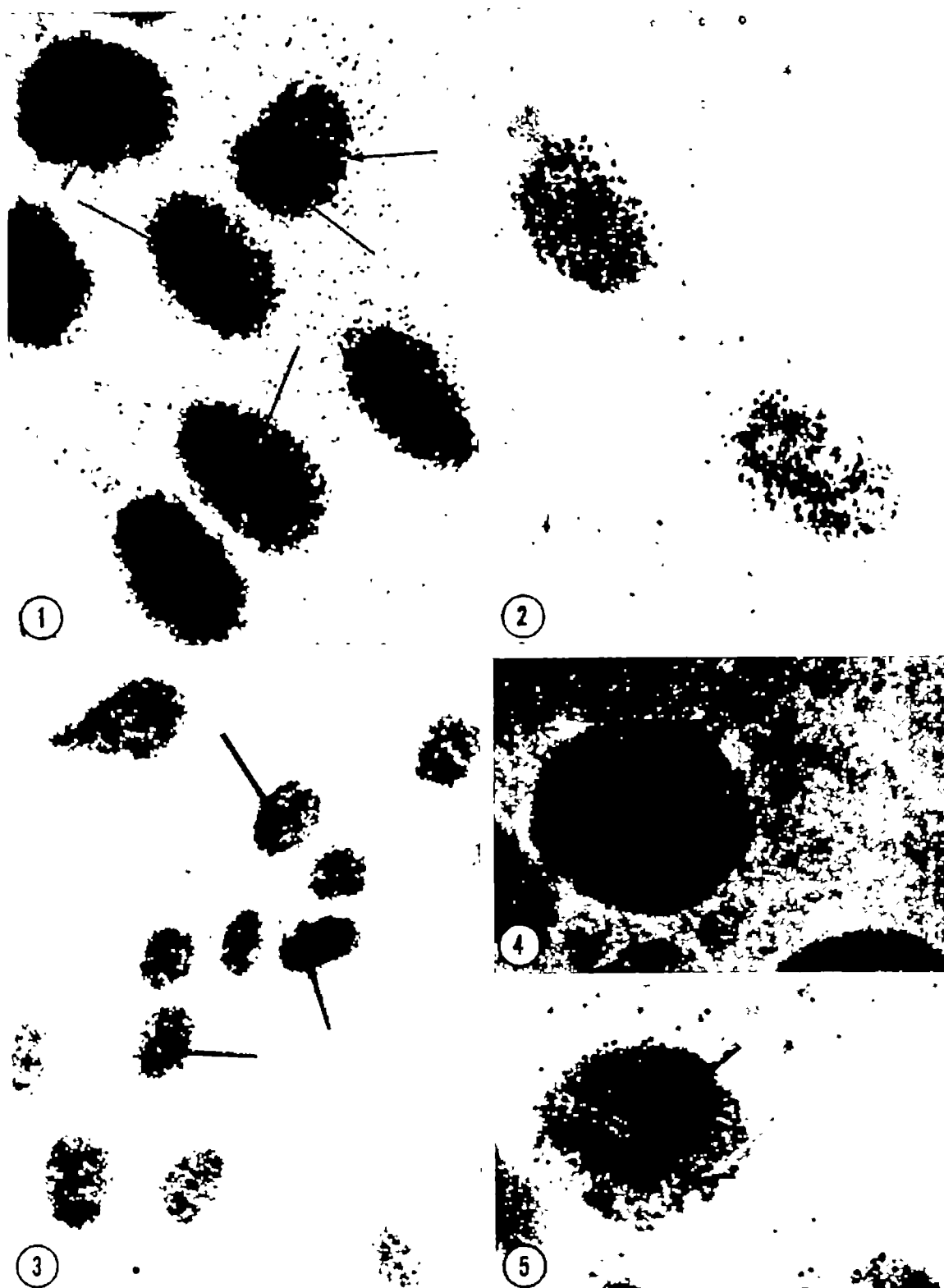
The mitotic rate in untreated randomly growing cells was 3 per cent. This population of interphase cells exhibited a uniformly heavy nucleolar and a moderate nuclear labelling in approximately 90–95 per cent of all cells; cytoplasm was lightly labelled (Fig. 1). The density of label in nucleolus, nucleus and cytoplasm was approximately in the proportions of 6:3:1 respectively. It was not possible to make exact grain counts because of the intense labelling, but the intracellular pattern of incorporation was evident. There was no labelling in mitotic cells. Thymidine-blocked cells had a mitotic rate of 0.3 per cent—10 times less than controls, demonstrating an almost complete premitotic block. Arrested cells appeared cytologically normal except for averaging larger nuclei than controls. Nuclear RNA labelling density was somewhat less than controls, but there was a complete absence of uridine incorporation into the nucleoli and cytoplasm (Figs. 2, 3). Phase-contrast observations were made under high magnification to verify that unlabelled areas resembling nucleoli in size and distribution were, in fact, these structures (Figs. 4, 5). More than 96 per cent of the labelled nuclei contained unlabelled nucleoli and cytoplasm. In the remaining 4 per cent, all nucleoli in each cell were equally labelled and to the same density as in controls. Blockage of RNA synthesis was reversible, as demonstrated by the fact that when excess thymidine medium was replaced by normal medium, there began a rapid incorporation of uridine into nucleoli during the first hour, amounting to about 75 per cent of controls. The amount of labelled RNA in the nucleoli and cytoplasm reached normal levels within 1–2 h.

Bootama *et al.*<sup>7</sup> state that cells blocked with excess thymidine are in the *S* phase of the cell cycle. Their results appear inadequate to support this conclusion since their evidence is based on the fact that cells were actually labelled with tritiated thymidine after being returned to normal medium—in effect, after the block was removed. They also observed a peak mitotic burst 6 h earlier than expected if the blockage was actually in early *S* phase.

In a recent abstract, Puck<sup>8</sup> also stated that thymidine-blocked *S3* HeLa cells are accumulated at the beginning of *S*. In support of the views of the foregoing authors is the finding that excess thymidine inhibits DNA synthesis<sup>11</sup>. In our experiments, cells are blocked with 2.5 mM of thymidine. When tritiated thymidine is added to such blocked cells in labelling doses, it is diluted 1,000 times by unlabelled thymidine. Under these conditions it would be surprising to see any evidence of DNA synthesis. In fact, we do not observe any labelled nuclei in blocked cells after exposure to 'diluted' tritiated thymidine for 1 h (New England Nuclear Corp. lot 108-55-1A-114, 8.7 c./mM, 12.5 μc./ml.). It should be possible to circumvent this difficulty and decide unequivocally whether DNA synthesis occurs in double-blocked cells by employing other DNA precursors besides thymidine. Feulgen cytophotometry may also help in clearing up this point. Judging from the timing of the peak mitotic burst observed in our experiments, the blockage appears to be in the *G1* or very early *S* phase. A more definitive conclusion as to the nature of the block must await further investigation as already indicated here. For the present we can assume, for theoretical considerations, that there is a complete cessation of DNA synthesis.

The accumulation of cells in the *G1* or early *S* phase by excess thymidine is the most obvious effect displayed. A second, and what appears to us to be a more significant action, is the selective inhibition of uridine incorporation into nucleolar RNA and a lack of cytoplasmic labelling. Chromosomal RNA synthesis is depressed slightly. These results, in regard to nucleolar and cytoplasmic RNA inhibition, are similar to those obtained with actinomycin *D* (ref. 2). The mimicry of results should not be construed to mean that the molecular mechanism of action is necessarily the same in both cases. On one hand, actinomycin *D* combines with the DNA template<sup>1</sup> and prevents the action of DNA-dependent RNA polymerase<sup>12,13</sup>, while excess thymidine is thought to inhibit DNA synthesis by blocking the formation of deoxycytidine triphosphate from cytidine-5'-phosphate<sup>8</sup>. The fact that nucleolar RNA synthesis is selectively blocked demonstrates, in accord with previous observations<sup>2,14-16</sup>, that chromatin RNA synthesis is largely independent of nucleolar RNA synthesis and that there exist at least two different extracytoplasmic fractions. Since tritiated uridine was available for 1 h in control cells of our experiments, there was sufficient time for nucleolar RNA to become labelled and to provide ribosomal components of cytoplasmic RNA (ref. 17). The fact that both nucleolar and cytoplasmic labelled RNA are eliminated by excess thymidine is probably due to the failure of nucleolar RNA to be formed which, in turn, allows no ribosomal RNA to be produced. These experiments do not exclude the possibility that excess thymidine acts as a competitive inhibitor for uridine or that cytoplasmic RNA synthesis is blocked independently of nucleolar RNA.

According to Smellie<sup>18</sup>, two different enzyme systems are found in nuclei that catalyse the incorporation of all 4 ribonucleotides into RNA. Both systems require a DNA primer and one also requires an RNA primer. Furthermore, RNA polymerase, a nucleolar constituent in animal cells<sup>19</sup>, appears to be naturally associated with DNA (ref. 20) and is required for RNA synthesis. Since the actinomycin *D*-DNA complex inhibits both RNA polymerase and DNA polymerase<sup>20</sup>, there is a quantitatively selective effect on RNA synthesis. It is suggested that nucleolar DNA synthesis triggers nucleolar RNA synthesis and also chromatin DNA synthesis. There is recent evidence that early replicating DNA of the nucleus plays a vital part in cell survival<sup>21</sup>. If it should be demonstrated that early replicating DNA is associated with the nucleolus, the argument will be further strengthened. Our observation of complete nucleolar and cytoplasmic RNA inhibition in thymidine-blocked cells (when DNA synthesis is inhibited) is consistent with the hypothesis



Figs 1-5. CMP cells exposed to tritiated uridine at  $20 \mu\text{g/ml}$  for 1 h and fixed in acetic alcohol (1-3). Developed *AR 10* autoradiographs, stained lightly with haematoxylin and eosin and photographed by bright-field except for Fig. 4. (Figs. 1, 2, 4-5,  $\times 1700$ ; Fig. 3  $\times 660$ ).

Fig. 1. Control cells demonstrate intense tritiated-uridine incorporation into nucleoli (see arrows for some examples); somewhat less uptake into chromatin RNA, and light cytoplasmic RNA labelling.

Figs. 2 and 3. Double thymidine-blocked cells. Note lack of nucleolar and cytoplasmic RNA labelling. Slight background labelling.

Fig. 4. Phase-contrast photomicrograph of a labelled cell which had been blocked with thymidine. Focal level is just below film. Arrow directs attention to nucleolus (nu).

Fig. 5. Same cell as in Fig. 4 but in bright-field. Focal level is on silver grains. Arrow points to unlabelled nucleolus (nu).

that nucleolar RNA synthesis is dependent on DNA synthesis and specifically the DNA localized within the nucleolus.

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## CHEMICALLY INDUCED DELAYED GERMINAL MUTATION IN *Drosophila*

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IT has long been recognized that there is a marked similarity of genetic effects in the production of gene mutation and chromosome breakage by X-irradiation and chemical mutagens. However, one notable difference between them was soon shown to be the high frequency of visible-mosaics (mainly for bristle mutations) produced after chemical treatment of *Drosophila*<sup>1</sup>.

As discussed here, the phenomenon of mosaicism is to be understood as the co-existence of somatic and/or germinal cells of different genetical constitution (for a mutation) in one and the same individual organism. The organism is said to be somatically-mosaic when mutation affects a proportion of the somatic cells, and germinally- (or gonadically-) mosaic when a proportion of the germ cells is affected.

This article reports the induction of germinal-mosaicism for sex-linked recessive lethal mutations after treatment of spermatozoa of *Drosophila melanogaster* by adult feeding of two monofunctional ethylating agents (diethyl sulphate and ethyl methanesulphonate), and *N*-nitrosomethylurea; mosaicism is afterwards detected by investigating the distribution of sex-linked recessive lethal mutations in the germinal tissue of the embryos produced from fertilization of untreated ova by the treated spermatozoa. In particular, ethyl methanesulphonate and *N*-nitrosomethylurea are found to produce the highest rates of mutation yet recorded in *Drosophila*, giving frequencies of sex-linked recessive lethal mutations of about 70 per cent. Formaldehyde, which was also tested, was found not to produce a significant increase in germinal sex-linked recessive lethal-mosaicism when administered by a larval feeding method.

The ethylating agents were selected for study, since previous tests for the induction of visible sex-linked mutations after treatment of *Drosophila* spermatozoa with diethyl sulphate indicated that mutations were originating mosaically<sup>2</sup>. *N*-nitrosomethylurea was selected for investigation, because tests showed that it was an extremely potent mutagen. Formaldehyde was examined because previous tests<sup>3</sup> on the induction of visible sex-linked recessive mutations after larval feeding of formaldehyde have shown that approximately 50 per cent of these mutations arose as complete (or whole-body) mutations, whereas the remainder showed somatic-mosaicism for the mutation.

### The Genetical Basis for Lethal-mosaicism

One of the most objective means (and, therefore, the standard test)<sup>4</sup> for the quantitative detection of genetic

mutation in *Drosophila* is to examine for sex-linked recessive lethal mutations arising in the germinal tissue of the male. Since the male is hemizygous for the X chromosome, the male cannot be a carrier of a pre-existing sex-linked lethal, and the frequency of lethals arising 'spontaneously', or induced by treatment, can be conveniently estimated from a sample of the germ cells of the male.

The procedure used here is to mate wild-type males individually for three days to two virgin females which are homozygous for a specially-constructed X chromosome (the Muller-5 chromosome). The *F*<sub>1</sub> female progeny from this mating are consequently heterozygous for the paternal wild-type X chromosome under test and a maternal Muller-5 X chromosome: each *F*<sub>1</sub> female represents one individual wild-type X chromosome donated by one individual spermatozoon.

The *F*<sub>1</sub> female progeny (or a sample of them) arising from each male are then mated individually to an *F*<sub>1</sub> Muller-5 brother. The importance of the Muller-5 chromosome is seen here, for it contains a complex inversion which prevents crossing-over between the two X chromosomes in the *F*<sub>1</sub> female, and thus keeps the wild-type chromosome intact. The Muller-5 chromosome also carries a dominant mutation, bar-eye, and a recessive mutation, apricot eye, and these markers allow easy recognition of the two classes of male and female in the *F*<sub>2</sub> culture.

An *F*<sub>2</sub> culture without a lethal contains two phenotypically distinguishable types of male and female: thus it contains about 50 per cent Muller-5 males and 50 per cent wild-type males; and it contains about 50 per cent homozygous Muller-5 females and 50 per cent heterozygous wild-type females. (The heterozygous wild-type females, being heterozygous for the bar-eye gene, exhibit phenotypically a kidney-shaped red eye.) An *F*<sub>2</sub> culture with a lethal is detected by the complete absence of wild-type males in the culture, which also contains both types of female as well as Muller-5 males.

Since the test detects a sex-linked recessive lethal in the *F*<sub>2</sub> culture by the complete absence of males which carry the paternally-derived wild-type X chromosome, it follows that the gonad of the *F*<sub>1</sub> female carries the paternal X chromosome only in a lethally mutated condition. However, if the gonad of an *F*<sub>1</sub> female carries the paternal X chromosomes in both the non-mutated and a lethally mutated condition (that is, the female is germinally mosaic for the paternal X chromosome), then the *F*<sub>2</sub> culture may contain some males carrying the non-mutated paternal X

chromosome, and be classified as a non-lethal, or possibly as a semi-lethal, culture.

Operationally, the sex-linked recessive lethal test thus detects mutational events which produce  $F_1$  females carrying the paternal X chromosome only in a lethally mutated condition (complete lethal), and misses  $F_1$  females which are germinally mosaic for the non-mutated X chromosome and for a lethally mutated X chromosome derived from it (lethal-mosaic).

However, it is possible to detect  $F_1$  females which are germinally-mosaic for a lethal by sampling several heterozygous  $F_1$  virgin females from a non-lethal (or apparently non-lethal) culture, and mating these individually to Muller-5 males. Since each  $F_1$  female receives only one paternal X chromosome from the  $F_1$  female (which may be either non-mutated, or lethally mutated when it arises from an  $F_1$  female germinally mosaic for a lethal),  $F_1$  lethal-mosaicism is detected by the presence of both non-lethal and lethal cultures in the  $F_2$  sample from a non-lethal  $F_1$  culture. By the same procedure, it is possible to test for lethal-mosaicism in  $F_2$  females by proceeding to the  $F_3$  generation, or for  $F_2$  lethal-mosaics by proceeding to the  $F_3$  generation, and so on.

Table 1 illustrates the complete and mosaic sex-linked recessive lethal frequencies after treatment of *Drosophila* males with diethyl sulphate or formaldehyde. The  $F_2$  complete lethal frequencies are those mutations which are apparently established throughout the germinal tissue of the  $F_1$  female; the  $F_1$  lethal-mosaics are mutations occurring after a delay (from a premutational event), and are established in only a fraction of the germinal tissue of the  $F_1$  female.

The diethyl sulphate treatment is by adult feeding\* for 24 h, and the formaldehyde treatment by larval feeding on a yeast-glucose medium over the entire larval life\*; controls were by adult feeding of males in the case of diethyl sulphate, and by larval feeding of females with formaldehyde for the formaldehyde control. (Formaldehyde is not mutagenic towards female larvae.) The concentration of diethyl sulphate was adjusted to give a comparable  $F_2$  complete lethal frequency with that of formaldehyde.

Since it is usually difficult to sample from all non-lethal  $F_2$  cultures, several  $F_2$  heterozygous wild-type/Muller-5 females are collected as virgins from each of a sample of non-lethal  $F_2$  cultures, and mated individually to Muller-5 males to give the  $F_3$  generation cultures. Care was taken to sample non-lethal  $F_2$  cultures derived from each  $P_1$  male.

Details are listed in Table 1 of the number of non-lethal  $F_2$  cultures examined, and the number of males from which these are derived, the average number of females

examined from each non-lethal  $F_2$  culture, and the number of  $F_3$  cultures which later show mosaicism for a lethal in the  $F_3$  generation. The percentage of  $F_1$  lethal-mosaics is calculated from the number of non-lethal  $F_2$  cultures which yield one or more lethal cultures in their  $F_3$  sets out of the total number of  $F_2$  sets examined.

A significant increase in the frequency of  $F_1$  lethal-mosaics is observed after adult feeding of diethyl sulphate compared with the controls, while no statistically significant increase in  $F_1$  lethal-mosaics is detectable after larval feeding of formaldehyde. An increase in the frequency of  $F_2$  lethals is observed only after diethyl sulphate treatment; the formaldehyde treatments show no significant difference from the controls.

The average number of lethal cultures defining the  $F_1$  lethal-mosaics after each treatment can be estimated from the number of  $F_2$  lethal-bearing females (detected in the  $F_3$  generation). For example, the diethyl sulphate treatment gives 17  $F_1$  lethal-mosaics and there are 48 lethal-bearing  $F_2$  females distributed among them. Consequently, the average number of lethal cultures from each  $F_1$  lethal-mosaic is (48/17) or 2.8 from an average of four females from each non-lethal  $F_2$  culture (see Table 1). This figure allows an estimation of 70 per cent (2.8/4  $\times$  100) as the average mutated fraction of  $F_1$  lethally-mosaic gonads after diethyl sulphate treatment; this compared with 25 per cent for the adult feeding control, 32 per cent and 15.5 per cent for the respective formaldehyde treatments of males, and 11 per cent for the formaldehyde-treated female control.

In some cases, however, an  $F_1$  female may not be germinally-mosaic for a lethal but produce a lethal gamete spontaneously during germ cell development. Thus, if an  $F_1$  female gives one lethal  $F_2$  culture in a sample of ten, the lethal need not necessarily have arisen from a lethally-mosaic  $F_1$  gonad; only if such cases occur clearly in excess of the number expected from spontaneous mutation can they be ascribed to  $F_1$  lethal-mosaicism. In general, the more lethals present in an  $F_2$  set the less are they likely to be due to spontaneous mutation; this criterion is complicated, however, when a spontaneous mutation occurs early in germ cell development, since the resulting clone of mutant cells could be taken to be a lethal-mosaic.

It should be noted that by the larval feeding method the mutagenic activity of formaldehyde is effective through the pre-meiotic primary spermatocyte stage, and the subsequent meiotic divisions of this cell may allow the complete establishment of a mutation in the spermatozoon, even if the origin of the mutation is delayed: the higher frequency of  $F_1$  lethal-mosaics from post-meiotic stages compared with pre-meiotic stages has already been

Table 1. COMPLETE AND MOSAIC SEX-LINKED RECESSIVE LETHAL FREQUENCIES AFTER TREATMENT OF *Drosophila* MALES (AND, IN ONE CASE, OF FEMALES) WITH DIETHYL SULPHATE OR FORMALDEHYDE

		Control	Diethyl sulphate	Formaldehyde	Formaldehyde	Formaldehyde
						(females treated)
$F_2$	Molar concentration	—	$2.8 \times 10^{-4}$	$4.8 \times 10^{-3}$	$4.8 \times 10^{-3}$	$4.8 \times 10^{-3}$
	Type of treatment	Adult feeding	Adult feeding	Larval feeding	Larval feeding	Larval feeding
	Survivors (%)	100	97	82	86	83
	No. males examined	84	46	86	45	23
	No. chromosomes tested	308	344	388	302	408
	Complete lethals (%)	0.0	13.0	9.8	0.5	0.24
$F_1$	No. non-lethal $F_2$ cultures examined	143	104	63	91	78
		(arising from 34 ♂♂)	(arising from 46 ♂♂)	(arising from 36 ♂♂)	(arising from 47 ♂♂)	(arising from 23 ♀♀)
	Av. No. $F_2$ females examined/non-lethal culture	4	4	7	5	0
	No. of $F_2$ cultures yielding at least one lethal in the $F_3$ set	2	17	4	5	2
	$F_1$ lethal-mosaicism (%)	1.4	16.3	4.3	5.5	2.6
	Total No. $F_2$ females tested	568	414	651	819	702
$F_3$	No. lethal-bearing $F_2$ females	2	48	9	7	2
	Lethals in $F_3$ (%)	0.35	11.6	1.38	0.85	0.28
	Total lethal frequency (%) (complete lethals + $F_1$ lethal-mosaics)	1.4	27.9	15.6	15.0	2.84
	No. non-lethal $F_2$ cultures examined	40	58	80	—	—
		(arising from 25 ♂♂)	(arising from 46 ♂♂)	(arising from 30 ♂♂)	—	—
	Average No. $F_2$ females examined/non-lethal culture	6	4	7	—	—
$F_4$	No. of $F_2$ cultures yielding at least one lethal in $F_4$	1	2	2	—	—
	$F_2$ lethal-mosaicism (%)	2.5	3.4	2.5	—	—
	Total No. $F_2$ females tested	240	222	560	—	—
	No. lethal-bearing $F_2$ females	1	2	2	—	—
	Lethals in $F_4$ (%)	0.42	0.86	0.36	—	—
		(arising from 25 ♂♂)	(arising from 46 ♂♂)	(arising from 30 ♂♂)	—	—

reported after chemical treatments of *Drosophila* males<sup>6,7</sup>. This consideration applies only to the formaldehyde experiments, since the other types of treatment reported here are effects on spermatozoa, which require fertilization for the establishment of a mutation the origin of which is delayed.

The estimation of  $F_1$  lethal-mosaics after diethyl sulphate treatment and the adult feeding control (see Table 1) are based on small  $F_1$  sample sets (average of 4) and do not detect  $F_1$  lethal-mosaics which contain less than 25 per cent of mutant X chromosome. The  $F_1$  sets of the other treatments (Tables 1 and 2) have been increased to about ten cultures, and here the sample size does not detect  $F_1$  mosaic-gonads containing less than 10 per cent mutant X chromosome. These sampling procedures thus miss those  $F_1$  lethal-mosaics which are fractionally less than are covered by the  $F_1$  sample sets, and thus they correspondingly underestimate the frequency of mutations which occur after a delay.

The adult feeding control, the diethyl sulphate treatment, and one of the male formaldehyde treatments have been taken to an  $F_2$  generation following the same procedure described for the  $F_1$ . No significant lethal-mosaicism is detectable from  $F_2$  females either after treatment compared with the control, and none of the  $F_2$  lethal frequencies differs significantly from any other.

Since all progenies are traceable back to individual  $P_1$  males, it is possible to determine whether  $F_1$  females tend to show mosaicism for a lethal if the male from which the female is derived also gives complete lethals in the  $F_1$  generation; that is, whether the  $F_1$  lethally mosaic female comes from a lethal line or, alternatively, from a non-lethal line. The experiments presented in Table 1 are found to give lethal-mosaics only from non-lethal lines for both controls, mosaicism only from non-lethal lines in the first formaldehyde experiment, and 60 per cent mosaicism from non-lethal lines and 40 per cent from lethal lines in the second formaldehyde experiment. The diethyl sulphate treatment is found to give 37.5 per cent mosaicism from non-lethal lines and 62.5 per cent from lethal lines. All  $F_1$  'lethal-mosaics' (detectable in the  $F_2$  generation) arise from completely non-lethal lines; that is, the males gave no lethals in the  $F_1$  or  $F_2$  generations.

Table 2 lists the complete and mosaic sex-linked recessive lethal frequencies arising from *Drosophila* spermatozoa after adult feeding treatments<sup>8</sup> with ethyl methanesulphonate and *N*-nitrosomethylurea. Both compounds produce very high frequencies of complete sex-linked recessive lethal mutations, and since these frequencies represent the highest induced mutation rates yet recorded in *Drosophila*, additional data are given of the sterilities in the  $F_1$  and  $F_2$  cultures. The fertility of  $F_1$  cultures after treatment can be judged from the average number of chromosomes it has been possible to examine per male, since all females recovered from  $F_1$

cultures were examined; the control figure (10) is an arbitrary sample, since more than 10 females could have been examined per male.

Treatment of spermatozoa with either ethyl methane-sulphonate or *N*-nitrosomethylurea is found to produce lethally mosaic  $F_1$  gonads at significantly high frequencies compared with the control. The average number of lethal cultures in each  $F_1$  set is 1 for the control, 5.4 for ethyl methanesulphonate, and 6.3 and 7.3 for the respective *N*-nitrosomethylurea treatments. Both the ethyl methane-sulphonate and *N*-nitrosomethylurea treatments produce clearly significant increases in  $F_1$  lethals compared with the control.

It has so far been assumed that when more than one lethal is present in an  $F_1$  set, the lethals are identical with one another. However, this may not be true as a general rule, especially after such effective mutagenic treatments, where the possibility of two or more different delayed mutations being established in the gonad must be considered. To decide this condition, it is necessary to detect autosomal recessive lethal mutations since sex-linked recessive lethals cannot be tested for identity ('allelism').

An examination for lethal line or non-lethal line origin of lethal-mosaics in these experiments (Table 2) is found to give a non-lethal line origin for the control, and half-and-half origins for the *N*-nitrosomethylurea treatment ( $4.85 \times 10^{-4}$  M). However, the higher concentrations of the *N*-nitrosomethylurea and ethyl methanesulphonate, where all non-lethal  $F_1$  cultures were examined, gave only lethal-bearing  $P_1$  males, and all the  $F_1$  lethal-mosaics are thus from lethal lines; apparent discrepancies in the number of males tested for the  $F_1$  and in the number of males used for sampling of non-lethal  $F_1$  cultures are due to some males giving only complete lethal cultures in the  $F_1$  generation.

The misclassification of an  $F_1$  culture as a complete lethal is statistically possible, though the culture may be, in fact, either  $F_1$  lethally mosaic, semi-lethal, or even completely non-lethal. Although a complete lethal is usually confirmed by crossing a single  $F_1$  heterozygous female from the culture to a Muller-5 male, an  $F_1$  mosaic-ally lethal or a semi-lethal culture could still register as a complete lethal. Occasionally, an apparently complete  $F_1$  lethal behaves as a non-lethal on retesting; and this may well be due to the misclassification of an  $F_1$  lethal-mosaic as a complete lethal, and the chance sampling of an  $F_2$  female for retest which carries the non-mutated paternal X chromosome. Accordingly, all complete  $F_2$  lethals which occurred at the higher concentrations of ethyl methanesulphonate and *N*-nitrosomethylurea were examined for possible  $F_1$  lethal-mosaicism by sampling 20 heterozygous  $F_2$  females from each complete lethal culture. The majority of  $F_2$  complete lethals gave only lethal cultures in their  $F_2$  sets, but five cultures were

Table 2. COMPLETE AND MOSAIC SEX-LINKED RECESSIVE LETHAL FREQUENCIES ARISING FROM *Drosophila* SPERMATOZOA AFTER ADULT FEEDING TREATMENTS WITH ETHYL METHANESULPHONATE (HMS) AND *N*-NITROSOMETHYLUREA (NMU)

	Control	HMS	HMS	NMU	NMU
Concentration	—	0.25% $1.95 \times 10^{-4}$ M	0.4% $3.12 \times 10^{-4}$ M	0.1% $9.7 \times 10^{-5}$ M	0.05% $4.85 \times 10^{-5}$ M
Duration of treatment (h)	24	24	24	24	24
Survivors (%)	100	76	71	88	98
Sterile $F_1$ cultures (%)	0.0	8.6	7.2	43.3	23.7
Sterile $F_2$ cultures (%)	0.5	10.7	19.0	13.2	8.8
No. males examined	40	28	45	12	80
Average No. chromosomes examined/male	10	9.5	8.5	2.8	6.7
$F_1$ No. chromosomes tested	398	259	150	23	536
No. lethal chromosomes	0	149	72	16	133
Complete lethals (%)	0.0	41.5	48.0	44.5	24.8
No. non-lethal $F_1$ cultures examined	80	—	74	17	91
(arising from 40♂♂)	—	—	(arising from 87♂♂)	(arising from 9♂♂)	(arising from 71♂♂)
$F_2$ Average No. $F_2$ females examined/non-lethal culture	10	—	10	16.3	10.8
No. $F_2$ cultures yielding at least one lethal in the $F_2$ set	1	—	18	3	12
$F_2$ lethal-mosaicism (%)	1.25	—	24.3	17.6	13.3
Total No. $F_2$ females tested	802	—	745	277	968
No. lethal-bearing $F_2$ females	1	—	97	19	88
Lethals in $F_2$ (%)	0.12	—	13.0	6.8	8.9
Total lethal frequency (%) (complete lethals + $F_2$ lethal-mosaics)	1.25	—	72.3	66.1	33.6

found to show mosaicism for a lethal; the  $F_1$  females from which this mosaicism arose were thus reclassified as  $F_1$  lethal-mosaics.

### The Embryological Basis of Lethal-mosaicism

Although the experiments (Tables 1 and 2) clearly show delay in the origin of sex-linked recessive lethal mutations after treatment of spermatozoa with *N*-nitrosomethylurea, diethyl sulphate, and ethyl methanesulphonate, their  $F_1$  lethal-mosaic frequencies give no real indication of the proportion of induced mutations which are expressed after a delay. Some, or even all, of the complete mutations may have arisen after a delay; that is, the mutation is not immediately established in the  $F_1$  female zygote, but occurs later in embryonic development. The gonadal tissue of *Drosophila* is derived from about three to seven cells which are situated at the posterior end of the embryo, and partitioning of these so-called pole cells to form the polar cap does not occur until about the ninth synchronous nuclear division (512 embryonic cells present)<sup>8</sup>. Since the sex-linked lethal test detects only those mutations which are present in the germinal tissue of the  $F_1$  female, a lethal may conceivably be established after a delay of several (up to about seven or eight) divisions and still be detectable as a complete mutation if the presumptive gonadal cells receive only a lethally-mutated X chromosome. Alternatively, an  $F_1$  female may be gonosomically-mosaic; that is, while carrying the non-mutated X chromosome in the gonadal cells, a lethally mutated X chromosome is present in the majority of the somatic cells. The process of gonadal embryogenesis adds a further complication to the assessment of mutational delay, since investigations on the subsequent embryonic migration of pole cells reveal that not all pole cells are incorporated into the gonad to function as primordial germ-cells, and no information is available as to a random or a non-random utilization of those pole cells (about 40 in the male, 8-12 in the female) which eventually form the gonad.

### Mosaicism for Visible Mutations

The examination of delayed germinal mutation can thus establish little in the timing or in the overall recognition of mutational delay. It would seem better to analyse such a problem by the use of visible mutations which phenotypically affect the majority of the somatic cells of the adult  $F_1$  female. Sex-linked recessive genes affecting body colour (such as yellow body) or bristle morphology (such as singed or forked bristles) when induced on a gametic X chromosome of the treated male are widely revealed in the somatic cells of an  $F_1$  female when the  $P_1$  male is crossed to a female homozygous for these genes. The induction of somatic effects involving complete mutation, or fractional mutations (such as half-and-half mosaics, quarter mosaics, etc.) can thus be used to measure the timing of mutational expression by the distribution of mutant tissue in the  $F_1$  females. However, when mutant expression is delayed more than one or

two nuclear divisions, the detection and the estimation of the fractional distribution of mutant tissue becomes difficult.

Investigations have established the whole-body character of most (93 per cent) X-ray-induced visible mutations in *Drosophila*, compared with the relatively high percentage (30/67) of fractional visible mutations arising spontaneously and after chemical treatments<sup>9</sup>; in all three series, the mutant tissue of the detected fractionals seems to involve half the body (not a quarter or less). This type of fractional mutation undoubtedly forms a large proportion of those sex-linked recessive lethals which are detected as germinally complete lethals. However, germinal-mosaicism also detects a group of delayed mutations which would not be recognized by phenotypic (somatic) effects alone. The reported absence of germinal lethal-mosaicism in *Drosophila* after X-irradiation<sup>10</sup> and after ultra-violet irradiation<sup>11</sup> of spermatozoa demonstrates the absence of a long delay in the origin of X-ray and ultra-violet induced mutation, in contrast to the prolonged delay which is found after chemical treatments.

Another consideration, arising from investigations of the induction of the autosomal visible mutation, dumpy wing (which has a restricted phenotypic distribution), after treatment of spermatozoa with a monofunctional quinacrine mustard<sup>12</sup>, is that five times as many spermatozoa carried this mutational event as would have been detected by examination of the germinal tissue alone. It has been reported<sup>1</sup> that most of the quinacrine mustard-induced phenotypic (somatic) dumpy wing-mosaics are germinally non-mutant, and this observation, besides supporting the embryological evidence that the gonad is produced from few embryonic cells, suggests that many lethal mutations must remain undetected, since they are included only in somatic tissue.

In spite of the difficulties involved in the overall assessment of delayed germinal mutation, the extension of the routine sex-linked recessive lethal test to include the detection of lethally mosaic gonads is clearly essential for a proper estimation of the effects of chemical mutagens; this consideration is of particular importance if a chemical mutagen produces a high frequency of germinal-mosaicism and a low frequency of germinally complete mutations.

I thank Prof. J. M. Thoday for supplying the laboratory facilities, and the British Empire Cancer Campaign for Research for financial support.

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## PHENOLS AS PLANT GROWTH REGULATORS

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THE discovery<sup>1</sup> of indolyl-3-acetic acid (IAA) as a plant growth hormone in 1934 led to the examination of compounds having a similar structure. Some of these were also found to promote cell elongation in the plant tissues used in the standard tests for growth-regulating activity, such as the wheat cylinder, pea segment, pea

curvature and tomato leaf epinasty tests<sup>2</sup>. They are active in producing morphogenic abnormalities in plants, for promoting the rooting of cuttings, for setting fruit in the absence of pollination and producing other physiological effects as does IAA. Furthermore, the more active of these compounds, for example 2:4-dichlorophenoxyacetic

acid (2:4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA), when applied to plants at certain dose rates, were found to destroy some species and not others. Such chemicals have been developed for use in agriculture as selective weed-killers.

The important synthetic plant growth regulators fall into three groups: (a) aryloxyalkancarboxylic acids, for example 2:4-D and MCPA; (b) arylalkancarboxylic acids, for example  $\alpha$ -naphthylacetic and certain chloro-substituted phenyl-acetic acids; (c) certain halogen derivatives of arylcarboxylic acids, for example 2:3:6-trichlorobenzoic acid. Much attention has been directed to establishing relationships between chemical structure and plant growth-regulating activity for compounds within these groups. It is of interest that substitution of chlorine into the 2- and 6-positions in the ring of phenoxyacetic acid confers only slight growth-regulating activity, whereas similar substituents with phenylacetic and benzoic acids give compounds with high activity<sup>3,4</sup>. Several theories attempting to account for such behaviour have been suggested.

Compounds outside the foregoing three groups have also been shown to exhibit growth-regulating activity. These include simple molecules such as ethylene, propylene and acetylene, and certain dithiocarbamate derivatives<sup>5</sup>. A number of other unrelated substances show slight activity in the wheat cylinder test. Since this activity is only evident at concentrations just below those which produce toxic symptoms, these compounds are not regarded as true growth substances. Their apparent activity could arise from damaging effects on the cell-wall which lead to changes in its plasticity<sup>6</sup>.

Among phenolic compounds, *trans*-caffeic acid was reported by Vendrig and Buffell<sup>7</sup> to be active in the *Avena* coleoptile test, but Thimann *et al.*<sup>8</sup> found it possessed negligible activity in this test and was inactive in the pea curvature test; we have shown it to be inactive in the wheat cylinder, pea segment and pea curvature tests. The compound acted as a synergist, however, when tested with IAA (ref. 8) as did a number of other phenolic compounds examined by Nitsch and Nitsch<sup>9</sup>.  $\alpha$ - and  $\beta$ -Naphthols have been reported to be slightly active in promoting rooting of cuttings of *Phaseolus vulgaris*<sup>10</sup>.

Work by one of us led in 1959 to the discovery of a new highly effective contact herbicide, 3:5-diiodo-4-hydroxybenzonitrile, which was given the common name 'ioxynil'<sup>11</sup>. Since that time, the compound has received considerable attention here, and much is now known of its mode of action. In a study of analogues, 2:4:6-triiodophenol, in which the nitrile group of ioxynil is replaced by iodine, was examined. The compound was found, over some weeks, to produce morphogenic effects in tomato plants when added to the soil in which they were growing. Since this was a response similar to that induced by synthetic plant growth-regulating substances, we decided

to make a systematic investigation of halogen-substituted phenols as growth substances. It has now been found that some of these molecules are markedly active in this respect.

Phenol itself,  $\alpha$ - and  $\beta$ -naphthol, and all the chloro- and dichloro-phenols were first examined in the wheat cylinder, pea curvature, pea segment, and tomato leaf epinasty tests. All were inactive with the exception of 2:6-dichlorophenol. Solutions of the sodium salt of this compound were active in the wheat cylinder test and highly active in the two pea tissue tests (Table 1). In the tomato epinasty test when applied at 2 per cent in lanolin a strong and typical growth-substance response was obtained.

Further experiments with trichloro-, tetrachloro- and pentachloro-phenols revealed the known high phytotoxicity of these compounds; 2:3:6-trichlorophenol, however, was found to possess significant growth-regulating activity (Table 1). The relatively unstable 2:6-dichlorothiophenol was slightly active, but 2:6-dibromophenol showed high, and the 2:6-diiodophenol very high, activity in the pea and tomato tests (Table 1); 2:6-dimethyl- and 2:6-dinitrophenols and 2-chloro-, 2-bromo- and 2-iodo-phenols were all inactive.

Within 24 h of the application of solutions of 2:6-dichloro-, dibromo- or diiodo-phenols to the soil of potted tomato plants, severe epinastic responses were obtained. This reaction was particularly severe and persistent with the dibromo- and diiodo-compounds; plants treated with the dichloro derivative in this way tended to recover after some days.

As with the benzoic and phenylacetic acids which show growth-promoting activity, these active phenols possess a weakly acidic grouping with two *ortho*-substituted halogen atoms in the ring. The pattern of activity exhibited by these phenols, however, is of interest in that the compounds are much more active in the pea curvature and pea segment tests than in the wheat cylinder test (Table 1), a type of behaviour which is not usually observed with synthetic plant growth-regulators. On the other hand, there are further similarities between the benzoic acids and phenols. In the 2:6-disubstituted benzoic acids, the activity is removed when a *para* substituent is introduced<sup>3</sup> and our results indicate that this same structural requirement operates also with phenols. Thus, 2:6-dichloro- and dibromo-phenols are active in the wheat and pea tests and 2:4:6-trichloro- and tribromo-phenols are inactive. Again, while 2:6-diiodophenol is active in these tests, substitution of OH, I, NO<sub>2</sub>, CN or COOH in the 4-position destroys activity completely. These and other observations indicate that phenols and benzoic acids may have a similar mode of action in plant growth regulation.

In view of the extensive investigations which have been carried out on plant growth substances during the past thirty years, the high activity of certain halogen-substituted phenols, here reported, must be regarded as an important

Table 1. GROWTH-REGULATING ACTIVITY OF CERTAIN HALOGEN-SUBSTITUTED PHENOLS ASSESSED IN THE WHEAT CYLINDER, PEA SEGMENT AND PEA CURVATURE TESTS

Compound	Test	Concentration $\times 10^{-4}$ molar									
		1	2.5	5	10	25	50	100	250	500	1,000
2:6-dichlorophenol	Wheat cylinder	—	—	—	99	99	101	104	109†	118†	T
	Pea segment	—	—	—	100	104	112†	122†	126†	126†	T
	Pea curvature	—	—	—	0	4	5	5	5	4	T
2:6-dibromophenol	Wheat cylinder	100	103	101	101	105*	111†	120†	119†	T	T
	Pea segment	100	100	100	102	106*	121†	124†	129†	125†	T
	Pea curvature	0	0	0	3	6	6	4	T	T	T
2:6-diiodophenol	Wheat cylinder	100	100	101	103	106*	108†	108†	T	T	T
	Pea segment	101	103	106*	124†	131†	133†	131†	123†	115†	102
	Pea curvature	0	1	4	2	4	6	6	T	T	T
2:3:6-trichlorophenol	Wheat cylinder	101	101	100	100	100	102	101	T	T	T
	Pea segment	101	101	102	100	100	106	103*	114†	111†	T
	Pea curvature	0	0	0	4	4	4	T	T	T	T
2:4-dichlorophenoxy-acetic acid	Wheat cylinder	118†	116†	150†	150†	161†	162†	161†	167†	146†	125†
	Pea segment	119†	120†	131†	125†	126†	134†	121†	120†	121†	126†
	Pea curvature	4	5	6	6	6	6	6	6	6	6

Results in the wheat cylinder and pea segment tests are expressed as the percentage of the final length of the control segments. Figures significantly different from control are indicated: \* at 5 per cent level, † at 1 per cent level, ‡ at 0.1 per cent level. Results in the pea curvature test are assessed on an arbitrary scale of 0 (inactive) to 6 (highly active). T signifies that toxic effects were observed.



finding which opens up many possibilities for future research. Some of these are now under investigation in this laboratory.

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## REACTION OF AMINO-ACIDS AND PEPTIDE BONDS WITH FORMALDEHYDE AS MEASURED BY CHANGES IN THE ULTRA-VIOLET SPECTRA

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AS part of an investigation of cross-links produced in proteins by formaldehyde<sup>1</sup> it was observed that solutions of proteins, peptides and amino-acids exhibit appreciable changes in their ultra-violet spectra in the 210–250 mμ range when formaldehyde is added to them<sup>2</sup>. Since the observation of Einhorn<sup>3</sup> that the *N*-alkyl amides of fatty acids are very unreactive, it has been generally accepted that the peptide bonds of proteins react with formaldehyde very slowly or not at all<sup>4,5</sup>. The observed spectral changes demonstrate that the peptide bond of certain peptides can in fact react with formaldehyde and suggest that similar reactions can occur in proteins. Fraenkel-Conrat and Oloott<sup>6,7</sup> have accumulated considerable evidence to prove that many side-chain groups can react. And although model investigations have indicated that some of these reactions are also accompanied by spectral changes<sup>8</sup>, this article will be limited to consideration of reactions involving only amino-groups, unsubstituted amido-groups and substituted amido or peptide-groups.

To force the reactions to equilibrium in a reasonable period of time and to minimize reactions among the molecules of the compounds themselves, high formaldehyde concentration (3 M) somewhat elevated pH (pH 10) and temperature (40° C) and very dilute amino- or amido-solutions (0.0005–0.001 M) were chosen initially. However, many measurements were also made at 25° C and at lower pH. Reagent grade formalin containing 37.1 per cent formaldehyde and 14 per cent methanol was diluted to the proper concentration before use. Formalin containing less than 1 per cent methanol, supplied by the Celanese Chemical Co., was found to give essentially the same results when tested in some of the reactions, although the rates were somewhat elevated. All reactions were carried out in buffered solutions: 0.02 M borate buffer above pH 8 and 0.038 M phosphate buffer below pH 8.

Absorbance changes at 220 mμ and constant temperature were followed with either a Beckman model DU or a time-driven model DK-2 spectrophotometer. The absorbance at 220 mμ of 3 M formaldehyde is not too high with the buffers used if the pH is kept below 10.5. Above pH 10.5 the much more highly absorbing anionic formaldehyde exists in appreciable concentration and readings must be made at longer wave-length with higher compound concentration. To compensate for changes in the absorption of the formaldehyde on standing, both reference and sample cuvettes always contained equal concentrations of formaldehyde initially. Some minor errors in the early report<sup>1</sup> attributable mainly to this effect are corrected here.

**Reactions of mono- and di-amino-acids with formaldehyde.** The addition of formaldehyde to neutral or alkaline solutions of free amino-acids at 40° C has the immediate effect of increasing the absorptivity 2–3-fold (Figs. 1 and 2), the increase depending on the concentration of

formaldehyde, the pH and the structure of the amino-acid. Glycine and threonine behave like the amino-acids indicated in Fig. 1. The increase may be reversed by acidifying the solutions to pH 1–2 whereupon the absorptivity drops to that of unassociated cationic amino-acid. It is usually assumed that reaction of formaldehyde with amino-acids is nearly instantaneous, but Baur<sup>9</sup> reported an initial change in conductivity lasting for as long as 2 min when glycine was mixed with formaldehyde. If absorptivity measurements are made rapidly at 25° C, the results indicated in Fig. 1 (inset) are obtained.

In harmony with the theories of Levy and Silberman<sup>10</sup> explaining their titration curves the initial rapid increase is attributed to monomethylolamine formation, while the subsequent decrease observed at higher formaldehyde concentrations to dimethylolamine formation. If the Levy-Silberman<sup>10</sup> theory regarding the reaction of formaldehyde

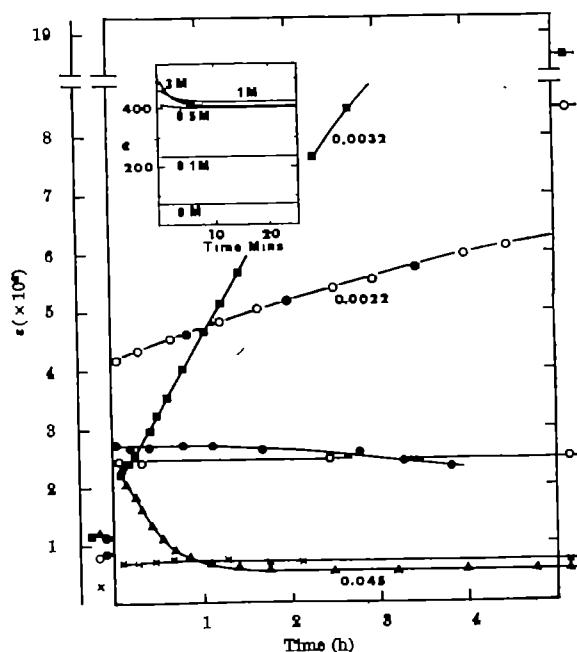


Fig. 1. Molar absorptivity changes at 220 mμ produced by the reaction of formaldehyde with mono- and di-amino acids. All curves on the large graph were obtained at 39 ± 1° C and 3 M formaldehyde at pH 9.5 with 0.001 M amino-acid. The 6-aminocaproate points are crosses (x), the L-2,4-diaminobutyrate points solid squares (■), the L-2,3-diaminopropionate points solid triangles (▲), the L-lysine points dotted circles (○), the L-proline points solid circles (●). The points to the left of the graph represent the absorptivity values before the addition of formaldehyde; the points to the right of the graph represent the absorptivity values when the reaction has reached equilibrium. In a few instances a small correction is made for a much slower secondary reaction. The numbers under the curves are apparent first-order reaction rate constants in units of min<sup>-1</sup>. The curves in the inset were obtained at 25° C with 0.001 M L-leucine at the indicated formaldehyde concentration in 0.02 M tetraborate buffer at pH 9.7 ± 0.3.

with primary amino-groups is accepted, it is possible to calculate, from this and previously published data<sup>8</sup>, the association constants of the amino-group with one and two molecules of formaldehyde, and to assign absorptivity values to the monomethylolamine and dimethylolamine respectively.

Like the monoamino-acids, the diamino-acids exhibit the initial changes in absorption with formaldehyde attributable to mono- and di-methylolamine formation. But in addition they exhibit a secondary change much slower even at 40° C than any observed with monoamino-acids (Fig. 1). Apparent first-order kinetics is obeyed throughout most of this phase of the reaction. French and Edsall<sup>4</sup> have suggested *N,N'*-methylene bridge formation for lysine. And although no attempt has been made to isolate any of the reaction products, the present observations lend some support to this possibility: (1) First-order kinetics for the secondary change is consistent with an intramolecular rather than an intermolecular reaction. (2) Unlike methylolamine compounds the lysine-formaldehyde reaction product does not return to an unassociated state at pH 1-2. This is indicated by an absorptivity value of about 400 for the acidified reaction solution instead of an expected value of about 50 for unassociated dicationic lysine and also by the very rapid return to high absorptivity on re-alkalinization. (3) The observed rate constants appear to correlate to some extent with the expected probabilities of formation of an 8-membered ring with lysine, a 6-membered ring with 2,4-diaminobutrate and a 5-membered ring with 2,3-diaminopropionate.

**Reaction of unsubstituted amides with formaldehyde.** Unlike amino-groups, unsubstituted amido-groups exhibit only a slow increase in absorption with formaldehyde at pH 10 represented by curves for acetamide and *N*-acetylaspargine in Fig. 2. At the high formaldehyde concentration used the reaction obeys apparent first-order kinetics. There is a strong pH dependence indicated

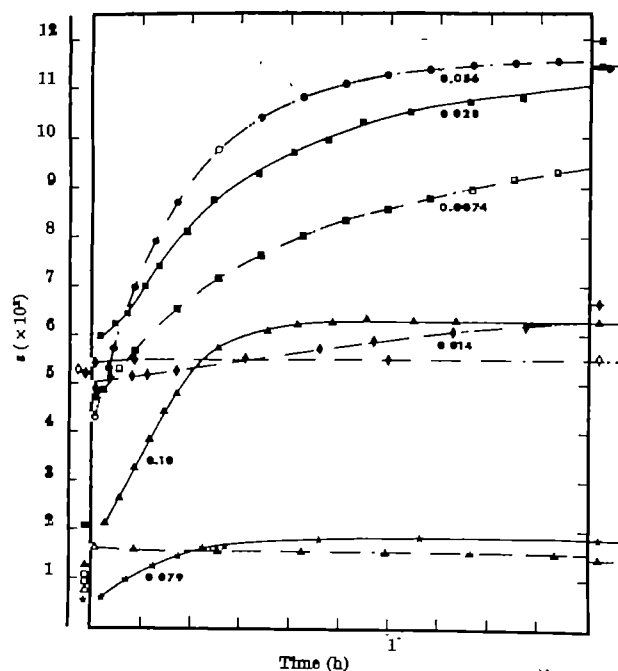


Fig. 2. Molar absorptivity changes at 230 m $\mu$  produced by the reaction of 3 M formaldehyde with unsubstituted amide groups. All reactions were carried out at either 20.3  $\pm$  0.7° or 24.7  $\pm$  0.8° C represented by broken lines and at either pH 9.0  $\pm$  0.3 represented by solid symbols or pH 6.0 represented by open symbols. Here the acetamide points are stars, the *N*-acetyl-L-asparagine points are diamonds, the L-asparagine points are squares, the L-glutamine points are circles, and the glycine points are triangles. As in Fig. 1 the points to the left of the graph represent the absorptivity values before addition of formaldehyde; the points to the right of the graph represent the absorptivity values when the reaction has reached equilibrium. The numbers under the curves are apparent first-order reaction rate constants in units of min<sup>-1</sup>.

by the absence of absorption change of *N*-acetylaspargine at pH 7 (Fig. 2) within 20 h. However, unlike the reaction of formaldehyde with the amino-group, the reaction with the amido group is not reversed by lowering the pH. Methylolamide formation by reaction of formaldehyde with acid amides has been known since the early researches of Einhorn<sup>9</sup> *et al.* using preparative procedures. Later Crowe and Lynch<sup>10</sup> using a polarographic method found that the reaction is second order for amide and formaldehyde concentrations, greatly increases in rate when the pH is varied from 8.6 to 12.7, and yields an association constant of about 25 over the entire pH range already indicated here. It is fairly certain that the absorption increase observed at pH 10 (Fig. 2) is caused by methylolamide formation.

The amino-acid amides treated with formaldehyde at pH 10 exhibit both the rapid initial increase in absorption characteristic of amino-groups and the slow subsequent increase characteristic of amido groups (Fig. 2). But at pH 7 only asparagine and glutamine exhibit the slow reaction whereas glycine does not (Fig. 2). From the disappearance of nitrous acid reactive groups and measurement of pH changes Levy and his colleagues<sup>11</sup> have concluded that asparagine and glutamine undergo rapid mono- and di-methylolamine formation, followed by slow methylolamide formation and *N,N'*-methylene bridge ring closure. Their results indicate that methylolamide formation limits the subsequent rate of ring closure. It is possible that the absorption changes observed at pH 7 indicate that asparagine and glutamine can form the *N,N'*-methylene bridge whereas glycine cannot. In any event, methylolamide formation does not appear to be the rate-limiting step because *N*-acetylaspargine, which has a free amido group, fails to react at pH 7. No explanation is at hand for the much greater reactivity of glutamine compared with asparagine.

**Reaction of *N*-alkyl substituted amides with formaldehyde.** The change in absorption observed with certain substituted amides, for example *N*-methylacetamide and *N*-acetyl glycine (Fig. 3), is similar to that observed with unsubstituted amides. On the other hand, the immediate increase observed with glycine anhydride is different (Fig. 3). It was erroneously stated earlier that glycine anhydride exhibits no change in spectrum<sup>4</sup>. Presumably the products of all these reactions are methylolamides. This conclusion is in agreement with those of other laboratories based on preparative methods starting with glycine anhydride<sup>12</sup> and *N*-methylacetamide<sup>13</sup> and measurement of bound formaldehyde<sup>14</sup>, but is contrary to that of Einhorn<sup>9</sup> already mentioned. The strong dependence of the rate of the reaction on pH may be seen by comparing the curves for *N*-acetyl glycine at pH values of 10 and 7 in Fig. 3. However, above about pH 9, where the reaction occurs at a readily measurable rate, the completeness of the reaction, as measured by the equilibrium absorptivity, appears not to depend on pH. Both the rate of attaining equilibrium and the completeness of the reaction do depend on formaldehyde concentration. Thus, the rate constants in Figs. 2 and 3 are only approximately comparable because, when the measurements were made, the importance of precise pH control was not realized. By measuring the equilibrium absorptivity at more than one concentration of formaldehyde, it is possible to calculate both the absorptivity of the methylolamide and the equilibrium constant of the reaction. None of these reactions is reversed by lowering the pH.

The effect of the *N*-alkyl substituent on the equilibrium point was determined by comparing the reactivity of various *N*-acetyl amino-acids. Unlike *N*-acetyl glycine, the *N*-acetyl derivatives of leucine (Fig. 3), alanine and glutamic acid exhibit only a slight rise in absorptivity at pH 10 or even higher. For the reaction to approach completion, two or more hydrogen atoms are required on the alkyl carbon atom bonded to the nitrogen atom. There is further evidence for this in the appreciable

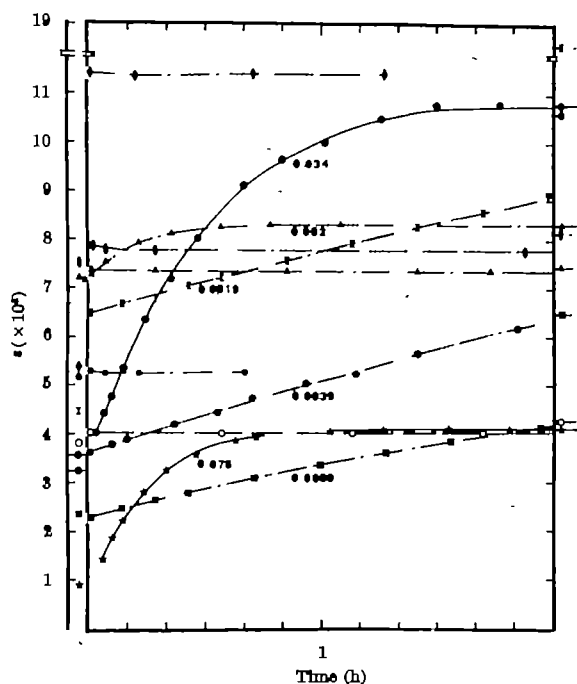


Fig. 3. Molar absorptivity changes at 220 m $\mu$  produced by the reaction of 3 M formaldehyde with substituted amide groups. The *N*-acetyl-glycine points are squares, the *N*-acetyl-L-leucine points hexagons, the *N*-acetyl-L-leucylglycine points triangles, the glycine anhydride points diamonds, the glycylglycine points circles, the glycyl-L-leucine points ellipses, the L-leucylglycine points double triangles ( $\nabla$ ), the *N*-methyl-acetamide points stars. As in Fig. 1 the points to the left of the graph represent the absorptivity values before addition of formaldehyde, the points to the right of the graph represent the absorptivity values when the reaction has reached equilibrium. The numbers under the curves are apparent first-order reaction rate constants in units of min<sup>-1</sup>. As in Fig. 2 the reactions were carried out at either 30  $\pm$  0.7° C represented by solid lines or 24.7  $\pm$  0.3° C represented by broken lines and at either pH 9.9  $\pm$  0.8 represented by solid symbols or pH 6.9 represented by open symbols.

increase in absorptivity observed when the *N*-acetyl derivatives of  $\beta$ -alanine and 6-aminocaproic acid are treated with formaldehyde at pH 10. The increase in absorptivity observed with *N*-acetylleucylglycine at pH 10 (Fig. 3) can then be explained in terms of appreciable methylation of the *N*-leucyl bond but not the *N*-acetyl bond.

The true peptides, possessing a terminal amino-group, may or may not exhibit the initial increase in absorption depending on the relative absorptivities of the dimethylol-amino and monomethylolamino derivatives and the concentration of formaldehyde. The slow subsequent increase observed with some of the *N*-acetyl amino-acids is likewise observed with the corresponding peptides. Thus, glycylglycine like *N*-acetylglycine undergoes a slow increase to

high absorptivity at pH 10 and appears not to react appreciably at pH 7 within 20 h (Fig. 3). Similarly, leucylglycine (Fig. 3), alanyl-glycine<sup>1</sup> and prolylglycine<sup>12</sup> all undergo slow increases to high absorptivity at pH 10. Glycylleucine, on the other hand, like *N*-acetylleucine, exhibits only a slight increase in absorptivity at pH 10 (Fig. 3). Of course, glycylproline with no replaceable hydrogen on the peptide bond nitrogen exhibits only the initial increase in absorptivity but no subsequent increase<sup>1</sup>.

With the examination of more peptides and with the great prolongation of the period of observation at different pH levels (see legend to Fig. 3) the parallel behaviour of peptides and *N*-acetyl amino-acids begins to disappear. The *N*-terminal amino-group, the side-chains on the acyl residues of the peptide and the length of the peptide chain all affect the reactivity of the peptide bonds in a discernibly systematic way, particularly at pH 7. The formation of a methylene bridge between the amino-nitrogen and a peptide-nitrogen has been suggested by French and Edsall<sup>4</sup>. Their suggestion was based in part on the work of Wadsworth and Pangborn<sup>14</sup>, who demonstrated the irreversible bonding of formaldehyde coupled with the disappearance of amino-nitrogen when alanyl-glycine was treated with 1 mole of formaldehyde at pH 8. The spectrophotometric studies lead to a similar conclusion. The complete evidence is based on the examination of a large number of additional peptides and is beyond the scope of this article. However, the results presented here clearly demonstrate that peptide bonds react with formaldehyde and provide some basis for concluding that part of the absorption change observed with insulin<sup>3</sup> and other proteins, including gelatine on formaldehyde treatment<sup>15</sup> is attributable to the reaction with certain peptide bonds.

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## BROAD-BANDED STRIATED BODIES IN THE SENSORY EPITHELIUM OF THE HUMAN MACULA AND IN NEURINOMA

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IN a recent publication Lowenstein and Osborne<sup>1</sup> described an interesting striated structure in the sensory hair cells of the labyrinth of the ammocoete larva of the lamprey, *Lampetra fluviatilis*. The authors have suggested that no such organelles have been described in the labyrinth of any other animal and a brief description of our own findings in the human labyrinth and elsewhere might be of some interest.

We have studied eleven specimens of the human utricle removed at operation to cure Ménière's disease. The

specimens were promptly fixed in iced buffered osmic acid in the first three cases and embedded in methacrylate, and in 4-5 per cent buffered glutaraldehyde<sup>2</sup> in the next eight cases, then embedded in 'Araldite'<sup>3</sup>. Ultra-thin sections were cut on a Huxley-type ultramicrotome, stained with Karnovsky's lead monoxide and examined under a Siemens Elmiskop I electron microscope.

There was more or less widespread degeneration affecting many cells of the macula showing vacuolation of the cytoplasm and large numbers of fat droplets. Moreover



Fig. 1. Degenerating cell of human macula (Ménière's disease) with large numbers of fat droplets (F). Note laminated inclusion (LI) ( $\times 16,800$ )

the cup-shaped nerve endings of Wersäll embracing the degenerating hair cells have undergone degenerative changes affecting both the numerous mitochondria and the nerve tissue proper. Such calyces appeared as darkly stained rings round cross-cut hair cells. The surface was usually covered with some amorphous degenerated cellular matter in which there were embedded remnants of the ciliary apparatus extensively damaged in all cases.

Two unusual structures have been observed: (a) intracellular laminated structures, (b) extracellular broad-banded filaments (? long-spacing collagen).

**Laminated structures.** These are polygonal or rhomboid crystalline structures 0.5–1.5  $\mu$  wide, formed by alternating dark and less dark lines (Fig. 1). The dark lines or bands are 100 Å wide and repeat approximately every 1,000–1,500 Å to form a macroperiod. Each major period is subdivided by a less dense line or band about 70–80 Å wide and they may be linked by thin fibrils.

The common site of the crystalline inclusions found in eight specimens (Table 1) seems to be the apical and perinuclear zones of the hair cells. There are, however, similar striated structures within the cuticle close to the rootlets of the stereocilia where their position suggests some functional association with the cilia. The inclusions may be present in well-preserved cells, but more frequently

appear to be associated with fat droplets, degenerating mitochondria or some amorphous matter probably of cytoplasmic origin. The inclusions may be single or form groups (Fig. 2), especially in degenerated parts of the epithelium where numerous laminated structures grouped together often form a 'parquet-floor' pattern (Wetzstein *et al.*<sup>4</sup>).

**Broad-banded filaments (long-spacing collagen 'Luse body').** Apart from the laminated inclusions, broad-banded filaments were seen in five specimens (Table 1) underneath the basement membrane of the neuroepithelium closely associated with myelinated nerve fibres (Fig. 3) or scattered between the numerous non-myelinated nerve axons of the utricular branch of the vestibular nerve. The broad-banded filaments form long chains or fusiform bodies. The intervals between the bands measure approximately 1,000 Å. There is little similarity between these extracellular filamentous structures and the intracellular laminated material described here. The long-spacing fibres are particularly abundant among the terminal branches of the vestibular nerve itself and can readily be distinguished from native collagen.

**Neurinoma (neurilemmoma).** Similar striated filamentous structures may be found in some tumours arising from Schwann cells. We have found numerous striated fusiform structures in thin sections of a neurilemmoma presenting as a nasal polyp (Fig. 4) similar to those first described in neoplasms of the eighth nerve by Luse<sup>5</sup> and by Raimondi *et al.*<sup>6</sup>; and more recently by Pillai<sup>7</sup> in the experimentally constricted tibial nerve of rats. The fusiform or comma-shaped striated bodies in the nasal tumour are closely associated with the basement membrane and seem to emerge from it.

Unusual laminated or striated structures have been described in various tissues by several authors; some of them are listed in Tables 2 and 3.

Unusual laminated inclusions in the sensory epithelium of the macula of the utricle of a patient operated on to



Fig. 2. Macula of the human utricle (Ménière's disease). Apical zone of sensory cell with laminated structures forming a semi-circle around a group of vacuolated mitochondria. Case 11. ( $\times 18,000$ )

Table 1. MENIERE'S LABYRINTHOTOXICITY FOR MENIERE'S DISEASE (11 PATIENTS)

Sex	Age	Duration of symptoms	Electron microscopy Laminated inclusions	"Long-spacing collagen"
M	57	6 years	+	+
M	40	2 years	+++	—
M	54	6 months	—	—
M	55	3 years	+++	—
F	51	7 years	+++	+++
M	65	4 years	+	+
M	55	4 years	—	++
F	16	1 year	+++	—
Deaf 16 years (mumps)				
M	43	4 months	+	—
M	53	2 years	—	—
M	51	2 years	+++	—

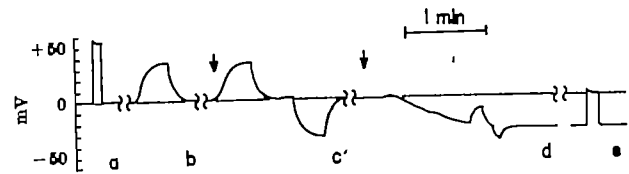


Fig. 1. a, Calibration pulse, 54 mV; b, testing pulse at 1,000-megohm input impedance: membrane resistance 15 megohm/cm<sup>2</sup>; c, addition of 0.4 c.c. 0.4 M KCl inside, producing a final concentration inside of 0.004 M KCl. Concentration ratio inside/outside cell is now 10-fold. Testing pulse: resistance unchanged; d, 0.04 c.c. of a 5 × 10<sup>-2</sup> per cent w/v 'Ultrawet' solution added inside. Testing pulse at 5 megohm input impedance: membrane resistance 11 k ohm/cm<sup>2</sup>; e, circuit open: potential developed reads 31 mV

tolerated by the membrane. Only those were used which brought down the resistance to 10 per cent of the starting value within 5-10 min.

Fig. 1 illustrates a typical experiment in which an anionic detergent developed a cationic resting membrane potential. Addition of the detergent lowered the initially high resistance by several decades. The input impedance was kept high in all cases with respect to the total membrane resistance, so that the potential was accurately recorded. The experiments were followed by controls to rule out potentials generated by the detergents themselves without a salt gradient. To exclude the effect of H<sup>+</sup>-ions the pH was adjusted before and tested after every experiment. No deviation from the adjusted buffer value was detected.

All four types of detergents lower the membrane resistance and develop potentials to varying degrees, at least transiently. Only the anionic detergents produce prolonged steady effects. It may be noted that amphoteric detergents could only be tested at a pH close to 6.8, since the membranes tended to break at higher values. The isoelectric points of these amphoterics usually lie far on the acidic side and so they only showed anionic properties. No qualitative difference in their behaviour from anionic detergents could be found.

All the detergents in the concentrations listed in Table 1 decreased the membrane resistance within a few minutes by at least 10<sup>3</sup> ohms. In the case of anionics and cationics the resistance stayed at low values or continued to decrease slowly during experiments that lasted for more than 1 h. The initial decrease of resistance produced by non-ionics, however, was followed in turn by a spontaneous increase, the resistance approaching the original high state again within minutes. This transient action apparently depended on the concentration of detergent used: the higher the concentration, the slower the lowered resistance reversed.

Potential differences across the membrane are produced by detergents in two ways: (a) there are potentials generated by the detergents themselves in the absence of additional salt gradients; (b) there are potentials generated by the detergents by virtue of an additional salt gradient.

All types of detergent produced potentials of varying magnitude, *per se*, in the absence of a salt gradient. The sign of these potentials was the same as the sign of the detergent molecule itself and not the sign of its counterion, non-ionic detergents acting like anionic detergents. Thus, anionic and non-ionic detergents showed a small anionic potential immediately after adding them to the system. This potential was small and did not exceed 4 mV; it decayed within seconds. On the other hand, cationic detergents developed a large cationic potential, *per se*, which increased with increasing concentration of the detergent and persisted for some time. For example, cetyl-pyridinium chloride in a final concentration of 10<sup>-2</sup> per cent w/v developed a cationic potential of 50-60 mV in a few minutes, which steadily decayed to zero over 5-10 min.

In the presence of a salt gradient (high concentration inside) anionic and non-ionic detergents generated a cationic potential. Only with anionics did a steady potential difference persist across the membrane, in many experiments for more than 1 h. The potential generated

Table 1

Detergent type and formula	Concentration used to lower R from > 10 <sup>6</sup> to < 10 <sup>4</sup> ohm cm <sup>2</sup>	Resistance and potential effects due to detergents					
		Resistance decrease		Potential developed			
		Transient	Steady	Zero salt gradient		With salt gradient	
				Transient	Steady	Transient	Steady
Anionic: Sodium N-methyl-N-octyl-taurate (Gen. Aniline, 'Igepon T 35')	5 × 10 <sup>-4</sup> ± 10 <sup>1</sup> % v/v	None	For more than 1 h, by more than 10 <sup>6</sup> ohms	Max in 10-30 sec, decay in 30-60 sec, less than 4 mV anionic	None	None	Within 1-2 min, for hours, cationic, large
Dodecyl-benzene Na-sulphonate (Atlantic Ref. Co., 'Ultrawet XX')	5 × 10 <sup>-4</sup> ± 10 <sup>1</sup> % w/v						
Tetradecyl sodium sulphate (K and K Laboratories)	1 × 10 <sup>-4</sup> ± 10 <sup>1</sup> % v/v						
Cationic: Cetyl-pyridinium chloride	1 × 10 <sup>-4</sup> ± 10 <sup>1</sup> % w/v	None	For more than 1 h, by more than 10 <sup>6</sup> ohms	Max. in 2-5 min, decay in 5-15 min, more than 60 mV cationic	None	None	None
Dodecyl-benzene trimethyl-ammonium chloride (Inter Chem. Co., 'Interman ABM 50')	1 × 10 <sup>-4</sup> % v/v						
Non-ionic: Polyoxyethylene-polyoxypropylene (Wyandotte Chem. Corp., 'Pluronic')	1 × 10 <sup>-4</sup> ± 10 <sup>1</sup> % w/v	Max. in 10-30 min, recovery in 20-60 min, by more than 10 <sup>6</sup> ohms	None	Max. in 10-30 sec, decay in 30-60 sec, less than 4 mV anionic	None	Max. in 10-30 min, decay in 20-60 min, large, cationic	None
	F 68						
	L 63						
	L 101						
Amphoteric: Dimethum N-lauryl β-amino dipropionate (Gen. Mills, 'Daciphat 160')	5 × 10 <sup>-3</sup> ± 10 <sup>1</sup> % w/v	Like anionics		Like anionics		Like anionics	
Decyl betaine (du Pont, Prod. 'BDO')	1 × 10 <sup>-3</sup> ± 10 <sup>1</sup> % v/v						

\* At pH 6.8.

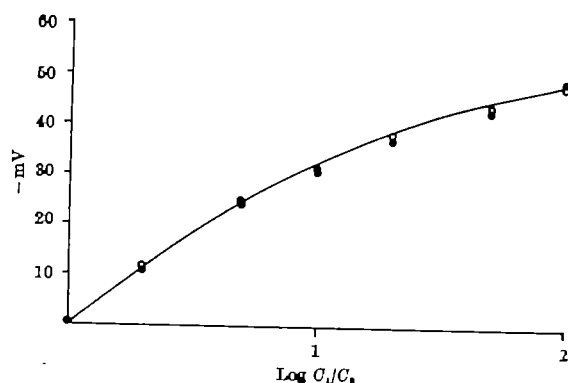


Fig. 2. Potential as function of KCl gradient. O, 'Ultrawet KX' (anionic),  $5 \times 10^{-3}$  per cent w/v final concentration, ●, 'Pluronic L 101' (non-ionic),  $4 \times 10^{-4}$  per cent v/v final concentration.

by non-ions in the presence of a salt gradient decayed within 20–60 min as the membrane resistance recovered. By contrast, cationic detergents lowered the membrane resistance considerably, but no potential difference was produced by a salt gradient. Introduction of a gradient during and after all phases of the self-generated cationic potential remained without additional effect on the potential. The potential developed across the membrane as a function of a salt gradient is shown in Fig. 2 for an anionic and a non-ionic detergent.

Saponin, a surface-active molecule with haemolytic activity, was found to act like an anionic detergent in a concentration of  $5 \times 10^{-3}$  per cent w/v.

The mode of action of detergents on lipid bilayers as described herein could be explained as a modification of the lamellar membrane structure by a phase transition to localized 'micellar' arrangements (cf. ref. 7). The structure of the membrane is sufficiently flexible to adopt these different configurations. The 'liquid' state of the lipid hydrocarbon chains in the lamellar phase<sup>8</sup>, that is, their

relatively free mobility, would permit the rearrangement of the lipid membrane structure. Localized lipid micelles with a negative charge would result and form a fixed-charge, cation-selective pore of the Teorall-Meyer-Sievers type. The degree of vibrational freedom across the bilayer of the 'fixed' sites (that is, restricted to the lipid phase) is unknown. If great enough, the fixed sites would become carrier-like.

This hypothesis could serve as a model for the specialized lipid-protein interaction developing channels in cell membranes. Proteins, for example, EIM, with their surface-active properties<sup>9</sup>, might modify the molecular configuration of the lipid membrane in a similar way as proposed for detergents. An illustration of such a hypothesis was recently given by Trams<sup>10</sup>.

Further investigations of molecularly defined surfactants with synthetic unsaturated lipid membranes may lead to information as to the atomistic arrangements of structural units in the cell membrane during different functional states.

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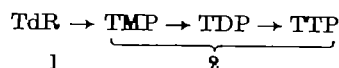
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## VARIATIONS IN PHOSPHOKINASE ACTIVITIES DURING THE CELL CYCLE IN SYNCHRONOUS POPULATIONS OF HELA CELLS

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KNOWLEDGE of the sequence of events in the replication cycle of mammalian cells would be of great importance in elucidating the control mechanisms involved. Up to the present, the process which has been most investigated in this connexion is the synthesis of DNA, which has been found to occur continuously between divisions in bacteria<sup>1</sup> but is restricted to the so-called S-phase in mammalian cells. Few observations have been made of the events in the cell cycle such as the levels of particular enzyme activities. The enzymes involved in the production of the immediate precursors of DNA synthesis are of special interest and have been widely studied in asynchronous cell populations, for example, the thymidine phosphokinase reactions:



which we have now examined in a synchronously dividing population of HeLa cells. For the purposes of the work recorded here, reaction 2 was taken as a single reaction, since, although the diphosphate has been shown to be an intermediate in some cell systems<sup>2</sup>, under the conditions

of assay used the formation of the diphosphate as a precursor of the triphosphate could not be detected.

The difficulty in examining these reactions at various stages in the cell cycle has been that of obtaining a homogeneous synchronous population of cells in sufficient quantity for the assay of these enzymes. Some previous work on the changes of level of the thymidine phosphokinases has been carried out using regenerating rat liver<sup>3</sup>; however, this tissue is unsatisfactory since less than 10 per cent of the cells take part in the burst of cell division following hepatectomy. Synchrony in mammalian cells has been obtained by a number of different methods<sup>4</sup>. In most of these, induction of synchrony is accompanied by a metabolic disturbance to the cells and may result in unbalanced growth of the culture. The technique of selecting cells from a randomly dividing population does not appear to be open to this criticism.

In the investigation reported here a reasonably synchronous population was obtained, in sufficient quantity for enzyme assay, by a method essentially that of Robbins and Marcus<sup>5</sup>. Mitotic cells were selected by washing a monolayer culture with Eagle's medium deficient in  $\text{Ca}^{++}$ . The cells were routinely grown as monolayers and in

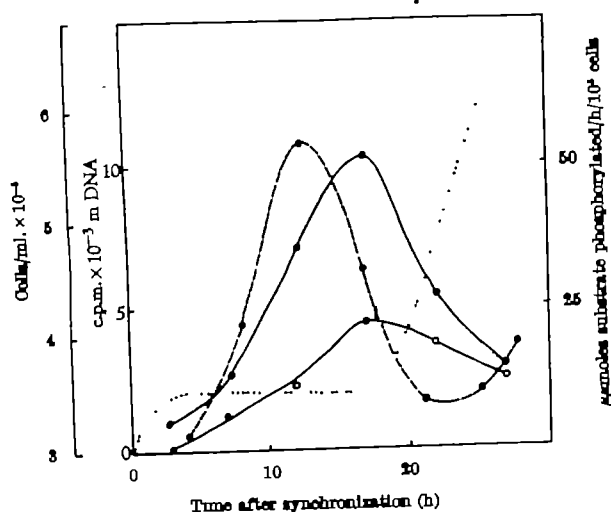


Fig. 1. Synchronous growth of HeLa cells through one division cycle after collection of mitotic cells at zero time. Cell number (.....) was measured by haemocytometer. The rate of DNA synthesis (●—●), given as c.p.m. in DNA, was determined by the incorporation of tritiated thymidine at each time by an aliquot of cells during a 15-min pulse. Thymidine phosphokinase activity (○—○), given as  $\mu$ moles TdR phosphorylated/h/10<sup>4</sup> cell equivalent, and thymidylate phosphokinase activity (□—□), given as  $\mu$ moles TMP phosphorylated/h/10<sup>4</sup> cell equivalent, were determined in the supernatant fractions.

suspension in Ca<sup>++</sup>-deficient Eagle's medium supplemented with 5 per cent pig serum and 2 per cent calf serum.

The degree of synchrony was determined by measuring the mitotic index of a stained preparation of cells at the time of collecting. This varied from 50 to 85 per cent. The cell number was followed during the subsequent cycle by counting cells in selected fields on Petri dishes<sup>4</sup> or by haemocytometer counting (Fig. 1).

The rate of DNA synthesis throughout the cell cycle was measured in two ways. First, by scoring the percentage of cells labelled in autoradiographs of coverslip cultures which had been pulsed with tritiated thymidine. Secondly, cells in suspension were pulsed with tritiated thymidine and then collected on 'Millipore' filters under suction, washed with about 20 ml. of phosphate-buffered saline and fixed by washing with a similar volume of 5 per cent trichloroacetic acid. The filter was then dissolved in scintillation liquid and counted in a Packard liquid scintillation counter. The incorporations thus measured were in good agreement with measurements of incorporation of tritiated thymidine into the hot trichloroacetic-soluble material of the cell. The curves for the rate of DNA synthesis during the cell cycle are similar to those described by Terasima and Tolmach<sup>7</sup> (Fig. 1).

For enzyme assays, 2–3 × 10<sup>7</sup> synchronous cells were maintained in magnetically stirred suspension throughout a complete cell cycle. Aliquots of the suspension, equivalent to 2–3 × 10<sup>4</sup> cells, were taken at intervals for preparation of an enzyme fraction. The cells were washed in ice-cold phosphate-buffered saline and then in a sucrose-potassium chloride medium at pH 8 (ref. 8) prior to sonication in a small volume (0.5 ml.) of the latter. The supernatant fraction obtained by centrifuging at 100,000g for 40 min was stored at –20°C and used for enzyme assays.

Thymidine kinase was assayed by incubating 0.05 or 0.1 ml. of the supernatant for periods up to 1 h at 37°C with an equal volume of an incubation medium containing 10 mM ATP, 10 mM MgCl<sub>2</sub>, 12 mM 3-phosphoglycerate and 160 mM tris-HCl, and about 700  $\mu$ moles tritiated thymidine of high specific activity (14 c./mmole, Radiochemical Centre, Amersham). The reaction was stopped by adding 100 per cent w/v trichloroacetic acid to a final concentration of 10 per cent. 20  $\mu$ l. of the supernatant, containing the phosphorylated products of thymidine, was chromatographed on Whatman No. 1 paper, using a

solvent system comprising butanol, acetone, acetic acid, ammonium hydroxide. The distribution of the phosphorylated derivatives was measured using a Nuclear Chicago windowless chromatogram scanner.

The percentage conversion of thymidine to TMP and TTP was plotted against incubation time—the slopes of these plots being taken as the relative enzyme activities of the supernatant preparations at different times during the cell cycle.

It was found that the level of thymidine phosphokinase (reaction 1) is low immediately after division (G1 phase), rises during the S period, reaches a maximum about 5 h after the peak of DNA synthesis and falls rapidly during the subsequent division (Fig. 1). The curve of activity for the thymidylate phosphokinase (reaction 2), assayed by substituting tritiated TMP for tritiated TdR in the substrate, is similar to that for thymidine phosphokinase. Preliminary assays have also shown a similar pattern for deoxycytidine phosphokinase.

These results show conclusively that the thymidine phosphokinases go through a sequence of appearance and disappearance during the cell cycle as assayed in the supernatant. The peak in enzyme activity occurs somewhat later than the peak of DNA synthesis as was also found in the case of regenerating liver<sup>8</sup>. Since these enzymes must have been present in sufficient quantity in the cell at the beginning of the S period, in order to produce the precursors required for DNA synthesis, it must be concluded that they are present in the cell, at such times, in a form which is not apparent on assay of the enzyme activity in the supernatant.

Two possible explanations of this finding can be suggested: (1) The enzymes are present before and during DNA synthesis in the form of a complex with the nuclear particulate fraction of the cell, and the observed increase in activity represents their release to the supernatant fraction when their function has been completed. (2) The observed increase in activity is due to actual enzyme synthesis and the subsequent decrease due to the binding of this enzyme to a nuclear particulate fraction, preparatory to its functioning in the next cell cycle. Such a mechanism has been postulated by Maxia<sup>9</sup> for DNA polymerase in embryos of sea urchins.

However, it has been shown for several cell systems that the lifetimes of the phosphokinases in the cell are considerably less than one cell cycle<sup>10</sup>. It therefore seems more probable that these enzymes will be formed shortly before they are to be used by the cell. In order to distinguish between these two hypotheses it will be necessary to determine whether the rise in enzyme-levels observed (Fig. 1) is due to new protein synthesis at this time or at some earlier stage, information which can only be obtained indirectly on the small samples available. These possibilities are being further examined.

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## LETTERS TO THE EDITOR

## RADIO ASTRONOMY

## Radio Brightness Contours of 3C273

PREVIOUS observations of the radio source 3C273 by the method of lunar occultations have shown that it consists of two separate components the positions of which were determined with an accuracy of 1 arc sec<sup>1</sup>. Component *A* coincides closely with a jet-like nebula and component *B* with a nearby object of stellar appearance<sup>2</sup>. This communication presents a contour map of the source, constructed from the analysis of five complete occultations.

The observations were made with a parabolic antenna 85 ft. in diameter, which tracked the source position for the duration of the occultation. The receiver was a switched type, without image rejection, operating at a frequency of 950 Mc/s. Its intermediate frequency was 30 Mc/s, band-width 5 Mc/s, and noise figure 3.6 dB. Figs. 1*a* and *b* show analogue records of the emission and immersion of the source at one occultation. The output time constant was 1 sec for all observations except for the data reproduced in Fig. 1.

Table 1 lists the dates of the occultations and the position angles at which they occurred. The position angle indicates the direction in which the Moon's limb advanced or receded across the central region of each component. The records were analysed on a digital computer with a programme<sup>3</sup> based on the restoration process of Scheuer<sup>4</sup>. This programme convolves the observed data with a restoring function and derives the brightness distribution as it would be observed with a gaussian fan beam scanned over the source along the position angle of the occultation. The observations were all restored with an effective beam-width of about 1.5 arc sec, the limit set by signal-to-noise ratio<sup>5</sup>. Examples of data restored by this method are plotted in Figs. 1*c* and *d*.

The five occultations yielded ten scans across the source, from which the radio-brightness contours shown in Fig. 2 were determined by the following procedure. The peaks of each restored scan were assumed to coincide with the centres of the components of the source. Points corresponding to 0.8, 0.6, 0.4 and 0.2 of the peak intensity of component *B* were then marked off along the appropriate position angle. Lines were drawn through these points

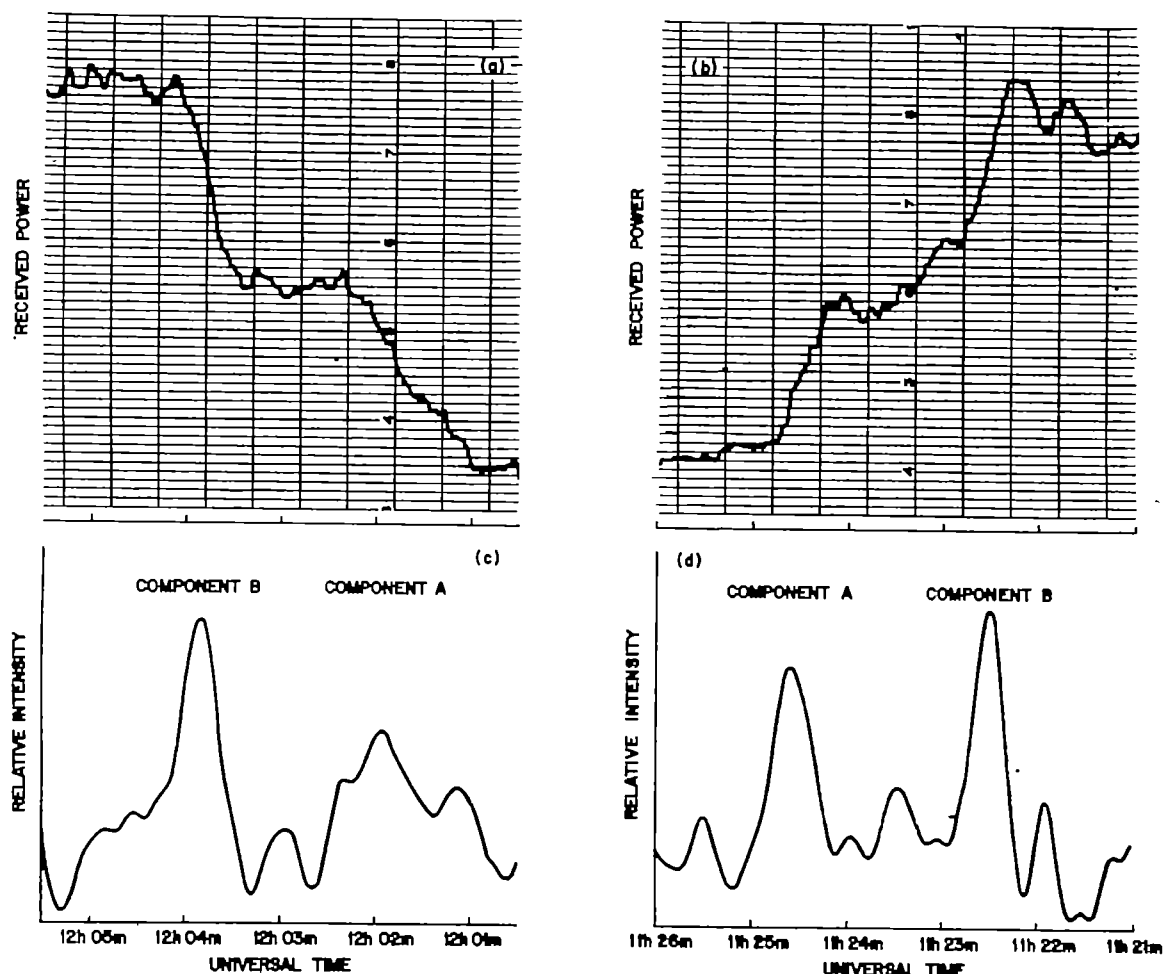


Fig. 1. Lunar occultation of December 9, 1963. Analogue records with receiver time constant of 14 sec: (a) emission; (b) immersion. Brightness distributions restored with an effective beam-width of 2.4 arc sec: (c) emission; (d) immersion.

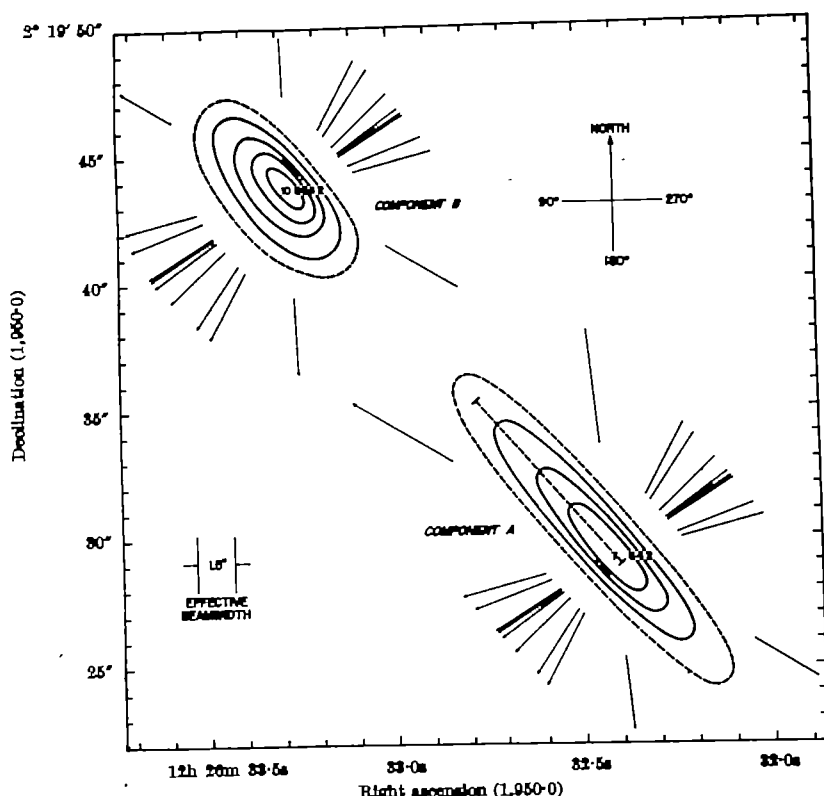


Fig. 2. Brightness contours of 30273 determined from occultation observations restored with an effective beam-width of 1.5 arc sec. Numbers on the contours indicate intensity on a scale of 10 relative to the central maximum of component B. The straight, dashed line indicates the extent of the optical jet. Arrows show the directions in which the Moon's limb crossed the components.

perpendicular to the direction of the occultation. A first approximation to the contours was obtained by inscribing smooth curves inside the envelopes determined by the lines. The contour map thus formed was divided into strips 1.5 arc sec wide perpendicular to the position angle of a given occultation. By integrating along these strips a scan across the source was simulated. The observed and simulated scans were compared and the locations of the contours adjusted to minimize their differences. This procedure was repeated for several position angles. The dashed contour delineates the region beyond which there was no detectable signal.

Component A is narrow and elongated along the axis of the components. At right angles to this axis it remains unresolved with the present resolution of 1.5 arc sec. Component B also shows elongation along the line joining the components. By contrast, observations at 410 Mc/s suggest that component B may be elongated perpendicular to the axis<sup>1</sup>. The limitations inherent in strip scans must be borne in mind when one examines the contour diagram. In particular, the increased separation between contours of component B near position angle 150° could in part be attributed to confusion with the extended region of component A.

The record of Fig. 1a is of particular interest because the Moon's limb receded along position angle 239°, which is only 15° from the line joining the centres of the components. The extension of component A can be seen quite clearly on the record as a relatively slow rise with no

associated diffraction pattern. The plateau between 12h 02.4m and 12h 03.5m U.T. indicates that component A ends approximately 10 arc sec from the centre of component B. This feature of the radio emission shows remarkable correspondence with the optical data<sup>2</sup>.

The centre positions of the two components have been calculated from the universal times corresponding to the peaks on the restored scans. The mean right ascensions and declinations (Epoch 1950) together with the root mean square errors are:

Component A: 12h 26m 32.42s ± 0.04s + 02° 19' 29".2 ± 0".4.  
Component B: 12h 26m 33.27s ± 0.04s + 02° 19' 43".7 ± 0".8.

The radio position for component B is 1.5 arc sec north-west of the corresponding optical position<sup>3</sup>. If we ascribe this difference to systematic causes and assume that the stellar object lies at the centre of component B, then the position of the optical jet is that indicated in the contour diagram by the straight, dashed line.

The ratio of the flux of component A to that of component B is 1.00 : 0.92. The combined flux of the two components at 950 Mc/s is  $4.5 \times 10^{-26}$  M.K.S. units (assuming  $M_{87} = 290 \times 10^{-26}$  units). Hence, the flux of component A at this frequency is  $2.7 \times 10^{-26}$  units and that of component B is  $2.1 \times 10^{-26}$  units. These

values are in accord with fluxes predicted from previous observations<sup>1,4,5</sup>.

I thank Dr. S. von Hoerner of the National Radio Astronomy Observatory for supplying his Fortran computer programme and Dr. W. Nicholson of H.M. Nautical Almanac Office for predicting the occultations and calculating the source positions. Mr. J. Ball assisted with the computations.

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## ASTRONOMY

### Correlation between the Twelve High-velocity Neutral Hydrogen Clouds and a Feature of the Sky in the Radio Continuum

Hulsbosch and Raimond<sup>1</sup> have discovered a region, 100° in diameter, which contains twelve clouds of neutral hydrogen distinguishable from the general background emission by their exceptionally high Doppler shifts. These shifts correspond to velocities from -85 km/sec to -175 km/sec relative to the Sun. It is interesting that there appears to be a relationship between these peculiar neutral hydrogen clouds and the features of the radio continuum emission from the high galactic latitudes.

Table 1. POSITION ANGLES OF OCCULTATIONS

Date of occultation	Position angles			
	Component A		Component B	
	Immersion	Emergence	Immersion	Emergence
Dec. 9, 1963	186°	239°	183°	241°
April 24, 1964	110°	325°	109°	325°
July 14-15, 1964	182°	305°	181°	304°
Sept. 7, 1964	154°	303°	183°	304°
Nov. 23, 1964	104°	332°	108°	334°



The existence of the zinc blende structure at these three points in the triangle leads us to expect adamantine solid solution at all compositions inside the triangle. The ease with which equilibrium can be attained will, it is to be hoped, stimulate work on the many similar systems that can be postulated from known adamantine compounds.

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## GEOFYSICS

### Airborne Observations of Natural Radioactivity in the Atmosphere

MANY observations of natural radioactivity in the atmosphere have been made<sup>1</sup>, especially at ground-level. Radon and its decay products predominate<sup>2</sup>.

This communication describes some measurements with a scintillation detector of background counting rates at altitudes up to 3.6 km. The day-to-day variations in counting rate were surprisingly large, and were found to occur mainly in the atmosphere between the ground and temperature inversion layers. The variations are due principally to changes in concentration of natural radioactivity in the atmosphere.

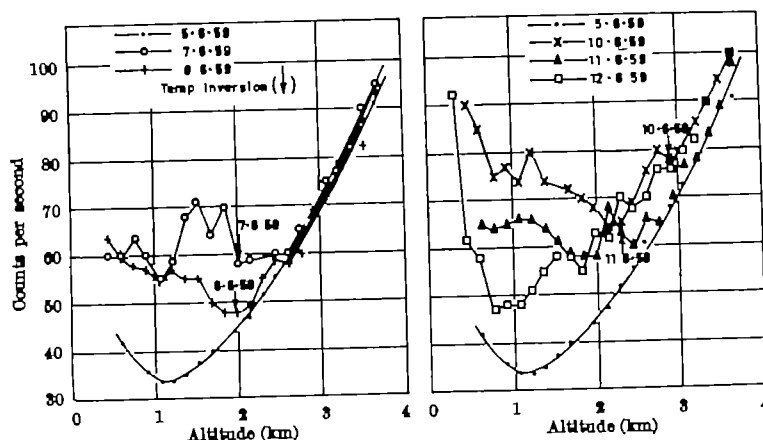


Fig. 1. Counting rate versus altitude

The detector, a scintillation unit with a cylindrical sodium iodide crystal of diameter 10 cm and height 7.5 cm, was mounted behind the tailplane of a DO 3 aircraft and could detect  $\gamma$ -rays coming from all directions. The flights were made near Gatton (elevation 100 m), Queensland, 85 km inland from the eastern coast of Australia. The airstreams were essentially from the east (maritime) for the four days immediately before the day of the first flight (June 5). Wind directions changed on June 6, and, for the subsequent days on which flights were made, the airstreams were essentially from the west (continental).

Counting rates were measured at intervals of 150 m altitude and the values obtained for the six flights are plotted against altitude (Fig. 1). The altitudes of temperature inversion are noted. The counting rates observed on June 5, in the maritime airstream, were the lowest of the series, and varied smoothly with altitude. The initial decrease in counting rate with altitude was due to

absorption by air of  $\gamma$ -rays from radioisotopes in the ground<sup>3</sup>. At higher altitudes the counting rate increases with altitude due to cosmic rays<sup>4</sup>. Counting rates, especially at heights less than 2 km, were higher on days of continental airstream (June 7-12) and varied more abruptly with altitude. The main increases in counting rates, as compared with results on June 5, occurred below the temperature inversion layers. This indicates that the increases were due to radioactivity in the atmosphere, and that it probably originated from the ground.

Simultaneous measurements of counting rate and relative humidity were made at 1-min intervals during several flights at constant altitude. During one flight the aircraft passed through successive moist and dry air regions at about 2-min intervals, and higher counting rates were recorded in the moist air than in the dry. These observations were made in clear air about 10-15 min before cloud formation was observed. The moist air pockets, which were not found an hour earlier at this altitude, may have been carried up by convection, thereby containing more natural radioactivity than the surrounding air.

It was probably mainly radon and its decay products which were detected. The principal  $\gamma$ -rays originate from lead-214 and bismuth-214 (ref. 5). The scintillation detector 'sees'  $\gamma$ -rays emitted by these isotopes distributed in the sphere of radius approximately 300 m about the aircraft. It has been estimated that the present detector will record 8 c.p.s. as a result of a uniform concentration of  $10^{-17}$  curies/c.c. of both lead-214 and bismuth-214 in the atmosphere at an altitude of about 2 km. The calculation<sup>6</sup>, allowing for the build-up of  $\gamma$ -ray quanta in air, gives a counting rate about eight times that for calculations based on exponential absorption of  $\gamma$ -rays. The

maximum increase in counting rate above the results of June 5 was about 46 c.p.s., that is, a concentration of about  $6 \times 10^{-17}$  curies/c.c. This is well within the range of concentrations found by other investigators<sup>7</sup>.

These observations show that by using a scintillation detector mounted on an aircraft, instantaneous measurements can be made of the natural radioactivity in the atmosphere. An air mass may be identified as being of essentially continental origin when the concentration of natural radioactivity is high. Estimation of the efficiency of scouring of radioactive aerosols by rain, and the degree of mixing across a stable interface between two fluids such as at the top of stratocumulus cloud, are two problems which may merit investigation with this technique.

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### Possible Destruction of Ozone by Volcanic Material at 50 mbar

VERTICAL ozone distributions over Boulder, Colorado ( $40^\circ$  N.), showed a persistent dip in the ozone concentration at 50 mb over the period from March 9 to April 10, 1964. At the middle of the period the dip reached a maximum amplitude of about 50 micro-mbar less than the neighbouring ozone partial pressures, and a 'half-width' of about 5 to 10 mb. The rise in ozone concentration below the 50-mb level was appreciably steeper than the lines of equal mixing ratio, even in the mean of all 23 soundings taken during the above period.

Fig. 1 shows the mean plot of partial pressure of ozone against total pressure for the nine consecutive soundings from March 16 to 27, 1964, over Boulder.

These observations appear to require an 'ozone sink' at 50 mb at the time and place of observation. It is tentatively suggested that such a sink could have been present in the form of a concentrated layer of volcanic dust. Meinel and Meinel<sup>1,2</sup> reported such dust, attributed to the eruption of Mt. Agung on Bali on March 17, 1963, as late as November 1963. Similar observations have been reported from India, Australia and South Africa.

Mossop<sup>3</sup> gives estimates of mean concentration and diameter of samples collected over Australia, while Bigg (personal communication) has furnished photographs showing patches of volcanic dust as far south as  $41^\circ$  S. and having a vertical thickness of the order of one kilometre or less.

From the persistence of the 'ozone hole' over Boulder and the winds then prevailing at the 50-mb level, which were very light, it is estimated that the patch of dust must have extended over horizontal distances of the order of a few thousand kilometres. Confirmation from stations in the United States Air Force ozone-sonde network gives support to this picture. A similar 'ozone hole' was found in soundings from Fort Collins, Colorado, on March 18 and March 25; from Albuquerque, New Mexico, on March 18 and April 8; and from Bedford, Massachusetts, on April 1 and April 2.

A model of such a dust layer has been drawn up and the resulting ozone profile computed. This model requires a thin layer having a vertical mixing coefficient more than an order of magnitude less than current estimates of the mean value. On the model chosen a destruction rate of one ozone molecule in every  $3.5 \times 10^7$  per sec is required to give rise to the observed ozone profile. As the mass of ozone per unit volume at 50 mb is several orders of magnitude greater than the estimated mass of dust per unit volume, such destruction must be catalytic in nature, most likely due to the adsorption of ozone molecules on the surface of the dust particles with subsequent decomposition on collision with a second ozone molecule.

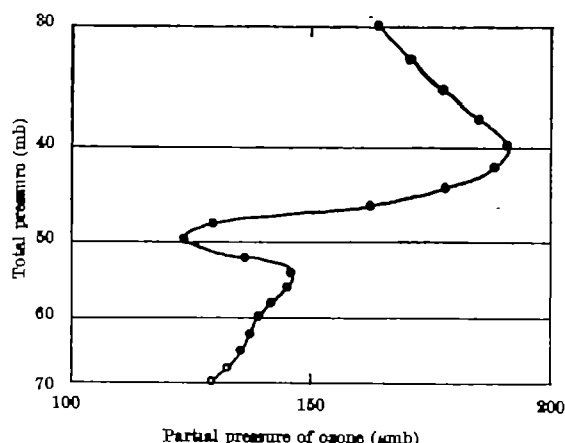


Fig. 1. Mean of nine consecutive ozone soundings, March 16-17, 1964

In conclusion, it is emphasized that this model is put forward to explain a very particular event and does not necessarily imply that dust is normally present in sufficient concentration to appreciably affect the ozone distribution at these levels. Nevertheless, the implications regarding circulation and vertical eddy diffusion require further investigation, as does the possibility that a concentration mechanism operated to produce the high dust concentrations required here (and observed over Australia).

I thank my colleagues at the National Center for Atmospheric Research, members of the U.S. Air Force ozone-sonde network, and Dr. E. K. Bigg for their assistance in this work.

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## METALLURGY

### Effects of Silver and Two-step Ageing Treatments on a Weldable Alloy of Aluminium, Zinc and Magnesium

POLMEAR and co-workers<sup>1-3</sup> have shown that small additions of silver to Al-Zn-Mg alloys can bring about increases in strength, and, by retarding over-ageing, allow alloys to be aged at higher temperatures. When silver was added to 'DTD 683' (ref. 3) the increase in hardness at ageing temperatures up to  $135^\circ$  C was small, the main advantage being that high hardness levels were maintained when ageing at temperatures up to  $180^\circ$  C.

Taylor<sup>4</sup> has shown that to produce the highest mechanical properties in a reasonably short time in weldable Al-Zn-Mg alloys a 2-step ageing treatment is required. The alloy is first aged at a temperature below  $100^\circ$  C, and then aged at or above  $120^\circ$  C, when reversion is followed by rapid ageing to give higher strengths than could be obtained by ageing directly at the higher ageing temperature. Taylor's work does not show whether the use of 2-step ageing treatments allows high hardness levels to be maintained at temperatures much more than  $135^\circ$  C. The use of high ageing temperatures could be an advantage in weldable Al-Zn-Mg alloys, since the heat-affected zone should be narrower the higher the ageing temperature.

Experiments to evaluate the effect of silver and the effect of 2-step ageing treatments on commercially produced weldable Al-Zn-Mg alloys have been carried out on two alloys containing nominally 5.0 per cent (weight) Zn, 1.3 per cent Mg, 0.1 per cent Cr, and 0.2 per cent Mn, one of which contained an addition of 0.45 per cent Ag. In the first instance hardness has been used as a measure of strength, though it is recognized that hardness is not a fundamental property.

Specimens of both alloys were solution-treated at  $460^\circ$  C for 30 min and water quenched. For each alloy specimens were first aged for 7 days at  $20^\circ$  C, 7 days at  $70^\circ$  C and 7 days at  $100^\circ$  C, before being aged at  $120^\circ$  C,  $140^\circ$  C,  $160^\circ$  C and  $180^\circ$  C. In addition some specimens were aged directly at the final ageing temperatures without a preliminary low-temperature age. The initial low-temperature ageing treatments were chosen arbitrarily.

In the silver-free alloy, 2-step ageing treatments resulted in higher peak hardness values at all ageing temperatures between  $120^\circ$  and  $180^\circ$  C, the effect being more marked the higher the final ageing temperature. The best results were obtained when the first ageing was for 7 days at  $70^\circ$  C.

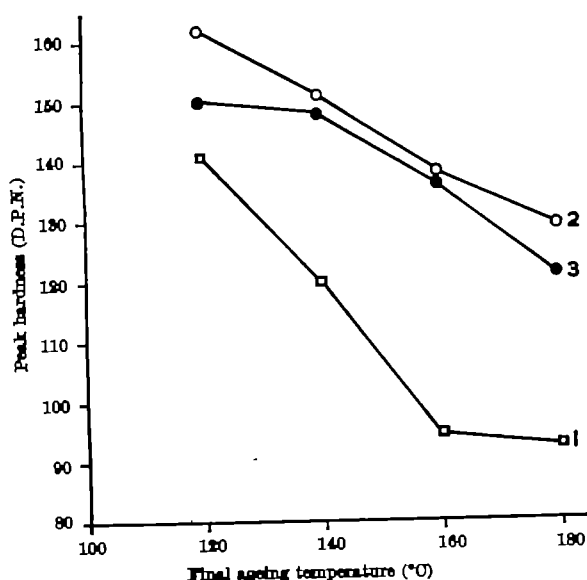


Fig. 1. Peak hardness against final ageing temperature for (1) 5.0 per cent Zn, 1.8 per cent Mg alloy aged directly at the final ageing temperature; (2) The same alloy first aged for 7 days at 70° C; (3) 5.0 per cent Zn, 1.8 per cent Mg, 0.45 per cent Ag alloy aged directly at the final ageing temperature

In the silver-containing alloy the peak hardness obtained at the final ageing temperature was effectively the same, whether the specimens were given a preliminary period of ageing at low temperature or not.

The effect of the silver addition on the silver-free alloy was similar to the effect of preliminary ageing at low temperature, in that both increased the peak hardness at the final ageing temperature, and both had the greatest effect when the final ageing temperature was high. These results are summarized in Fig. 1, where peak hardness is plotted against the final ageing temperature (1) for the alloy of 5.0 per cent Zn and 1.8 per cent Mg, aged directly at 120°–180° C; (2) for the same alloy first aged for 7 days at 70° C; (3) for the alloy of 5.0 per cent Zn, 1.8 per cent Mg and 0.45 per cent Ag, aged directly at 120°–180° C.

The results show that for the composition investigated, an addition of silver brings about a small increase in strength when ageing at 120° C, but is a distinct advantage in allowing the use of higher ageing temperatures. However, the same advantage can be obtained by using a two-step ageing treatment.

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### Influence of Zirconium Additions on the Epsilon Phase of the Titanium-Aluminium System

THE TiAl phase ( $\epsilon$ ) of the aluminium-titanium system exists as a single-phase field and can be achieved as a high-temperature phase without a polymorphic phase transformation. Results of an early investigation by the Armour Research Foundation indicated that an alloy of 36 per cent aluminium with 64 per cent titanium had superior oxidation resistance at 1,500° F, good hot hardness, and low density; however, it lacked any degree of room-temperature or elevated-temperature ductility<sup>1</sup>. The cause of this brittleness appeared to be the ordered face-centred tetra-

gonal lattice structure, in which [002] planes of aluminium atoms were alternated with titanium atoms. It was assumed that if the  $c/a$  ratio could be reduced to unity, and the atomic arrangement could be disordered, the ductility of the  $\epsilon$  phase could be improved. Keesler and McAndrews<sup>2</sup> tried to reduce the  $c/a$  ratio to unity by the additions of 13 different elements. Davies<sup>3</sup> in 1959 suggested the addition of Zr as a means of reducing the  $c/a$  ratio. Sandlin and Klug<sup>4</sup> and Troup<sup>5</sup> continued to study the effect of Zr additions to the TiAl binary. The purpose of our investigation was to refine the 2,325° F  $\epsilon$ -phase boundary of the Al-Ti-Zr system outlined by the earlier investigators and to determine the influence of zirconium on the  $c/a$  ratio within the  $\epsilon$  phase at this temperature.

Eighteen experimental alloys were prepared from high-purity aluminium (99.99 per cent), titanium (99.99 per cent), and zirconium (99.72 per cent Zr containing 0.21 per cent Hf). Twenty-gram button specimens of each composition, as indicated in Fig. 1, were arc-melted in a high-purity argon-helium atmosphere. Each button was melted six times and inverted between each melt to ensure homogeneity. Weight losses during melting did not exceed 0.69 per cent. Nominal and analysed compositions agreed within 1 per cent. The specimens were wrapped in tantalum foil, encapsulated in quartz, and homogenized at 2,325° F for 24 h. After heat treatment, the quartz capsules were quenched in brine to preserve the high-temperature phase/s present.

Filings for X-ray analysis were passed through a 325-mesh screen. This powder was wrapped in tantalum foil and subjected to a low-temperature anneal in evacuated 'Vycor' tubes containing spectroscopically pure argon at a pressure of less than one micron. The latter operation was necessary to remove the effects of cold work.

Powder studies were conducted using a Siemens 114.6 mm camera with Straumanis mounting. Copper K $\alpha$  radiation was filtered with nickel. The  $d$ -values taken from the film, corrected for shrinkage, were compared with the  $d$ -values of TiAl, TiAl<sub>3</sub>, Ti<sub>3</sub>Al, Al-Zr phases, and possible contaminants. Specimens 1, 2, 3, 5, and 7 were in a two-phase region of TiAl and Ti<sub>3</sub>Al. Specimens 15, 16, 17 and 18 were in a two-phase region of TiAl and TiAl<sub>3</sub>. Specimens 4, 6, 8, 9, 10, 11, 12, 13, and 14 were within the boundary of the TiAl phase. The maximum intensity  $d$ -values of the contaminants Ti<sub>3</sub>O<sub>5</sub> and Al<sub>2</sub>O<sub>3</sub> were identified in some films. Fig. 1 presents the TiAl phase boundary determined during this study as compared with the investigation by Troup.

The quenched specimens were also examined metallographically to determine their metallurgical structure.

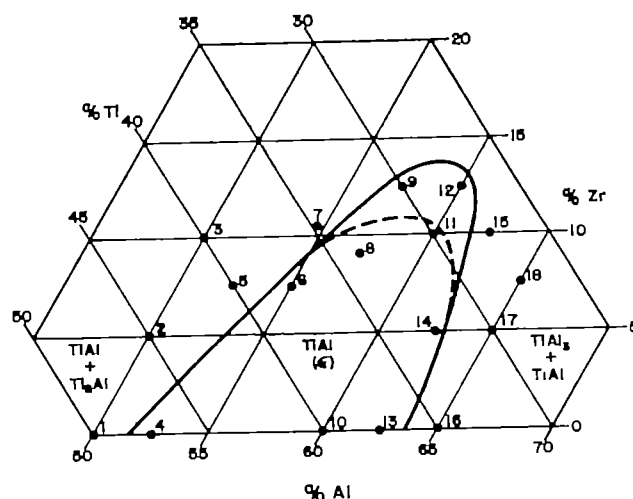


Fig. 1. Nominal compositions and TiAl phase boundary at 2,325° F; —, present work; ---, after Troup; solid circles represent compositions investigated

Two etching procedures were used to obtain photomicrographs of homogenized titanium-rich samples: (1) Kroll's etch (6 ml.  $\text{HNO}_3$ , 3 ml.  $\text{HF}$ , 91 ml.  $\text{H}_2\text{O}$ ) with 10-sec immersions, and (2) electrolytic etch (60 ml.  $\text{HClO}_4$ , 350 ml.  $\text{C}_2\text{H}_5\text{OC}_2\text{H}_4\text{OH}$ , 360 ml.  $\text{CH}_3\text{OH}$ ) using a stainless steel cathode for various times at 20 V and a current density of 780 m.amp/cm<sup>2</sup>. Aluminium-rich samples were etched with an etchant commonly used for aluminium (45 ml.  $\text{H}_2\text{O}$ , 9 ml.  $\text{HNO}_3$ , 9 ml.  $\text{HF}$ , 30 ml.  $\text{H}_3\text{PO}_4$ , 6 ml.  $\text{HC}_2\text{H}_3\text{O}_2$ , 5-10 ml.  $\text{H}_2\text{O}_2$ ) by immersion for 3-6 min. The metallographic data supported the results of the X-ray examination.

The  $c/a$  ratios of specimens in the TiAl single phase region were computed by the Myers and Davies<sup>4</sup> graphical method of precise lattice-parameter determination. The 20 values within the 60°-160° range were used for these computations. The binary limits of the TiAl phase were found to be from about 51.5 to 63.5 atomic per cent aluminium.

The average  $c/a$  ratio for the intermediate phase TiAl was computed to be 1.019. The  $c/a$  ratio was found to increase slightly as the zirconium substitutionally replaced the titanium within the TiAl lattice. The  $c/a$  ratio at the maximum solubility limit of zirconium within the single phase was 1.026. The  $c/a$  ratio did not approach unity with Zr additions as had been previously reported but actually was found to increase.

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## CHEMISTRY

### The System Dicalcium Silicate-Merwinite

It was reported earlier<sup>1</sup> that a new compound of the approximate molar composition  $(2\text{CaO} \cdot \text{SiO}_2)_{1.4}(\text{3CaO} \cdot \text{MgO} \cdot 2\text{SiO}_2)_{0.6}$  had been located in the system  $2\text{CaO} \cdot \text{SiO}_2 - 3\text{CaO} \cdot \text{MgO} \cdot 2\text{SiO}_2$ . This system, relevant to processes in the iron, slag and slag cement industries, has now been fully explored and the stable phase assemblages between the new compound and adjoining compounds have been established. The results are presented in the phase diagram given in Fig. 1.

The compositions studied were prepared from crushed quartz (99.95 per cent pure),  $\text{CaCO}_3$  (99.9 per cent pure) and  $\text{MgO}$  (99.9 per cent pure) by ignition at 1,250° C in platinum boats in a platinum/rhodium resistance furnace. The fineness of the reaction mixture is of importance to the formation of the new compound, and during a heat treatment of 10 days duration the mixtures were ground frequently to pass a 300 mesh sieve.

The liquidus, covering the temperature range 1,800° C-2,130° C, was established by means of special techniques of high-temperature microscopy<sup>1</sup> which showed dicalcium silicate to be the primary phase throughout the system.

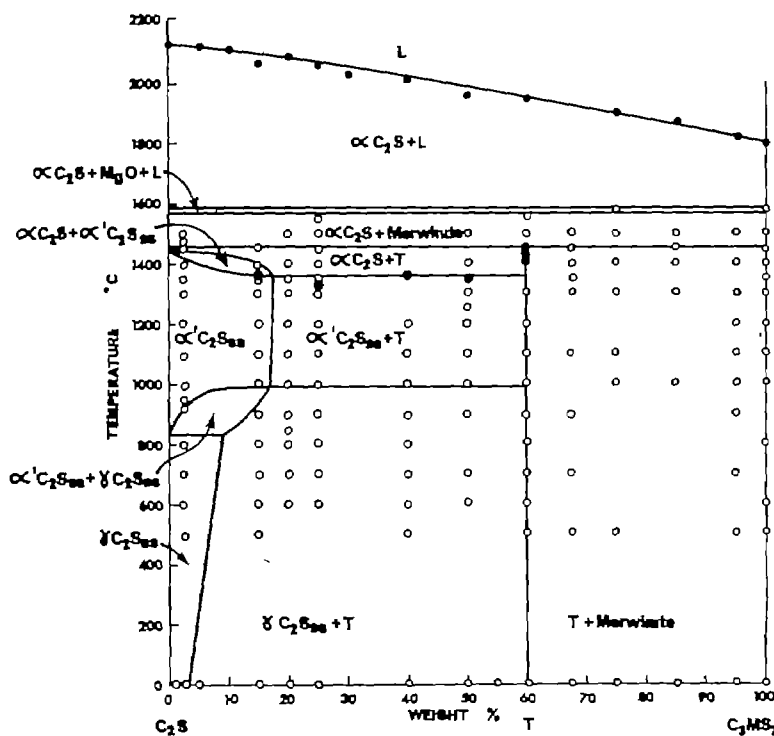


Fig. 1 The system  $2\text{CaO} \cdot \text{SiO}_2 - 3\text{CaO} \cdot \text{MgO} \cdot 2\text{SiO}_2$ . ●, Liquidus temperature; ○, points at which determinations were made with the high-temperature X-ray camera; T, new calcium magnesiosilicate; ss, solid solution; ⊕, temperature of  $\alpha \rightarrow \alpha'$  transition of  $2\text{CaO} \cdot \text{SiO}_2$  by optical determination; —, phase boundary; C,  $\text{CaO}$ , S,  $\text{SiO}_2$ ; L, liquid; M,  $\text{MgO}$ .

First liquid is formed throughout the system at 1,575° C, where merwinite decomposes to give  $\alpha 2\text{CaO} \cdot \text{SiO}_2$ , periclase ( $\text{MgO}$ ) and liquid. At 1,590° C,  $\text{MgO}$  dissolves leaving dicalcium silicate and liquid. The presence of periclase in the system within this narrow range of temperature was established by X-ray analysis of quenched products. These findings are compatible with the accepted view<sup>2</sup> of the melting behaviour of pure merwinite, and of the position of the invariant point<sup>3</sup> in the system  $\text{CaO}-\text{MgO}-\text{SiO}_2$  at which  $2\text{CaO} \cdot \text{SiO}_2$ , periclase and merwinite co-exist with liquid.

In the region in which periclase is present, the system  $2\text{CaO} \cdot \text{SiO}_2 - 3\text{CaO} \cdot \text{MgO} \cdot 2\text{SiO}_2$  ceases to be binary.

The subsolidus regions in which the system is binary were explored systematically, by means of high-temperature X-ray analysis<sup>1</sup> at ascending temperatures of preparations first annealed at 500° C.

The new calcium magnesiosilicate decomposes into  $\alpha 2\text{CaO} \cdot \text{SiO}_2$  and merwinite at 1,460° C. It was detected in the system over the wide range of compositions between 5 and 95 per cent of merwinite.

Magnesium ions enter into solid solution in the structures of the  $\gamma$ - and  $\alpha'$ -polymorphs of dicalcium silicate whereas the  $\alpha$ -polymorph does not appear to form a solid solution. The  $\beta$ -polymorph of dicalcium silicate does not form a part of the stable assemblages. The solubility of Mg ions in the  $\gamma$ -structure increases with temperature and reaches a maximum equivalent to approximately 9 per cent (weight per cent) merwinite at 830° C. The temperature of the inversion  $\gamma 2\text{CaO} \cdot \text{SiO}_2 \rightarrow \alpha' 2\text{CaO} \cdot \text{SiO}_2$  is raised from 830° for pure  $2\text{CaO} \cdot \text{SiO}_2$  to 990° C for the maximum  $\gamma 2\text{CaO} \cdot \text{SiO}_2$  solid solution. The temperature of the  $\alpha 2\text{CaO} \cdot \text{SiO}_2 \rightarrow \alpha' 2\text{CaO} \cdot \text{SiO}_2$  inversion is lowered to 1,360° C as the proportion of merwinite increases. The composition of the maximum solid solution of Mg ions with  $\alpha' 2\text{CaO} \cdot \text{SiO}_2$  occurs at 82.5 per cent  $2\text{CaO} \cdot \text{SiO}_2$ , 17.5 per cent  $3\text{CaO} \cdot \text{MgO} \cdot 2\text{SiO}_2$ . The  $\alpha \rightarrow \alpha'$  inversion is accompanied by changes in birefringence observable by high-temperature microscopy and the locus of the  $\alpha \rightarrow \alpha'$  inversion was determined by this method as well as by X-ray analysis. The new calcium magnesiosilicate



co-exists with the polymorphs of  $2\text{CaO} \cdot \text{SiO}_2$  and with merwinite, and throughout the system its X-ray diffraction pattern (already published<sup>1</sup>) remains unaltered in terms of reflexions present and their relative intensities. This is consistent with the view that it is a singular compound.

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### Electronic Spectrum of $\text{IO}^-$ in Solution

Compounds of iodine in the +1 oxidation state are unstable in aqueous solution, and for this reason their chemistry is still not clearly defined. Recording the absorption spectrum of  $\text{IO}^-$  should be helpful for a systematic investigation of this group. Conflicting reports have been published on this subject<sup>1,2</sup>. Apart from the instability of  $\text{IO}^-$ , the main difficulty arises from the overlap of its spectrum with that of other iodine compounds, in particular that of  $\text{I}_2^-$ . High alkalinity and low  $\text{I}^-$  concentration are desirable for suppressing the formation of  $\text{I}_2^-$  and other +1 iodine compounds as  $\text{HIO}$ ,  $\text{I}_2\text{OH}^-$ ,  $\text{I}_2\text{O}$  (ref. 1). Here we report the results of a spectrophotometric examination of iodine in highly concentrated alkali solutions.

Solutions of iodine in 4 N sodium hydroxide were prepared by rapidly adding 10 ml. of 4 N sodium hydroxide solution to weighed quantities of iodine in 'low actinic' flasks. The sodium hydroxide solution was previously kept at 25° C, its delivery time was relatively short and the flask was shaken to ensure the fast dissolution of iodine in the alkali. Some solution was then rapidly transferred to a 1-cm silica cell situated in the thermostated cell compartment (25° C) of a Hilger 'Uvispek' spectrophotometer. The absorbance was measured against a reference solution of 4 N sodium hydroxide. Care was taken not to expose the cell to light apart from the short periods required for the spectrophotometric measurements. This was done because the solutions appeared to be sensitive to light. Measuring the absorbance ( $A$ ) at a fixed wave-length  $\lambda$ ,  $A$  was found to decrease with time.

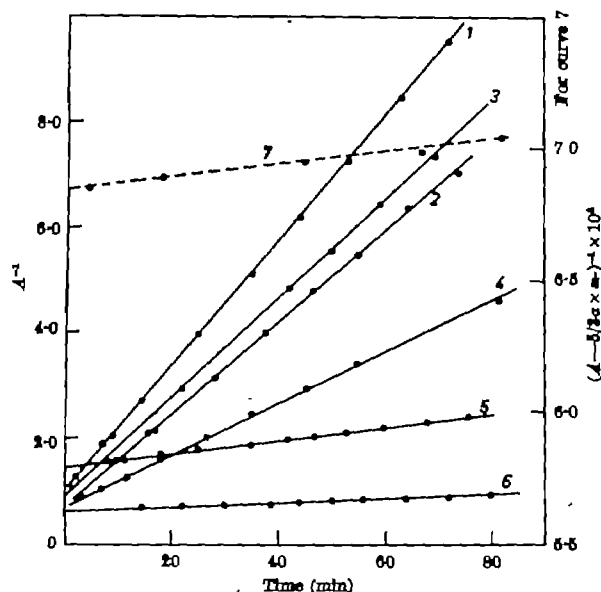


Fig. 1. Kinetic curves for the disproportionation of  $\text{IO}^-$  in 4 N sodium hydroxide. Curves 1, 2 and 3, ( $\text{I}_2$ ) =  $4.25 \times 10^{-4}$  M,  $\lambda$  = 290, 295 and 320 m $\mu$  respectively. Curve 4, ( $\text{I}_2$ ) =  $3.23 \times 10^{-4}$  M,  $\lambda$  = 300 m $\mu$ . Curves 5 and 6, ( $\text{I}_2$ ) =  $3.84 \times 10^{-4}$  M,  $\lambda$  = 280 and 260 m $\mu$ , respectively. Curve 7, ( $\text{I}_2$ ) =  $4.3 \times 10^{-4}$  M,  $\lambda$  = 248 m $\mu$  (before measuring  $A$  each sample was diluted with 4 N sodium hydroxide by a factor of 1/100).

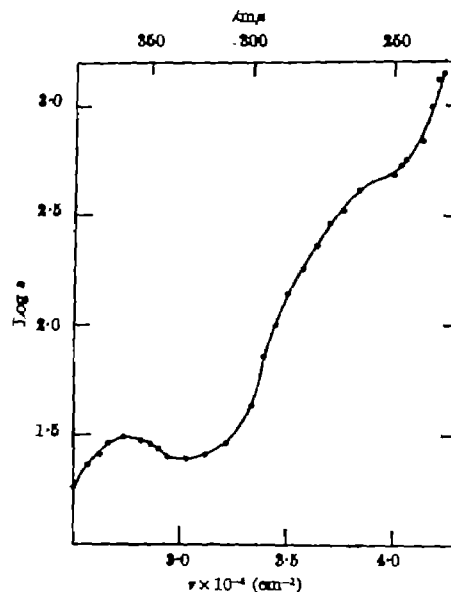
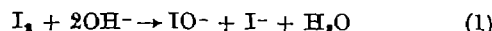
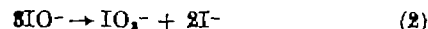


Fig. 2. The electronic spectrum of  $\text{IO}^-$  in solution

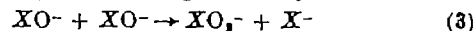
Fig. 1 shows plots of  $1/A$  against time at few wave-lengths, where  $\text{I}^-$  and  $\text{IO}_3^-$  scarcely absorb (curves 1-6). The light-absorbing species obviously decays by a second-order reaction. We identify this species with  $\text{IO}^-$ , which is produced by the relatively fast reaction:



and which undergoes the overall reaction:



The analogous reactions for  $\text{ClO}^-$  and  $\text{BrO}^-$  are also of second order<sup>3,4</sup>. The rate-determining step for the decomposition of the hypohalites is presumably:



From the relation  $A = \epsilon_{\text{IO}^-}(\text{IO}^-) + \epsilon_{\text{I}^-}(\text{I}^-) + \epsilon_{\text{IO}_3^-}(\text{IO}_3^-)$  and the stoichiometry of reactions (1) and (2) we derive:

$$[A - a(5/3 \epsilon_{\text{I}^-} + 1/3 \epsilon_{\text{IO}_3^-})]^{-1} = \frac{k}{\Delta \epsilon} t + \frac{1}{\Delta \epsilon a} \quad (4)$$

where  $a$  is the initial concentration of iodine,  $k$  is the apparent rate constant and  $\Delta \epsilon = \epsilon_{\text{IO}^-} - 2/3 \epsilon_{\text{I}^-} - 1/3 \epsilon_{\text{IO}_3^-}$ .  $\epsilon_{\text{I}^-}$  and  $\epsilon_{\text{IO}_3^-}$  were determined by measuring the spectra of  $\text{I}^-$  and  $\text{IO}_3^-$  in 4 N sodium hydroxide solution (in the spectral region examined the correction for  $\text{IO}_3^-$  could be ignored). To test the validity of equation (4) and determine  $\epsilon_{\text{IO}^-}$  in the region where  $\text{IO}^-$  and  $\text{I}^-$  strongly absorb, samples of  $\text{IO}^-$  solution were withdrawn at appropriate times from the thermostatic flask and rapidly diluted with 4 N sodium hydroxide by a factor of 100. The solutions thus obtained were relatively stable and their spectra were measured. Curve 7 (Fig. 1) exemplifies the validity of equation (4). From the intercepts and slopes of the lines the rate constant  $k$  and  $\epsilon_{\text{IO}^-}$  could be determined.  $k$  appears to be constant, irrespective of  $\lambda$ , at  $(4.0 \pm 0.4) \times 10^{-3} \text{ M}^{-1} \text{ sec}^{-1}$ . Fig. 2 shows the absorption spectrum of  $\text{IO}^-$ . Raising the concentration of  $\text{OH}^-$  to 10 N had scarcely any effect on the spectrum, but  $k$  decreased to some extent: its value was  $(2.7 \pm 0.2) \times 10^{-3} \text{ M}^{-1} \text{ sec}^{-1}$  at 6 N sodium hydroxide and remained nearly the same at 10 N. This may be due to the presence of little  $\text{HIO}$  in the 4 N sodium hydroxide solution. On the other hand, at  $(\text{OH}^-) = 2 \text{ N}$  the spectrum was considerably changed, probably due to the formation of other iodine compounds.

The weak absorption band of  $\text{IO}^-$  has already been reported but with somewhat different shape and intensity ( $\epsilon_{\text{max}} = 60$ ) (ref. 1). This is probably due to the relatively low  $\text{OH}^-$  concentration used in that work (1 N). More-

over, no kinetic investigation was carried out and the extrapolation to zero time was performed graphically on the  $A-t$  curves. The spectrum at  $\lambda < 280$  m $\mu$  was not recorded earlier. It displays two absorption bands, one appearing as a shoulder at about 255 m $\mu$  and the other is of high intensity with  $\lambda_{\text{max}} < 235$  m $\mu$ .

The low-intensity bands of ClO $^-$ , BrO $^-$  and IO $^-$  appear at 290 $^{\circ}$ , 331 $^{\circ}$  and 365 m $\mu$ , respectively, with  $\epsilon_{\text{max}}$ : 360, 326 and 81 M $^{-1}$  cm $^{-1}$ , respectively. The valency shell of the hypohalite ions is isoelectronic with that of the halogen molecules: (s $\sigma$ ) $^2$  (y $\sigma$ ) $^2$  (z $\sigma$ ) $^2$  (w $\pi$ ) $^4$  (v $\pi$ ) $^4$ , in the molecular orbital treatment. The low-energy band is probably due to the excitation  $^3J_g^+ \leftarrow ^1\Sigma^+$  which involves the transfer of the antibonding v $\pi$  electron to the more strongly antibonding w $\sigma$  orbital. As in the case of the halogen molecules this excitation is likely to bring about the dissociation of the hypohalite ions. The dissociation energy in the ground state  $D^{\circ}(\text{XO}^-)$  can be estimated from that of the XO radical, since XO $^-$  differs from XO by having one more antibonding v $\pi$  electron. This reduces the bond order from 3/2 to 1, that is,  $D^{\circ}(\text{XO}^-) \sim 2/3 D^{\circ}(\text{XO})$ . The estimated values for ClO $^-$ , BrO $^-$  and IO $^-$  are thus: 42, 37 and 28 kcal, respectively. The ground state probably correlates with O( $^3P_{2/2}$ ) and X( $^3P_{3/2}$ ), whereas the  $^1\Pi$  excited state may decompose to yield O( $^3P_2$ ) and X( $^1S_0$ ). If this is correct, then the corresponding potential curves should cross, since the electron affinity  $E(\text{O})$  is smaller than  $E(\text{X})$ . High kinetic energies are thus imparted to the dissociation products formed by irradiating the hypohalite ions in their low intensity bands. This may explain their high photochemical sensitivity\*. The subsequent reaction is probably:  $\text{O} + \text{XO}^- \rightarrow \text{XO}_2^-$ .

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### An Effect of Ultrasonic Standing Waves on Electrodeposition

In the course of an investigation carried out here into the possibility of producing fine line patterns by using ultrasonic standing waves to control physical or chemical processes, the following electrochemical effect was observed.

Ultrasonic standing waves were set up in the electrolyte of a small plating cell, using a barium titanate disk transducer and, 2.5 cm from it, a glass block as a reflector. In the gap between the reflector and transducer was a sheet nickel cathode, 1.5 cm  $\times$  1 cm, arranged so that the standing waves were perpendicular to its surface. Parallel to the cathode and about 5 cm from it was a copper anode 2.5 cm $^2$ . When small amounts of copper were plated on the cathode by passing about 8 m.amp through the cell, it was found that the deposit was formed in regular lines corresponding to the standing waves in the electrolyte. Heavier deposition caused the pattern to disappear. The best electrolyte found was 1 g/l. copper sulphate pentahydrate; the addition of complexing agents had no observable effect. Patterning was found at 2, 6 and 10 Mc/s, giving 27, 80 and 134 lines/cm, but becoming very faint at the highest frequency.

No reference has yet been found to this effect in the literature, but it may be related to the Debye potentials observed by Yeager *et al.*<sup>1</sup>. These workers found and measured the variation in the potential of a small platinum

electrode as it was moved through standing waves at 265 kc/s in various electrolytes. This was in the region of 10  $\mu$ V.

Unsuccessful attempts have been made to measure the difference in current passed by two wire cathodes spaced a quarter of a wave-length apart on the standing wave field; but in view of the very small voltages found by Yeager *et al.*, it is to be expected that more sensitive experimental techniques would be needed than were available.

It is not known whether, at the ultrasonic power and frequencies used, the Debye voltage is sufficient to cause the variation in plating thickness observed, or whether some other mechanism is responsible.

This work is the subject of a provisional patent.

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### Structural Differences between the Alkali Imidodisulphates

It has long been known from goniometric measurements<sup>1</sup> that there are morphological similarities between ammonium and potassium imidodisulphates (or iminodisulphonates), NH(SO $_3$ NH $_2$ ) $_2$  and NH(SO $_3$ K) $_2$ . The crystal structure of the potassium salt<sup>2</sup>, which has recently been refined<sup>3</sup>, conforms accurately to the space-group C 2/c. Preliminary X-ray examination of single crystals at room temperature revealed<sup>4</sup> that ammonium imidodisulphate differs somewhat in structure from the potassium salt in that its true unit cell has a *b*-axis three times as long ( $3 \times 7.74 = 23.2$  Å) as a pseudo-cell isomorphous with the potassium salt. Rubidium imidodisulphate appears to have a five-fold superlattice since, on *b*-axis photographs, a few reflexions can be seen on up to four additional weak layer-lines between adjacent strong layer-lines. In both salts, reflexions on the extra layer-lines are of very low intensity. We have investigated these structural irregularities by means of X-ray diffraction and broad-line nuclear-magnetic-resonance spectroscopy.

Negative piezo-electric tests<sup>5</sup> imply that at room temperature the structure of neither ammonium nor rubidium imidodisulphate departs significantly from centrosymmetry. From X-ray diffraction examination, the pseudocell [110] axis of ammonium imidodisulphate shows at 300° K a true repeat distance of  $3 \times 7.50 = 22.5$  Å; the  $(3n+1)$  and  $(3n+2)$ th layer-lines are always very weak (where  $n = 0, 1, 2, \dots$  is the layer-line number of the pseudocell). This is consistent with the triple-cell picture in which the true cell is C-face centred. At 80° K, the same axis exhibits only one weak layer-line between each pair of strong ones and the axial length is  $2 \times 7.49 = 15.0$  Å. This suggests that at low temperatures there is a transition from the triple unit cell, which exists at room temperature, to a cell having dimensions comparable with those of the potassium salt but in which true C-centring is replaced by pseudo C-centring.

From an approximate least squares X-ray refinement<sup>6</sup> of the ammonium imidodisulphate structure, carried out on the pseudocell reflexions only, and with the assumption of isomorphism with potassium imidodisulphate, it appears that the structure in the pseudocells is closely related to that of the potassium salt. Under similar conditions, the rubidium imidodisulphate structure did not refine satisfactorily. In this salt, the few exceedingly weak extra reflexions on *b*-axis X-ray photographs at room temperature are confined to the  $(5n \pm 1)$ th layer-lines ( $n = 0, 1, 2, \dots$ ), where the true axis length is  $5 \times 7.68 = 38.4$  Å; low-temperature photographs suggest that the quintupled cell persists down to 80° K. On room tempera-

ture rubidium salt rotation photographs about the  $5 \times 7.54 = 37.7 \text{ \AA}$  long [110] axis of the pseudo-cell, regarded as [510] of the true cell, a few extra reflexions were detected on the  $(5n+2)$  and  $(5n+3)$  layer-lines but none on the  $(5n+1)$  or  $(5n+4)$  layers. Neither at  $80^\circ$  nor at  $300^\circ \text{ K}$  were any such pronounced intensity differences apparent in ammonium imidodisulphate between one weak layer-line and another.

Attempts to prepare thallous imidodisulphate by double decomposition of the fairly soluble (41 g in 100 g water at  $20^\circ \text{ C}$ ) ammonium imidodisulphate were unsuccessful; possibly the Capetan<sup>8</sup> method, via the sulphamate, or a method analogous to that due to Baumgarten<sup>9</sup> for the preparation of ammonium and potassium imidodisulphates, would be better. Morphological evidence<sup>10</sup> indicates that the ammonium, potassium, and thallium salts of methionic acid,  $\text{CH}_3(\text{SO}_3\text{H})_2$ , which is iso-electronic with imidodisulphuric acid, are isomorphous with the imidodisulphates. While potassium methionate<sup>11</sup> is isostructural with potassium imidodisulphate, no superlattice structure analogous to that in ammonium imidodisulphate has been seen in ammonium methionate<sup>4</sup>.

Proton magnetic resonance spectra of powdered ammonium, potassium, and rubidium imidodisulphates were recorded at  $28.6 \text{ Mc/s}$  and with an  $H_1$  field of about 2 mG on a broad-line proton magnetic resonance apparatus<sup>12</sup> based on the Robinson<sup>13</sup> detector. Despite the small structural change revealed by X-rays, there was no significant change in the proton magnetic resonance line-width,  $\Delta H_{\text{m.s.l.}}$ , of  $4.8 \pm 0.5 \text{ G}$  when ammonium imidodisulphate was cooled from  $283^\circ$  to  $90^\circ \text{ K}$ . For the rubidium salt, there was no change between  $268$  and  $80^\circ \text{ K}$  in the line-width of  $2.6 \pm 0.5 \text{ G}$ . For potassium imidodisulphate, on the other hand, there was a possibly significant broadening in  $\Delta H_{\text{m.s.l.}}$  from  $2.3 \pm 0.5 \text{ G}$  at  $293^\circ$  to  $3.3 \pm 0.5 \text{ G}$  at  $80^\circ \text{ K}$ .

We thank Dr. W. G. Perdok for carrying out and communicating the piezo-electric measurements, Mr. R. Steadman for making X-ray equipment available, and Dr. M. R. Truter for help with low-temperature X-ray measurements.

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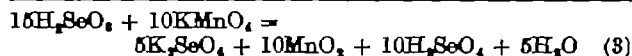
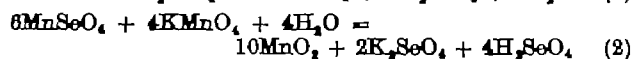
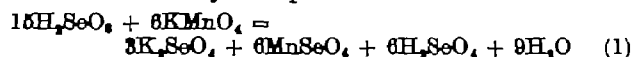
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### Reaction of Selenious Acid with Potassium Permanganate

ALTHOUGH the reaction of selenious acid with potassium permanganate has been used in estimating selenium<sup>1-3</sup> it has not been investigated in detail. According to Charlot<sup>4</sup> selenious acid reduces permanganate to manganese dioxide, which separates from the solution. But it is observed that when potassium permanganate is added to a large excess of selenious acid solution, the pink colour due to permanganate is discharged and the formation of precipitates of  $\text{MnO}_2$  does not take place. If selenious acid is not in large excess, only the precipitates of  $\text{MnO}_2$  appear shortly after the addition of permanganate. This

suggests that selenious acid first reduces permanganate ion to manganous ion which reacts with permanganate to form manganese dioxide. In the present investigation the reaction between selenious acid and potassium permanganate has been studied by physico-chemical methods.

The chemical analyses of the solution and the solid obtained by mixing solutions of selenious acid and potassium permanganate in different proportions reveal that, when selenious acid and permanganate are mixed in the molar ratio of 5:2, only a portion of selenious acid is oxidized to selenic acid. Complete oxidation of selenious acid occurs within 40 min if the molar ratio of  $\text{H}_2\text{SeO}_3$  to  $\text{KMnO}_4$  is equal to or less than 3:2. The net reaction between selenious acid and potassium permanganate in acidic medium may be represented as:



The reduction of permanganate by reaction (2) may be assumed to be negligible immediately after the addition of permanganate to selenious acid solution. Under such conditions the rate of oxidation of selenious acid is equal to  $5/2$  times the rate of reduction of permanganate which is directly related to the rate of disappearance of pink colour due to permanganate. The reduction of permanganate by selenious acid in the presence of sulphuric or phosphoric acid has been followed by measuring absorbance due to permanganate at different times (up to 90 sec) before the appearance of precipitate. The reaction (1) leading to the oxidation of selenious acid is found to be first order with respect to  $\text{H}_2\text{SeO}_3$ , first order with respect to permanganate, and zero order with respect to hydrogen ion. The rate law for the oxidation of selenious acid is given by the expression:

$$\frac{-d(\text{H}_2\text{SeO}_3)}{dt} = K(\text{H}_2\text{SeO}_3)(\text{MnO}_4^-)$$

The value of the rate constant  $K$ , calculated from the molar extinction coefficient of permanganate and optical density data taken with equimolar solutions of potassium permanganate and selenious acid (in  $0.6 \text{ N H}_2\text{SO}_4$ ) in the range  $(4.445 - 26.67 \times 10^{-4} \text{ N})$  is  $26.70 \pm 0.7 \text{ mol}^{-1} \text{ litre sec}^{-1}$  at  $26 \pm 0.2^\circ \text{ C}$ . The energy of activation evaluated from the values of  $K$  at different temperatures between  $20^\circ$  and  $32^\circ \text{ C}$ , is found to be  $20 \pm 0.5 \text{ kcal}$ .

Absorption measurements have been taken with the Spectronic '20' and the Beckman model 'D' spectrophotometer at  $520 \text{ m}\mu$  using a cell of  $1 \text{ cm}$  light path. We thank Dr. G. M. Naber and Dr. B. N. Ghosh, of the University of Bombay, for allowing us to use these instruments.

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### Some Properties of Silicon Difluoride

GASEOUS silicon difluoride has been observed in spectral studies<sup>1</sup>, and polymers of composition  $(\text{SiF}_2)_n$  were prepared by Schmeisser<sup>2</sup> from magnesium and dibromodifluorasilane. However, a convenient preparation of both silicon difluoride gas and polymer was first described by

Pease<sup>3</sup> in 1958, in which silicon tetrafluoride was passed at low pressures over silicon at temperatures above 1,050° C. Pease showed that gaseous silicon difluoride would polymerize at low temperatures to form a rubbery polymer, and that it could be reacted with halogens or unsaturated organic molecules to give compounds containing SiF<sub>3</sub> groups.

We have extended these investigations and have found that both SiF<sub>2</sub> gas and its polymer (SiF<sub>2</sub>)<sub>n</sub> have some quite unusual properties.

In our experiments silicon tetrafluoride was passed over granular silicon at 1,150° C and the pressure after the hot zone was maintained at 0.1–1.0 mm by pumping. A mass spectrometric study showed that the gas emerging from the furnace contained only SiF<sub>2</sub> and SiF<sub>4</sub>.

Cooling the gas mixture to –196° C gave a red condensate which became a white rubbery solid at room temperature. We found that this polymer could be destructively distilled at 200°–350° C to give all the perfluorosilanes Si<sub>n</sub>F<sub>2n+2</sub> from SiF<sub>4</sub> through Si<sub>10</sub>F<sub>22</sub> (ref. 4). The compounds SiF<sub>4</sub>, Si<sub>2</sub>F<sub>6</sub>, Si<sub>3</sub>F<sub>8</sub>, and Si<sub>4</sub>F<sub>10</sub> have been isolated from the pyrolysis products, other compounds being identified by their mass spectra. Perfluorotrisilane and higher perfluorosilanes have not previously been described. They have similar boiling points to the corresponding silanes, but much higher melting points, for example, Si<sub>4</sub>F<sub>10</sub>, m. pt. 67° C, b. pt. 85° C; Si<sub>4</sub>H<sub>10</sub>, m. pt. –88° C (ref. 5), b. pt. 107° C (ref. 6).

The solid polymer reacts with 20 per cent aqueous hydrofluoric acid to give a mixture of silanes from SiH<sub>4</sub> to at least Si<sub>10</sub>H<sub>22</sub>. The yields are 25–35 per cent theoretical and the relative abundances of the species are similar to those obtained from silicon monoxide and HF (ref. 7) although less monosilane is formed. The polymer also reacts with amines and ethers to give complex mixtures of products, some of which retain long silicon chains.

Silicon difluoride is rather stable in the gas phase, apparently decaying only by collisions with the walls of the containing vessel. For example the pressure of SiF<sub>2</sub> fell from 0.1 mm to 0.05 mm in 2.5 min in a 5-l. flask at 25° C. No gaseous polymers were detected by mass spectrometry, but a polymer film appeared on the walls of the flask. Similarly, SiF<sub>4</sub> did not react in the gas phase with oxygen, boron trifluoride, phosphorus trifluoride or carbon monoxide, although in the case of oxygen the rate of decay increased greatly due to reaction on the walls of the flask. However, if silicon difluoride was condensed with any of these four gases at –196° C, reaction occurred and new products were obtained on warming to room temperature.

Under these conditions boron trifluoride formed a white silicon–boron–fluorine polymer, and two new compounds SiF<sub>2</sub>SiF<sub>2</sub>BF<sub>2</sub> and SiF<sub>2</sub>SiF<sub>2</sub>SiF<sub>2</sub>BF<sub>2</sub> (ref. 8) have been isolated. With phosphorus trifluoride the polymer was red and a mixture of volatile compounds, presumably of type Si<sub>n</sub>F<sub>2n+1</sub>PF<sub>3</sub>, was obtained which readily decomposed to perfluorosilanes and black PF polymers. Oxygen reacted with the silicon difluoride to give a clear liquid polymer mixed with a little (SiF<sub>2</sub>)<sub>n</sub>, and carbon monoxide gave a white, brittle solid polymer. Heating either the oxygen or carbon monoxide polymers gave a whole range of silicon oxyfluorides not previously described.

Thus, condensing silicon difluoride gas with another compound causes it to exhibit a far greater level of reactivity than is shown by the uncondensed gas or the polymer (SiF<sub>2</sub>)<sub>n</sub>. Preliminary work on the infra-red spectrum of SiF<sub>2</sub> trapped in an argon matrix suggests that at 20° K there is already some polymerization of the SiF<sub>2</sub> and that this is more extensive at 40° K. The spectrum is similar to that obtained by Milligan<sup>9</sup> for the dimerization of CF<sub>2</sub> to C<sub>2</sub>F<sub>4</sub> in a matrix, and is different from that observed for the (SiF<sub>2</sub>)<sub>n</sub> polymer at room temperature and the perfluorosilanes. Hence a type of unsaturation may exist in the low temperature con-

densed silicon difluoride polymers which could cause the observed high reactivity.

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## BIOCHEMISTRY

### Stimulation of Steroid C-11 $\beta$ Hydroxylation in Adrenal Mitochondria by Cyclic 3',5'-Adenosine Monophosphate

ADENOSINE-3',5'-monophosphate (cyclic 3',5'-AMP) has been shown to stimulate steroid C-11 $\beta$  hydroxylations in rat adrenal homogenates<sup>1</sup> as well as the production of corticosterone from endogenous precursors in surviving rat adrenal sections<sup>2</sup>. In the latter instance, the effect of the cyclic nucleotide appeared to be related to enhancement of  $\alpha$ -glucan phosphorylase activity<sup>3</sup>, consequent NADPH production, and, possibly, to increased provision of endogenous steroid substrate<sup>4</sup>. In homogenates, however, stimulation of steroid C-11 $\beta$  hydroxylation did not seem to be dependent on glycogen phosphorylation, NADPH generation, or the availability of endogenous precursors<sup>5</sup>. Since steroid C-11 $\beta$  hydroxylase activity is associated with adrenal mitochondria<sup>6</sup>, experiments were undertaken to determine whether cyclic 3',5'-AMP was effective in isolated mitochondria. The data presented here reveal that the cyclic nucleotide was capable of stimulating C-11 $\beta$  hydroxylation of added progesterone and 11-deoxycorticosterone in purified adrenal mitochondria fortified with NADPH.

Adrenal glands were obtained under light 'Nambutal' (sodium pentobarbital) anaesthesia from young male rats (130–150 g) of an inbred Sprague-Dawley strain. The glands were immediately placed in a Petri dish kept on ice, dissected free of fat and connective tissue, and weighed. All subsequent procedures involved in the isolation of mitochondria were conducted at 0°–4°. Approximately 500 mg adrenal tissue were homogenized for 0.5 min in 8 ml. of 0.25 M sucrose using an all-glass homogenizer with a loose-fitting pestle. The sucrose was adjusted to pH 7.1 by addition of 40 mM *tris*-hydrochloride buffer (pH 7.4) to a final concentration of 0.8 mM. The suspension was then centrifuged at 700g for 10 min. Centrifugations were carried out in plastic tubes in a Sorvall refrigerated centrifuge. The supernatant was carefully removed with a dropping pipette and recentrifuged at 5,000g for 10 min. The supernatant and loosely-packed material from this centrifugation were discarded. The pellet was resuspended in the *tris*-sucrose medium with 8 strokes of a loose-fitting 'Teflon' pestle and centrifuged again at 5,000g for 10 min. The supernatant and loosely-packed material were again discarded. Thus far, the isolation procedure

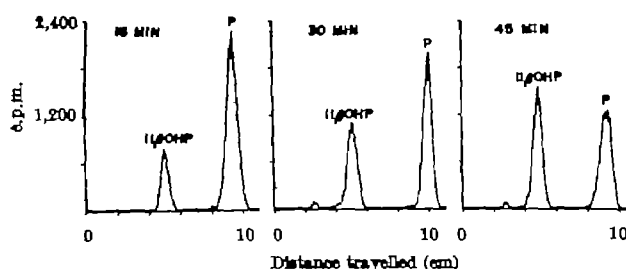


Fig. 1. Radioactivity scanning patterns of thin-layer chromatograms, showing conversion of (4-<sup>14</sup>O)-progesterone to 11 $\beta$ -hydroxyprogesterone during incubation with rat adrenal mitochondria for 15–45 min. The incubation medium (3 ml) contained 0.2  $\mu$ mole progesterone (0.06  $\mu$ Ci), 1  $\mu$ mole NADPH, and mitochondrial suspension equivalent to 70–80 mg of adrenal tissue. Chromatographic origin is represented by 0 on abscissa. P, progesterone; 11 $\beta$ OHP, 11 $\beta$ -hydroxyprogesterone.

was similar to that described by Brownie and Grant<sup>7</sup>. The pellet was then resuspended in *tris*-sucrose and centrifuged at 10,000*g* for 10 min and the supernatant was removed as described before. Electron microscopy revealed that this pellet was composed mainly of intact mitochondria. Finally, the pellet obtained at 10,000*g* was resuspended in a buffer composed of 13 parts of 0.154 M NaHCO<sub>3</sub> and 37 parts of 0.154 M KCl. Protein was measured in aliquots of the mitochondrial suspension by the method of Lowry, Rosebrough, Farr, and Randall<sup>8</sup>.

The incubation mixture contained 0.2 ml. of mitochondrial suspension, 1  $\mu$ mole NaNADPH, 0.2  $\mu$ mole (0.05  $\mu$ Ci) of (4-<sup>14</sup>O)-progesterone or (4-<sup>14</sup>C)-11-deoxycorticosterone in 0.018 ml. 95 per cent ethanol, 48  $\mu$ moles NaHCO<sub>3</sub>, and other additives, made up to a final volume of 2 ml. with 0.154 M KCl. The amount of mitochondrial suspension added to each sample was equivalent to approximately 3–4 mg wet wt. mitochondria (0.5 mg mitochondrial protein), derived from 75 mg adrenal tissue. Methods of incubation, extraction, chromatography on thin-layer plates, measurement of radioactivity, and identification of the separated steroids have been described earlier<sup>1,9</sup>. Recovery of radioactive steroids carried through the extraction procedure exceeded 95 per cent.

The adrenal mitochondrial preparations were active in *O*-11 $\beta$  hydroxylations only when NADPH or NADP, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were added. In incubation periods varying in length from 15 to 45 min, the major metabolite formed from (4-<sup>14</sup>C)-progesterone was 11 $\beta$ -hydroxyprogesterone (Fig. 1). After 80 min, very small amounts of a more polar substance, which migrated more rapidly than corticosterone, were also noted. This may have been a *C*-18 hydroxylated product<sup>10</sup>. The absence of 11-deoxycorticosterone and corticosterone indicated that significant contamination with microsomal *O*-21 hydroxylase activity had not occurred. Cyclic 3',5'-AMP consistently and rapidly enhanced *O*-11 $\beta$  hydroxylation of progesterone in adrenal mitochondria. The conversion of progesterone to 11 $\beta$ -hydroxyprogesterone was stimulated to a greater degree by the cyclic nucleotide (2 mM) after incubation for 15–30 min than after 45 min, averaging 30 per cent in the former instances and only about 10 per cent in the latter (Table 1).

Since progesterone has been reported to inhibit *O*-11 $\beta$  hydroxylase activity in adrenal mitochondrial preparations<sup>11</sup>, the utilization of 11-deoxycorticosterone was also investigated. The major steroid formed was the *O*-11 $\beta$  hydroxylated product, corticosterone (Fig. 2, control). Subsequent experiments revealed that a portion of the radioactive material included under the corticosterone peak was 18-hydroxy-11-deoxycorticosterone. At a substrate concentration of 100  $\mu$ M and with an incubation period of 15 min, the basal rates of hydroxylation were similar for progesterone and 11-deoxycorticosterone; that is, about 20  $\mu$ moles  $\times 10^{-3}$  steroid converted per 100 mg adrenal tissue/h. However, the

Table 1. EFFECT OF CYCLIC 3',5'-AMP ON *O*-11 $\beta$  HYDROXYLATION OF (4-<sup>14</sup>O)-PROGESTERONE IN RAT ADRENAL MITOCHONDRIA

Incubation time (min)	Control 11 $\beta$ -hydroxyprogesterone formed ( $\mu$ moles $\times 10^3$ /100 mg adrenal)	Cyclic 3',5'-AMP 11 $\beta$ -hydroxyprogesterone formed ( $\mu$ moles $\times 10^3$ /100 mg adrenal)	Per cent increase	P value
15	3.04 $\pm$ 0.24	5.18 $\pm$ 0.29	21	< 0.01
15	5.22 $\pm$ 0.24	6.88 $\pm$ 0.27	22	< 0.01
30	8.27 $\pm$ 0.22	15.20 $\pm$ 1.10	33	—
30	9.64 $\pm$ 0.50	15.50 $\pm$ 1.10	27	—
45	14.49 $\pm$ 0.79	16.28 $\pm$ 0.22	11	0.05

The incubation medium (3 ml.) contained 0.2  $\mu$ mole progesterone (0.06  $\mu$ Ci), 1  $\mu$ mole NADPH, and 4  $\mu$ moles cyclic 3',5'-AMP (where indicated) in the presence of mitochondria from 70–80 mg adrenal tissue. The means  $\pm$  S.E.M. are shown where there were 4 samples, otherwise individual values are given. In the former instances, the P values are for the differences between control values and those obtained with the cyclic nucleotide.

stimulatory effect of cyclic 3',5'-AMP was greater with 11-deoxycorticosterone, varying from 50 to 75 per cent above the control level during a 15-min incubation period (Figs. 2 and 3). Furthermore, significant enhancement of corticosterone formation from 11-deoxycorticosterone could be produced with relatively low levels (0.5 mM) of the cyclic nucleotide (Fig. 3). This concentration appears to be lower than that required for stimulation of corticosteroid production in intact adrenal cells from the rat<sup>2</sup>.

The specificity of action of cyclic 3',5'-AMP has not as yet been thoroughly examined. The available data indicate that 5'-AMP also has a stimulatory effect on

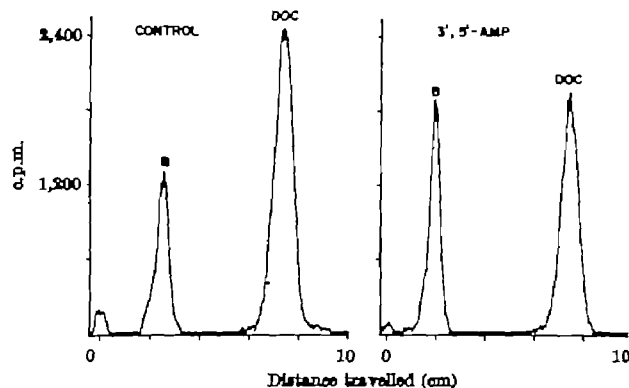


Fig. 2. Radioactivity scanning patterns of thin-layer chromatograms showing conversion of (4-<sup>14</sup>O)-11-deoxycorticosterone to corticosterone during incubation with rat adrenal mitochondria for 15 min. The incubation medium (3 ml.) contained 0.2  $\mu$ mole 11-deoxycorticosterone (0.06  $\mu$ Ci), 1  $\mu$ mole NADPH, and mitochondrial suspension equivalent to 80 mg of adrenal tissue; 4  $\mu$ moles of cyclic 3',5'-AMP were added where indicated. DOC, 11-deoxycorticosterone; B, corticosterone.

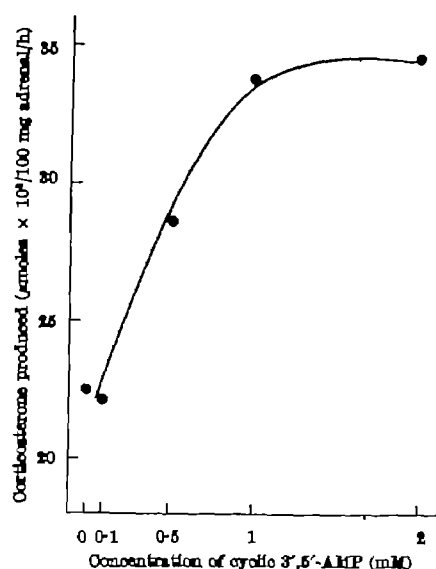


Fig. 3. Influence of varying concentrations of cyclic 3',5'-AMP on conversion of (4-<sup>14</sup>O)-11-deoxycorticosterone to corticosterone during incubation with rat adrenal mitochondria for 15 min. Each point represents the average of 3 values. See legend to Fig. 2 for additional explanations.

steroid  $C-11\beta$  hydroxylations in adrenal mitochondria. Stimulation could not be produced with 2 mM 5'-ATP. The latter nucleotide has recently been shown to inhibit the stimulatory effect of mitochondrial swelling agents on pregnenolone synthesis from endogenous sources in adrenal mitochondria<sup>18</sup>.

The mechanism whereby cyclic 3',5'-AMP enhances steroid  $C-11\beta$  hydroxylations in adrenal mitochondria is unknown. Stimulation of NADPH generation has been suggested as a basis for the steroidogenic action of the cyclic nucleotide in the intact adrenal cortical cell<sup>8</sup>. However, steroid hydroxylation was not observed in adrenal mitochondria or homogenates<sup>8</sup> in the absence of NADPH or an NADPH-generating system, even when cyclic 3',5'-AMP was added in high concentration (4 mM). Possible explanations for the action of cyclic 3',5'-AMP in adrenal broken cell preparations include stimulation of the transport of steroid or co-factor across the mitochondrial membrane and direct activation of the steroid  $C-11\beta$  hydroxylase enzyme system. Effects of cyclic 3',5'-AMP on biological transport and on enzyme activities in other tissues have been described<sup>19</sup>. The relationship of the stimulation by cyclic 3',5'-AMP of  $C-11\beta$  hydroxylations in adrenal mitochondria and homogenates to the action of the cyclic nucleotide in the intact cell remains to be explored.

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### Heterotopic Calcification of Human Nail and Hair

THE deposition of calcium salts in tissue other than osteoid, known as heterotopic calcification, may conveniently be divided into two distinct types: dystrophic calcification of localized areas which occurs in dead or degenerate tissue following injury, for example, mosquito oostegons, and metastatic calcification of apparently normal tissue due to a generalized upset of calcium and phosphorus metabolism and the draining of calcium from the bones as occurs in hyperparathyroidism.

In view of the discovery during the past few years that a wide variety of hard keratins may exist in a calcified form<sup>1-4</sup>, an examination has been made of human nail and hair for evidence of heterotopic calcification.

Using as a test for calcification the presence of calcium orthophosphate spacings in the X-ray diffraction pattern of keratin, 110 specimens of finger nail and 40 of hair

were examined for calcification, 50 of the nails and all the hair, with one exception, were selected from geriatric patients and the remaining nail specimens from persons who could be divided roughly into three categories: (a) healthy males and females 5-45 years of age whose nails appeared normal; (b) persons of category (a) but whose nails were abnormal in appearance, for example, in shape or contained white flecks, or were mechanically weak; (c) persons of category (a) but whose nail or nail-bed had suffered injury.

Although 16 per cent of the nails from geriatric patients showed strong calcium phosphate reflexions, these reflexions were absent in the X-ray diffraction patterns of all the hair samples. A beard hair, however, which emerged at intervals from an inflamed follicle (chin area of healthy male aged 33 years) showed strong calcium phosphate reflexions with considerable orientation (Fig. 1).

Nails from categories (a) and (b) showed no calcification, although sometimes very poor keratin reflexions were observed in (b). An examination of white flecks in nails, which are often associated with low serum albumin, supported the view that they are not due to calcification<sup>5</sup>. In category (c), three cases of calcification due to injury were found, an outstanding example being studied in greater detail. The nail (female aged 4 years), which, together with the bed, had been injured by crushing, was allowed to grow until it dropped off. The appearance of the nail at the point of discontinuity of growth was quite distinct, as is shown in the electron micrograph (Fig. 2). The X-ray diffraction photograph of this material showed very strong calcium phosphate reflexions, which were absent in undamaged nail of the same subject, and an electron diffraction pattern of calcium phosphate also was obtained. The amount of calcified keratin present was sufficient to enable a micro calcium determination to be made with a flame photometer, the results being as follows (per cent calcium): Normal nail of subject, 0.20; nail at point of severance, 7.2; control 1 (male aged 33 years), 0.26; control 2 (female aged 7 years), 0.35.

It is generally assumed that the calcium salt responsible for calcification is hydroxyapatite, and the percentage of calcium in the damaged nail corresponds to 18 per cent of this substance.

It has been established, therefore, that dystrophic calcification of nail and hair can occur as the result of damage to the nail bed or hair follicle. Calcification on ageing, however, does not occur in hair but is limited to

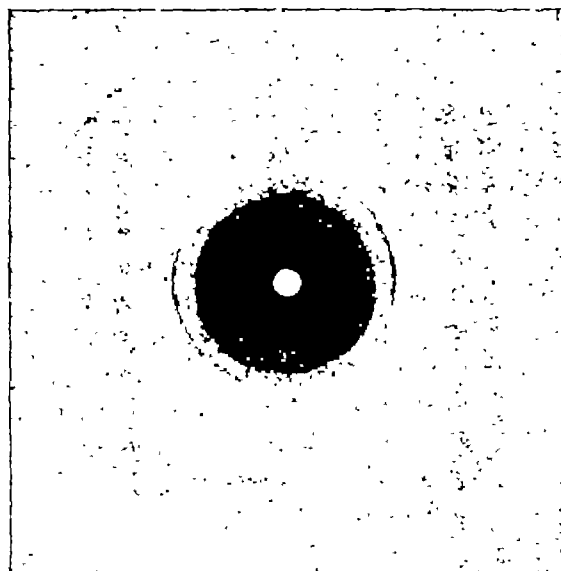


Fig. 1. X-ray diffraction pattern of hair from a beard. Copper  $K\alpha$  radiation. Distance 4.0 cm



Fig. 2. Electron micrograph of damaged nail showing calcified material on the right ( $\times 6,150$ )

nails, and, in view of the fact that bones can decalcify with age, could possibly be metastatic in nature.

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### Metabolic Activity of Monolayer Tissue Cultures of Human Thyroid

STUDIES of iodine metabolism by dispersed human thyroid cells have been reported by Pulvertaft *et al.*<sup>1</sup> Pastan<sup>2</sup> and Tong *et al.*<sup>3</sup> have reported similar studies on calf and sheep thyroid cells, respectively. The latter authors' findings suggest that the follicular structure of intact thyroid tissue is not indispensable for the formation of thyroid hormones. Kerkof *et al.*<sup>4</sup> have reported their interesting observation of the re-organization of the monolayers of thyroid cells in tissue culture into a structural pattern resembling cross-sections of intact thyroid tissue when thyroid stimulating hormone was included in the medium.

Initially, the present work was concerned with the question of the indispensability of the follicular organization of the thyroid cells, not only with regard to the formation of thyroid hormones, but also with the formation in the culture medium of larger molecules (peptide or protein) which show the immunological activity of thyroglobulin in the tanned red-cell agglutination inhibition test. At the same time, the specificity of such activities was tested by using an established cell-line as a control (ERK cells). Coombe *et al.*<sup>5</sup> have shown that the ERK cells are of human origin.

Thyroid glands were manipulated within a few hours after surgical removal. The cells were dispersed by trypsin according to the method described by Pulvertaft *et al.*<sup>1</sup> with minor modifications. The cells were cultured in 'Pyrex' babies' bottles using the following media with and without the addition of 10 per cent calf serum (containing 11.6  $\mu$ g iodine/100 ml.): (a) TCM-199 (Glaxo), which is iodine-free; (b) a mixture composed of balanced electrolyte solution, tryptic meat broth and lactalbumin hydrolysate (3.9–7.8  $\mu$ g iodine/100 ml.). Thyroid cells could not survive more than 3 passages, partly because of their increasing stickiness to glass and our consequent inability to strip them off with trypsin without causing cellular damage. Although some of the cultures survived for nearly 2 months, an established cell line was never obtained from the 20 human thyroids used in this work. Pulvertaft *et al.*<sup>1</sup> are the only authors who claim to have done so.

All the cultures were derived from pathological material, obtained at operation. Nine cases were classified as toxic diffuse goitre, 5 as toxic nodular goitre, 5 as non-toxic nodular goitre and one as Hashimoto's thyroiditis. It was noted that poor growth resulted from 2 toxic diffuse goitres, one toxic nodular goitre and the case of Hashimoto's thyroiditis. Thus the most consistent growth was obtained from the 5 cases of non-toxic goitre, but as the numbers were small this may not be a significant finding.

Five of the above thyroid cell cultures were used for metabolic studies (2 diffuse toxic, 1 nodular toxic, 1 nodular non-toxic, 1 auto-immune thyroiditis of the Hashimoto type).

When the monolayers had been developed (2–3 days), they were washed several times with buffered electrolyte solution and fresh medium was added. A number of bottles were kept for the detection of stable thyroglobulin 0, 24 and 72 h after this change. An increase in the tanned red-cell agglutination inhibition titre was taken as an indication of the synthesis of thyroglobulin-like material. The results of this titration were, however, doubtful because positive results were obtained in only a small number of the bottles where calf serum was added, but not in protein-free medium. On the other hand, evidence was obtained of the presence of proteolytic activity in thyroid cell culture medium as indicated by the breakdown of <sup>125</sup>I-labelled human thyroglobulin added *in vitro* (Assem<sup>6</sup>). The labelled material was incubated with samples of tissue culture medium TCM-199 (pH 7.1–7.4) in which thyroid cells had been growing, for 24 h at 37°C. The breakdown products were separated by dialysis at 4°C against tap water for 16 h and original and residual radioactivity was counted in a well-type scintillation counter. The results of a typical experiment are given in Table 1. The addition of denatured ox haemoglobin (1 per cent) and 'Trasyol', 100  $\times$  i. units/ml. (a proteinase inhibitor, Farbensfabriken Bayer A.G.) induced a little reduction in this proteolytic activity. The ERK medium, however, showed a similar activity. The occurrence of a small degree of degradation of labelled thyroglobulin in the controls which were unused media could be explained by the contamination of this preparation with proteolytic enzymes originating from thyroid tissue from which it was prepared.

Only one culture (derived from a toxic diffuse goitre), of 5 tested in this way, showed ability to concentrate <sup>125</sup>I when added to the medium as iodide, after 2–3 days growth. In this culture the ratio of cell to medium radioactivity was 1.6:1, while the ratio of cell radioactivity to number of cells was 8 times greater than in ERK

Table 1. DIALYSISABLE <sup>125</sup>I, AS PER CENT OF TOTAL RADIOACTIVITY AFTER INCUBATION OF <sup>125</sup>I-LABELLED THYROGLOBULIN FOR 24 h AT 37°C

Additions	Medium of ERK cells	Medium of thyroid cells Primary cultures	Sub-cultures	Control (unused culture medium)
None	43	40	33	1.5
'Trasyol'	33	28	20	13
Denatured ox haemoglobin	24	30	20	14



cultures. In contrast, the remaining thyroid cultures gave cell/medium radioactivity ratios from 0.1:1 to 0.4:1; not significantly different from that of the ERK cells (0.2:1). However, the cells of two of the thyroid cultures with low  $^{131}\text{I}$  uptake contained traces of thyroxine and probably triiodothyronine (as well as mono- and di-iodotyrosine), when examined by paper chromatography after pancreatic digestion. The culture media in which both thyroid and ERK cells had been growing contained minute traces of  $^{131}\text{I}$  in organic form (mainly as iodotyrosines).  $^{131}\text{I}$ -labelled thyroglobulin was detected in the medium of only one thyroid culture. This material gave a radioactive peak in the alpha-globulin region on electrophoresis, which was displaced towards the cathode on adding dilute Hashimoto serum containing thyroglobulin antibody. This particular culture was grown in the medium containing calf-serum, and the cells concerned did not concentrate  $^{131}\text{I}$ .

There was therefore suggestive but not conclusive evidence that the thyroid cells were making thyroglobulin-like compounds. A more definite finding was that these cells produced enzymes with proteolytic activity. Ability to make these enzymes was not, however, specific to thyroid cells, since they were also found in the ERK cultures. The presence of proteolytic activity in the medium would inevitably make the detection of thyroglobulin difficult by any technique.

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### Nutritive Value of Nigerian Water Melon

WATER melon (*Citrullus vulgaris*) is cultivated in Nigeria principally for the semi-drying oil which is extensively used for cooking and may be used as a substitute for cotton seed oil. The seeds are also used as vegetable soup, but the flesh is never eaten.

The present work was undertaken to investigate the nutritive value of the seeds as a basis for future work. The analytical work has been concerned with proximate analysis, mineral composition and the determination of oxalic, hydrocyanic and phytic acid content.

The seeds were bought at different times from the market, dried overnight at 100° C, and finely ground. Crude protein, crude fibre, ash, ether extract and hydrocyanic acid were determined according to the Association of Official Agricultural Chemists' *Methods of Analysis*<sup>1</sup>. Potassium was estimated by flame photometry, other minerals by means of a photoelectric spectrometer, oxalic acid by the method of Dye<sup>2</sup> and phytic acid by the method of McCance and Widdowson<sup>3</sup>.

Table 1 shows a typical proximate analysis of the seeds. Table 2 gives the distribution of the major inorganic constituents and Table 3 the minor inorganic constituents, while Table 4 shows the oxalic, hydrocyanic and phytic

Table 1. PROXIMATE ANALYSIS OF NIGERIAN WATER MELON

Dry matter %	Crude protein %	Ether extract %	Crude fibre %	Carbohydrate %	Ash %	Calories %
91.93	31.88	57.06	8.24	4.37	0.17	565.66

Quantities are given as percentage fresh weight.

Table 2. MINERAL STATUS OF NIGERIAN WATER MELON; MAJOR ELEMENTS

N %	P %	K %	Na p.p.m.
5.10	1.456	0.54	164

Table 3. MINERAL STATUS OF NIGERIAN WATER MELON; MINOR ELEMENTS

Ca %	Mg %	Mn p.p.m.	Fe p.p.m.	Cu p.p.m.	B p.p.m.	Zn p.p.m.	Mo p.p.m.	Al p.p.m.
0.13	0.53	42	75	42.2	12.5	50	1.0	68

Table 4. OXALIC, HYDROCYANIC AND PHYTIC ACID CONTENTS OF NIGERIAN WATER MELON

Oxalic acid %	Hydrocyanic acid mg %	Phytic acid P mg %	Phytic acid P as % of original P
0.8	0	000	41

acid contents. All determinations are based on percentage of dry matter.

The seeds are very rich in protein and serve as a protein supplement, forming one of the most important sources of protein in the Nigerian diet. Nearly 60 per cent of the seed consists of oil. The oil is used as a substitute for palm oil in cooking, and after the oil has been extracted the residue is wrapped in leaves and steamed to give 'oaks'.

The seed is a good source of energy, 100 g supplying about 566 calories. The ash content is high. The important dietary constituents of the ash are the calcium, phosphorus and iron contents. It is poor in calcium and iron but relatively rich in magnesium. The phosphorus content is very high. Of all the Nigerian seeds and vegetables analysed, water melon contains the highest phosphorus content. This gives a Ca:P ratio of 1:11. Although the ratio is unbalanced (1:2 is the satisfactory ratio), since it is taken along with other foodstuffs the satisfactory ratio could easily be met.

It is generally accepted that, under certain conditions, dietary calcium is precipitated in the intestine, resulting in reduced calcium availability, which restricts growth and even causes rickets. The two acids that are supposed to be responsible for this are oxalic and phytic acids. Furthermore, the lowering of the calcium by excretion as calcium oxalate takes place through the kidneys. Crystals of calcium oxalate are deposited in the uriniferous tubules in the form of granules or stones, leading to oxaluria.

Calcium determinations in foodstuffs are therefore of little significance from the nutritional point of view, unless taken in conjunction with oxalic or phytic acid content or both. Water-melon seeds contain negligible amounts of oxalic acid, but nearly half the phosphorus occurs as phytic acid. Nevertheless, the amount of non-phytic phosphorus is still appreciable.

Cyanogenesis has been extensively studied in many plants, chiefly because of the toxic effect that the deadly poisonous hydrocyanic acid produced could have on livestock. Hydrocyanic acid does not occur free, but combined with sugars to form an insoluble, non-toxic compound known as cyanogenetic glycoside. The water-melon seed does not contain any hydrocyanic acid.

The plant samples were analysed by Dr. A. L. Kenworthy, Horticulture Department, Michigan State University, East Lansing, and this work was supported, in part, by a Michigan State University International Programmes—Ford Foundation grant.

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### Rat Liver Sorbitol Dehydrogenase

THERE has been considerable interest in the effect of various diets and hormones on liver enzymes. One of the problems related to the interpretation of the results is the dependence upon the method of reporting the data. This problem arises because various treatments cause changes in liver nitrogen content, liver cellularity, body-weight, and ratio of liver-weight to body-weight. Several considerations related to the reporting of results obtained after dietary and hormonal treatment have been discussed previously<sup>1,2</sup>. It would therefore be interesting if the

activity of one enzyme were to remain unaffected by these many treatments using any given method of reporting results. Such an enzyme might be useful as a control. This report will present the effects on the activity of liver sorbitol dehydrogenase activity of several treatments known to affect many liver enzyme activities. Sorbitol dehydrogenase does not appear to be related to any apparently important normal metabolic function of the liver during most regimens or hormonal treatments, and it may therefore serve as a control enzyme activity for most treatments.

All animals (male Sprague-Dawley rats) were maintained on diet or treatment for 5 days before being killed, when they weighed between 100 and 200 g. The basic diets have been described previously<sup>1</sup> and the hormone treatments were as follows: cortisone (5 mg/rat/day), hydrocortisone (5 mg/rat/day), and thyroxine (1 mg/rat/day) injected interperitoneally. All rats receiving hormone treatment were fed the glucose diet (85 per cent glucose, 25 per cent casein, 5 per cent corn oil, 4 per cent salts, 1 per cent vitamins). The rats were killed and bled, and the liver was removed and rapidly chilled. A portion of the liver was homogenized in 4 volumes of 0.14 M KCl centrifuged for 30 min at 20,000g at 0-4° C. The resulting supernatant solution was used for a protein determination<sup>2</sup>, thus both a total and soluble protein content were determined. The sorbitol dehydrogenase was assayed as follows: 1.0 ml. of *tris* buffer (0.1 M, pH 7.4), 0.8 ml. of water, 1.0 ml. of D-fructose (1.0 M), 0.1 ml. of DPNH (7.5  $\mu$ moles/ml.) and 0.1 ml. of enzyme (a '1 to 10' dilution of supernatant solution obtained as above). All activities were corrected for endogenous DPNH oxidation. All assays were conducted at 25° C using a Gilford Model 2000<sup>3</sup> multiple absorbance recorder. The results are reported as units per 100 g body weight, per g liver, and per mg liver protein (both soluble and total). One unit of activity is the amount of enzyme required to cause the oxidation of one  $\mu$ mole of DPNH in one minute at 25° C under the prescribed assay conditions. All reactions obeyed zero order kinetics up to five times the greatest activities reported.

In this investigation 32 liver enzymes were examined, but only sorbitol dehydrogenase behaved consistently in all treatments. Two liver phosphatases, six enzymes related to glycogenolysis, one enzyme related to lipid metabolism, five enzymes linked to the tricarboxylic acid cycle, three pentose phosphate shunt enzymes, three transaminases, three enzymes related to aromatic amino-acid metabolism, four urea cycle enzymes and four other enzymes related to amino-acid metabolism were measured in addition to sorbitol dehydrogenase. The sorbitol dehydrogenase activity of rats on various diets or hormonal treatments is reported on the basis of specific activity (activity per g liver protein), per g liver, and per 100 g body weight (Table 1). A high protein diet which has been shown to increase the activity of many liver enzymes<sup>4-6</sup>, had no effect on sorbitol dehydrogenase activity per 100 g body weight. The same was true of a high fructose diet<sup>4,6,10</sup>. The administration of the hormones which have been shown to cause marked alterations in liver enzyme activity, cortisone and hydrocortisone<sup>6-7,11-14</sup>, and thyroxine<sup>6,12-16</sup>, had no effect on sorbitol dehydrogenase.

It is interesting to note that none of the treatments studied affected the activity of liver sorbitol dehydrogenase per 100 g body weight. The fructose diet would be considered the most likely to affect the activity of this

enzyme, since fructose is a substrate for this enzyme, but this treatment had no effect. One or more treatments caused significant alterations in the sorbitol dehydrogenase activity compared with the high glucose diet when the results are reported as activity per g soluble liver protein, per g total liver protein, or per g liver. Thus, it appears that when the most desirable method of reporting results is per 100 g body weight, sorbitol dehydrogenase is an excellent reference enzyme to show that the changes in activity were not due to non-specific overall changes in all enzyme activities. In cases where the enzyme being measured is related to a function performed by the liver for the entire body, such as gluconeogenesis, urea synthesis, aromatic amino acid metabolism, etc., activity per 100 g of body weight may possibly be the best method of reporting results as it may reflect the relationship between need and activity<sup>1,2</sup>.

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## Isoenzymatic Nature of L-Glutamic Dehydrogenase of Higher Plants

ELECTROPHORESIS of albumins of mature seeds of *Vicia faba* on polyacrylamide gel, with subsequent location of L-glutamic acid dehydrogenase (L-glutamate:NAD oxidoreductase (deaminating) EC 1.4.1.2) (GDH), using a tetrazolium procedure, revealed that this enzyme could be separated into a number of isoenzymes. A preliminary investigation has been made of these isoenzymes together with an examination of their distribution within the seeds and seedlings of *Vicia faba* (broad bean) and *Pisum sativum* (pea).

Protein extracts were prepared from the sterile seeds, embryos or seedlings using 5 per cent (w/v) potassium sulphate-0.1 M phosphate buffer, pH 7.2 (2°); the supernatant from this extract was then saturated with ammonium sulphate (4°). The precipitate which appeared was redissolved in 5 per cent (w/v) potassium sulphate-0.1 M phosphate buffer, pH 7.2, and then dialysed against tap water for 18 h. The supernatant from this dialysis was then subjected to disk electrophoresis<sup>1</sup> using 6 per cent acrylamide as previously described<sup>2</sup>. GDH activity was detected by incubating the gels under aerobic conditions at 35° for various times in a solution containing the following, per ml.: L-glutamic acid, 5 mg; nicotinamide adenine dinucleotide (NAD), 1 mg; 5-methyl phenazine methosulphate (PMS), 0.1 mg; 3-(4,5-dimethyl thiazolyl-

Table 1. EFFECTS OF DIETS AND HORMONES ON ACTIVITY OF LIVER SORBITOL DEHYDROGENASE

Treatment	No. animals	Activity (mean value $\pm$ S.E.M.)			
		per g liver	per g liver protein total	per g liver protein soluble	per 100 g body wt.
Glucose diet	10	5.46 $\pm$ 0.22	19.0 $\pm$ 0.8	30.3 $\pm$ 1.9	26.0 $\pm$ 1.4
Fructose diet	6	4.37 $\pm$ 0.13	14.5 $\pm$ 0.4	30.0 $\pm$ 1.2	27.3 $\pm$ 0.5
Protein diet	6	6.02 $\pm$ 0.56	18.7 $\pm$ 1.3	34.4 $\pm$ 2.0	27.7 $\pm$ 2.2
Hydrocortisone	6	3.74 $\pm$ 0.16	14.8 $\pm$ 0.5	25.5 $\pm$ 1.3	25.7 $\pm$ 1.0
Cortisone	6	4.89 $\pm$ 0.13	16.7 $\pm$ 0.5	28.5 $\pm$ 0.5	28.4 $\pm$ 0.6
Thyroxine	8	6.17 $\pm$ 0.35	20.1 $\pm$ 0.8	30.8 $\pm$ 1.6	24.9 $\pm$ 1.4

2)-2,6-diphenyl tetrazolium bromide (MTT), 0.5 mg; phosphate buffer 0.1 M, pH 6.5.

Electrophoresis of albumins of mature seeds of *Vicia faba* on polyacrylamide gel, followed by detection of GDH activity using the procedure described, revealed the presence of seven formazan bands, five strong and two weak with respect to formazan deposition (Fig. 1); the two weaker formazan bands are not seen on the photograph. Similar results have been obtained with albumins extracted from mature seeds of *Pisum sativum*.

The electrophoretogram shown in Fig. 1 raises two important issues: first, do the formazan bands represent GDH activity; and secondly, are these isoenzymes formed by the electrophoretic separation or during the extraction procedure?

Evidence that these bands do represent GDH activity can be summarized as follows: (1) formazan deposition does not occur on gels boiled after electrophoretic separation of the albumins; (2) if NAD, PMS or L-glutamic acid is omitted from the incubation mixture formazan deposition does not occur; (3) only very weak formazan bands develop when NADP is substituted for NAD in the incubation mixture, the GDH of corn leaves has been reported to be NAD specific<sup>4</sup>; (4) formazan deposition, due to NADH<sub>2</sub>-MTT-tetrazolium reductase (diaphorase), occurs at different sites on the gel to those described here for GDH activity and then only with prolonged incubation times. These results strongly suggest that formazan deposition is due to the initial activity of GDH.

Evidence, which is described here, suggests that the pattern shown in Fig. 1 is not formed as a result of the electrophoretic procedure used in the separation of the isoenzymes described. Isoenzymes 1-5 have been separated, located and afterwards eluted off polyacrylamide gel; these eluates, after concentration and a second electrophoresis, revealed only one strong band of GDH activity on the gel and they did not reproduce the full pattern shown in Fig. 1.

Attempts to alter the isoenzyme pattern of the mature seed by altering the extraction conditions have so far

failed. Extraction of mature seeds with either citrate, veronal or *tris*-hydrochloric acid buffers instead of phosphate buffer, followed by electrophoresis, show an isoenzyme pattern similar to that of Fig. 1. Crude phosphate buffer extracts, pH 7.2, of mature seeds or extracts, purified by dialysis or ammonium sulphate precipitation, likewise show a pattern identical to that of Fig. 1.

Of the amino-acids tested as substrates instead of L-glutamic acid, only L-aspartic acid, L-valine and L-threonine produced a pattern similar to that shown in Fig. 1, but formazan deposition was extremely weak in these instances.

Using the tetrazolium procedure already described changes in the isoenzyme pattern of the cotyledons, radicle and shoot have been detected as germination proceeds; these changes have been followed using sterile germinating seeds of *Pisum sativum*. The dormant cotyledons (embryo-free) possess six isoenzymes, 2-7 (see Fig. 1 for isoenzyme numbers). Isoenzyme 2 is characterized by weak formazan deposition, isoenzymes 3-7 showing strong formazan deposition. As germination proceeds the cotyledons show a gradual loss of activity, judged by formazan deposition, of the more positively moving isoenzymes which leads eventually to the presence of only isoenzyme 7, 21 days after planting. The dormant radicle possesses isoenzymes 1-7, all isoenzymes showing strong and equal formazan deposition; this contrasts with the pattern of the dormant cotyledons in which isoenzyme 1 is not detected and isoenzyme 2 is only weakly active as judged by formazan deposition. As germination proceeds, formazan deposition by the more positively moving isoenzymes of the radicle decreases to give only two formazan bands at 66 h after planting; eventually only one formazan band remains 21 days after planting: this band probably corresponds in position to isoenzyme 7 of the pattern of the mature seed. The dormant shoot, like the dormant radicle, also possesses all seven isoenzymes. In this case isoenzyme 7 is characterized by stronger formazan deposition than the other six. Again, as germination proceeds the more positively moving isoenzymes show a loss of formazan deposition which eventually leads to only isoenzyme 7 remaining 21 days after planting.

These results suggest that the GDH described here can be separated into a number of isoenzymes and that these different forms of the enzyme are not formed as a result of the methods of extraction or separation which have been employed. Different parts of the pea seed appear to have a specific GDH isoenzyme pattern which can change with age.

A better understanding of the part played by GDH in the maturing and germinating seed may give some clue as to the function of the GDH-isoenzymes in the various parts of the seed.

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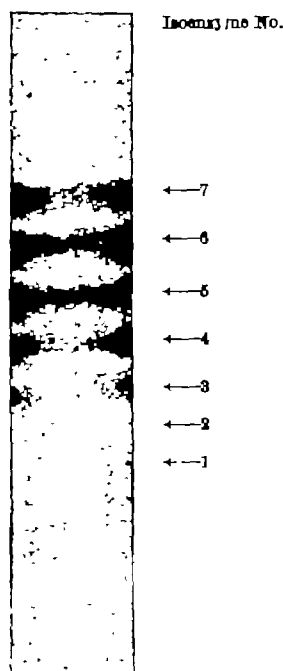


Fig. 1. GDH isoenzyme pattern of the albumins extracted from mature seeds of *Vicia faba*. Isoenzymes 1 and 2 are not visible on the photograph; for details of electrophoretic separation and location of GDH activity see text.

### Transfer of Radioactivity of Cholesterol-7 $\alpha$ -<sup>3</sup>H to Fatty Acids of Tissue Lipids *in vivo*

When randomly labelled cholesterol (either with carbon-14 or tritium) was fed to rats, a small but appreciable amount of the administered radioactivity could be recovered in the carcass fatty acids<sup>1</sup>. In both cases it was assumed that the label was due to incorporation of the

carbon atoms of the isopropyl group of the cholesterol side-chain into the isolated fatty acids. In the course of recent experiments involving cholesterol metabolism in baboons we administered cholesterol- $6\alpha$ - $^3\text{H}$  (5 mc.) to a male baboon (18 kg) which was killed 3 days later. The lipids of a number of tissues (liver, spleen, kidney, lung, adrenal) were extracted with chloroform-methanol 2:1 (ref. 2), separated by chromatography on silicic acid\* and assayed for radioactivity by liquid scintillation spectrometry.

An appreciable amount of radioactivity was detected in all the lipid classes. While most of the tritium was found to be present in cholesterol and cholesterol ester, as would be expected, we also detected considerable radioactivity in most of the other lipid fractions. Table 1 presents the percentages of recovered tritium radioactivity present in a few representative tissues. Maximum activity was usually found in the triglyceride or phospholipid fraction. The purity of the individual lipid fractions was established by thin-layer chromatography on 'Silica gel G'. In order to ascertain the site of the radioactive hydrogen, several of the more radioactive fractions of the liver and kidney lipids were subjected to trans-esterification by heating with 5 per cent  $\text{H}_2\text{SO}_4$  in methanol at  $70^\circ\text{C}$  for 4 h. The fatty acid methyl esters were extracted from the trans-esterification mixture with hexane and the absence of contamination by the original lipid fraction was confirmed by thin-layer chromatography. The data (Table 2) indicate that the  $7\alpha$  hydrogen of cholesterol had been transferred to the fatty acids of the various tissue lipid fractions being studied. Experiments are in progress to extend this observation to other species and to attempt to elucidate the mechanism of the transfer.

Table 1. RECOVERY OF TRITIUM IN TISSUE LIPID FRACTIONS

Lipid fraction	Percentages of recovered tritium in:				
	Liver	Kidney	Aorta	Lung	Adrenal
Cholesterol ester	62.7	11.4	15.5	—	60.2
Triglyceride	17.6	1.0	3.8	6.0	13.0
Free fatty acid	8.0	18.9	4.0	0.3	1.0
Cholesterol	5.4	61.0	50.0	90.0	1.0
Diglyceride	0.9	1.0	2.3	1.0	0.5
Monoglyceride	0.9	1.4	5.8	0.5	5.8
Phospholipid	3.8	5.1	14.2	2.1	7.1

Table 2. RADIOACTIVITY RECOVERED FROM FATTY ACIDS AFTER TRANS-ESTERIFICATION

Lipid fraction	Percentage of $^3\text{H}$ radioactivity recovered as fatty acid methyl ester in:	
	Liver	Kidney
Triglyceride	—	94
Diglyceride	—	78
Monoglyceride	57	61
Phosphatidic acid	71	—
Cardiolipin	76	71
Leucithin	42	70
Sphingomyelin	45	—
Cerebroside	—	79

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## Growth of *Escherichia coli* NCTC 5928 in Relation to Enzymes in Vitamin B<sub>6</sub> Metabolism

Results have been obtained which indicate the presence in *Escherichia coli* NCTC 86 of pyridoxamine phosphate oxidase<sup>1</sup> and of pyridoxine phosphate oxidase<sup>2</sup>. These enzymes had previously been demonstrated in rabbit liver preparations<sup>3-5</sup>. These enzymes in *E. coli* NCTC 86 have been investigated in aqueous extracts obtained from acetone-ether dried cells (kindly supplied by the Lister Institute of Preventive Medicine, Elstree, Herts.), which had been grown for 18 h at  $37^\circ\text{C}$  on a casein-digest medium solidified with agar<sup>6</sup>.

The culture media were as follows:

A.  $\text{KH}_2\text{PO}_4$ , 9 g/L; Difco bactocaseitone, 5 g/L; trace of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ; distilled water; sufficient 5 N NaOH to bring the pH to 7.4.

B.  $\text{KH}_2\text{PO}_4$ , 9 g/L; Difco caseamino acids, 5 g/L; trace of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ; distilled water; sufficient 5 N NaOH to bring the pH to 7.4.

C. Simple salts medium<sup>7</sup>:  $\text{KH}_2\text{PO}_4$ , 9 g/L; ammonium sulphate, 2 g/L; D-glucose, 1 g/L; sufficient 5 N NaOH to bring the pH to 7.0.

In all cases,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.2 g/L, was added after pH adjustment and sterilization.

The *E. coli* NCTC 5928 cells were produced at  $30^\circ\text{C}$  under aeration, collected, and afterwards dried with acetone-ether<sup>8</sup>. Aqueous extracts were prepared by extracting the cells overnight with distilled water (20 mg cells/ml. water) at  $3^\circ\text{C}$ , after which the suspension was centrifuged at 12,000g for 30 min at  $0^\circ\text{C}$ . The enzyme activities were determined on the supernatant cell-free extract.

To determine enzyme activity, reaction mixtures were made up with pyridoxamine phosphate (Pam P) or pyridoxine phosphate (Pin P) as substrate. The pyridoxal phosphate (Pal P) produced was determined by the phenylhydrazine procedure<sup>9</sup>, and protein was determined by a modification of the method of Lowry *et al.*<sup>10</sup>. Specific enzyme activities were expressed in  $\mu\text{g}$  pyridoxal phosphate formed/h/mg protein.

Table 1

Culture medium	Phase of growth	Specific activities ( $\mu\text{g}$ Pal P formed/h/mg protein)		Pin P ox. Pam P ox.
		Pyridoxamine phosphate oxidase measured at pH 10.1	Pyridoxine phosphate oxidase measured at pH 10.1	
Caseamino acids	Log	1.54	1.54	1.00
	Decl. growth	1.43	1.93	1.35
	Stat.	1.53	1.48	0.97
Simple salts	Log	0.92	1.92	2.09
	Decl. growth	1.50	3.25	1.96
	Stat.	1.50	2.75	1.65

It was found that cells of *E. coli* NCTC 5928 grown in an amino-acid medium (caseamino acids), containing an acid hydrolysate of casein as the sole source of carbon and nitrogen, possessed a significant amount of pyridoxamine phosphate and pyridoxine phosphate oxidase activities, whereas cells grown in a similar medium (bactocaseitone), consisting of a pancreatic digest of casein, had no similar activity. Cells grown in the simple salts medium were found to have marked activities for these enzymes.

The reason for these differences is a matter for speculation. Caseamino acids consist of a mixture of amino-acids, commercial casein having been completely hydrolysed by HCl, with the destruction of the tryptophan originally present in the casein<sup>11</sup>. Bactocaseitone contains a mixture of peptides and amino-acids resulting from the hydrolysis of casein by proteolytic enzymes from the pancreas<sup>12</sup>, with a high proportion of tryptophan<sup>13</sup>. Bactocaseitone also contains a number of substances, including pyridoxine, which originate from the non-protein impurities of the pancreatic enzyme preparations. These substances are destroyed in the HCl hydrolysis procedure.

It appears that the oxidases can be produced in cells grown in a medium containing either a mixture of amino-acids without tryptophan, or with  $\text{NH}_4^+$  ions as nitrogen source and D-glucose as carbon source. It is possible that tryptophan, at relatively high concentrations in the growth medium, may act as a competitive homologue for the formation of the active sites in the enzyme protein.

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## PHYSIOLOGY

### Reflex Innervation of the Ankle Joint

In spite of its obvious importance in postural physiology and lower limb surgery, no specific study of the innervation of the ankle joint in relation to mechanoreceptor reflexes elicitable therefrom has been reported previously. For this reason, we have examined the innervation of 58 ankle joints in the cat with macroscopic, microscopic and neurophysiological techniques similar to those used previously in this laboratory for the knee<sup>1</sup>, temporo-mandibular<sup>2</sup> and laryngeal<sup>3,4</sup> joints.

**Extrinsic innervation.** The articular tissues on the posterior aspect of the ankle joint (capsule, ligaments and fat pad) have the densest innervation—through articular branches of the long saphenous, posterior interosseous and anterior tibial nerves; from the intramuscular nerves in the flexor digitorum muscle; and from a nerve plexus in the deep posterior fascia of the lower leg. The anterior aspect of the joint is supplied from the anterior tibial nerve. The medial aspect (and the deltoid ligament) is supplied from the posterior tibial nerve. The lateral aspect (and the related collateral ligaments) is supplied from the anterior tibial and interosseous nerves.

**Articular nerve endings.** Microscopic examination (with gold chloride, frozen silver and methylene blue techniques) of the terminations of the articular nerves in the tissues of the ankle joint reveals the endings to be similar in type to those in the knee joint<sup>1</sup>, although different in their relative proportions. Myelinated afferent fibres of medium size (8–12 $\mu$ ) in the articular nerves terminate in Type I and Type II corpuscles that are located on all aspects of the fibrous capsule of the joint. In the ankle, however, the Type II end-organs are relatively more numerous than in the knee joint<sup>1</sup>, so that the numerical relations of the Type I and Type II corpuscles are more like those in the temporo-mandibular joints<sup>2</sup>. Type II corpuscles are present also in the posterior fat pad at the back of the ankle joint, as is the case with the other joints<sup>1,2</sup>. Myelinated afferent fibres of larger diameter (up to 17 $\mu$ ) in the articular nerves innervate Type III corpuscles, which are confined to the joint ligaments (as in other joints<sup>1,2,5</sup>). Plexuses and free nerve terminals, present throughout the fibrous capsule, fat pad and the walls of the articular blood vessels at the ankle, constitute the Type IVa variety of articular nerve ending, and are supplied by small (less than 5 $\mu$ ) myelinated and unmyelinated afferent fibres in the articular nerves. Type IVb endings, confined to the

tunica media of the articular blood vessels, probably represent the terminations of unmyelinated, post-ganglionic sympathetic vasomotor fibres in the articular nerves.

No nerve endings of any type are present in the synovial tissue of the ankle joint—as is the case with all the other joints we have examined<sup>1–3,6</sup>. Likewise, there are no Pacinian corpuscles in the articular tissues of the ankle (or other<sup>7</sup>) joints.

**Mechanoreceptor reflexes.** Neurophysiological studies<sup>4,8</sup> show that the corpuscular end-organs are articular mechanoreceptors with differing behavioural characteristics. Type I corpuscles are slowly-adapting and Type II corpuscles are rapidly-adapting—both types having low thresholds. The Type III corpuscles are high threshold, slowly-adapting mechanoreceptors. The non-corpuscular Type IVa terminations provide the articular pain receptor system.

Passive movements of the skinned and tenotomized ankle joint in the intact, lightly anaesthetized animal activate the Type I and Type II corpuscles to produce reciprocally related polysynaptic reflex changes in motor unit activity in the leg muscles, identified by multi-channel electromyography<sup>4,8</sup>—dorsiflexion of the foot provoking gastrocnemius facilitation and tibialis anterior inhibition. The motor unit responses are rapidly adapting from the Type II corpuscles, and slowly adapting from the fewer Type I corpuscles. The Type III corpuscles are not activated, except at the extremes of joint displacement. Painful stimulation of the capsular tissues of the joint activates the Type IVa endings to produce polysynaptic reflex spasm of both flexor and extensor muscles in the leg.

The articular origin of the reflex responses in the leg muscles is confirmed by their suppression following articular neurectomy, local anaesthesia (with 1 per cent 'Lignocaine' solution) of the joint capsule, and electrocoagulation of the joint capsule. Similar reflex responses in the leg muscles can be provoked by direct mechanical stimulation of the joint capsule with varying intensities. With increasing barbiturate anaesthesia, the articular reflexes in the leg muscles are abolished before the monosynaptic stretch reflexes, elicited in the same muscles, disappear; and they can only be demonstrated at the early stages of such anaesthesia. The articular mechanoreceptor reflexes appear to operate polysynaptically through the  $\gamma$ -motoneurone loop, contributing thereby to the co-ordination of limb muscle tone in posture and movement.

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### Basal Metabolic Rate in Developing Renal Hypertension

THE development of renal hypertension in the rat, induced by means of partial occlusion of one renal artery and contralateral nephrectomy, has been reported to be associated with transient elevation of cardiac output<sup>1</sup>. The experiments reported here were designed to test the possibility that this transient rise in cardiac output is due to a concurrent increase in metabolic rate.

A spirometer was constructed to hold two rats in separate compartments, each of which could be coupled independently to a closed circuit which included an air pump and a carbon dioxide absorber. The gas within the circuit was pumped round at a rate of 1 l./min. After initial washing through of the system with oxygen, further oxygen was introduced into the system in successive 5-ml. amounts and the absorption time measured by observing through a microscope eyepiece the movement of a fine wire attached to a rubber tambour, 13 cm in diameter, included in the circuit. Negligible pressure changes occurred in the closed circuit. The whole apparatus was immersed in a water bath and the observations made at a temperature of between 26° and 29° C, since it had been shown that oxygen uptake was constant above 26° C. Female Wistar albino rats weighing initially 200-220 g were conditioned in pairs daily to the spirometer for seven days. Three measurements of uptake of oxygen were then made on separate days over a week, at the end of which time the animals were subjected to an operation consisting of the application of a silver clip to the right renal artery together with removal of the left kidney. The clip in the case of one member of a pair had a gap 0.25 mm wide (experimental group) and in the other 0.5 mm (control group). Serial observations of uptake of oxygen were then made every 2-3 days for four weeks. After each measurement of uptake of oxygen the systolic blood pressure was measured by a tail plethysmographic method under light ether anaesthesia.

Eight rats in the control group remained normotensive throughout (blood pressure, less than 140 mm mercury), whereas ten rats in the experimental group developed mild to severe hypertension (blood pressure, 140-260 mm mercury) (Fig. 1). A slight post-operative fall in mean oxygen uptake occurred in both groups but there was no significant difference between the groups at any time after operation. Statistical analysis of the figures showed that the scatter was such that a difference of 8 per cent in oxygen uptake between the experimental and control groups would have been detected. No difference between the weight changes in the two groups occurred.

These results indicate that the mean increase of 25 and 35 per cent in cardiac output observed in rats developing this form of renal hypertension in the first two five-day periods after operation<sup>1</sup> is most unlikely to be due to an increase in tissue oxygen requirements. The further possibility has been suggested by Borst<sup>2</sup> that haemodilution could be responsible for the increase in cardiac output. The haematocrit in these animals developing hypertension is not reduced by more than 0.5 per cent below that in the control animals<sup>3</sup> and it is very improbable that such small changes could be responsible. Thus the transient increase

in cardiac output observed in the development of renal hypertension is not readily explicable on the basis either of increased metabolic rate or of haemodilution. Such an increase beyond the metabolic requirements of the tissues might be expected to stimulate autoregulation of peripheral tissue perfusion and may be of importance in the pathogenesis of renal hypertension as suggested by Ledingham and Cohen<sup>4</sup>.

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### Endothelial Vesicles and Protein Transport

THE physiological mechanism involved in the transfer of water, solutes and macromolecules across endothelium remains obscure<sup>1,2</sup> though it has been subjected to considerable investigation. When permeability is increased, endothelial gaps, believed to be intercellular, are found in the endothelium<sup>3-5</sup>. In electron microscopic examinations, tracer particles, injected into the blood stream, aggregate in the gaps, which are the sites of plasma leakage and the stigmata and stomata of light microscopy<sup>6,7</sup>. However, in physiological states, with the exception of lymphatics<sup>8,9</sup> and such special vascular beds as the sinusoidal vessels of the liver<sup>1,10-12</sup>, spleen<sup>14</sup>, bone marrow<sup>13,15</sup> and frog heart<sup>16</sup>, gaps do not occur in vascular endothelium. This report concerns the finding of a new type of vesicle in endothelium, and it is considered that these specialized micropinocytotic vesicles could be related to protein transport in the lymphatic and vascular endothelium of frog tissues.

Queensland green tree frogs (*Hyla caerulea*) were pithed or decapitated prior to the removal of the interdigital webs of the hind limbs, and portions of the tongue, mid-gut, lung and abdominal skin. Tissues were cut into small blocks, fixed for 2-18 h in 1 per cent osmium tetroxide, buffered according to Zetterqvist's method<sup>17</sup> and then dehydrated in ethanol and embedded in 'Araldite'. The sections were cut on an LKB (type 4800 A) ultramicrotome, mounted on copper grids, stained with lead<sup>18</sup> and 1 per cent uranyl acetate in ethanol and then examined in a Siemens Elmiskop I electron microscope.

In the webs, large cleft-like lymphatic spaces which never contained blood were regularly found. They were lined by a layer of endothelial cells with an incomplete basement membrane. The ultrastructure of the endothelium was similar to previous descriptions<sup>9,19</sup>. An intercellular gap was observed only once.

Two morphologically distinct types of vesicle were present in the endothelium (Figs. 1 and 2). The micropinocytotic vesicles and caveolae, first described by Palade<sup>20</sup> in vascular endothelium, were relatively numerous; these vesicles were round or oval and for the most part 600-1000 Å in diameter, though more elongated or even multilocular forms were observed. These vesicles and pits were bounded by a well-defined membrane immediately within which a light grey zone of variable thickness faded centripetally. The thickness and density of this zone often varied around the circumference of the vesicle.

Of much less frequent occurrence were the dense micropinocytotic vesicles. These were round or oval and varied in size from 600 to 1600 Å, most being 800-1200 Å. The limiting membrane was frequently denser than the foregoing type of vesicle. Inside the limiting membrane was a zone of granular or fibrillary material 100-300 Å

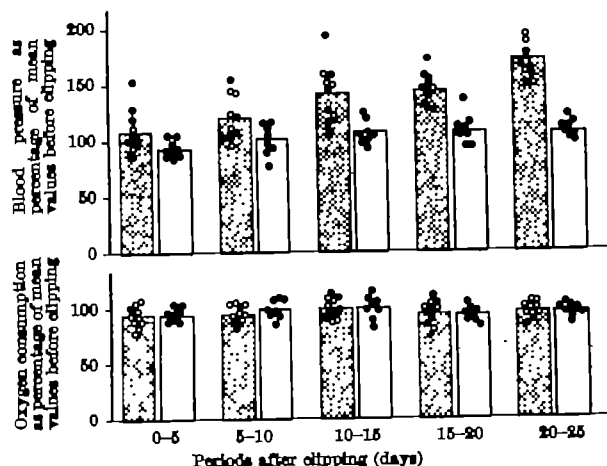


Fig. 1. Stippled, tight clip; white, loose clip



Fig. 1. Lymphatic endothelium containing a dense vesicle (DV) and an infolding of the basal surface of the plasma membrane of similar structure (DC). Vesicles (V) and caveolae (C) of light density are illustrated. ( $\times 51,500$ )



Fig. 2. Lymphatic endothelium with two dense caveolae (DC) on the luminal surface. Vesicles (V) and caveolae (C) of light density, an unidentified dense body (D), endoplasmic reticulum (R) and nucleus are illustrated. The lumen is at top. ( $\times 51,500$ )

thick. Yet the most striking feature was a layer of radially arranged fibrillary material approximately 150–200 Å wide on the cytoplasmic surface (Figs. 1 and 2). Small vesicles often had a wider zone of material lining the cavity, but they may not have been sectioned through the centre. At low magnifications they were recognisable because of this overall density which contrasted with the more numerous vesicles of low density. This specialized structure of the wall was seen in slight indentations of the plasma membrane as well as in distinct caveolae and vesicles. At times only part of the wall of a vesicle was so modified.

The endothelium of blood capillaries, venules, arterioles and small arteries also contained the two morphologically distinct types of vesicle. As in the lymphatic endothelium, dense vesicles were less frequent than the type originally described by Palade<sup>19</sup>.

The less-dense vesicles are well-documented structures and are known to occur in endothelium, pericytes and smooth muscle cells. They have been extensively investigated for the possibility of transfer of fluid, solutes and larger molecules across the vessel wall, but at present no conclusive evidence has been forthcoming<sup>1,2</sup>.

The dense vesicles have been observed in the cells of the proximal convoluted tubules in bat kidney, in the liver cells of rats and chickens and also in Kupffer cells<sup>20–22</sup>. Roth and Porter<sup>23</sup> specifically stated that the dense vesicles in these sites were different from and should not be confused with the pinocytotic vesicles of endothelium. They were under the impression that dense vesicles did not occur in endothelial cells, for the vesicles had not been observed previously in endothelium. In the past they could have been overlooked in mammalian tissues, for they may

not be so numerous as in amphibia which have a proportionately greater daily turnover of fluid and protein from blood to lymph<sup>22–24</sup>.

As yet, the functional significance of the two types of vesicle in lymphatic and vascular endothelium is uncertain. However, Roth and Porter have provided evidence that dense vesicles play an important part in the uptake of protein by the developing oocyte of the mosquito in the formation of yolk granules<sup>24–25</sup>. It was suggested that the bristle border on the cytoplasmic surface was responsible for the invagination of the plasma membrane in the formation of pits and vesicles or, what is more likely, for the selectivity of uptake and carriage by the vesicles<sup>24</sup>. So, if these vesicles are specifically concerned with the uptake of protein in other sites, they probably have a similar function in endothelium. Moreover, in endothelial cells they could be concerned with protein transfer across the vessel wall, be it vascular or lymphatic.

The present unconvincing evidence for the role of pinocytotic vesicles (of the less-dense variety) in the transfer of tracer particles across endothelium<sup>1,2</sup> may be due in part to the selectivity of vesicles and to the physical and chemical nature of the injected particles. Therefore the permeability of lymphatic and blood vessels should be investigated with the view to determining the functional significance and selectivity of these two types of endothelial vesicle.

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### Anti-progestational Activity of Rutin on the Rabbit Uterus

A DIET containing 0.4 or 1.0 per cent of rutin, a flavonol glycoside with vitamin P activity, has been shown to have no effect on reproduction<sup>1,2</sup>. However, Cutting *et al.*<sup>3</sup> observed that a diet containing 0.1 per cent rutin significantly impaired the fertility of female mice. Therefore,



Table 1. EFFECT OF SUBCUTANEOUS ADMINISTRATION OF ROUTIN ON PROGESTERONE-INDUCED ENDOMETRIAL CARBONIC ANHYDRASE INCREASE

Dose (mg/animal)	Rutin	No. of rabbits	Carbonic anhydrase content (enzyme unit/g tissue)
0	0	9	51 ± 4
2.0	0	7	583 ± 34
1.0	0	8	307 ± 39
2.0	10.0	5	573 ± 59
2.0	2.0	4	553 ± 54
2.0	1.0	5	565 ± 41
2.0	0.2	3	534 ± 61
2.0	0.1	5	346 ± 28*
2.0	0.02	3	373 ± 18*
2.0	0.01	4	419 ± 27*
2.0	0.001	4	499 ± 31
1.0	10.0	5	300 ± 57
1.0	0.5	5	430 ± 58
1.0	0.05	5	341 ± 24†
1.0	0.005	5	337 ± 60
0	10.0	4	65 ± 7
0	1.0	4	49 ± 5

\* P < 0.01 compared with enzyme response to 2 mg of progesterone alone.  
† P < 0.01 compared with enzyme response to 1 mg of progesterone alone.

Table 2. EFFECT OF ORAL ADMINISTRATION OF ROUTIN ON PROGESTERONE-INDUCED ENDOMETRIAL CARBONIC ANHYDRASE ACTIVITY

Dose (mg/animal)	Rutin	No. of rabbits	Carbonic anhydrase content (enzyme unit/g tissue)
0	0	5	55 ± 3
2.0	0	4	543 ± 38
2.0	100.0	5	547 ± 25
2.0	10.0	5	437 ± 59
2.0	1.0	5	532 ± 68
2.0	0.1	4	613 ± 76

using carbonic anhydrase determination in the uterine endometrium as an indicator of the progestational proliferation in Clauberg rabbits<sup>4</sup>, an attempt was made to elucidate whether or not rutin is capable of affecting the progestational proliferation of the rabbit endometrium.

Experiments were performed on immature albino female rabbits weighing approximately 1.5 kg. Following the Clauberg method<sup>4</sup>, all the animals were primed with a subcutaneous injection of 5 µg oestradiol once daily for 6 days. This was then followed by simultaneous administration of progesterone and rutin once daily for 5 days. Progesterone dissolved in sesame oil was given subcutaneously and rutin was administered either subcutaneously or orally as a thin alkaline saline solution. The standard dose of progesterone was 1.0 or 2.0 mg per animal and the rutin doses varied as indicated in Table 1. The rabbits were killed two days after the last dose. The endometrium was dissected from the isolated uteri and homogenized in cold distilled water. The supernatant of the homogenate was tested for carbonic anhydrase activity by the method of Miyake and Pincus<sup>5</sup>, the activity being expressed in terms of enzyme units/g wet weight of the endometrium<sup>7</sup>.

As indicated in Table 1, a standard dose of progesterone (2 mg or 1 mg) alone exerted a typical activation of endometrial carbonic anhydrase. However, when given subcutaneously, with 2 mg progesterone, rutin in amounts ranging from 0.01 to 0.1 mg appeared significantly to inhibit the endometrial response; 0.01 mg causing a 39 per cent, 0.02 mg a 33 per cent, and 0.1 mg a 28 per cent inhibition. Likewise, a total dose of 0.05 mg rutin also appeared unequivocally to prevent the effect of 1.0 mg progesterone. However, in doses totalling more than 1.0 mg, there was no significant difference between the endometrial enzyme responses to progesterone with and without rutin. In an attempt to show the effect of rutin itself on endometrial enzyme activity, rutin alone was injected subcutaneously in doses of 1.0–10.0 mg per animal. No significant increase in the endometrial enzyme above the control was found. In other experiments (Table 2), when rutin was administered orally in doses of 0.01–100 mg per animal along with 2 mg of progesterone, rutin-treated animals responded by showing a marked increase

in the activity of endometrial enzyme. The degree of increase was essentially the same as that of the enzyme activation characteristic of progesterone alone.

From the present finding that in the rabbit rutin exhibited a weak inhibition of the increase in endometrial carbonic anhydrase caused by progesterone, it seems that rutin acts as an antiprogestational agent, presumably due to a direct action of rutin itself on the uterus.

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## PHARMACOLOGY

### Metabolic Investigations on Pristinamycin

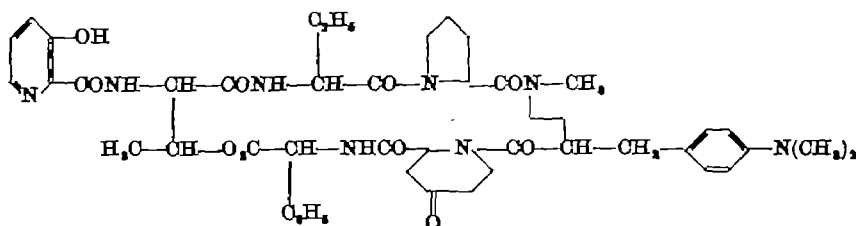
PRISTINAMYCIN is a new antibiotic obtained from *Streptomyces pristinae spiralis*, a *Streptomyces* isolated from a soil sample taken at San Carlos (Cordoba) in Argentina<sup>1</sup>. It is made up of several components and belongs to the family of synergistic antibiotics which includes streptogramin, stephydomycin and ostreogrycin. Chromatographic analysis has shown that pristinamycin is a mixture of two groups of components, the proportions of which vary within certain fixed limits:

Group I	{ Pristinamycin I <sub>A</sub> Pristinamycin I <sub>B</sub>	15–30 per cent 5–10 per cent
Group II	{ Pristinamycin II <sub>A</sub> Pristinamycin II <sub>B</sub>	45–60 per cent 5–10 per cent

Some of these components resemble those of the other antibiotics mentioned, but pristinamycin may be differentiated from these by its antibiotic activity, which results from the whole complex of different constituents<sup>2</sup>.

Pristinamycin, which is administered orally, is highly active against *Staphylococcus aureus*, including strains resistant both to benzylpenicillin and to erythromycin, *Streptococcus pyogenes* and *Streptococcus pneumoniae*. It also shows high activity against strains of *Streptococcus faecalis* and *Haemophilus influenzae* and against *Neisseria* resistant to lincomycin<sup>3</sup>.

Clinical use<sup>4–6</sup> of pristinamycin has prompted us to examine its degradation in the human body, and as a first approach we tried to identify any metabolite originating from one of the main components of pristinamycin which is called constituent I<sub>A</sub> (or 12,535 E.P.), a cyclopeptide of the formula:



This compound is identical to factor B in the ostreogrycin complex, described by Eastwood, Snell and Todd<sup>1\*</sup>.

For the systematic investigation of the degradation products which may be excreted in human urine, it has been necessary to synthesize and to resolve the unusual amino-acids present in constituent I<sub>A</sub>; furthermore, the

Table 1.  $R_F$  VALUES OF SYNTHETIC PEPTIDES RELATED TO PRISTINAMYCIN

Peptide	$R_F^1$	$R_F^2$	$R_F^3$
Pic-Thr	0.20	0.79	0.50
Pic-Thr-But	0.25	0.45	0.53
Pic-Thr-But-Pro	0.48	0.80	0.54
Pic-Thr-But-Pro-Damaphic	0.40	0.61	0.69
Pic-Thr-But-Pro-Damaphic-Pip-Phegly	0.34	0.74	0.59
Pic-Thr-O(Phegly)	0.30	0.50	0.62
Thr-But	0.52	0.15	0.18
Thr-But-Pro	0.15	0.22	0.25
But-Pro-Damaphic	0.22	0.22	0.21
Damaphic-Pip-Phegly	0.51	0.50	0.59
Pip-Phegly	0.22	0.09	0.17
Pic-Gly	0.75	0.41	0.33
Pic-Thr-Gly	0.48	0.55	0.45

The following abbreviations are used: Pic, 3-hydroxypicolinic acid; Thr, L-threonine; But, D- $\alpha$ -aminobutyric acid; Pro, L-proline; Damaphic, L-para-dimethylamino  $\beta$ -methylphenylalanine; Pip, 4-oxopipicolic acid; Phegly, L- $\alpha$ -phenylglycine; Gly, glycine.  $R_F^1$ :  $R_F$  by thin-layer chromatography on silica gel with methanol.  $R_F^2$ :  $R_F$  by paper chromatography with butanol-acetic acid-water (7-1-2).  $R_F^3$ :  $R_F$  by paper chromatography with isoamyl alcohol-pyridine-water (25-25-50).

linear heptapeptide corresponding to the opening of the aforementioned cyclic lactone, and several oligopeptides, as indicated in Table 1, have been synthesized<sup>11</sup>.

The search for the metabolites in urine was performed as follows:

(1) For the untransformed constituent  $I_A$ .

This investigation was carried out by thin-layer and paper-chromatography as well as by bioassay<sup>12</sup>.

(2) For compounds arising from possible degradation of the cyclopeptide.

The search for the amino-acids was performed by chromatography of the urines by means of a Technicon automatic amino-acid analyser. Synthetic amino-acids and urine collected immediately before the ingestion of pristinamycin were used for reference purposes.

The assay, of peptides which could occur from cleavage of the cyclopeptide, was carried out by two techniques: paper chromatography was used for comparison of urines with blanks containing synthetic peptides, and thin-layer chromatography on silica gel was used for a systematic search for peptides. Comparative chromatograms of the urines to be investigated, blanks, and urines with added pristinamycin, were developed. Parallel areas were cut out on each plate, silica gel corresponding to each area was collected and eluted and the eluate was hydrolysed. Quantitative assays of the hydrolysates were then performed in order to determine the presence or absence of peptides and to evaluate their qualitative composition.

(3) For modified hydrolysis products.

The results of the foregoing investigation suggested that a search be made for compounds the structure of which resulted from a modification of the metabolites derived from the cyclopeptide. Paper chromatography, especially on anion exchange paper, was used for this work.

A complete survey of the urines of volunteers, who ingested 4 g of pristinamycin over 32 h in four administrations, gave the following results:

(1) About 10 per cent of constituent  $I_A$  of pristinamycin was excreted without transformation.

(2) No single amino-acid derived from constituent  $I_A$  was excreted in any detectable amount in urine.

(3) No peptide metabolite could be identified by comparison with the synthetic peptides listed in Table 1. These include the linear heptapeptide which would result from opening the macrolactone and correspond to theoretical hydrolysis fragments of constituent  $I_A$ .

(4) However, some metabolites could be detected in small amounts; ultra-violet absorption and fluorescence showed that they were related to 3-hydroxypicolinic acid. The main product was identified as a derivative of 3-hydroxypicolinylglycine, but even this peptide dis-

appears from the urine 6 h after the absorption of pristinamycin.

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### Significance of Smoking in Investigations of Urinary Excretion Rates of Amines in Man

In the determination of amphetamine ( $T_R$  9 min) by a gas-liquid chromatographic method<sup>1</sup>, an additional peak ( $T_R$  26 min) was observed in the extracts from urine of some but not all subjects; only subjects who smoked gave this latter peak. In smokers, large differences were observed in the 'apparent amphetamine' content of the urine determined by a methyl orange complexing method (using 1:2 dichloroethane as the extracting solvent, a solvent wash with borax pH 9.2 and measurements read at 540 m $\mu$ ), and the amphetamine content determined by gas-liquid chromatography<sup>1</sup> (Fig. 1).

The additional component with a  $T_R$  of 26 min in the urine of smokers was shown to be nicotine by gas-liquid chromatography, thin-layer chromatography and also by the infra-red spectrum of the isolated base.

The sum of the amphetamine content and the nicotine content, both determined by gas-liquid chromatography, gave values comparable to the 'apparent amphetamine' content determined by the methyl orange technique.

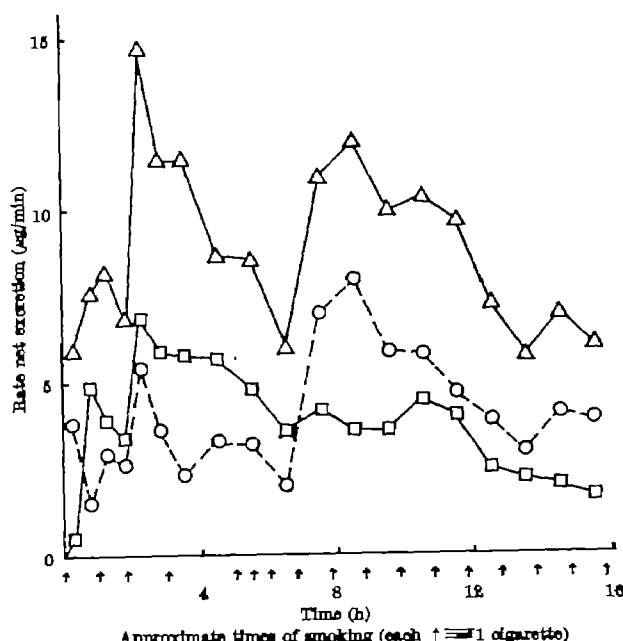


Fig. 1. True ( $\square$ ) and apparent ( $\Delta$ ) rates of amphetamine excretion in the urine of a smoker as determined by gas-liquid chromatography for the former, and by the methyl orange procedure for the latter. The rate of nicotine excretion ( $\circ$ ) in the same urines is also indicated

The formation of complexes with methyl orange is a commonly used method for the determination of amines in urine and other biological fluids<sup>1-4</sup>. However, there seems to be little indication that only non-smokers have been used for investigations in man, although it has been reported<sup>1</sup> that nicotine complexes with the dye in the methyl orange technique. We have found that in many of the diverse methyl orange methods<sup>1-4</sup> the nicotine-methyl orange complex partitions into the organic phase and thus interferes with the determination of amines in biological fluids by these methods.

Using a gas-liquid chromatographic method for the determination of nicotine, we found that the rate of nicotine excretion in urine is influenced by the pH of the urine (see also ref. 9). Subjects smoking 20 cigarettes a day at fairly regular intervals excreted 0.1-4.3 (mean 1.0)  $\mu\text{g}$  nicotine per min under normal conditions, 0.4-13.0 (mean 5.0)  $\mu\text{g}$  nicotine per min when the urine was acidic after the oral administration of ammonium chloride, and less than 0.1  $\mu\text{g}/\text{min}$  when the urine was alkaline after the oral administration of sodium bicarbonate.

Usually 'blank' values for urine by the methyl orange method are obtained before the method is used for the determination of drugs in urine. Obviously the 'blank' value for a smoker will depend on his smoking habits and the pH of the urine at the time of collection; this value may have little significance when applied as a correction at the time of the determination of the drug.

It is our opinion that smokers should not be used in urine excretion investigations of basic drugs unless nicotine has been shown not to interfere with the assay procedure adopted.

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### Biological Half-lives of the Antibiotic Lincomycin Observed in Repetitive Experiments in the same Subjects

SOME information on the disappearance half-time of lincomycin (the registered trade-mark of the Upjohn Company is 'Lincocin') has been reported by Ma, Lim and Nodine<sup>1</sup> and by Wagner and Alway<sup>2</sup>.

We now wish to report biological half-lives of lincomycin estimated from serum levels obtained after oral administration of lincomycin hydrochloride in doses equivalent to 0.5 g lincomycin base. In three experiments (I, II and III) doses were administered to the same panel of six adult male subjects at 0, 7, 28, 35, 84 and 91 days. In experiment IV a panel of sixteen adult male subjects received doses one week apart. Table 1 lists the formulations and their code numbers used in the four experiments.

Experiments II, III and IV were cross-over studies in which half the subjects received one formulation and the other half received the other formulation the first week; the formulations being reversed with respect to

Table 1

Expt.	Code	Type
I	1	Hard-filled capsule containing only lincomycin hydrochloride.
II	1	Ibid.
II	2	Aqueous solution formed by dissolving the above capsule contents in one-half glass of water.
III	3	Hard-filled capsule containing lincomycin hydrochloride, lactose, talc, and magnesium stearate.
III	4A	Pediatric solution containing lincomycin hydrochloride, preservatives, sweetening agents and flavouring agents.
IV	3	Ibid.
IV	4B	Pediatric solution—same formula as 4A but different lot.

subjects the next week. In each experiment blood samples were obtained at zero time (just before administration) and at 1.5, 3, 4.5, 6, 8, 10, 12 and 14 h after administration. Serum was collected and frozen. Antibiotic concentrations in serum were determined by the agar diffusion method with *Sarcina lutea* ATCC 9341 as the test organism. The method was the standard cylinder plate procedure of Hanks *et al.*<sup>3</sup>, as modified by Vavra *et al.*<sup>4</sup>.

Disappearance rate constants were calculated in the usual way<sup>5</sup> by first plotting the serum levels against time on semilogarithmic graph paper. All terminal points which appeared randomly distributed about a straight line were used. The method of least squares was employed using an IBM '1620' computer programme. The computer converted the serum levels to their logarithms (base 10) and printed out the slope of the least squares line. Division of the slope into 0.301 ( $\log_2$ ) gave the biological half-life,  $t_{1/2}$ .

The biological half-lives calculated from serum levels observed in repetitive studies in the same six subjects are shown in Table 2.

The biological half-lives calculated from serum levels observed in Experiment IV are shown in Table 3. The median half-life is 4.8 h, which agrees quite well with the overall median half-life of 4.6 h obtained in the other three experiments.

Analysis of the data shown in Tables 2 and 3 provides little, if any, evidence that the biological half-life of lincomycin changes significantly with time when repetitive oral doses are given at weekly or less frequent intervals.

Ma, Lim and Nodine<sup>1</sup> reported a longer disappearance half-time of lincomycin after the last dose than that observed after the first dose when lincomycin hydrochloride was administered intramuscularly every 12 or 24 h to human subjects on a multiple-dose regimen. The difference in apparent average half-lives was not statistically significant, but they suggested that "further studies or the further analysis of available data should be carried out elucidating this point".

However, 'disappearance rates' and 'half times' reported by these authors following multiple doses were estimated from serum concentrations observed at 1 and 12 h or 1 and 24 h after administration. Our analysis of lincomycin serum levels following intramuscular administration indicates that part of the dose is absorbed over at least a 12 h period. Hence, Ma, Lim and Nodine estimated half-lives from data containing points in the absorption-tissue distribution phase, and not really a set of points randomly

Table 2. BIOLOGICAL HALF-LIVES (IN HOURS) OF LINCOMYCIN OBSERVED IN REPETITIVE STUDIES IN THE SAME SIX ADULT MALE SUBJECTS

Study	Date	Subject						Date Median
		H	B	O	K	B	P	
I	18/3/64	—	0.13*	—	5.35*	—	4.63*	5.26
	25/3/64	13.8*	—	5.37*	—	4.47*	—	5.37
II	24/3/64	4.00†	4.24†	4.43*	5.23*	5.23*	4.51†	4.47
	31/3/64	5.90*	5.02*	4.80†	4.55†	4.10†	4.56*	4.68
III	19/5/64	7.86†	8.43†	5.15†	4.24†	4.59†	4.47†	4.87
	26/5/64	9.09†	4.90†	4.22†	4.09†	4.18†	4.14†	4.20
Subject median		7.86	5.52	4.80	4.56	4.47	4.51	4.60

\* Formulation 1 (median half-life = 5.29 hours)

† Formulation 2 (median half-life = 4.38 hours)

‡ Formulation 3 (median half-life = 4.36 hours)

§ Formulation 4A (median half-life = 4.37 hours)

Table 2. BIOLOGICAL HALF-LIVES (IN HOURS) OF LINCOMYCIN OBTAINED IN CROSS-OVER EXPERIMENT IV IN SIXTEEN ADULT MALE SUBJECTS

Group of subjects	Cross-over No. 1	
	19/5/64	20/5/64
	3*	4B*
1-4	5.87	5.40
	6.63	4.43
	3.77	3.76
	4.19	3.62
	4B	3
5-8	4.93	5.46
	5.31	5.24
	4.78	4.74
	3.44	4.41
Median half-life	4.86	4.58
Cross-over No. 2		
	20/5/64	27/5/64
	3	4B
9-12	4.25	4.18
	4.50	4.81
	4.02	3.77
	5.27	7.64
	4B	3
13-16	5.71	11.5
	4.62	5.81
	5.20	5.13
	8.80	3.52
Median half-life	4.66	5.31

\* Formulation code; see Table 1.

distributed about a straight line on semilogarithmic paper. If absorption is still proceeding when  $t_1$  is estimated, the estimated  $t_1$  will be higher than the true value<sup>1</sup>. Re-evaluation of their data does not suggest an increase in half-life following a multiple-dose regimen.

Assuming a disappearance rate constant estimated from data obtained after the first dose, Wagner and Alway<sup>2</sup> made predictions of serum levels of lincomycin which agreed reasonably well with serum levels observed after multiple intravenous injections.

Further examination of Tables 2 and 3 shows that median half-life is essentially independent of the specific formulations involved. The rate constant for disappearance (or the biological half-life) of a drug in the body would be expected to be independent of dosage form effects if the data were properly collected and interpreted<sup>3</sup>. After the solution and capsule treatments, the median biological half-lives were: Experiment II, 4.38 and 4.55; Experiment III, 4.87 and 4.86; and Experiment IV, 4.80 and 4.99, respectively. Hence, in two of three experiments, the capsule yielded a very slightly greater median half-life.

In addition, fitting of the serum level data to a three-compartment model (Model I, ref. 5) indicated that first-order rate constants for appearance of lincomycin in the blood were not significantly different after the capsules, compared with the solutions. These additional analyses gave us confidence that absorption was, for all practical purposes, complete at the times the half-lives were estimated.

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### Influence of the Substantia Nigra on the Concentration of 5-Hydroxytryptamine and Dopamine of the Striatum

EVER since the finding that certain of the basal ganglia contain relatively high concentrations of dopamine (3-hydroxytyramine)<sup>1,2</sup> there has been a growing interest in the possible role of this amine in the functioning of these structures of the extrapyramidal system<sup>3</sup>. Hornykiewicz *et al.* have demonstrated the virtual absence of this amine from the caudate nucleus and putamen of patients dying from Parkinson's disease<sup>4,5</sup> and, even though the concentrations of noradrenaline<sup>6</sup> and 5-hydroxytryptamine<sup>6</sup> in these regions of the brain are substantially below normal values, the decrease in the concentration of dopamine is greater and, in contrast, uninfluenced by administration of monoamine-oxidase inhibitors<sup>7</sup>.

In work concluded some time ago in our laboratories it was possible to show that experimental lesions in the ventromedial tegmental area of the brain-stem of rhesus monkeys result eventually in the loss of dopamine and noradrenaline from the corpus striatum<sup>8,9</sup>. Only those lesions that resulted in a major loss of cells of the substantia nigra produced a fall in the concentration of the catecholamines. The present work was undertaken to determine whether 5-hydroxytryptamine (found in the striatum of man in concentrations of about 0.5-1.0 µg/g<sup>10</sup>) is also influenced by a nigral lesion. The technique was the same as in our previous work: an electrolytic lesion was placed along the dorsomedial edge of the substantia nigra of seven monkeys with the aid of a stereotaxic instrument; lesions were made on the left side, the right side remaining untouched. After 108-126 days during which neurological observations were made, the animals were killed under deep 'Nembutal' anaesthesia, and the brains were removed. The caudate nucleus and putamen of each side were dissected, weighed and stored in the frozen state until the chemical analyses were performed. The remainder of the brain-stem was placed in 10 per cent neutral formalin, in preparation for histological examination. The parts of the brain to be analysed were homogenized in three parts of 0.1 N hydrochloric acid. Half this homogenate was then treated for determination of 5-hydroxytryptamine by the method of Bogdanski, Pletscher, Brodie and Udenfriend<sup>10</sup>. Dopamine was determined in the other half by the procedure of Sourkes and Murphy<sup>11</sup>, after extraction by the method of Shore and Ohm<sup>12</sup> for noradrenaline. Sections of the brain-stem were stained<sup>13</sup> and then examined to identify the site of the lesion and its effect, if any, on the cellularity of the substantia nigra. In this series, four of the seven monkeys showed a great loss of cells from the pars compacta on the operated side; the side opposite to the lesion had the usual degree of cellularity seen in sections taken from unoperated monkeys. This result is illustrated in Fig. 1.

A summary of the amine analyses is given in Table 1. It is evident that those animals that showed considerable loss of cells from the substantia nigra also lost much of the dopamine from the striatum of the same side; indeed, the loss amounted to 81-100 per cent of the dopamine, compared with the corresponding control structure. This, then, is in confirmation of earlier findings<sup>8,9</sup>. On the other hand, in those monkeys whose substantia nigra had a normal cellularity there was no difference between the striata of SP9 or between the caudate nuclei of SP1. The caudate nuclei of monkey SP2 and the putamenal nuclei of SP1 and SP2 showed large differences as between the operated and intact (control) sides, but these were not consistent in the direction of the differences.

In regard to the concentration of 5-hydroxytryptamine, the values for the nuclei of the operated side in the three animals retaining a normal complement of nigral cells (SP1, SP2 and SP9) ranged from 86 to 179 per cent of the values found in the corresponding parts on the intact side of the brain. The lower and higher extremes were found

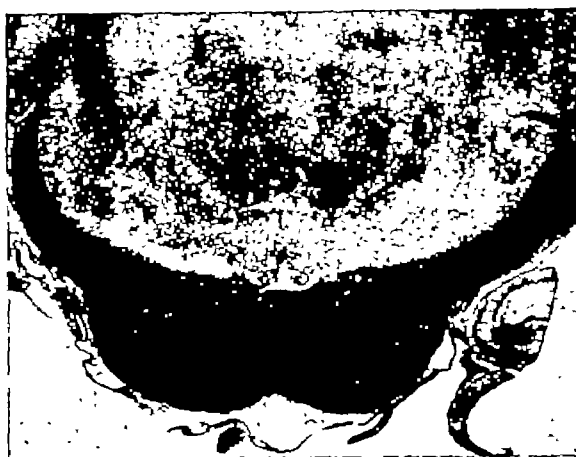


Fig. 1. Transverse section through midbrain of monkey SP7 showing complete cell loss in the pars compacta of the substantia nigra on the side on which an electrolytic lesion had been made. The lesion, which interrupted the corresponding nigrostriatal fibres, was rostral to the level of the section shown

in the caudate nuclei of SP9 and SP1, respectively. Of the group of monkeys that had histological evidence of cellular loss on the operated side, SP8 had a greater concentration of 5-hydroxytryptamine on the operated side, but the others (SP7, SP10 and SP12) had 23-33 per cent less than in the corresponding parts of the intact side.

The concentration of dopamine in the brain has been previously shown to be sensitive to experimental lesions of the brain. Lesions of the substantia nigra cause a loss of dopamine from the striatum of the monkey, as determined by direct chemical analysis<sup>10,11</sup> or of the rat, as detected by a histological fluorescence technique<sup>12</sup>; lesions in the globus pallidus of the rabbit result in a decrease of dopamine of the caudate nucleus, beginning between the fourth and seventh days following operation<sup>13</sup>. These results are borne out by the results recorded here obtained with a new series of monkeys. It is noteworthy, however, that the changes in concentration of 5-hydroxytryptamine in the present series bear no obvious relation to those of dopamine. Of the four animals with nigral loss of cells resulting from the lesion, three showed a decrease in the 5-hydroxytryptamine concentration of the striatum on the operated side, but the fourth animal did not. This decrease was moderate (23-33 per cent), compared with the change in dopamine in the same structures or compared to the decrease in the concentration of 5-hydroxytryptamine seen in patients with Parkinson's disease (50-70 per cent)<sup>14</sup>. Thus, the amine changes following the nigral lesion are analogous to those observed in Parkinson's disease with respect to dopamine but, on the basis of the four monkeys in the present series that showed nigral loss of cells, the analogy cannot be extended to 5-hydroxytryptamine.

Table 1. DOPAMINE AND 5-HYDROXYTRYPTAMINE IN MONKEYS WITH UNILATERAL LESION OF THE BRAIN-STEM

Substantia nigra	No.	Structure	Dopamine*		5-Hydroxytryptamine*	
			Operated side	Intact side	Operated side	Intact side
Normal No. of cells on side of lesion	SP1	caudate	5.87	6.03	0.66	0.48
		putamen	2.38	6.28	0.99	1.00
	SP2	caudate	2.73	1.59	0.84	0.35
Decreased No. of cells on side of lesion	SP7	caudate	0.82	3.81	0.08	0.80
		putamen	0.40	3.53	0.56	0.77
	SP8	caudate	0.07	3.99	0.60	0.80
	SP10	caudate	0.94	4.30	0.30	0.58
		putamen	1.35	5.53	0.34	0.44
	SP12	caudate	0.96	3.02	0.30	0.54
		putamen	0.30	2.55	0.31	0.41

\* µg/g wet weight of tissue.

The influence, if any, of nigrostriatal fibres<sup>1</sup> on the concentration of this amine in the brain is being studied further.

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## Anti-arrhythmic Action of Bretylium

THE anti-arrhythmic action of guanethidine which was found to prevent a type of clinical paroxysmal ventricular tachycardia<sup>1</sup> and prevented experimental atrial fibrillation<sup>2</sup> suggested that other agents which interfere with the release of catecholamines might exhibit similar effectiveness.

The procedure used to test this possibility was derived from a method which I developed earlier. In this, after control observations which eliminate animals that occasionally exhibit susceptibility to acetylcholine-induced atrial fibrillation, insulin and glucose are administered. This causes a drop in plasma potassium concentration to a low stable level which occurs in about thirty minutes and remains relatively stationary for about ninety minutes. During this time, dogs are highly susceptible to atrial arrhythmias. This is called the 'susceptible period'.

During the susceptible period, at 15-20-min intervals, the dogs were administered acetylcholine intravenously in doses starting with 0.3 mg/kg. Previous studies have shown that with an unprotected dog such a dose will provoke atrial fibrillation almost routinely (98 per cent) and the fibrillatory dose is usually 0.1 mg/kg or less<sup>3</sup>.

In the initial investigations, the test drug was administered immediately after the test subject had exhibited episodes of acetylcholine-induced atrial fibrillation at least twice at 0.3 mg/kg or less. After administration of the test drug, a latent period of 10 min or more was permitted. Then the preparation was challenged as before with incremental doses of acetylcholine to the maximum dose of 0.5 mg/kg. In later experiments, longer latent periods (up to 18 h) were allowed before challenge. The challenge period, 30-120 min after insulin-glucose administration, was rigidly maintained regardless of the time of administration of the test drug.

Atrial fibrillation at any dose was deemed 'lack of protection', but 'protection' means that the test drug protected at the highest dose (0.5 mg/kg) and all lower doses. The highest dose was administered three or more

Table 1. EFFICACY OF BRETILIUM TO PROTECT AGAINST EXPERIMENTALLY INDUCED ATRIAL FIBRILLATION (Some groups have been combined when the data obtained indicated close agreement of the parameter under observation)

1 Experimental series	2 No. of experiments	3 Dose of bretylium (mg/kg)	4 Route of administration	5 Delay before challenge (h)	6 Protected challenges/total challenges	7 Range of protection (individual) (%)	8 Percentage protected
d	6	5	I.V.	0.5-2	3/25	0-100	12
e	4	5	I.V.	1-3	8/17	0-100	47
f	10	8, 10(2)†	I.V.	0.5-3	19/30	0-100	63
g	7	8, (2)†, 10	I.V. (2)† and S.C.	4-6	10/27	0-100	70
h	9	5(1)†, 8(2)†, 10	I.V. (2)† and S.C.	18-19	0/23	0	0
i	2	2.5	S.C.	2-8	0/5	0	0
j	2	2.5	S.C.	4-6	8/6	0-75	50
k	2	5	S.C.	2-8	0/6	0	0
l	9	5	S.C.	4-6, 7-9	22/32	0-100	69
m	4	5, 10(1)†	S.C.	12-15	4/13	0-80	33
n	7	8(2)†, 10	I.V. (2)† and S.C.	4-6	19/27	0-100	70
o	4	10	S.C.	6-7	6/15	0-100	40
p	9	5(1)†, 8(2)†, 10(6)†	I.V. (2)† and S.C.	18-19	0/23	0	0
q	11	20	S.C.	4-6	28/43	0-100	65
r	4	20	S.C.	8-9	5/16	0-60	31
s	3	30	S.C.	4-6	11/16	43-100	69
t	6	30	S.C.	18-19	7/17	0-100	41
u	4	30	S.C.	18-19	8/8	100	100

\* See both series.

† (1)†, (2)†, etc., indicate number of dogs of different dose or route than the principal series but that the end results were similar.

‡ Same experiment, different challenge dose of acetylcholine to indicate partial protection.

§ ACh dose 0.5.

¶ ACh dose 0.4.

times and a percentage was calculated from the protected challenges divided by the total number of challenges. These are the figures shown in columns 6 and 7 of Table 1.

In addition to the specific tests to determine anti-fibrillatory activity of bretylium, four other related determinations were made: (1) An attempt was made to determine the latent period before activity of the drug after intravenous and subcutaneous administration. (2) The period of maximum effectiveness of the drug was sought. (3) The duration of the drug action was ascertained for various dose levels. (4) An attempt was made to determine a dose-effect relationship at the previously found time of maximum effectiveness. To do this, dogs which were not protected at low doses were allowed rest periods of a week, then they were administered a higher dose of bretylium and retested. The dosage range for this series was 2.5-30.0 mg/kg.

In all, 108 experiments were performed, involving 20 dogs. The pertinent experiments are presented in Table 1. All the dogs used exhibited susceptibility to atrial fibrillation in separate control experiments before and after experimental runs with bretylium.

Bretylium exhibited an anti-fibrillatory effect in a reasonably constant fashion which was dose-dependent when the drug was administered subcutaneously. Intravenous administration of bretylium led to inconsistent results unless the drug was infused over 10-15 min. Rapid renal excretion is assumed to be the factor concerned with this decrease in efficacy. Both rapid and prolonged injections are presented together in Table 1.

An examination of the results obtained yields the following conclusions: (1) Doses of 2.5 mg/kg or less were almost ineffective. (2) Some dogs were protected at all doses above 2.5 mg/kg. (3) A period of about 4 h was necessary for maximum effectiveness. (4) The effect of the drug was greatly diminished at 8 h. (5) Nearly all the dogs were protected at 30.0 mg/kg or less (18 of 20). (6) At the time of maximum effectiveness (4-5 h), with the highest dose (30.0 mg/kg), only two dogs were not fully protected (series x). (7) No anti-arrhythmic effect was found at 18 h except with the highest dose (30.0 mg/kg) (series z).

It became evident during this work that the results followed an approximate linear log dose-response relationship, for with each increase of the dose of bretylium, more of the dogs were protected. The number of observations for each point is inadequate to define the curve specifically, but the hypothesis that the maximum dose (30.0 mg/kg) would be completely protective was tested. With experiments at the highest dose (30.0 mg/kg), three dogs which

were unprotected at lower doses were retested. With this dose of bretylium, one dog was totally protected and two others were partially protected—that is, protected against 0.4 mg/kg but not against 0.5 mg/kg acetylcholine. The value of continuing with greater dosage of bretylium was dubious.

Previous reports which relate this anti-arrhythmic activity have not been found. Boura and Green<sup>4</sup> have reported local anaesthetic action which may be operant here. However, it is surprising that a 4-h delay is necessary before activity is demonstrated and intravenous injections have very low immediate efficacy, since one would expect local anaesthetic action to have less latency.

The diminished action after 12 h may suggest that the action herein reported may not be related to prevention of catecholamine release since such a mechanism has been shown to be maximum at about 20 h<sup>5</sup>. The similarity of pharmacological action for bretylium and previously reported for guanethidine implies a related mechanism.

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## HAEMATOLOGY

### Blood Preservatives for the Glutathione Stability Test

WHEN red cells deficient in glucose-6-phosphate (G-6-PD) are incubated with acetylphenylhydrazine under standardized conditions, there is a marked drop in concentration of reduced glutathione (GSH). Under the same conditions, the GSH of normal cells is relatively unaffected<sup>1</sup>. This procedure, the glutathione stability test, has been widely used in the diagnosis of G-6-PD deficiency<sup>2</sup>.

Recently, Davies and Gower<sup>3</sup> reported that ACD (acid-citrate-dextrose), used as a blood preservative for carrying out the GSH stability test, was unsatisfactory unless its pH was adjusted to 7.6. This finding was quite surprising

Table 1

Day of storage	pH 5.0 system		pH 7.6 system	
	Initial GSH (mg/100 ml. RBC)	After 2 h incubation with APH (mg/100 ml. RBC)	Initial GSH (mg/100 ml. RBC)	After 2 h incubation with APH (mg/100 ml. RBC)
0	57.0	56.31	51.1	39.5
6	50.23	44.70	55.16	43.96
13	46.45	47.0	53.71	52.76
20	53.24	41.08	51.55	30.23
27	48.29	41.94	54.37	16.82
35	45.33	36.00	45.39	13.82

to us, since both Szeinberg *et al.*<sup>4</sup> and McGovern *et al.*<sup>5</sup> had reported that the glutathione stability test gave satisfactory results for up to 4 weeks when blood was stored in ordinary ACD solution, and their results were recently confirmed by Palek and Volek<sup>6</sup>. Many other investigators have used ACD as a preservative solution for the purpose of this test. We have, therefore, compared the GSH stability of normal blood preserved in ACD, National Institutes of Health formula B, at the usual pH 5.0, and as recommended by Davies and Gower<sup>3</sup> at pH 7.6. The GSH stability test was carried out as originally reported<sup>1</sup>, except that the DTNB method<sup>7</sup> was used for the determination of glutathione.

Several samples of blood were examined. The results of a typical experiment are summarized in Table 1. When blood was preserved in ordinary ACD solution glutathione stability was essentially unaltered for approximately 4 weeks. When blood was stored in ACD solution the pH of which had been adjusted to 7.6, GSH was abnormally unstable initially, and markedly so after 3 weeks.

Careful examination of the studies of Davies and Gower clarifies the reason for the discrepancy between their findings and those of others. It is apparent that Davies and Gower have carried out the glutathione stability test in a manner very unlike that originally reported<sup>1</sup> and used by other investigators. They have pre-incubated their blood samples at 37° C for 24 h prior to carrying out the glutathione stability test. Studies carried out in this laboratory have recently shown that ACD solution, with pH values of 5.0 are very poor preservatives of red cell ATP (adenosine triphosphate) at 37° C, when compared with solutions of pH 7.6 (ref. 8). Adenosine triphosphate is required for the utilization of glucose by red cells. It might, therefore, not be surprising that some red cells would manifest better GSH stability after prolonged incubation at 37° C in such a preservative solution than in the ordinary ACD solution. It must be emphasized, however, that the glutathione stability test is not carried out by pre-incubating blood at 37° C for 24 h. Only a 2-h incubation is involved. As shown in Table 1, when the test is carried out in the conventional manner, ACD solution pH 5.0 is clearly superior to ACD solution pH 7.6. The latter solution cannot, therefore, be recommended as a preservative for the glutathione stability test.

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## PATHOLOGY

## 'Yellow Fat' in the Wild Rabbit

DURING investigations into the population dynamics of the European wild rabbit (*Oryctolagus cuniculus* L.) on the island of Skokholm, Pembrokeshire, an abnormal condition of adipose tissue was observed. The condition is apparently unknown in rabbits elsewhere, except on the adjacent island of Skomer, and is hitherto undescribed.

During the severe winter of 1962-63 about half the Skokholm population of approximately 6,000 rabbits became emaciated and died. Healthy, dead and dying rabbits were collected and examined in February and March 1963. Necropsy showed that about half of these had no fat deposits, but most of the remainder had abundant fat of a yellow-brown colour around the kidneys. It extended along the para-vertebral areas into the pelvis to enclose the bladder ventrally. Normal fat was observed in very few animals. In animals where the change was slight, dark brown or yellow pin-point spots could be seen; in late winter and spring, both yellow and brown patches were commonly seen in the same animal. The 'yellow fat' is characteristically marbled or mottled in appearance, varies in colour from orange-yellow to dark brown, and unlike normal fat is hard and firm in the live animal; the surface of severely mottled fat is usually smooth, but it may occasionally be rough and uneven. The incidence of moderate and severe mottling is greatest during periods of reduced food quality, and, in females, during pregnancy and lactation. The condition has been seen in animals as young as six weeks.

Histological examination of the fat showed adipose tissue cells of variable size, some small ones bordered by giant cells and small clusters of histiocytes. The fat was divided into small lobules by strands of fibrous tissue, which contained occasional clusters of granules of a brown pigment. Staining by Nile-blue sulphate, sudan dyes, perchloric acid naphthoquinone<sup>1</sup> and the O.T.A.N. methods<sup>2</sup> demonstrated the presence of abundant neutral fat, together with free fatty acids and some phospholipid in the region of the giant cells. Cholesterol was not demonstrated, though birefringence was seen where fatty acids occurred. Much of the fatty material present was not soluble in lipid solvents; extraction of carbowax sections with chloroform-methanol (2:1) failed to remove any appreciable quantity of sudanophilic material, and sections of tissue embedded in paraffin wax also showed abundant material stainable with the sudan dyes.

The brown pigment was not acid-fast, but was positive to Schmorl's reaction. It was not autofluorescent. Prolonged oxidation of unsaturated fats can produce such a pigment. The condition is similar to lipogranulomatous conditions such as sclerema neonatorum, which occurs in man; similar conditions have been described in mink<sup>3</sup>, cat<sup>4</sup>, pig<sup>5</sup> and rat<sup>6</sup>. In these cases, a high intake of polyunsaturated fat (for example, cod liver oil) in the presence of a relative deficiency of vitamin E has been implicated. It is likely that the rabbits we describe, living on a small island inhabited by numerous sea-birds and with a sparse growth of grass, are ingesting some such fish oils. In the absence of vitamin E, unsaturated fats can give rise to unstable peroxides, and thus to the brown oxidation products which are described here. These can stimulate an inflammatory reaction in the tissues where they occur.

Seito *et al.*<sup>7</sup> stated that 'yellow fat' in pigs was more fluid than normal fat. We have not found this to be so in the rabbit, probably because the fat is interspersed with fibrous tissue and resinous oxidation products. The 'yellow fat' does not seem able to act as a reserve food store, as does normal fat. The rabbits, dying of starvation, still possess appreciable perirenal adipose tissue, though of this altered nature.



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### High Antihæmophilic Factor In Multiple Myeloma

An investigation of antihæmophilic activity in 18 patients with multiple myeloma showed a high antihæmophilic factor (AHF) in 10 cases. The elevated AHF values were not related to hyperglobulinaemia or any other factor which could be determined.

AHF assay was performed by the method of Bergna<sup>1</sup>. Assays on 52 normal plasmas ranged from 50 to 200 per cent of the group mean. Assays were performed on 18 patients with multiple myeloma in various stages of the disease. The blood was drawn at 10.00 a.m. and at bed rest except for one ambulant patient. Symptoms of fever and pain and medications were noted. No patients were studied directly after transportation to the hospital, and none had acute fractures.

The AHF assays of the 18 patients were examined in relation to the age and sex of the patient; total serum protein and albumin and globulin fractions; serum and urine electrophoresis with quantitation of serum proteins by electrophoresis using the Beckman/Spinco 'Analytrol'; immuno-electrophoresis<sup>2</sup> using anti-gamma 2 and anti-gamma<sub>1</sub> A sera; content of plasma cells in particle smears of aspirated marrow; therapies employed in treatment; and the clinical state of the patient.

Twelve patients had elevated AHF values. Nine were over 300 per cent of the normal group mean, the highest value recorded being 630 per cent. Eleven of these 12 cases with elevated AHF had hyperglobulinaemia, and one had a normal serum globulin level with gamma globulin in the urine. The patient with the highest AHF assay was untreated and had normal total protein and a normal serum globulin fraction by chemical determination, but had an elevated beta fraction by electrophoresis, and gamma<sub>1</sub> A by immuno-electrophoresis. Of the remaining six cases, five had normal AHF levels and marked hyperglobulinaemia. One patient, untreated, aged 74, was lowest in AHF (30 and 60 per cent) and had a very high gamma of 77.2 per cent with a total protein of 10.1 g per cent.

The plasma and serum of one patient, whose plasma contained 390 per cent AHF, were studied in the thromboplastin generation test<sup>3</sup>. His plasma, diluted with 7 parts of hæmophilic plasma, produced a normal TGT, while normal plasma diluted with 3 parts of the same hæmophilic plasma give an abnormal TGT. The patient's serum showed no thromboplastic effect. Cohn 'Fraction I' prepared from this patient assayed at 85 per cent of the original plasma AHF. Later, 160 ml. of the same patient's plasma containing 2.92 units of AHF in 1 ml. (1 unit = 1 ml. of normal plasma assaying 100 per cent AHF activity) was fractionated by the method of Cohn<sup>4</sup>. 'Fraction I', dissolved in 15.5 ml., had an assay of 22.7 units per ml., representing 75 per cent of the original activity.

Pitney and Elliott<sup>5</sup> demonstrated that Australian Aborigines, who have hyperglobulinaemia, have a higher plasma AHF than white Australians. They also found elevations of AHF in white Australians who had hyperglobulinaemia associated with lymphosarcoma, autoimmune disease, hepatic cirrhosis, nephrosis and multiple myelomatosis. They thought the rise in AHF might be related to the increased globulin production. Pitney *et al.*<sup>6</sup> studied AHF in normal families and concluded that there was no relation to sex, but there was an increase with age. They found no relation between concentrations of AHF and plasma proteins, particularly gamma globulin, in their normal individuals. Amundsen *et al.*<sup>7</sup> reported elevated AHF levels in patients with carcinoma. Ingram<sup>8</sup> found an increase in AHF following infusion of adrenaline, and Iatrides<sup>9</sup> demonstrated elevated AHF after exercise. Increase in AHF after trauma and childbirth has been shown by Davidson and Tomlinson<sup>10</sup>. Increase in AHF in pregnancy has been reported by Strauss and Diamond<sup>11</sup>.

In the present study there was no consistent correlation of the AHF activity with age, sex, therapy, hyperglobulinaemia, type of abnormal globulin (gamma 2 or gamma<sub>1</sub> A), degree of plasma cell hyperplasia in the bone marrow, or clinical state of the patient. No patients had any recognised thrombotic complications.

The site of origin of AHF is not known. One wonders if the abnormal protein production by the plasma cells in multiple myeloma could include AHF or AHF activator<sup>12</sup> in some cases.

The high AHF values in patients with multiple myeloma are a good source of this factor for experimental purposes. The highly concentrated AHF from such plasma is being used in an attempt to produce anti-AHF antibody in the experimental animal.

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### Gel-filtration of Isotopically labelled Ferritin from HeLa Cells

HeLa human cancer cells have been shown to produce ferritin when grown in the presence of iron<sup>1</sup>. HeLa ferritin is electrophoretically heterogeneous; its properties have been compared with those of ferritin isolated from other cells grown *in vitro* and from human and animal tissues<sup>2</sup>. On DEAE chromatography of homogenates of HeLa cells grown in the presence of FeSO<sub>4</sub> (<sup>57</sup>Fe), the distribution of the radioactivity was practically the same as the distribution of ultra-violet-absorbing components in the case of purified horse ferritin; the assignment of the activity to ferritin has been confirmed by paper electrophoresis<sup>3</sup>. The use of radio-iron in such experiments results in great sensitivity and specificity. We now report the behaviour of HeLa ferritin on gel-filtration.

HeLa cells were grown in bottles, and the well-developed monolayers incubated for 24 h with  $10 \mu\text{g FeSO}_4$  and  $0.3\text{--}0.5 \mu\text{Ci } ^{55}\text{Fe}$  per ml., as described previously<sup>3</sup>. The cells were collected and homogenized, and the microsomal and the 105,000g supernatant fractions were obtained as before<sup>3</sup>. The microsomal pellet and the supernatant were diluted with buffer to give solutions with an absorbancy of 30–50 at 210 m $\mu$ , and 0.3–0.5 ml. of these solutions was placed on the column. Usually columns  $55 \times 1$  cm were used; they were loaded to a height of 50 cm with 'Sephadex G-200' (Pharmacia, Uppsala), pretreated with 0.15 M NaCl + 0.02 M phosphate buffer. However, satisfactory separations were also obtained with shorter columns ( $35 \times 1$ , and even  $20 \times 1$  cm). Care has to be taken not to use too concentrated a solution of whole homogenate or of the microsomal fraction, or the flow may be too slow. Elutions were carried out with 0.15 M NaCl + 0.02 M phosphate buffer (pH 7.3–7.4) at a flow rate of 10 min per 1.5-ml. fraction. The absorbancy at 210 and 260 m $\mu$  was measured against a blank of the eluting solution after suitable dilution of the fractions<sup>4</sup>. The activity was determined with a well-type scintillation gamma-spectrometer.

Typical 'fritograms' of the HeLa whole cell homogenate show three peaks (Fig. 1). The pattern is general for cell homogenates; similar fritograms have been obtained with human fibroblasts and liver cells (unpublished results). With the microsomal fraction one symmetrical sharp peak is found (Fig. 2). This fraction is almost free from components of molecular weight below 200,000. The great absorbancy at 260 m $\mu$  indicates a high content of nucleic acids, and the variation in the ratio of the absorptions at 210 and at 260 m $\mu$  between different parts of the peak

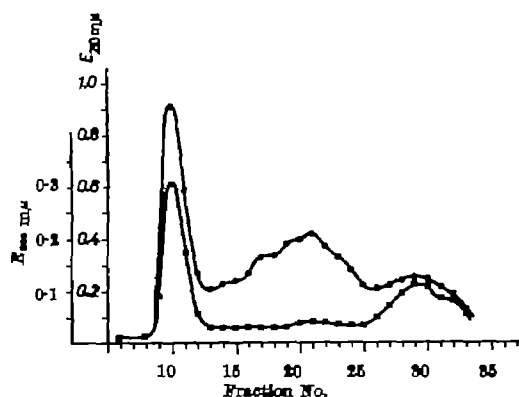


Fig. 1. Gel-filtration of whole cell homogenate. 1 ml solution (absorbancy 30 at 210 m $\mu$ ) was placed on the column ( $35 \times 1$  cm) loaded to a height of 30 cm. The flow rate was 1 fraction (1 ml.)/5 min. Absorbancy at 210 m $\mu$ , O, at 260 m $\mu$ , ■.

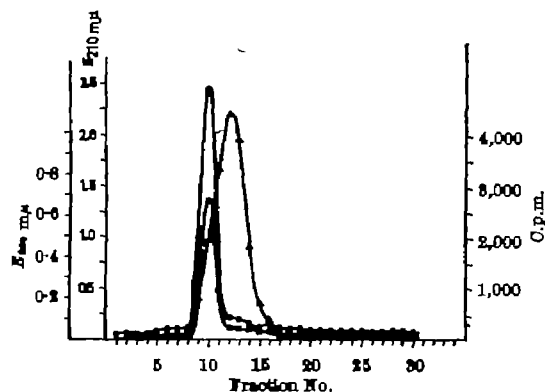


Fig. 2. Gel filtration of microsomal fraction. The column dimensions were  $55 \times 1$  cm and the flow rate 1 fraction (1.5 ml.)/10 min. Absorbancy at 210 m $\mu$ , O, at 260 m $\mu$ , ■; radioactivity, Δ.

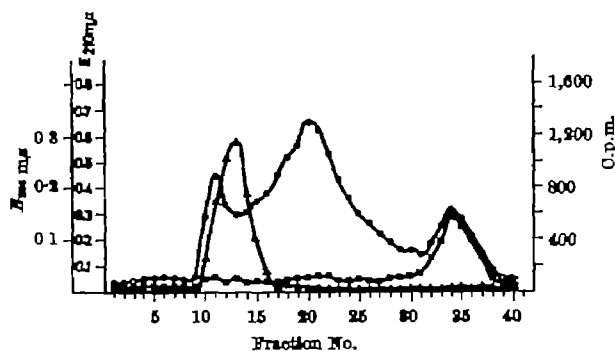


Fig. 3. Gel-filtration of the 105,000g supernatant. Column and signs as in Fig. 2.

suggests the presence of molecular species with unequal nucleic acid contents.

A different pattern is obtained with the 105,000g supernatant (Fig. 3). Small amounts of material passing the column without entry into the gel are noted. Most of the material of the 105,000g supernatant emerges in the second peak. This is asymmetrical; presumably it could be resolved further at slower flow rates. The third peak consists of low-molecular components with high absorption at 260 m $\mu$ ; it is missing from cell homogenates dialysed before gel-filtration. To judge from the 210/260 ratio, the first and second peaks, although macromolecular, are chemically different from the microsomal peak. Values of the ratio between 12 and 25 were obtained for the 105,000g supernatant fractions, and only between 2.8 and 3.1 for the corresponding microsomal fractions.

The distribution of the radio-iron in the microsomes as well as in the 105,000g supernatant is interesting. In both fritograms a fairly sharp peak appears that is distinct from the microsomal peak (Fig. 2) and also from the first peak of the 105,000g supernatant (Fig. 3). The appearance of the radioactivity in the range of the material excluded from the gel is consistent with the known values of the molecular weight of ferritin. Apoferritin has a molecular weight of 465,000 (ref. 5) and that of ferritin itself is up to 747,000, depending on the iron content<sup>6,7</sup>. The displacement of the radioactivity peaks as compared with the absorption peaks suggests the possibility of differentiation of macromolecules with weights greater than 200,000, the approximate exclusion limit from 'Sephadex G-200'. The discrimination by 'Sephadex' columns of molecules heavier than 200,000 is also reflected by the differences of the 210/260 ratio between different parts of both the microsomal peak and the first peak of the 105,000g supernatant.

Purified horse spleen ferritin<sup>8</sup> behaved on gel-filtration on 'Sephadex G-200' like the radioactive component of homogenates of HeLa and of fibroblasts grown *in vitro*<sup>8</sup>. When  $^{55}\text{Fe}$ -labelled homogenate of fibroblasts was mixed with horse ferritin and run through the column, the position of the radioactivity peak was almost the same as that of the absorbancy peak. It thus appears that the molecular size of ferritin of the cells grown *in vitro* is similar to that of ferritin in animal tissues.

The results obtained demonstrate the suitability of gel-filtration for the analysis of cell homogenates. Macromolecular components can rapidly be separated from low-molecular substances. The method is also well suited to the separation of the microsomal fraction from smaller macromolecules and for the differentiation of components of the 105,000g supernatant. The effectiveness of the separation is well illustrated by the sharpness of the ferritin peak in the fritogram, as measured radiochemically.

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## IMMUNOLOGY

### Specifically Increased Graft-versus-Host Reactivity of Thymus Cells from Immunized Mice

It has recently been shown that thymus cells from immunized mice are capable of transferring immune reactivity. Sub-lethally irradiated recipient mice exhibit recall tetanus antitoxin production after they have been injected with thymus cells from mice immunized with tetanus toxoid<sup>1</sup>. Similarly, thymus cells from mice immunized with bovine serum albumin are capable of transferring antibody formation capacity when injected into non-irradiated recipients<sup>2</sup>. The work recorded here was undertaken to determine whether thymus cells from mice immunized to  $F_1$  hybrid histocompatibility antigens would exhibit specifically increased graft-versus-host immunological reactivity after injection into  $F_1$  hybrid mice of the immunizing strain. We will show that immunization does specifically increase the graft-versus-host reactivity of thymus cells, and that the relative increase in reactivity is similar to that observed with injection of spleen cell populations from similarly immunized animals.

$A$ -strain mice 45 days of age were divided into three groups. One group of mice received an immunizing injection of 50 million ( $A \times O3H$ ) $F_1$  hybrid spleen cells intraperitoneally. Another group received an immunizing injection of 50 million ( $A \times O57BL/1$ ) $F_1$  hybrid spleen cells intraperitoneally, and a third group was not immunized. Foreign pure strain cells were not used for immunization for two reasons. First, injected lymphoid cells have been shown to enter the thymus<sup>3</sup>, and therefore pure strain cells might persist there long enough to be able to give a graft-versus-host reaction of their own in our assay system. Secondly, injected pure strain cells might exert a graft-versus-host reaction against the injected  $A$ -strain mice, thus interfering with the immunological apparatus of the injected mice<sup>4</sup>. Between 10 and 21 days after immunization, mice were killed under ether anaesthesia, their thymuses removed, and thymus cell suspensions prepared in lactate-Ringer's solution, using a loose-fitting Potter-Elvehjem glass homogenizer. The immunological capacity of these thymus cell suspensions was evaluated by the graft-versus-host assay of Simonsen *et al.*<sup>5</sup>. This assay measures the degree of spleen enlargement induced in young  $F_1$  hybrid recipients of lymphoid cell suspensions from one of the parental strains. Litters of 6-8-day-old ( $A \times O3H$ ) and ( $A \times O57BL/1$ ) $F_1$  hybrid mice were used for all assays. Each suspension of thymus cells was assayed by injecting 60 million thymus cells intraperitoneally into ( $A \times O3H$ ) $F_1$  hybrid recipients, and 10 million thymus cells intraperitoneally into ( $A \times O57BL/1$ ) $F_1$  hybrid recipients. Negative controls consisted of non-injected littermates of the injected  $F_1$  hybrid recipients.

Eight days after cell suspension administration the hybrids were killed, their body and spleen weights determined, and the relative spleen weight (mg spleen/10 g body-wt.) calculated. Finally, in each litter the 'spleen index' was determined by dividing the mean relative spleen weight of the mice receiving thymus cell suspensions by the mean relative spleen weight of the non-injected control mice. In an attempt to quantitate the increase of reactivity of thymus and spleen cells resulting from immunization, we have also compared the immunological reactivity of spleen and thymus cells from immunized donors with the reactivity of spleen and thymus cells from non-immunized donors. The graft-versus-host assays of spleen and thymus cells from normal donors were performed and evaluated exactly as already described here, and the data obtained represent a portion of another study which is as yet unpublished.

It is apparent that thymus cells derived from  $A$ -strain mice immunized with ( $A \times O3H$ ) $F_1$  hybrid cells show increased graft-versus-host reactivity in ( $A \times O3H$ ) $F_1$  hybrid recipients (Table 1). Similarly, thymus cells from  $A$ -strain mice immunized with ( $A \times O57BL/1$ ) $F_1$  hybrid cells show increased reactivity in ( $A \times O57BL/1$ ) $F_1$  hybrid recipients. The specificity of this increased reactivity is shown by the failure of thymus cells from immunized donors to exhibit increased reactivity in the test strain to which they were not immunized. The comparison between cell suspensions from immune and non-immune donors is shown in Table 2. It can be seen that the reactivity of 10 million thymus cells from immunized mice does not differ significantly from the reactivity of 20 million thymus cells from non-immune donors, and similarly the reactivity of 1 million spleen cells from immunized mice is approximately equivalent to the reactivity of 2 million spleen cells from non-immune donors.

It can be concluded that the thymus participates in the process of immunization against histocompatibility antigens at least to the extent of containing cells which exhibit increased specific immune reactivity. Our results are similar to those of Fiore-Donati *et al.*<sup>6</sup>, who also showed increased graft-versus-host reactivity after immunization. However, these authors did not show the specificity of the reaction, whereas our experiments demonstrate conclusively that the increased immunological reactivity is specific for the immunizing antigen. There are

Table 1. SPECIFICITY OF INCREASED IMMUNOLOGICAL REACTIVITY EXHIBITED BY THYMOCYTES FROM IMMUNIZED  $A$ -STRAIN MICE

Donor	No. donors assayed	Strain of recipient animal	No. thymus cells injected ( $\times 10^6$ )	Mean spleen index* obtained $\pm S.E.$
Normal $A$	7	( $A \times O3H$ ) $F_1$	60	1.19 $\pm$ 0.05
$A$ immune to ( $A \times O3H$ ) $F_1$	8	( $A \times O3H$ ) $F_1$	60	1.58 $\pm$ 0.07
$A$ immune to ( $A \times O57BL/1$ ) $F_1$	7	( $A \times O3H$ ) $F_1$	60	1.14 $\pm$ 0.04
Normal $A$	8	( $A \times O57BL/1$ ) $F_1$	10	1.17 $\pm$ 0.04
$A$ immune to ( $A \times O57BL/1$ ) $F_1$	12	( $A \times O57BL/1$ ) $F_1$	10	1.51 $\pm$ 0.08
$A$ immune to ( $A \times O3H$ ) $F_1$	7	( $A \times O57BL/1$ ) $F_1$	10	1.26 $\pm$ 0.07

\* Spleen indexes were calculated by dividing the relative spleen weight (mg spleen/10 g body-wt.) of the experimental group by the relative spleen weight of the negative control group (non-injected litter-mates).

Table 2. IMMUNOLOGICAL REACTIVITY OF THYMUS AND SPLEEN CELLS FROM NORMAL AND IMMUNE  $A$ -STRAIN MICE

Donor	No. donors assayed	Type of cells injected	No. cells injected ( $\times 10^6$ )	Mean spleen index* in ( $A \times O57BL/1$ ) $F_1$ hybrids $\pm S.E.$
Normal $A$	9	Spleen	2	1.83 $\pm$ 0.11
$A$ immune to ( $A \times O57BL/1$ ) $F_1$	7	Spleen	1	1.29 $\pm$ 0.14
Normal $A$	9	Thymus	1	1.91 $\pm$ 0.13
$A$ immune to ( $A \times O57BL/1$ ) $F_1$	11	Thymus	20	1.65 $\pm$ 0.12
Normal $A$	7	Thymus	10	1.20 $\pm$ 0.08
$A$ immune to ( $A \times O57BL/1$ ) $F_1$	12	Thymus	10	1.51 $\pm$ 0.08

\* Spleen indexes were calculated by dividing the relative spleen weight (mg spleen/10 g body-wt.) of the experimental group by the relative spleen weight of the negative control group (non-injected litter-mates).

at least two hypotheses which can account for the presence of sensitized cells in the thymus of the immunized animals. On one hand, the cells exhibiting increased reactivity may have migrated to the thymus after being sensitized in the peripheral lymphoid organs. Alternatively, the immunizing cells or antigens may have entered the thymus and sensitized the thymus cells directly. Both hypotheses are consistent with the findings from parabiosis<sup>7</sup> and spleen cell injection<sup>8</sup> experiments showing that lymphoid cells can enter the thymus. These experiments have also shown that the relative increase of immunological reactivity after immunization is approximately as great for thymus cells as for spleen cells. This finding suggests that the immunologically responsive cells of the thymus participate in the development of immunity as represented by the graft-versus-host reaction to approximately the same extent as do the responsive cells of the spleen.

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## Distribution of Agglutinating Antibodies to *Listeria monocytogenes* among Vertebrates

INVESTIGATIONS of the host-parasite relationship of *Listeria monocytogenes* to man and to several species of domestic ungulates have revealed that serum antibodies reactive with the bacterium may be found in a high percentage of apparently normal individuals<sup>1</sup>. The proper interpretation of these immunological reactions has been a persisting question in serology, particularly since the species concerned are subject to clinical listeriosis. Do the antibodies demonstrate a high level of inapparent infection by a ubiquitous organism wherein few of the attacked individuals experience the clinical disease, or do they arise from antigenic stimuli other than *L. monocytogenes* and manifest cross-reactivity because heterogenetic antigen systems are involved?

Indirect evidence in answer to the question was sought by testing the sera of a number of vertebrates for agglutinating antibodies. The study involved reptiles and fish as

well as birds and mammals. Most of the serum samples were collected at the San Diego Zoo, California; others were obtained from animals in their natural habitat. The sera were tested in a somatic agglutinating system starting at the 1:25 dilution<sup>2</sup>. Antigen was prepared from a strain of *L. monocytogenes* belonging to serotype 4b. Three or more thermostable antigenic determinants occur on the organism, thus reactivity of antibodies from different sera are not necessarily directed against the same determinant group. Reactions were recorded by visual estimation as follows: 4+ = 100 per cent of cells agglutinated, 3+ = 75 per cent of cells agglutinated, 2+ = 50 per cent of cells agglutinated, 1+ = 25 per cent of cells agglutinated, and 0 = no cells agglutinated.

As shown in Table 1, only 15 of 118 mammalian sera tested failed to demonstrate some agglutinating activity. The ruminants were distinguished among the mammals by the greater percentage showing reactions and by the generally high titres. Likewise, among the birds it was found that a high percentage had antibodies. Eighty-four per cent of the birds reacted, although the titres tended to be somewhat lower than those obtained from mammals. Sera from 11 fish caught in the Pacific Ocean were tested and 7 contained demonstrable antibodies. The reactions were all obtained from bonita (*Sarda macleoti*), although the group included 1 barracuda and 1 kelp bass. Titres were low, but the presence of antibodies to *L. monocytogenes* in this environment was of special interest. Of the 31 reptiles tested, 2 were lizards and the rest were various species of snakes. As a group of vertebrates, these were the least reactive, since antibody was detected in only 35 per cent. Titres were low except in a few notable examples such as a copperhead snake (*Agkistrodon contortrix latrocinus*), a blotched water snake (*Natrix erythrogaster transversa*), and a monitor lizard (*Varanus exanthematicus albigularis*) which had titres of 1:200. Antibody was detected in 9 of the 17 species of reptiles tested.

The results suggest that sera can be expected to contain antibodies reactive with *L. monocytogenes* even though they are not stimulated by that organism. So far as is known, the ocean may be considered a *Listeria*-free environment, and yet antibodies were detected in both fish and mammals collected from the ocean. Seven of the 9 bonita and 2 of 3 sea-lions reacted in this series. Suggestions that *L. monocytogenes* shares 1 or more heterogenetic antigens with other micro-organisms seems logical, and in the instances cited here it is likely that some marine forms of microbial life have antigenic patterns related to those of *Listeria*. The concept of heterogenetic antigens is likewise strengthened by the presence of antibodies in terrestrial reptiles. It is conceivable that these species could encounter the bacterium by means such as ingestion of infected rodents, but the organism is not known to infect cold-blooded vertebrates, and it is not likely that the presence of antibodies in such hosts can be explained as *Listeria* infections.

The possibility must be considered that certain species might have antibodies which were not stimulated by antigens encountered in the environment. These could be called 'normal antibodies' which might relate to blood group systems or other cellular constituents of the host and have a genetic basis. This can be suspected in situations where a high percentage of individuals of one species react while those of a related species, in a similar environment, do not. This may apply among the reptiles,

Table 1. AGGLUTINATING ANTIBODY TITRES FOR *Listeria monocytogenes* AMONG VERTEBRATES

	Total tested	Zero at 1:25	1+ at 1:25	2+ at 1:25 or greater	2+ at 1:50 or greater	2+ at 1:100 or greater	2+ at 1:200 or greater	2+ at 1:400 or greater	2+ at 1:800 or greater
Ruminant mammals (25 species)	85	9 (11%)	11 (13%)	65 (76%)	61 (72%)	56 (66%)	34 (40%)	18 (21%)	2 (2%)
Non-ruminant mammals (31 species)	31	0 (0%)	8 (26%)	17 (55%)	12 (39%)	9 (29%)	5 (16%)	1 (3%)	1 (3%)
Birds (39 species)	67	9 (13%)	13 (20%)	35 (52%)	33 (49%)	23 (34%)	16 (24%)	9 (13%)	3 (5%)
Salt-water fish (3 species)	11	4 (36%)	2 (18%)	5 (45%)	4 (36%)	2 (18%)	0	0	0
Terrestrial reptiles (17 species)	31	20 (64%)	3 (10%)	8 (26%)	5 (16%)	3 (10%)	3 (10%)	0	0

for example, all the activity being recorded among 9 species, while 8 others, representing 45 per cent of the specimens, showed no reactions.

The prevalence of *Listeria* antibodies among ruminant mammals at the San Diego Zoo requires special consideration. Among the domestic animals, ruminants are most frequently associated with clinical listeriosis. It is therefore tempting to assume that much of the antibody activity encountered here was due to contact with *L. monocytogenes*. However, the authorities at the San Diego Zoo were not aware of any disease problem caused by this organism. The possibility that soil may serve as a habitat for the bacterium has been considered by some investigators<sup>1</sup>. Accordingly, soil samples were collected in nine enclosures at the Zoo where animals had high *Listeria* titres, but no isolations of the organism were made. It is likely that the ruminant animals in the Zoo were in an environment free of *L. monocytogenes* and that the high titres of antibody activity in their sera arose from other causes. Certain organisms peculiar to the ruminant digestive tract might well have antigens in common with *Listeria*, and this may be responsible for these confusing serological results. Neter<sup>4</sup> has reported that *L. monocytogenes* contains the so-called 'Rantz antigen', which has been described as a substance common to several Gram-positive bacteria<sup>5</sup>. This may well be a facet of the problem, but it would appear to be only a part of the explanation, since sera absorbed with various organisms containing 'Rantz antigen' are still reactive with *Listeria* cells.

This note is not to suggest that immune antibodies to *L. monocytogenes* do not occur. Rather, a definition of the problem of serodiagnosis in listeriosis requires that means be found to achieve recognition of those antibodies which arise from contact with antigens of *L. monocytogenes* against a frequent background of cross-reacting antigen-antibody systems.

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### Opsonic Requirements for Bacterial Phagocytosis

THAT specific antibody is required for the uptake of most, if not all, bacteria by phagocytic cells has been accepted since the early work on opsonins by Almroth Wright and Douglas. In recent years evidence has accumulated that complement may also be involved since partial removal of complement in a variety of phagocytic systems reduces the rate and amount of uptake<sup>1,2</sup>. The fragments of 7S  $\gamma$ -globulin which can be obtained by enzymatic digestion of antibody are reported to be unable to fix complement in their combination with antigen<sup>3</sup>, and recently Miescher *et al.*<sup>4</sup> have found that such fragments possess little or no activity in an *in vivo* opsonic system. Using a highly sensitive intraperitoneal test in mice we have confirmed that pepsin or pepsin-digest fragments of rabbit 7S antibody are only capable of opsonizing *S. adelaide* when used at a concentration one thousand times greater than the minimum effective dose of the original 7S  $\gamma$ -globulin. Quantitatively similar results with fragment I have recently been obtained by Shands and Suter<sup>5</sup>.

Fragments labelled with iodine-131 were found to combine with the bacterial surface in a manner quantitatively similar to the 7S  $\gamma$ -globulin. This suggests to us that complement-binding on the bacterial surface may be mandatory for phagocytosis to occur.

As a result of this conclusion we have attempted to construct a model whereby complement could be fixed at the bacterial surface in the absence of specific antibody, in order to see if this is the prime requirement for opsonization. The idea was to take normal human  $\gamma$ -globulin, previously adsorbed with *S. adelaide* to remove all specific antibody against the organism. This could then be aggregated by heating to 63° C for 15 min and purified according to the method of Christian<sup>6</sup>, to yield a fairly homogeneous and highly anti-complementary aggregate with a molecular size of approximately 1 million. Similar material has been shown by Laliker *et al.*<sup>7</sup> to adsorb strongly on to the surface of red cells, and there seemed a good possibility that it would attach similarly to bacterial cells and fix complement close to the bacterial surface in an analogous way to specific antibody. The question then arises whether these coated bacteria could be phagocytosed.

We prepared aggregated human  $\gamma$ -globulin in this way, and after labelling with iodine-131 the uptake of this aggregate by 10<sup>7</sup> *S. adelaide* was followed over a range of concentrations of 10  $\mu$ g–2 mg/ml. At all levels approximately 5 per cent of the proffered aggregate was adsorbed to the bacteria and could not be removed by repeated washing with saline. Incorporation of the aggregate alone or when adsorbed to bacteria, into a standard rabbit anti-sheep cell haemolysin complement titration using 6 O'H50 units of guinea-pig complement, showed that approximately 5  $\mu$ g of the aggregate could fix 1 O'H50 unit of guinea-pig complement. Phagocytosis tests were then carried out as follows: 10<sup>7</sup> *S. adelaide* were incubated for 10 min with amounts of aggregated  $\gamma$ -globulin ranging from 10  $\mu$ g to 10 mg/ml, and washed once in saline by centrifuging. The washed suspension was then diluted 1:50 in saline, and 0.1 ml. of this, containing approximately 10<sup>4</sup> organisms, was injected intraperitoneally into each of ten normal mice. At timed intervals thereafter the mice were killed, the surviving organisms recovered by washing out the peritoneum and viable counts made on agar<sup>8</sup>. In a parallel control experiment 1  $\mu$ g of 7S  $\gamma$ -globulin from *S. adelaide* rabbit antiserum was added to the 10<sup>7</sup> organisms before washing and diluting.

Coating with aggregated  $\gamma$ -globulin was completely ineffective and at the end of 90 min most of the injected organisms were recovered from the peritoneum, in sharp contrast to the control with specific antibody where only 0.1 per cent of the organisms were recoverable after this time.

It seemed possible that the aggregated  $\gamma$ -globulin might be inactive due to elution *in vivo*. We have shown that this is not so by following the effect of injecting aggregate-treated bacteria into mice previously immunized against the human  $\gamma$ -globulin. In this case there was rapid phagocytosis and killing presumably due to specific combination between the aggregated globulin on the bacterial surface and specific antibody in the presence of mouse complement *in vivo* (Fig. 1).

We deduce from these findings that, if the opsonic function of antibody requires the participation of complement, this function cannot be imitated merely by attaching complement near to the bacterial surface. Any satisfactory explanation of the mechanism of opsonization must then encompass two points. First, the specific attachment of complement by antigen-antibody complexes, and secondly, the fact that very few molecules of antibody may suffice to opsonize each bacterium. Approximately ten molecules of 19S antibody per bacterium will successfully opsonize *S. adelaide*<sup>9</sup>. This amount of antibody cannot occupy more than 0.1 per cent of the bacterial surface. The original conception of Almroth Wright of a layer of opsonizing protein around the bacteria which he likened

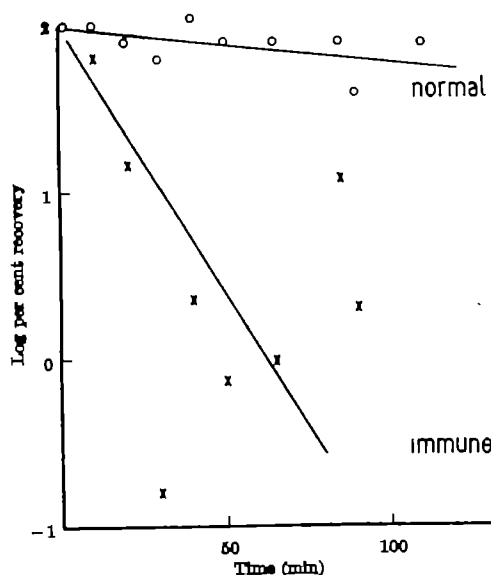


Fig. 1. *In vivo* opsonization of *S. adelaide* coated with aggregated human  $\gamma$ -globulin in normal mice (upper curve) and in mice previously immunized against human  $\gamma$ -globulin (lower curve). The logarithm of the percentage of injected micro-organisms recovered from the mouse peritoneum is plotted as a function of time.

to "buttering a bitter pill" is obviously inadequate. A possible explanation which must now be considered is one which involves production of a chemotactic or activating mediator released by the antigen-antibody combination with complement. Evidence for the existence of such a factor has been provided by Boyden<sup>3</sup> and by Shands and Suter<sup>4</sup>.

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## RADIOBIOLOGY

### Geographical Variation in the Total Body Burden of Caesium-137 in Japanese People as assessed by Blood Analysis

IN view of the complexity in the collection of muscle tissues from autopsies throughout the country to assess the total body burden of caesium-137 in Japanese people, an attempt was made to assess it by the analysis of whole blood<sup>1</sup>. This method presents the problem of the relation between the concentration of caesium-137 in blood and the total body burden; when an equilibrium condition is not attained between the total body and diet or blood.

Since the transition rate of caesium-137 from food to blood is considered to be much more rapid than that from blood to the total body, a change in the diet level will be

followed quickly by a change in blood. Therefore, when the level of caesium-137 in the diet is increasing, the factor relating the total body burden to blood concentration will be smaller than when it is decreasing.

This difficulty can be overcome by making parallel determinations on control human subjects of both blood and the total body levels. Such determinations have been made continuously since November 1963 on several members of the staff of this Institute, the main purpose being to determine the long-term variation in the factor relating blood and the total body levels<sup>1,2</sup>.

At the end of July 1964, five samples each of citrated whole blood (from males only) were collected from nine blood banks throughout the country. Each sample, 200 ml. in volume, was ashed after the addition of caesium carrier and its caesium-137 content was determined by a radiochemical method. This method comprises radiochemical separation by a combined ammonium molybdo-phosphate-dipicrylamine-chloroplatinic method and low-background  $\beta$ -counting in 4 $\pi$ -geometry, the chemical yield being a little less than 90 per cent on average, the counting efficiency 69 per cent and the background counting rate less than 1 c.p.m.<sup>3</sup>. This made measuring time less than one hour of the sample activity within a standard error of 10 per cent. The concentration of caesium-137 thus obtained in whole blood was corrected for the content of anti-coagulant. The result shown in Table 1 was averaged for each district and one standard deviation of the mean is also indicated.

The determination on laboratory personnel (males) of blood and the total body levels was made on July 21, 1964, the latter measurement being done at the National Institute of Radiological Sciences by Mr. T. Inuma, and the relation between the levels was found (Table 2).

Salo *et al.* measured the levels of caesium-137 in Finnish Lapps in May 1962, and tried to obtain the correlation between the body burden and the concentration in blood<sup>4</sup>. However, the correlation should be shown between the body burden per unit body-weight and the concentration in blood, because the body burden is a function of the body-weight while the concentration in blood is not. The mean body-weight of the laboratory personnel shown in Table 2 is 56 kg, and this by chance coincides with the average body-weight of Japanese men. Therefore, in this particular case, correction for the body weight was neglected in the assessment of body burden in Japanese. The factor relating the body burden (mc.) to blood concentration (pc./kg blood) was calculated to be 0.44, and this was multiplied by the local mean value of blood concentration shown in the last column of Table 1 to obtain the body burden (Fig. 1).

The figure shows a significant variation between localities, higher body burdens in districts in the north and near

Table 1. GEOGRAPHICAL VARIATION IN THE LEVELS OF CAESIUM-137 IN JAPAN

District	No. in Fig. 1	Milk <sup>1</sup> (pc./l.)	Polished rice (pc./kg)	Human blood (pc./kg)
Hokkaido	1	152	220	43.8 $\pm$ 6.9
Tohoku Pacific side	2	208	182	35.6 $\pm$ 7.9
Hokuriku	3	149	168	66.0 $\pm$ 23.8
Kan-to	4	82	101	38.0 $\pm$ 9.3
San-to	5	153	113	41.7 $\pm$ 2.3
Northern Kanto	6	106*	120	30.2 $\pm$ 5.9
Southern Kanto	7	105	30	26.6 $\pm$ 3.3
Kinki	8	77	23	25.1 $\pm$ 3.4
Seto-uchi	9	182	24	24.9 $\pm$ 9.5
Northern Kyushu				

\* Av. for Saitama and Kanagawa.

Table 2. RELATION BETWEEN THE LEVELS OF CAESIUM-137 IN BLOOD AND THE TOTAL BODY BURDEN IN THE CONTROL SUBJECTS (JULY 21, 1964)

Subject (male)	Age	Body weight (kg)	<sup>137</sup> Cs in blood (pc./kg blood)	Total body burden (mc.)
Ar	28	49.0	50.6	13.7
Ob	22	54.2	19.7	8.9
Ku	23	58.3	32.8	14.1
Iw	23	59.8	35.2	19.4
Su	24	55.5	29.9	17.2
Ya	44	55.5	29.1	18.8
So	28	52.0	29.5	10.7
Sa	20	64.0	44.0	16.4
Av.		56.0	33.9	14.0

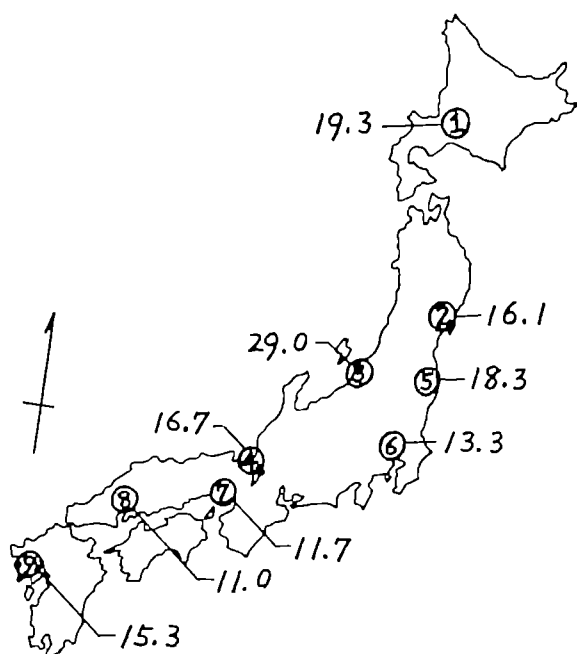


Fig. 1. Geographical variation in total body burden of caesium-137 in Japanese people as assessed by blood analysis, July 21, 1964. Amounts in nCi.

the Japan Sea, lower in districts in the south and near the Pacific. This pattern is very similar to that known for the fall-out deposition rate, and radioactivities in some crops. In Table 1 are shown the levels of caesium-137 in polished rice (crop in autumn 1963) and liquid milk<sup>1</sup> (average for the period spring 1963–spring 1964). Rice was taken because it is one of the main contributors of caesium-137 in Japanese diet, while milk was taken because it may represent locally consumed foodstuffs such as fresh leafy vegetables. The range of geographical variation in blood level is, however, smaller than those in foodstuffs. This may reflect an effect of distribution and consumption of foodstuffs throughout the country as well as the complexity of diet composition.

Measurements have conveniently been made of the total body burden of caesium-137 by using whole body counters. However, when the whole body counter is not available or quite limited in use for the assessment of population mean levels of caesium-137, blood analysis seems to be a promising substitution. The collection of human muscle tissues cannot always be carried out easily and the specimens from autopsies of hospitalized people is subjected to criticism because a bias may be introduced in the assessment for healthy live people. The analysis of muscle tissues obtained from a hospital in Tokyo indicated total body burdens of 10.3, 9.5, 10.3 and 8.4 nCi in April, May, June and July 1964, respectively<sup>2</sup>. The average body burdens of the laboratory personnel in this Institute were measured by a whole body counter as 12.3, 13.0, 14.5 and 14.9, respectively in the same month, given above. The result may indicate a negative bias of 30 per cent on the average when the assessment is based on the analysis of muscles of hospital origin.

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### Reversal of some Effects of Gamma-irradiation by the Ethyl Ester of Gibberellic Acid

THREE experiments concern the effect of gamma irradiation in retarding the sprouting of potato tubers and the ripening of tomato fruits.

**Potatoes:** Potatoes (variety 'Up-to-date') were received in the laboratory about 2 weeks after lifting and stored at room temperature (23°–35° C; relative humidity, 70–95 per cent). After being stored for 2½ months, potatoes that were free from sprouts were packaged individually in perforated polyethylene bags. Half of them were irradiated with 12.6 krad of cobalt-60 gamma rays at the rate of 2.1 krad/min. One day after irradiation, half the irradiated and the same number of control tubers were dipped in a solution (150 p.p.m.) of ethyl ester of gibberellic acid (GA) for 25 min. The remaining tubers, irradiated and control, were dipped in distilled water for the same period. The tubers were afterwards dried, re-packaged in perforated polyethylene bags and stored at 11°–12° C (relative humidity (R.H.) 85–90 per cent).

The relevant results are presented in Tables 1–3 and Fig. 1. All the irradiated, gibberellic acid-treated potatoes had sprouted after 25 days of storage; thereafter, the sprouts began to wither. No sprouting occurred in irradiated tubers treated with distilled water. Similar results were obtained by me in an experiment of a different design and conducted at a different temperature<sup>1</sup>. Gibberellic acid also hastened the sprouting of control tubers (Table 1). After 25 days of storage, the fresh weight loss was as follows: (i) Control, GA-treated—2.5 per cent, (ii) control, distilled water-dipped—1.9 per cent, (iii) irradiated, GA-treated—2.2 per cent, and irradiated, distilled water-dipped, 1.4 per cent. Treatment with gibberellic acid caused an increase in the number of buds growing per tuber as well as the number of buds growing on the stem and half of each tuber in irradiated as well as control lots (Table 2). None of the tubers decayed within 25 days of storage.

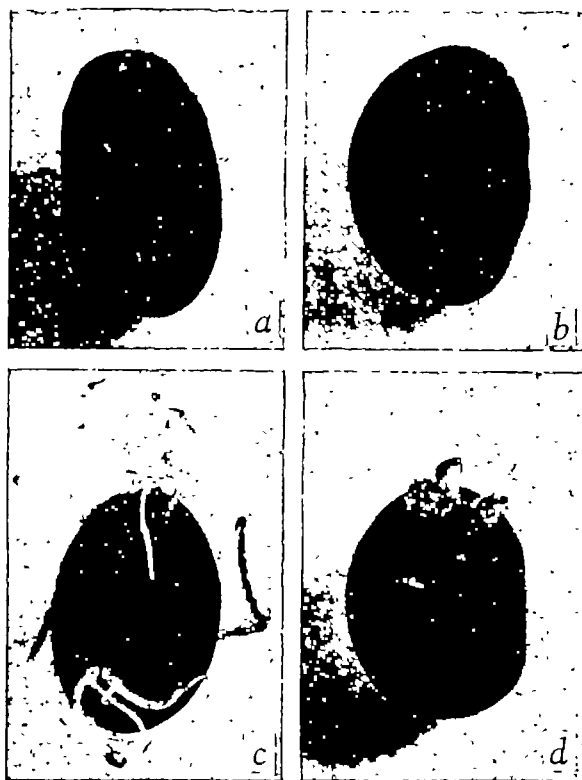


Fig. 1. Effects of gamma-irradiation and gibberellic acid treatment on potato tubers, photographed after 45 days of storage. (a) irradiated, treated with gibberellic acid, (b) irradiated, dipped in distilled water, (c) control, treated with gibberellic acid, (d) control, dipped in distilled water.



Table 1. NO. POTATOES SPROUTED OUT OF 40 IN EVERY TREATMENT

Storage period (days)	Control		Irradiated	
	GA	Distilled water	GA	Distilled water
5	27	22	12	nil
10	30	24	20	nil
15	24	27	27	nil
20	26	30	28	nil
25	27	31	40	nil

Table 2. SPROUTING AFTER 25 DAYS OF STORAGE

	Control		Irradiated	
	GA	Distilled water	GA	Distilled water
(1) No. of buds growing per tuber*	5.0	3.7	4.8	No sprouting
(2) No. of buds growing on stem-end half per tuber*	0.7	0.4	0.6	No sprouting

\* Means of 40 measurements.

Table 3. FRESH WEIGHT LOSS AND SPROUTING AFTER 45 DAYS OF STORAGE IN CONTROL SERIES

	GA	Distilled water
(1) Percentage cumulative fresh weight loss in potatoes*	5.1	3.8
(2) Length (cm) of longest sprout in a tuber†	2.8	1.9
(3) Length (cm) of longest internode in all the sprouts in a tuber†	1.6	0.9
(4) No. of nodes in the longest sprout in a tuber†	3.2	4.8

\* Determinations were made on samples of 5 tubers.

† Means of 40 measurements.

In control potatoes, treatment with gibberellic acid resulted in an increase in loss of fresh weight, an increase in the length of the sprout, an increase in the internodal length of the sprout and a decrease in the number of nodes in the sprout, after 45 days of storage (Table 3). Fig. 1 shows 4 potatoes, belonging to the 4 treatments, as photographed after 45 days of storage.

**Tomatoes:** Tomatoes (variety 'Marglobe') were picked in the green stage and stored at room temperature (24°–33° C, R.H. 70–80 per cent). After 5 days of storage, fruits that were still green were selected and packaged individually in perforated polyethylene bags. Half of them were irradiated with 12.6 kreds of gamma rays at the rate of 2.1 kreds/min. Three and a half hours after irradiation, half the irradiated and the same number of control fruits were dipped in a solution (150 p.p.m.) of ethyl ester of gibberellic acid for 25 min. Simultaneously, the remaining fruits from each of the irradiated and the control lots were dipped in distilled water for the same period. All fruits were afterwards dried, re-packaged in perforated polyethylene bags and stored at 11°–12° C (R.H. 85–90 per cent).

The results obtained are recorded in Tables 4 and 5. Treatment with gibberellic acid hastened the rate of ripening both in irradiated and control tomatoes (Table 4). After 25 days of storage, the fresh weight loss was as follows: (i) control, GA-treated—2.0 per cent; (ii) control, distilled water-dipped—1.5 per cent; (iii) irradiated, GA-treated—2.3 per cent; (iv) irradiated, distilled water-dipped—1.3 per cent.

Table 4. NUMBERS OF GREEN TOMATOES (OUT OF 40 IN EACH TREATMENT GROUP)

Storage period (days)	Control		Irradiated	
	GA	Distilled water	GA	Distilled water
5	30	26	22	40
10	31	27	23	34
15	11	14	8	23
20	8	10	6	13
25	nil	nil	nil	nil

Table 5. NUMBERS OF DECAYED TOMATOES (OUT OF 40 IN EACH TREATMENT GROUP)

Storage period (days)	Control		Irradiated	
	GA	Distilled water	GA	Distilled water
25	5	3	6	nil
30	7	4	9	nil
35	10	5	12	nil
40	13	7	14	3

The rate of decay was higher in gibberellic acid-treated, in irradiated as well as in control fruits (Table 5). The decay was mainly due to fungus diseases.

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## BIOLOGY

Divisions in Isolated Cells of Palisade Parenchyma of *Arachis hypogaea*

THE exclusive use, up to now, of isolated cells of angiosperms from mechanically separated callus of multicellular origin<sup>1-4</sup> carries the inherent shortcoming that such cells and tissues are patently of artificial origin, environment, and physiology. From every botanical viewpoint, it behoves the investigator of cell morphogenesis to start his experiments with a normal cell. Theoretically, one of the very best starting materials is the mesophyll cell of the angiospermous leaf, the use of which was attempted, unsuccessfully, by Haberlandt<sup>5</sup> more than 65 years ago. Though Haberlandt reported consistent failure in this early attempt, it is difficult to know the reasons for it, beyond the undeniable fact that axenic conditions were not attained, and indeed, were not considered essential by him. Bobiloff-Preisser<sup>6</sup> attempted to repeat Haberlandt's trials, with modifications, but had the same failures. Schmucker<sup>7</sup> reported that the very easily separated mesophyll cells of *Macleaya cordata* divided, yielding groups of a dozen cells in filter-sterilized grated sap of the same leaf. He published no further observations on it. His results, however, were confirmed by Fiedler<sup>10</sup> (p. 382), who presented no photographs. Kandler<sup>11</sup> doubted the foregoing reports and tried the same and other leaves without any success. Once again, Kohlenbach<sup>12</sup> reported the growth of these isolated mesophyll cells in White's medium with coconut water and 2,4-D. His photomicrograph shows a packet of 6–8 cells which he thought were produced by divisions from a single palisade cell. It appears to us that final proof of divisions in the single isolated mesophyll cell is possible only through a photographic record by time-lapse. This has been provided in the present communication.

We agree with the early authors that it is difficult to find leaves suitable for excision of intact, isolated mesophyll cells. We have, actually, tried the leaves of most of the plants growing on the grounds and in the greenhouses of this laboratory and have found that only a few are suitable. Nevertheless, a number of them not only give good excisions, but also show growth and division in some of their isolated cells. Our first success was preaged by the formation of variously-sized spheres of parenchyma in liquid shake cultures of excised mesophyll cells of the peanut, *Arachis hypogaea*. Excisions were then observed in liquid medium\* by time-lapse photomicrography through an inverted phase-contrast microscope at 100× and 200×. Mature leaflets were surface-sterilized in 2.5 per cent sodium hypochlorite, and rinsed in sterile distilled water. They were then drained of most adhering liquid and torn across to expose the mesophyll. Scrapings were

\* Minerals listed as macroelements by Heller<sup>13</sup> (p. 140); those as microelements were, in g per litre of medium: H<sub>2</sub>BO<sub>3</sub>, 0.000066; MnCl<sub>2</sub>·4H<sub>2</sub>O, 0.000086; ZnCl<sub>2</sub>, 0.000156; CoCl<sub>2</sub>, 0.00002; CuCl<sub>2</sub>·2H<sub>2</sub>O, 0.000084; NaMoO<sub>4</sub>·2H<sub>2</sub>O, 0.000025; disodium salt of ethylene dimethyltetraacetic acid, 0.0008; sucrose, 20; casein acid hydrolysate (vitamin-free, salt-free), 0.4; 2,4-dichlorophenoxyacetic acid, 0.001; myo-inositol, 0.1; sorbitol, 0.05; thiamin hydrochloride, 0.0001; pyridoxime hydrochloride, 0.0001; nicotinic acid, 0.0006; choline chloride, 0.01; riboflavin, 0.0001; ascorbic acid, 0.0001; calcium pantothenate, 0.0001; biotin, 0.00001; 'Pyrex'-distilled water to make 1 litre. All components of the liquid were filter-sterilized and never subjected to the heat of autoclaving.

made through the mesophyll by points of microscalpels (Ball<sup>13</sup>) and placed directly into liquid medium in the observation chamber (Ball<sup>14</sup>). The top cover glass was sealed into place and the preparation was placed upon the stage of the microscope. Intervals between motion picture frames were either 2 or 3 min. As in earlier work on *Sequoia*<sup>14</sup> the cells and cell groups soon adhered to the upper surface of the lower cover glass and could then be photographed from below under optimal optical conditions.

For 3-5 days after inoculation, the palisade cell (Fig. 1) showed streaming of cytoplasm which merely moved the large chloroplasts back and forth. Then a localized or overall enlargement occurred. The former often yielded a spherical structure with a filamentous end, the latter an egg-shaped or almost spherical body. Either type of increase caused a loss of the morphological polarity of the cell and of its identity as palisade parenchyma. During this time the chloroplasts increased in size and lost much of their green colour. Mitosis was always preceded by systrophy (cf. Senn<sup>15</sup>); this involved a symmetrical gathering of plastids around the nucleus (Fig. 2). Later, the cell plate developed outwardly from the centrally-located division figure and made contact with the original wall. The new wall had no consistent orientation with the original axis of the dividing cell and was often curved (Fig. 3). Divisions of the derivative cells were also without such orientation (Fig. 4) and the cell-mass produced showed no resemblance to an embryology. The maximum

growth of an isolated cell within the observation chamber yielded 20-30 cells during a period of 14 days. No derivative cell had any resemblance to the palisade parenchyma cell from which it was derived. Dividing cells were exclusively palisade; spongy mesophyll cells showed little change save plasmolysis. The shrinkage is undoubtedly stimulus plasmolysis, as pointed out by Kandler<sup>11</sup>. Boblloff-Preisser<sup>6</sup> (p. 251) stressed the use of the mature leaf in preventing plasmolysis of excised mesophyll cells.

Our results conclusively demonstrate the potential ability to divide in the palisade cell of at least one angiosperm. Similar results by time-lapse have also been achieved from leaves of *Aster* sp., and in shake cultures from leaves of *Erigeron* sp., *Gaillardia* sp., and *Oenothera* sp. Probably the most important prerequisite of successful culture is the facility of removing intact mesophyll cells from the torn-open leaf.

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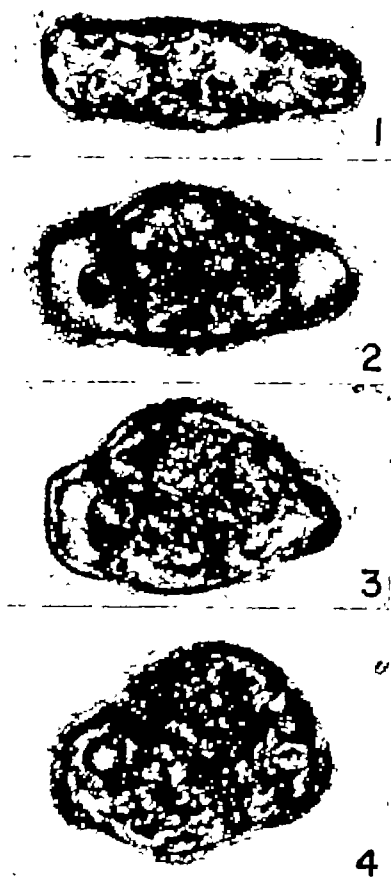


Fig. 1. View of palisade cell shortly after inoculation into observation chamber ( $\times$  c. 400)

Fig. 2. Same cell 71.4 h later, showing the enlargement on both sides and systrophy (arrangement of chloroplasts around nucleus) ( $\times$  c. 400)

Fig. 3. Same cell 96.8 h after inoculation, showing the division wall running diagonally in an S-form between the two cells adhered to the upper surface of the lower cover glass ( $\times$  c. 400)

Fig. 4. Same cell 118.5 h after inoculation, showing the division of the right derivative of the last figure and some enlargement of the left ( $\times$  c. 400)

### Resistance of Tobacco to Stem Infection by *Peronospora tabacina* Adam.

STEM infection with blue mould (*Peronospora tabacina* Adam.) is a serious problem of Australian tobacco crops. Many stem-infected plants are stunted in growth and/or fracture at the base and fall over before the leaves are harvested. Paddick and Hill (unpublished results) concluded from surveys of disease in Victoria that stem infection was a major cause of field loss.

Breeding lines, previously selected on the basis of seedling resistance to leaf infection, were grown at Myrtleford, Victoria, in the 1961-62 season. Many of these lines became stem-infected by blue mould. Thus, in breeding tobacco for resistance to blue mould, consideration should be given to resistance to stem infection as well as to resistance to leaf infection. Fourteen lines which differed in field incidence of stem infection were tested for stem resistance to mould under glass-house conditions. The objective was to assess whether artificial stem infection, as described by Mandryk<sup>1</sup>, could be used in screening breeding lines prior to more extensive field testing. Ten of the lines were normal day-neutral types. The other four were 'mammoth' types, with a short-day requirement for flowering. Seven lines, including one 'mammoth', had derived their resistance from *Nicotiana debneyi* Domin., and six, including three mammoth lines, from *N. goodenopendii* Wheeler by the back-crossing method of plant breeding. The remaining line owed its resistance to the radiation-induced mutation. A highly resistant species (*N. debneyi*) and a completely susceptible tobacco cultivar ('Virginia Gold') were also included in the test.

Seed was germinated on vermiculite, and the seedlings were grown and supplied with nutrients, as described by Hill and Mandryk<sup>1</sup>. Eight weeks after sowing, six plants of each line were stem-inoculated at the base by injecting a suspension of conidiospores of *P. tabacina*. As controls, six plants of each line were stem-injected with distilled water. Plants were kept for 48 h at a temperature of 20° C in an atmosphere nearly saturated with water vapour. After this initial incubation period, the temperature was increased to between 20° C and 29° C and the relative humidity reduced to 70 per cent. Three weeks after injection, the extent of stem infection was determined by measuring the lengths of macroscopically visible necrosis, which had spread from the point of injection, in the cortex, vascular bundles and pith. Except in the highly resistant lines *N. debneyi* and CA229, all the plants injected with spores exhibited stem necrosis.

A comparison of field and glass-house stem infection is shown in Table 1 for the lines with day-neutral flowering requirements.

Table 1. FIELD AND GLASS-HOUSE STEM INFECTION OF DAY-NEUTRAL BREEDING LINES

Code No. of line	Percentage of 100 plants naturally infected in the field	Mean length (cm) of necrosis in 6 plants injected in the glass-house		
		Pith	Vascular bundle	Cortex
<i>N. tabacum</i> cv.				
'V. Gold'	100	14.0	27.0	22.0
<i>N. debneyi</i>	0	0	0	0
CA696	100	3.8	28.8	0.8
M11	100	7.0	17.0	20.0
CA641	80	2.0	4.0	2.0
BA872	78	0.7	8.8	0
CA782	62	0.8	4.8	0
CA681	55	0.0	17.7	0
CA704	54	3.0	19.0	0.2
CA752	30	1.0	5.2	0
CA601	27	0.7	4.5	0
CA229	0	0	0	0

Four lines with high field incidence of stem infection developed appreciable necrosis in pith, vascular bundle, and cortex under controlled glass-house conditions. Five lines with moderate field infection showed necrosis in the pith and vascular bundle only; line CA229, which was not stem-infected in the field, did not develop stem necrosis in the glass-house test. The correlation coefficient of +0.78 (derived from the 12 lines (including 'Virginia Gold' and *N. debneyi*) listed in Table 1) between the percentage of plants infected in the field and the length of necrosis of the vascular bundle in the glass-house test suggests that the stem-injection test could be used as an indication of the susceptibility of breeding lines. There was less agreement between the field infection and the extent of necrosis in either the pith or cortex.

The four 'mammoth' lines showed a higher field incidence of stem infection than would be predicted from their performance in the glass-house test (Table 2). Because of their short-day requirement for flowering they remained in a susceptible condition in the field for a period of six months.

Table 2. FIELD AND GLASS-HOUSE STEM INFECTION OF 'MAMMOTH' BREEDING LINES

Code No. of line	Percentage of 100 plants naturally infected in the field	Mean length (cm) of necrosis in 6 plants injected in the glass-house		
		Pith	Vascular bundle	Cortex
M11	100	1.8	1.7	Tr*
CA694	100	0	8.7	0
DA687	100	0	6.0	0
AA352	87	Tr*	0	0

\* Tr, trace.

Resistance to both the stem and leaf phases of the disease should be considered in breeding tobacco for blue mould resistance. Except in the case of lines which remain vegetative for a long period, the extent of necrosis of the vascular bundle following stem injection under the conditions defined could be used for screening of breeding lines prior to more extensive field testing. One breeding line, CA229, with resistance to blue mould introduced

from *N. goodspeedii*, was shown to be highly resistant to stem infection.

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### Role of Growth Regulators in the Uptake and Metabolism of s-Triazine Herbicide by Tea Leaves

In many cases the effectiveness of herbicides has been increased by the addition of surface active agents to the spray<sup>1-4</sup>, but little information is available about the effects of growth regulators on the uptake and metabolism of herbicides by foliage. The object of this work was to investigate the role of gibberellic acid (GA<sub>3</sub>) and indolyl-3-acetic acid (IAA) on the absorption, diffusion, translocation, and metabolism of 2-chloro-4,6-bis(isopropylamino)-s-triazine (propazine) by the upper and lower surfaces of tea leaves, *Camellia sinensis*.

The methods used to study the absorption of <sup>14</sup>C-propazine by detached tea leaves were slightly modified from those described by Biswas<sup>1</sup>, and Biswas and Rogers<sup>2</sup>, to study the absorption of s-triazine herbicides and gibberellic acid respectively. One variety of tea, 'Nyasaaland', was grown at 65°-75° F in the greenhouse until the plants developed 20-25 leaves. At this stage the third leaf from the top was taken for this study. Four hundred ml. of a 0.5 per cent water-agar solution was added to 100 ml. of a solution of propazine, 100 p.p.m., of which 10 ml. was <sup>14</sup>C-labelled. The agar solutions and the herbicide were mixed thoroughly and then poured into petri dishes. The specific activity of water-agar solution containing propazine was 0.035 µc./ml. The total concentration of propazine in the water-agar solution was 20 p.p.m.

One tea leaf per plate was floated on the agar while it was still fluid. High-vacuum grease was applied at the detached end of each leaf to prevent absorption through the cut end. Either the upper or lower surface of the leaves was placed in contact with the agar and permitted to absorb for 24 h under a light intensity of 150 ft.-candles at 28° C. The amount of herbicide absorbed by the leaves in the water-agar solution was determined by the 'Gas Flow 2pi' internal proportional counter.

The leaf samples were cut into small pieces and homogenized in a 'Virtis' homogenizer for 10 min in 10 ml. absolute ethyl alcohol. The homogenate was filtered and one ml. of the filtrate was used for counting. Mixtures of labelled and non-labelled solutions of propazine were prepared and diluted to known concentrations for standard reference solution. The unknowns were calculated with reference to known concentrations of standard solution.

The method used to study the effects of GA<sub>3</sub> and IAA was the same as described above except that one ml. of growth regulator (100 p.p.m.) was added to the water-agar solution together with 50 ml. of a solution of <sup>14</sup>C-propazine, 100 p.p.m.

The diffusion of <sup>14</sup>C-propazine by the upper and lower surfaces of tea leaves with and without growth regulators was obtained by a simple method. The leaves were cut into small pieces and soaked for 24 h in a solution of 16 g ammonium oxalate, 4 g oxalic acid, and 980 ml. distilled water. After 24 h the leaf pieces were boiled over a low flame until the cuticle began to loosen, when the cuticle was removed with forceps. The tea cuticle comes off very easily and uniformly by this procedure. The separated cuticles were then stored in a refrigerator for 24 h and were examined for ruptures under a light microscope before use. The separated cuticle was placed between two rubber

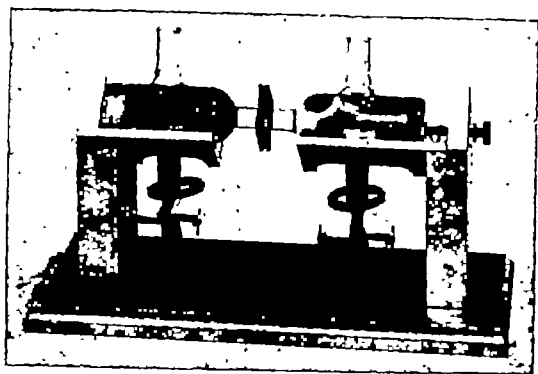


Fig. 1. Diffusion cell apparatus

gaskets under water in a beaker and then placed between two diffusion cells (Fig. 1). In filling the two cells, the outlets were first closed with clamps. One cell was filled with distilled water and the other cell with an equal amount of a 20 p.p.m. solution of  $^{14}\text{C}$ -propazine. To study the effects of growth regulators (GA<sub>3</sub> and IAA) the propazine solution was spiked with growth regulators by the method as described with regard to absorption. The concentrations of propazine which diffused through the separated cuticles were determined by the isotope counting procedure. The concentrations of the unknown samples were calculated from the known standard solutions.

The study of the translocation of propazine was made by autoradiographic techniques. Only the basipetal movement of propazine was studied in this particular experiment. In this case the leaves attached to the plants were floated in the water-agar solution containing 100 p.p.m. of  $^{14}\text{C}$ -propazine (specific activity 2.53  $\mu\text{C}/\text{ml.}$ ) for 40 h in the greenhouse. At the end of 48 h the leaves floated in agar were cut off from the rest of the plant. The plants were placed between blotting papers for 2 days for drying, and then wrapped in 'Cellophane' paper and exposed to X-ray film for 6 weeks.

To study the metabolism of  $^{14}\text{C}$ -propazine, the tea plants were grown in a water culture solution containing labelled propazine for 7 days. At the end of 7 days the plants were harvested, cut into small pieces and homogenized with absolute ethyl alcohol. The homogenate was then filtered and the filtrate was concentrated in a flash evaporator. The concentrated extract was then chromatographed with the original  $^{14}\text{C}$ -propazine as a reference in a solvent system consisting of butanol:acetic acid:water (120:30:50) using Whatman No. 1 paper. The chromatogram was then cut into half-inch sections and the activity of the paper strip was determined by the 'Gas Flow 2pi' internal proportional counter.

The differential absorption of  $^{14}\text{C}$ -propazine by the upper and lower surfaces of tea leaves showed a marked difference in absorption (Table 1). The upper surface absorbed only 3.5 p.p.m. of propazine, whereas the lower surface absorbed 15.0 p.p.m. With the addition of gibberellic acid, increased absorption was obtained in the upper surface, whereas only a slight decrease in absorption in the lower surface was noticed. But with the addition

Table 1. ABSORPTION OF  $^{14}\text{C}$ -PROPAGINE HERBICIDE BY THE UPPER AND LOWER SURFACES OF TEA LEAVES, WITH AND WITHOUT GROWTH REGULATORS

Propazine:	Absorption, p.p.m.					
	Without growth regulators		With gibberellic acid		With indolyl-3-acetic acid	
	Upper	Lower	Upper	Lower	Upper	Lower
	3.5	15.0	9.5	14.5	8.5	16.5

Table 2. DIFFUSION OF  $^{14}\text{C}$ -PROPAGINE BY THE UPPER AND LOWER SURFACES OF TEA LEAVES, WITH AND WITHOUT GROWTH REGULATORS DURING ONE HOUR

Propazine:	Diffusion, p.p.m.					
	Without growth regulators		With gibberellic acid		With indolyl-3-acetic acid	
	Upper	Lower	Upper	Lower	Upper	Lower
	0.23	1.45	0.09	0.27	0.06	0.06

of IAA, both upper and lower surfaces absorbed more as compared to the treatment without growth regulators. As shown in Table 1, both these growth regulators reacted more or less in the same pattern in their effectiveness in absorption.

The amount of propazine diffused through the separated cuticles by the upper and lower surfaces of tea leaves, with and without growth regulators, was more or less the same. In both cases (Table 2) a slight decrease in diffusion was obtained. These results are in agreement with the previous observation<sup>1</sup>. It seems to us from this study that the growth regulators decrease the process of diffusion.

The herbicide propazine, although absorbed in large quantities by tea leaves, is not translocated basipetally. This observation is in agreement with the previous work<sup>1</sup> on peanut plants. Therefore, the toxic effects of propazine seemed to be via root absorption, since this herbicide is absorbed freely through roots and translocated to the different parts of the plants.

In studying the absorption of propazine by the roots and its subsequent metabolism by the tea plant, it was noted that propazine is degraded to form two compounds with  $R_f$  of 0.24-0.28 and 0.46-0.50 respectively. The  $R_f$  value of non-metabolized propazine was 0.96-0.98. Further investigation is in progress to identify and test the bio-activity of these new products and will be reported at a later date. It seems that propazine is easily metabolized by tea plants, giving at least two or possibly more metabolites.

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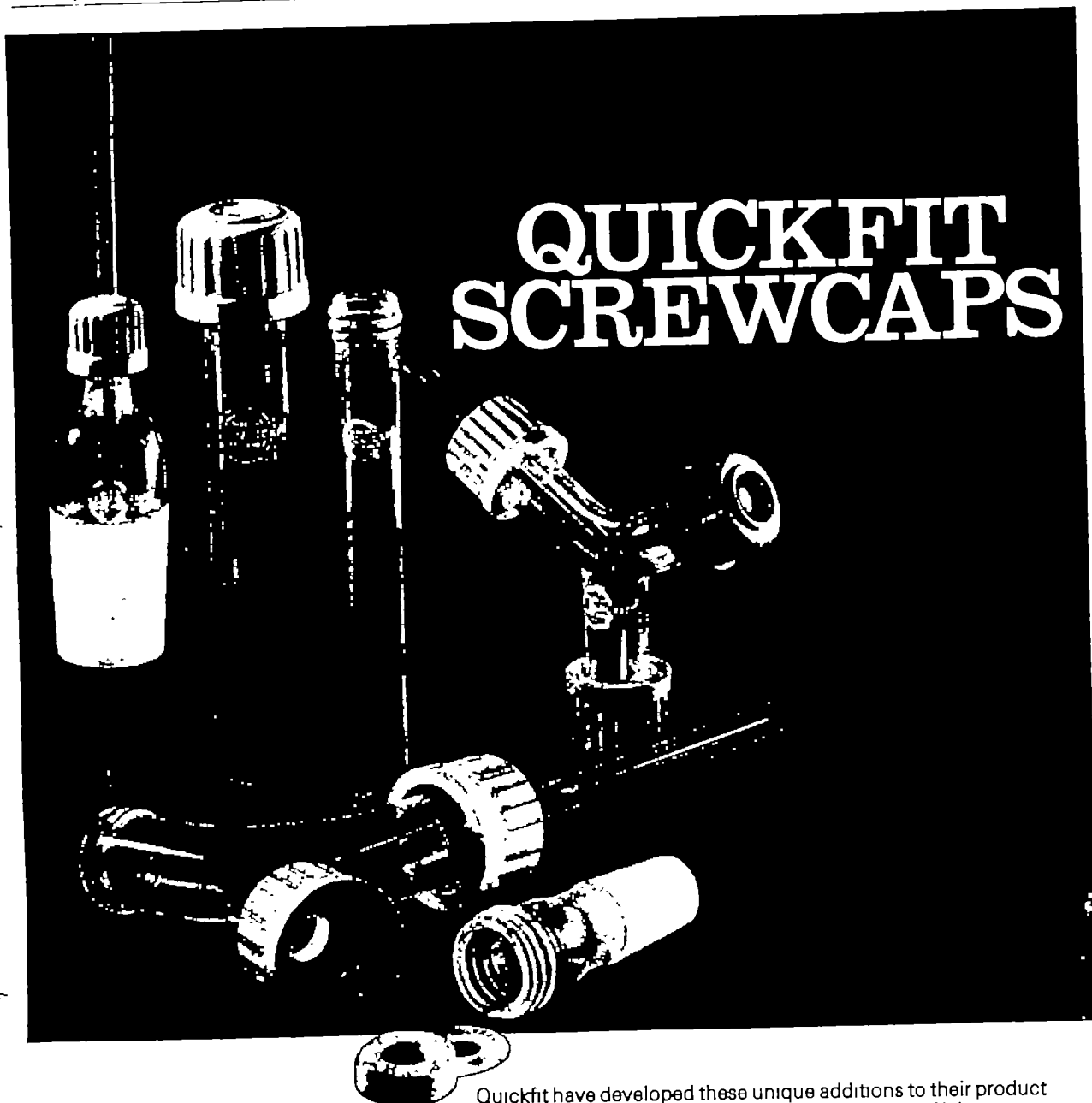
## New Method of Recording Germination Data

GERMINATION data are normally published in the form of 'percentage germination'. These are usually, but not always, accompanied by some information on the time taken for the stated percentage to be reached, a typical example being '91 per cent germination in 18 days'. This method is unsatisfactory for two main reasons: (a) It does not facilitate the comparison of germination data published by two authors. Thus, while it is satisfactory within a single investigation, percentage germination in  $x$  days is of little use in comparing different observations except in the unusual case where  $x$  is the same in both. (b) It does not indicate the rapidity of germination but only its final extent. Information which may be important from an ecological or horticultural point of view may thus be lost.

The method proposed is an attempt to record germination data so that a direct comparison between papers will be facilitated and to give in a single figure some indication of the speed of germination as well as its final extent.

The percentage germination is recorded every 24 h. At the end of some suitable time, which must depend to some extent on the species being studied, the results are summed. It has been found with weed species that 10 days is sufficient in that seeds which have not germinated after this time are normally dormant or dead. This gives a figure, recorded as  $\Sigma 10$ , which can vary between 0 (no germination) and 1,000 (100 per cent germination after 24 h). Usually the result is between the two extremes except

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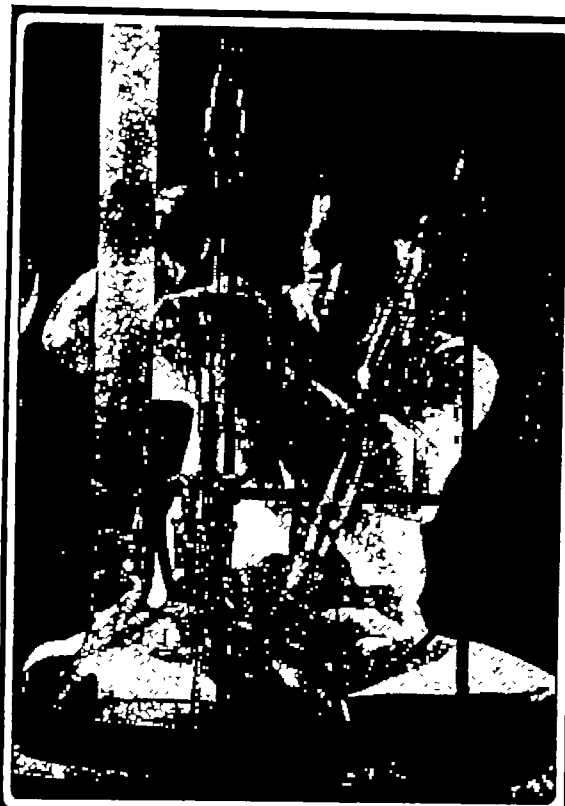


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with completely dormant seed or with seed with exceptionally rapid germination, such as cereals. With some seeds a longer or shorter period may be more suitable which can be recorded as  $\Sigma 20$  or  $\Sigma 5$ , etc.

The results obtained can be statistically analysed by the usual methods, such as chi-squared techniques. If several species are tested using  $\Sigma 10$ , then a direct comparison not only of their germination ability but also of their speed of establishment is obtained. This may be equally important in a study of species growing together since seedling establishment is often one of the most critical stages in the life of the plant.  $\Sigma 10$  will, therefore, often be a function of the competitive ability of the species at this stage. It should also be of use when the effects of dormancy breakers or inhibitors are being studied, since it will give an indication of the overall effect of a germination regulator.

While it is true that if authors recorded germination data on a wide variety of  $\Sigma$  systems comparison of papers would not be facilitated, it seems probable that  $\Sigma 10$  would be a suitable way of recording most data and if this became the convention it would be most useful. Furthermore, if an author found it necessary to record as  $\Sigma 20$  then it would be quite easy to determine  $\Sigma 10$  from this original records if this were later desired. In any event, if  $\Sigma 10$  became the normal method of recording germination data but  $\Sigma 20$  was found to be of greater use in a particular case, it would occupy but little extra space to publish  $\Sigma 10$  also.

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<sup>1</sup> Taylor C. A., *Plant Physiol.*, **24**, 93 (1949).

### Further Observations on the Caribbean Sponge *Cryptotethya crypta* (de Laubenfels)

RECENTLY, there has been some interest in the Caribbean sponge *Cryptotethya crypta* because of the presence, in extracts of this animal, of a number of unique nucleosides<sup>1,2</sup>: spongothymidine (3- $\beta$ -D-arabofuranosylthymine)<sup>3</sup>, spongouridine (3- $\beta$ -D-arabofuranosyluracil)<sup>4</sup>, and spongosine (9- $\beta$ -D-ribofuranosyl-2-methoxyadenine)<sup>4,5</sup>. During an investigation<sup>6,7</sup> to ascertain whether the sponge possessed an arabinose nucleic acid, it was shown that the ratio of the amounts of ribonucleic acid to deoxyribonucleic acid was the reverse of that found in other animal tissues. This result has been confirmed histochemically<sup>8</sup>, and evidence was presented to indicate the possible presence of an unusual nucleic acid. However, no definite evidence has come forth, as yet, for the existence of an arabinose nucleic acid in this sponge.

Interest in these arabinosides has recently been stimulated by speculations<sup>9</sup> concerning their role in cellular ageing and cancer chemotherapy. An integral part of these speculations is the presumption that an anaerobic mode of existence causes cells to produce arabinosides, which are then incorporated into various cellular components causing irreversible changes in metabolism. Because large amounts of arabinosides had been found in this sponge and, more particularly, because the sponge had been reported to grow more or less covered with sand, it was suggested that at least some anaerobic metabolism may take place in this animal. Our recent findings concerning the habitat of this sponge indicate that its requiring anaerobic metabolism is at least open to question, and some of the other speculations also may have to be re-evaluated.

*Cryptotethya crypta* was first described<sup>10</sup> from the waters around Bimini Island, British West Indies, where it was collected by dredging at a depth of about 5 metres. Search of the bottom using a diving helmet, at that time, showed no indication of its presence. It was concluded

from this, and the fact that the pores and oscules were minute, that the sponge grew under the surface of the sand.

We have collected this sponge on several occasions and have noticed that considerable quantities of algae are usually found growing on its upper surface. The lower surface generally shows signs of having been ripped from a larger piece or a surface to which the sponge had been attached. No specimens have been seen which gave the appearance of having grown unattached, as would be expected for a sponge growing loosely under the coral sand. Specimens without a covering of algal growth are also very rare.

In March 1964, further samples of the sponge were collected at Bimini. 'SCUBA' gear was used, and the area in which the sponge was known to be found by dredging was studied. The bottom is composed of coral ledges which are covered by a thick animal and plant growth. The ledges are interspersed with fairly wide areas of coral sand, the whole presenting a fairly level bottom. On close examination of the coral ledges, *Cryptotethya* was found growing among the algae, firmly attached to the rock, and covered with growth. The sponges, because they blend so extremely well with the surroundings, are very difficult to distinguish.

On dredging a bottom such as that described, the grab traverses both the sandy areas and the ledges. It usually comes up filled with a mixture of sand, algae, and sponges. It is probable that on crossing over the coral ledges, both the sponges and algae are scraped off. When these are then mixed with sand from the other areas, it would not be difficult to conclude that the sponges came from the sandy areas. Later, a few small specimens were found growing in the lagoon east of the island. These sponges, which were growing in relatively shallow water, were found growing in a manner similar to those in the open ocean.

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## FORESTRY

### Ultra-thin Sections of Softwood Tracheids and Paper

DURING work on the structure of paper a need arose to examine it in cross-section at high resolution. The sectioning of plant cell walls in their natural state, for example in wood, does not appear to present insuperable difficulties, and techniques based on the early work of Ribi<sup>1</sup> are routinely used. However, no work appears to have been done on the sectioning of pulped fibres, as used in paper, in their dry unswollen state without any pre-treatment. In the skilful work of Asunmaa<sup>2</sup> the fibres were pre-treated by swelling and reaction with thallium ethylate, and after drying from water were dried from benzene, to "open the structure".



We have found that sectioning of untreated paper, using standard techniques of methacrylate embedding, is highly dependent on the pulping process that the constituent fibres have undergone. In the case of paper prepared from pulps that have undergone only the grinding process of fibre separation, sections can be produced quite readily. In the case of paper made from pulps in which the fibre separation is provided chemically by partial extraction of the lignin and hemicelluloses, less satisfactory sections are produced, the least acceptable sections occurring with the most severely degraded pulps. Thus groundwood, consisting typically of 28 per cent lignin and 12 per cent hemicelluloses, produces good sections; a very high yield sulphate pulp produces satisfactory sections and lower-yield sulphate pulps gave rather less satisfactory results. A bleached sulphite pulp containing approximately 85 per cent alpha-cellulose was virtually impossible to section.

The major difficulty in satisfactory sectioning appears to be fragmentation, which appears to occur during the sectioning process. The micrograph of Fig. 1 shows a low-yield sulphite fibre, fragmented and revealing its microfibrillar structure. A possible explanation of this phenomenon lies in the mechanics of the sectioning process. During cutting, the section is constrained to deviate through a large angle and this causes both compression and shear in the section. Under this deformation a plastic material will cut with ease, whereas a brittle material will fragment. It is suggested that a fibre with a high lignin and hemicellulose content will be plastic during cutting, while fibres with a high alpha-cellulose content, consisting substantially of parallel crystalline microfibrillar rods hydrogen-bonded to each other, will be brittle and give rise to fragmentation. This explanation

accords with the recent work of Goring<sup>4</sup> on the thermal softening of lignin, hemicellulose and cellulose.

In an attempt to overcome this problem of fragmentation, a number of other embedding media have been tried. It was found that, using 'Aquan', fragmentation did not occur and satisfactory sections were produced from quite severely degraded pulps (Fig. 2). It was found, however, that by using 'Aquan' for embedding, it was not necessary to cut thin sections. Sections as thick as 1  $\mu$  could be shadowed and inserted into the electron microscope with satisfactory results. It would appear that the heating effect of the electron beam clears the specimen in this case, leaving the shadowing which is effectively a replica of the surface of the section. This clearing, which does not occur with cellulose embedded in methacrylate or any other media that we have tested, indicates that during embedding with 'Aquan' a chemical reaction occurs. The dodecenyl succinic and hexahydro phthalic anhydrides that are used to cross-link 'Aquan' in the embedding process apparently produce a mild esterification of the cellulose, giving a structure that is perhaps thermoplastic and thus does not fragment during sectioning, and one that is more volatile in the electron beam. Embedding with 'Aquan', while of value, cannot therefore be regarded as a method that is free from the criticism<sup>5</sup> that pre-treatment is involved.

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## ENTOMOLOGY

### Chemostimulation of Oviposition by the Cabbage Root Fly *Erioischia brassicae* (Bouché)

*Erioischia brassicae* is an important pest of cultivated brassicas, ovipositing in the soil close to host plants. Since all previously known host plants have been confined to the family Cruciferae<sup>1</sup> in which mustard-oil glucosides<sup>2</sup> are characteristically present<sup>3</sup>, it is of importance to know if these chemicals play a part in stimulating the flies to oviposit in the vicinity of the host. It has been shown that among pests of Cruciferae, *Plutella maculipennis* (Curt.) was stimulated to oviposit by a mustard oil<sup>4</sup>, and *Pieris brassicae* L. by an aqueous solution of a mustard-oil glucoside<sup>5</sup>; with both pests, however, the rate of oviposition was much lower than that on host-plant material.

Field and laboratory experiments were therefore carried out to discover whether *E. brassicae* would oviposit only near plants containing mustard-oil glucosides. Eight species of Cruciferae, eight species of other families also containing mustard-oil glucosides<sup>6</sup>, and six species lacking these substances were tested as possible hosts. *E. brassicae* oviposited near all the Cruciferae in the following list, of which only the first two species had previously been recorded as hosts: *Stenobrium officinale* (L.) Scop., *Sinapis arvensis* L., *Sinapis alba* L., *Rorippa nasturtium-aquaticum* (L.) Hayek, *Lepidium sativum* L., *Ocheiranthus cheiri* L., *Cardamine hirsuta* L., *Armoracia rusticana* Gaertn., Mey and Scherb. Flies also laid eggs near *Rosella odorata* L., *R. luteola* L., *Tropaeolum majus* L., *T. peregrinum* var. *canariense*, *Oenothera spinnosa* L., and *C. inornata* L., but no eggs were laid near two species reported as containing mustard-oil glucosides: *Limnanthes douglasii* R. Br. and *Plantago major* L. No eggs were found around the following species lacking mustard-oil glucosides: *Allium*

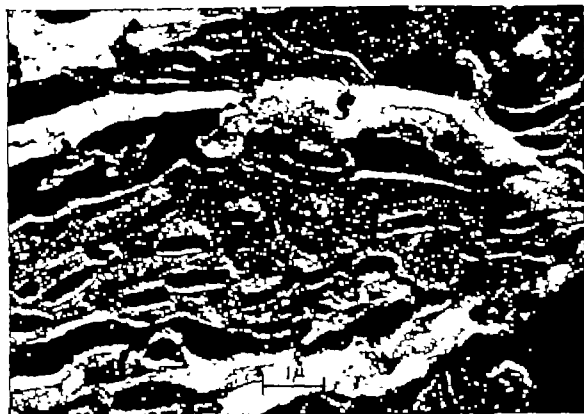


Fig. 1. Part of a fibre from a low-yield sulphite pulp, showing fragmentation.



Fig. 2. Fibre-to-fibre contact region in paper. 'Aquan' embedding.

*cepa* L., *A. porrum* L., *Beta vulgaris* L., *Vicia faba* L. and *Daucus carota* L. Thus *E. brassicae* oviposited not only near crucifers, including six species not previously recorded as hosts, but also near species of the families Rosaceae, Tropaeolaceae and Capparidaceae that, in common with Cruciferae, contain mustard-oil glucosides. Experiments were therefore made to determine whether a mustard-oil glucoside, and breakdown products associated with mustard oils in plants, stimulated oviposition in the absence of the plant.

The glucoside tested was sinigrin; the breakdown products allyl mustard-oil and  $\beta$ -phenylethylamine\*. Each was presented in aqueous solution between two concentric glass-tubes, thus exposing a meniscus 1 cm in diameter. The tubes were embedded in a jar of damp sand to provide a physical medium for oviposition and to complete the test-unit. One set of test-units containing a range of concentrations of solution were randomly sited, and replaced daily, in cages containing 25 newly emerged flies of each sex. The substances were tested at the following concentrations (p.p.m.):

Sinigrin	0, 25*, 250, 2,500†
Allyl mustard-oil	0, 100, 200*, 400
$\beta$ -Phenylethylamine	0, 10, 100†, 1,000

\* No. *E. brassicae* eggs laid significantly different from No. laid on distilled water test-units at  $P < 0.05$ .

† No. *E. brassicae* eggs laid significantly different from No. laid on distilled water test-units at  $P < 0.01$ .

Eggs were laid on each test-unit, but the following concentrations of chemicals provided more favoured sites: 25 and 2,500 p.p.m. sinigrin, 200 p.p.m. allyl mustard-oil and 100 p.p.m.  $\beta$ -phenylethylamine. These solutions were less effective, however, than juice squeezed from the 'root' of swede (*Brassica napus* L. var *napobrassica* (L.), Rehb.), a favoured host of *E. brassicae*.

These results support the hypothesis that the oviposition preferences of *E. brassicae* are correlated with the presence of mustard-oil glucosides in hosts, while the high rate of oviposition on test-units containing swede juice suggests that chemostimuli provided by plants might be more potent than the physical characteristics of hosts in eliciting oviposition. Each of the three chemicals tested appeared to provide only a partial explanation of the stimulating effect caused by swede juice, and so the stimulating effects of further substances, and mixtures of substances, are being tested.

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### Nitrogen Status of Sugar-cane Leaves and the Fecundity of a Hemipterous Pest

In Jamaica, cane-fly (*Saccharosydne saccharicora* (Westw.) Hom.: Delphacidae) has long been known as a pest of sugar-cane, with records of occasional outbreaks dating back to the nineteenth century. In recent years, however, it has become so prevalent in certain areas that outbreaks can be prevented only by resort to aerial spraying of insecticides on a large scale. Work on the epidemiology of cane-fly has shown that the initial build-up in population occurs most often in plant cane, despite the fact that in such cane the biological pressure of natural

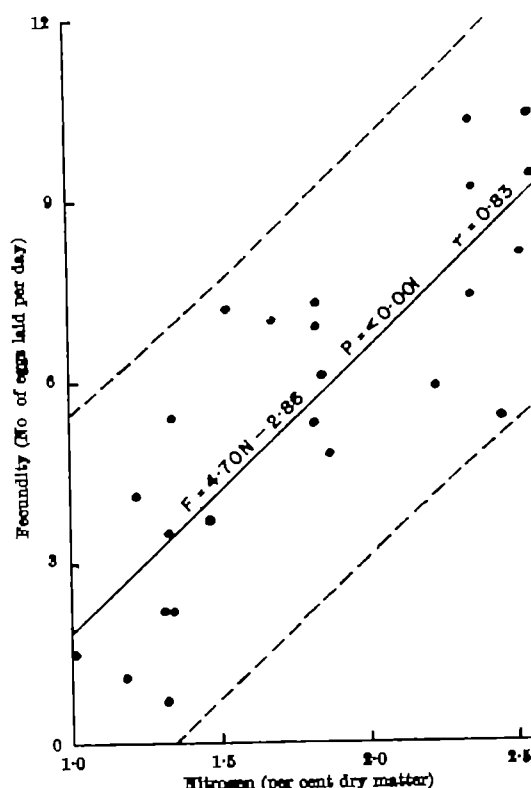


Fig. 1. Broken lines show 5 per cent fiducial limits

enemies is as great as elsewhere<sup>1,2</sup>. The tentative conclusion was, therefore, that the rate of reproduction is an important variant. It is well known that an adequate supply of organic nitrogen is essential for the reproduction of most insects<sup>3</sup>, and that the level of nitrogen in leaf lamina material changes with, *inter alia*, fertilizer treatments, the age of the leaf and the age of the plant<sup>4</sup>. Accordingly, an experiment was designed to determine whether the fecundity of cane-fly was related to the nitrogen content of the leaf.

The experiment was conducted in a glass-house, where sugar-cane was grown in drums which had received different amounts of sulphate of ammonia. Records of the fecundity of a total of 163 cane-fly and the total nitrogen in the leaf were kept for seven months. Newly matured females were caged individually on the third leaf (counting the spindle leaf as No. 1), and the number of eggs laid over a period of about 15 days was noted; fecundity was expressed as the number of eggs laid per day. Two males were caged with each female, and were replaced whenever necessary. Total nitrogen in the third leaf was estimated by the standard micro-Kjeldahl method, and expressed as percentage dry matter.

The results (Fig. 1) show that there is a very significant relationship between the nitrogen status of the leaf and the fecundity of cane-fly. (The scatter of points could probably be reduced by refinements in technique.) An increase from 1.5 to 2.5 nitrogen per cent dry matter, which is the range that is normally encountered under field conditions, results in an increase in fecundity of more than 100 per cent. The increasing importance of cane-fly, therefore, can be attributed at least in part to the improving standard of sugar-cane agriculture and the resulting higher nutritional status of the host plant. The relationship also explains many features of outbreaks, in particular the initial build-up in plant cane, the heavy infestations in young cane, and the population decline as the cane ages. Future work is planned to determine whether it is the amino-acid rather than total nitrogen content of the leaf which is critical, and the relevance

of this to observed differences in varietal susceptibility of sugar-cane to cane-fly.

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<sup>1</sup> Ann. Rep. Res. Dep. Sug. Manuf. Assoc. Jamaica 1963, 20.

<sup>2</sup> Ann. Rep. Res. Dep. Sug. Manuf. Assoc. Jamaica 1963 (in the press).

<sup>3</sup> Wigglesworth, V. B., *The Principles of Insect Physiology*, fifth ed., 476 (Methuen and Co., 1963).

<sup>4</sup> Burr, G., et al., *Ann. Rev. Plant Physiol.*, 8, 275 (1957).

## MICROBIOLOGY

### Serotypes of *Candida albicans*

THE classification of *Candida albicans* into two serological groups on the basis of agglutination tests was first reported by Hasenclever and Mitchell<sup>1</sup>, and both in the original and subsequent papers<sup>2-4</sup> these authors and their colleagues referred to group A and group B strains. The findings were confirmed by me<sup>5</sup>, using a double-diffusion method, and, for the sake of conformity, similarly using the word 'group' with reference to the serological division within the species. Nevertheless, it must be apparent that this terminology is not consistent with that relating to the serological classification of other microbiological species such as *Pneumococcus* and *Haemophilus influenzae*, where the word 'type' is employed, and that confusion may arise if the word 'group' is used to describe not only an antigen (group antigen) which is common to more than one species of a particular genus, but also the serological division within a species.

There is reason, therefore, for hoping that in any future contributions to this subject the word 'type' or 'serotype' will replace the word 'group'.

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<sup>1</sup> Hasenclever, H. F., and Mitchell, W. O., *Antigenic Studies of Candida*, I, *J. Bact.*, 88, 570 (1961).

<sup>2</sup> Hasenclever, H. F., Mitchell, W. O., and Loeve, J., *Antigenic Studies of Candida*, II, *J. Bact.*, 88, 574 (1961).

<sup>3</sup> Hasenclever, H. F., and Mitchell, W. O., *Antigenic Studies of Candida*, III, *J. Bact.*, 88, 578 (1961).

<sup>4</sup> Hasenclever, H. F., and Mitchell, W. O., *Antigenic Studies of Candida*, IV, *Serotypes*, 2, 201 (1963).

<sup>5</sup> Stallybrass, F. C., *Candida Protoplasma*, *J. Path. Bact.*, 87, 89 (1964).

<sup>6</sup> Stallybrass, F. C., *The Incidence of the Serological Groups of Candida albicans in Southern England*, *J. Hyg. (Camb.)*, 68, 395 (1964).

### Inhibitory Effect of Dipicolinic Acid on the Anaerobic Oxidation of Glucose by the Cell-free Extract from Vegetative Cells of *B. subtilis*

It has been reported that the dipicolinic acid content of several species of bacterial spores ranges from 5 to 10 per cent of the dry weight of spores<sup>1,2</sup>. The biological actions of dipicolinic acid in spores have been discussed in a few reports. Harrell<sup>3</sup> suggested that the release of dipicolinic acid from spores has some relation to the ability of the organism to oxidize glucose. Harrell, Doi and Halvorson<sup>4</sup> have further shown that dipicolinic acid has a stimulatory effect on glucose oxidation of the cell-free extract of *B. cereus* spores. The relationships of dipicolinic acid content to the heat stability<sup>5</sup> and to the stainability<sup>6</sup> of spores have also been studied.

We wish to report that dipicolinic acid has an inhibitory effect on the anaerobic oxidation of glucose by the cell-free extract from the vegetative cells of *B. subtilis*.

*B. subtilis* (PCI 219) was cultivated on meat extract agar at 37° C for 20 h. Sporulation is known to begin immediately after the 20th h of cultivation under the

conditions employed. The cells were gathered in M/50 phosphate buffer (pH 7.2), washed several times with sterilized phosphate buffer, and finally suspended in M/10 phosphate buffer. The bacterial suspension was then oscillated in a 10-ks sonic oscillator (Kubota 'KMS-100') for 30 min at 2°-3° C. After rupture the vegetative cell brei was centrifuged at 6,000 r.p.m. for 20 min. The supernatant cell-free extract was stored at -20° C before use in enzymatic studies.

The anaerobic oxidation of D-glucose was assayed by measuring the dehydrogenase activity using the Thunberg technique. The side-arm cap contained 0.5 ml. of the cell-free extract containing 2-4 mg protein/ml., 0.1 ml. of 1 M phosphate buffer (pH 7.2), 0.2 ml. of a neutralized solution of dipicolinic acid in varying concentrations, and 0.2 ml. water to make up a total volume of 1.0 ml. The concentrations of dipicolinic acid used were 0.5 M, 0.05 M and 0.005 M. The side-arm caps were incubated for 60, 120 or 180 min at 37° C in order to ensure enough contact of dipicolinic acid with the enzyme preparation before the reaction of the latter with the substrate. The main tubes contained 1.2 ml. 0.025 M glucose, 1.0 ml. 0.01 per cent methylene blue in 1/10 M phosphate buffer (pH 7.2), 0.5 ml. 1 mg/ml. NAD and 0.3 ml. 1 M phosphate buffer (pH 7.2). At the end of the pre-incubation period the tube was thoroughly evacuated, the contents were mixed, and the tube was again incubated at 37° C. The time for complete decolorization of methylene blue was then recorded. In each experiment two different types of control runs were made: (1) dipicolinic acid was omitted, and (2) dipicolinic acid was added to the main tube instead of to the side-arm. The latter type of control experiment was designed to determine the effect of a simultaneous contact of the cell-free extract with both dipicolinic acid and the substrate. The results obtained are summarized in Table 1.

From the results shown in Table 1, it is apparent that dipicolinic acid has an inhibitory effect on the anaerobic oxidation of glucose catalysed by the cell-free extract of *B. subtilis* in the presence of NAD and methylene blue. Two important features of the inhibition noted are: (a) the inhibitory effect is dependent on the concentration of dipicolinic acid (Expts. 1-3), a pronounced inhibition being observed at a concentration of dipicolinic acid as high as 0.1 M in the pre-incubation mixture; (b) the degree of inhibition is markedly increased by a longer contact of dipicolinic acid with the enzyme preparation before incubation with the substrate (Expts. 3-5).

We have further examined whether the inhibitory effect could be found only with dipicolinic acid or with a variety of pyridine mono- and di-carboxylic acids. The compounds tested were quinolinic acid (pyridine-2,3-dicarboxylic acid), lutidinic acid, isocinchomeronic acid (pyridine-2,5-dicarboxylic acid), phthalic acid, nicotinic acid, and isonicotinic acid. The experimental conditions were the same as those described here except that 0.2 ml.

Table 1. INHIBITORY EFFECT OF DIPICOLINIC ACID ON THE ANAEROBIC OXIDATION OF GLUCOSE BY THE CELL-FREE EXTRACT FROM VEGETATIVE CELLS OF *B. subtilis*

The cell-free extract was added to the side-arm cap, and glucose, methylene blue and NAD were added to the main tube

Experiment No.	Side-arm cap Pre-incubation (min)	Dipicolinic acid (μmoles)	Dipicolinic acid in main tube (μmoles)	Complete decolorization of methylene blue (min)
1	60	1.0	0	80
	60	0	0	28
	60	0	1.0	46
2	60	1.0	0	87
	60	0	0	34
	60	0	1.0	47
3	60	100	0	>150*
	60	0	0	37
	60	0	100	110
4	120	100	0	>150*
	120	0	0	32
5	180	100	0	>150*
	180	0	0	22
	180	0	100	65

\* Only partial decolorization was noted after 150 min.

VETERINARY SCIENCE

Seasonal Variation in the Breeding Activity of Rams

Most investigations into the breeding season of sheep in Britain have been concerned with the behaviour of females. Little attention has been paid to the desire or ability of males to mate at different times of the year, for it is generally considered that under temperate conditions there is no discernible non-breeding season in rams of the British breeds.

Present investigations on the natural breeding behaviour of the Clun ewes in the College Farm flock show that the female breeding season commences about the beginning of August and terminates during the second half of March, although there are considerable variations between individuals and between seasons.

On the other hand, a number of ewes have been served during their reputed anoestrous period from the end of March to the beginning of August. Depending largely on time of lambing and earliness of weaning, a proportion of these services have proved fertile, and in earlier observations<sup>1</sup> it was entirely on the basis of autumn lambing that such 'out-of-season' matings were recorded, for the rams then in use were not raddled outside the normal tupping period.

The rams included two Hampshires and one Kerry, and although all three had an equal opportunity to mate with the ewes concerned, every one of 43 late-summer and autumn-born lambs produced over four seasons up to 1962 by 30 ewes was clearly a Kerry cross-lamb.

Both the Hampshires and the Kerry had been effective during the normal tupping seasons, so that this suggested a real difference in the seasonal breeding behaviour of the rams, and possibly the breeds, concerned, but due to the lack of adequate data at the time of service, it was not clear whether the Hampshire rams failed to serve any ewes, or whether they did so but the matings were infertile.

However, since April 1962, when sufficient new rams were introduced to make up two replicates each of a Hampshire, a Suffolk and a Kerry, rams of the respective breeds have worn individual distinctive coloured crayons while accompanying ewes continuously throughout the year. Observations have been made, generally twice a day, of any ewes marked at service, and every 14 days such ewes have been washed clean. In this way separate and continuous service records have been kept for each individual ram.

During the 'anoestrous periods' of 1962-64 an aggregate scatter of 20 ewes were marked, but on every occasion except one the only crayon colour showing has been that worn by a Kerry ram.

This also suggests that while the ewes are 'anoestrous' there is a difference in libido between the Kerry rams and the others. It is possible that the Kerry rams tried to mount ewes whether they were in season or not, but this idea is not supported by the lambing evidence already mentioned.

Specific examples have been recorded among these cases of the Hampshire and Suffolk rams (even when close penned) showing no interest in a ewe which stood to be properly served by a Kerry ram. Nor did the removal of the Kerry, to preclude any possible dominance effect, arouse any interest in either ram of the other two breeds. Nor is the ewe preference factor considered important at this stage. The only ewe preferences recorded have, in fact, been a very few cases in favour of Suffolk rams during the normal tupping season.

During the autumns of 1962 and 1963 no lambings resulted from the recorded 'anoestrous' matings, but this may possibly be partly explained by the fact that during this period no early weaning took place, that is, this could be a ewe factor rather than a ram factor<sup>1</sup>.

Table 2. INHIBITORY EFFECTS OF CHEMICAL ANALOGUES OF DIPICOLINIC ACID ON THE ANAEROBIC OXIDATION OF GLUCOSE BY THE CELL-FREE EXTRACT FROM VEGETATIVE CELLS OF *B. subtilis*

Experiment No	Side-arm cap Pre-incubation (min)	Dipicolinic acid (μmoles)	Analogues of dipicolinic acid*	Complete decolorization of methylene blue (min)
6	60	—	—	25
	60	20	—	65
	60	—	Isoctinohomeric acid	47
7	60	—	—	23
	60	20	—	67
	60	—	Isidinic acid	45
8	60	—	—	23
	60	20	—	67
	60	—	Quinolnic acid	44
9	60	—	—	23
	60	20	—	50
	60	—	Phthalic acid	23
10	60	—	—	50
	60	20	—	29
	60	—	Nicotinic acid	20
11	60	—	—	57
	60	20	—	22
	60	—	Isonicotinic acid	—

\* Twenty μmoles of each analogue were added.

Table 3. EFFECT OF DIPICOLINIC ACID ON THE ANAEROBIC OXIDATION OF L-ALANINE BY THE CELL-FREE EXTRACT OF VEGETATIVE CELLS OF *B. subtilis*  
0.5 ml. of cell-free extract containing approximately 4 mg protein/ml. or 0.4 mg protein/ml. was used in Exp. No. 12 or No. 13, respectively

Experiment No.	Side-arm cap Pre-incubation (min)	Dipicolinic acid (μmole)	Substrate	Complete decolorization (min)
12	60	—	D-glucose	17
	60	20	D-glucose	43
13	60	—	L-alanine	11
	60	20	L-alanine	10

0.1 M neutralized solution of the compound to be tested was added to the side-arm cap. The results obtained are shown in Table 2. From the results shown in Table 2, it is apparent that the most potent inhibitor among the compounds tested is dipicolinic acid, while the other three different kinds of pyridine dicarboxylic acids have considerable inhibitory effect (Expts. 6-8). Pyridine monocarboxylic acids and benzene dicarboxylic acid showed little or no inhibition (Expts. 9-11).

We also wished to determine whether the inhibitory effect of dipicolinic acid is specific only for the glucose oxidation. A preliminary experiment of the inhibitory effect on the anaerobic oxidation of L-alanine by the same enzyme preparation was also carried out as an example. The results are shown in Table 3. The data shown in the table indicate that the oxidation of L-alanine is not inhibited by dipicolinic acid.

The results of the present experiment seem to indicate an induced inactivation of the glucose dehydrogenase system by dipicolinic acid of a high concentration *in vitro*, and are of great interest in view of the fact found *in vivo* that during the course of sporulation the accumulation of a tremendous amount of dipicolinic acid proceeds with the concomitant loss of the glucose-oxidizing activity of spores<sup>2</sup>.

Further investigation will be needed as to the mechanism of inhibition of the glucose oxidation by pyridine dicarboxylic acids, particularly by dipicolinic acid.

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<sup>1</sup> Powell, J. F., *Biochem. J.*, **54**, 210 (1963).

<sup>2</sup> Tinelli, R., *Ann. Inst. Pasteur*, **83**, 364 (1955).

<sup>3</sup> Hazrell, W. K., *Bact. Proc.*, **44** (1956).

<sup>4</sup> Hazrell, W. K., Dol, R. H., and Halvorsen, H. O., *J. App. Bact.*, **20**, xiii (1957).

<sup>5</sup> Church, B. D., and Halvorsen, H. O., *Nature*, **183**, 124 (1959).

<sup>6</sup> Hachisuka, Y., and Kuno, T., *Int. Symp. Physiol. Ecol. Biochem. of Germine-tion*, Greifswald, Germany, September 1963.

<sup>7</sup> Hachisuka, Y., Asano, N., Kaneko, M., and Kanbe, T., *Science*, **134**, 174 (1956).

Not only have the Kerry rams appeared to be the only active rams during the 'out-of-season' period but they have also appeared to be quicker off the mark at the onset of the normal breeding season. For example, in 1963, by the day on which all three rams had begun to work, a Kerry ram had already served seven ewes over a 10-day period and a Hampshire only one; while in 1964 a Kerry had already served eight ewes over a 14-day period and a Suffolk only two.

There thus appears in these particular rams to be a real difference, during the 'anoestrous period' of the ewes, between the sexual behaviour of the Kerry rams on the one hand, and of the Hampshires and Suffolks on the other. Either the Hampshires and the Suffolks have been almost totally lacking in libido at this time or the ewes concerned have not exhibited a degree of heat detectable by them though it has apparently been patent enough for the Kerrys.

It is not known whether these differences between the rams are associated with differences in semen quality, but semen samples have been collected by electroejaculation from two other replicates of similar groups of rams at fortnightly intervals from November 1963 to November 1964 and the results of this work are now being examined.

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<sup>1</sup> Lees, J. L., *Nature*, **208**, 1089 (1964).

## PSYCHOLOGY

### Substitution of Intracranial Electrical Stimulation for Photic Stimulation during Extinction Procedure

It has been demonstrated that a conditioned response to a peripheral physiological stimulus (CS) can later be evoked when electrical stimulation of the cortical or subcortical centres is substituted for the original conditioning stimulus<sup>1-3</sup>. The present experiment was designed to examine the degree to which electrical stimulation of the occipital cortex, during the extinction stage of an avoidance conditioning procedure, would elicit conditioned responses (CRs) in an animal previously trained to respond to light (CS).

Ten male albino rats, 150-180 days old, with bipolar electrodes chronically implanted in the occipital cortex, were used. The electrodes, insulated with 'Teflon' except at the very tip, were approximately 150 $\mu$  in diameter. Histological examination showed that the electrodes were in the visual cortex about 0.5-1 mm deep from the surface.

Initially, all animals were trained, in a Skinner box, to press a lever to avoid a mild shock to the feet when a light (CS) was presented. The floor of the box had grids through which the foot shock (US) was delivered. The light emitted by two 25-W bulbs mounted in the box was used as the CS. This light was sufficient to illuminate the interior of the box fairly uniformly. Each trial was initiated by the onset of the CS for 5 sec, after which the US was delivered. Both the CS and the US continued until the animal pressed the lever. If the animal failed to respond within 50 sec after the onset of the US, the CS and US were terminated automatically and it was counted as a trial.

The CR was a lever press within the initial 5 sec of the CS presentation. Forty trials were administered per day with intertrial intervals of 20-40 sec. Animals were trained to make 80 per cent CRs per day for two consecutive days. The day after the last conditioning day, the animals were tested for generalization to electrical stimulation of the occipital cortex. On the test day, the CS-US paired trial was administered for the first

No. conditioning trials	No. extinction trials to the test stimulus	No. extinction trials to the CS
160	8	—
240	20	—
400	0	—
880	12	—
440	0	—
240	16	72
160	13	49
480	0	286
160	25	57
160	40	80

20 trials. Immediately after the 20th trial, the extinction procedure (without a foot-shock) was started. In this procedure, electrical stimulation of the occipital cortex (test stimulus) was substituted for the original CS (light). The test stimulus consisted of a train of biphasic waves of 3 msec duration and a frequency of 35 c/s. The intensity varied among animals between 10 and 120  $\mu$ amp. The test stimuli were below the threshold for any visible behavioural signs such as a cessation of movements or the appearance of tremors. For each extinction trial, the test stimulus was presented for a maximum of 5 sec. A lever press within this time was scored as a CR. The extinction procedure was terminated when animals failed to make CRs for six consecutive trials. Immediately after extinction to the test stimulus, five animals were subjected to additional extinction trials to the original CS (light). The criterion for the extinction was again six consecutive trials without a CR.

Table 1 shows the number of conditioning trials (column 1), extinction trials to the test stimulus (column 2), and extinction trials to the original CS (column 3). Seven out of ten animals trained to the light stimulus showed generalization to the test stimulus. The degree of generalization varied considerably among the subjects (from 8 CRs to 40 CRs). For the group as a whole, there was no correlation between the intensity of the stimulating current and the number of the CRs elicited ( $P$ ,  $-0.31$ ). The three subjects which failed to show generalization to the test stimulus failed also to show any other detectable effects of the stimulation even when the intensity was raised to 120  $\mu$ amp. This may indicate that the failure of three animals to generalize was due to a lack of an adequate stimulation of the neural tissues. The five animals that were subjected to additional extinction trials using the original CS (light) elicited a large number of CRs. For these animals, there was no significant correlation between the number of extinction trials to the test stimulus and the number of extinction trials to the original CS ( $P$ ,  $-0.1$ ).

These findings agree with previous findings that the intracranial stimulation can be substituted as CS to evoke conditioned responses previously trained to a peripheral physiological stimulus. Studies on stimulus generalization have demonstrated that generalization (a) is a decreasing function of the differences between the original and the test stimulus, and (b) varies directly with the degree of original learning. The present results, however, indicate that the generalization to the intracranial stimulation used as the test stimulus is not related to the number of acquisition trials or to the intensity of the stimulating current. This may be due to the fact that the neural excitation produced by the direct intracranial electrical stimulation is quantitatively as well as qualitatively different from that produced by the peripheral physiological stimulation.

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<sup>1</sup> Nielsen, H. C., Knight, J. M., and Porter, P. B., *J. Comp. Physiol. Psychol.*, **55**, 168 (1962).

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## FORTHCOMING EVENTS

Saturday, July 17—Sunday, July 18

BRITISH ACADEMY OF FORENSIC SCIENCES (at the London Hospital Medical College, New Hall, Ashfield Street, London, E.1.)—Sixth Annual Scientific Meeting. Main theme—"Poisons and Poisoning".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT EXPERIMENTAL OFFICER (preferably with a knowledge of chromatographic techniques) to assist in developing and applying analytical techniques for insecticide residues, and to aid in studies on the behaviour of insecticides in soils and plants.—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (July 17).

ASSISTANT LECTURER or LECTURER AT THE TIDAL INSTITUTE AND OBSERVATORY for research on the mathematical theory and numerical analysis of tides and kindred phenomena.—The Registrar, The University, Liverpool, quoting Ref. OV/169/N (July 17).

ADMINISTRATIVE ASSISTANT (preferably with some familiarity with the notations and terminology of higher mathematics) IN THE DEPARTMENT OF MATHEMATICS.—The Registrar, The University, Leeds, 2 (July 19).

RADIATION PROTECTION OFFICER (physicist, physical chemist, biophysicist, health physicist, or other suitably qualified person).—The Registrar, The University, Nottingham (July 19).

LECTURER (with a good honours degree and experience in teaching, research or industry, or a postgraduate qualification) IN PHARMACOLOGY.—The Registrar, Brighton College of Technology, Moulsecomb, Brighton, 7 (July 20).

POST-DOCTORAL FELLOW (with experience in plasma physics, microwave techniques or electromagnetic theory and use of a computer) in a plasma physics group which is studying the scattering of microwaves by various plasma configurations.—The Registrar, University College of Wales, Aberystwyth (July 20).

DEMONSTRATOR IN THE DEPARTMENT OF VETERINARY SURGERY.—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh, 8 (July 21).

RESEARCH ASSISTANT (graduate in zoology) IN ZOOLOGY to undertake research on ecology of Collembola in woodland, probably in Huntingdonshire, starting September 1965.—Head of the Department of Botany and Zoology, Sir John Cass College, Jewry Street, London, E.C.3 (July 21).

UNIVERSITY DEMONSTRATOR (with a registrable qualification in veterinary medicine or an honours degree in pharmacology or a related subject) IN THE DEPARTMENT OF VETERINARY PHARMACOLOGY, Royal (Dick) School of Veterinary Studies.—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (July 21).

RESEARCH ASSISTANT (with a good honours degree, some postgraduate experience, and preferably an interest in physiological mycology and/or soil microbiology) IN THE DEPARTMENT OF BOTANY, Queen's College, Dundee.—The Secretary, University of St. Andrews, Queen's College, Dundee (July 24).

RESEARCH ASSISTANT (with a Ph.D. degree in chemistry) to work with Dr. J. S. Whitehurst on steroids.—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (July 24).

LECTURER (with an honours degree in physiology or closely allied subject, and preferably some research experience) IN THE DEPARTMENT OF PHYSIOLOGY.—The Secretary, The University, Edinburgh (July 26).

LECTURER (with previous experience in forensic medicine and general pathology) IN THE DEPARTMENT OF FORENSIC MEDICINE.—The Secretary, University of Edinburgh, Edinburgh (July 28).

RESEARCH ASSISTANT (with an honours degree in chemistry and an interest in biochemistry) IN THE DEPARTMENT OF FORENSIC MEDICINE, to assist in a programme of research into the development of rapid methods of toxicological analysis.—Dr. Harold V. Street, Department of Forensic Medicine, University New Buildings, Teviot Place, Edinburgh (July 30).

SECOND CHAIR OF CHEMISTRY (candidate's interests should be either in physical or inorganic chemistry).—The Registrar, The University, Hull (July 30).

SENIOR LECTURER or LECTURER and LECTURERS or ASSISTANT LECTURERS (3) IN GEOGRAPHY at the University of the West Indies (Mona).—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (July 30).

SENIOR LECTURER, LECTURERS or ASSISTANT LECTURERS (with an appropriate qualification and/or experience in one or more of the following fields: taxonomy, ecology, genetics, cytology or general botany) IN BOTANY at the University of Ife, Nigeria.—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (July 30).

ASSISTANT (B.Sc. honours biochemistry or with biochemistry in ordinary degree, familiar with fractionation techniques, and preferably experience in protein chemistry) for a study in bacterioid purification.—The Secretary, Glasgow Royal Infirmary, 84 Castle Street, Glasgow, G.4 (July 31).

ASSISTANT LECTURER (medically qualified or non-medically qualified) IN MEDICAL GENETICS to be responsible for cytogenetics.—The Registrar, The University, Manchester, 13, quoting Ref. 188/65 (July 31).

FAULTY LECTURESHIPS (2) IN MATHEMATICS (each lectureship will be held with a stipendiary fellowship at an Oxford college).—The Secretary of Faculties, University Registry, Broad Street, Oxford (July 31).

LECTURER or ASSISTANT LECTURER (preferably with interests in a branch of pure mathematics) IN MATHEMATICS IN THE SCHOOL OF MATHEMATICS AND PHYSICS.—The Registrar, University of East Anglia, Norwich Hall, Norwich, NR8 88c (July 31).

LECTURERS and ASSISTANTS IN ZOOLOGY.—Secretary of the University Court, The University, Glasgow (July 31).

SCIENTIFIC OFFICERS (2) (with a good honours degree, preferably in physiology or biochemistry and/or a veterinary qualification, 1965 graduates would be considered) IN THE PHYSIOLOGY DEPARTMENT to undertake work on (1) the endocrine control of mineral metabolism, and (2) the physiology of digestion in farm animals.—The Secretary, Rowett Research Institute, Bucksburn, Aberdeen, Scotland (July 31).

CHAIR OF AGRICULTURAL BIOLOGY at Makerere University College, Uganda.—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (August 15).

CHAIR OF BIOCHEMISTRY, CHAIR OF SOCIOLOGY, and SENIOR LECTURERS, LECTURERS or ASSISTANT LECTURERS IN AGRICULTURAL BIOCHEMISTRY and BIOCHEMISTRY at the University College of Rhodesia and Nyasaland.—The Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (August 15).

CHAIR OF SOCIAL ANTHROPOLOGY IN THE DEPARTMENT OF SOCIAL ANTHROPOLOGY AND AFRICAN ADMINISTRATION, University of the Witwatersrand,

Johannesburg, South Africa.—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, August 30).

LECTURER (graduate in science, medicine or veterinary science, and preferably possessing a higher qualification and experience in teaching and research in pharmacology) IN PHARMACOLOGY at the University of Queensland, Australia.—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, August 27).

CHAIR OF PHARMACOLOGY at the University of the Witwatersrand, Johannesburg, South Africa.—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, August 31).

LECTURER (preferably theoretical physicist with experience in crystals or solid state physics) IN PHYSICS.—The Secretary, University of St. Andrews, Queen's College, Dundee, Scotland (August 31).

LECTURER IN PSYCHOLOGY at the University of Canterbury, Christchurch, New Zealand.—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 31).

LECTURER (preferably theoretical physicist with experience in crystal or solid state physics) IN PHYSICS in Queen's College, Dundee.—The Secretary, University of St. Andrews, Queen's College, Dundee (August 31).

POST-DOCTORAL RESEARCH FELLOW IN THE DEPARTMENT OF INORGANIC CHEMISTRY, University of Melbourne, Australia.—The Registrar, University of Melbourne, Parkville, N.S. Victoria, Australia (August 31).

SENIOR LECTURER/LECTURER (with an honours degree in physics or textile physics or equivalent qualifications, and for the appointment as Senior Lecturer a higher degree is essential) IN THE SCHOOL OF TEXTILE TECHNOLOGY, University of New South Wales, Australia.—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 31).

SENIOR LECTURER or LECTURER IN EDUCATION at Victoria University of Wellington, New Zealand.—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 31).

LECTURER (with an honours degree in optometry or equivalent qualifications, some clinical experience, and preferably teaching and/or research experience) IN THE DEPARTMENT OF OPTOMETRY, School of Physics, University of New South Wales, Australia.—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 20).

SENIOR LECTURER (with an honours or higher degree in physics or metallurgy with research and/or teaching experience in the field of metal physics) IN METAL PHYSICS IN THE DEPARTMENT OF METALLURGY, University of Melbourne, Australia.—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London) (September 30).

SENIOR LECTURER (with special interests in physical oceanography, including dynamic oceanography and/or marine geology) IN OCEANOGRAPHY; and a LECTURER (preferably with a special interest in cosynology) IN BIOGEOCHEMISTRY.—The Registrar, University College of Swansea, Singleton Park, Swansea (October 1).

ASSISTANT CONSERVATOR OF FORESTS (national of the United Kingdom or the Republic of Ireland, with a degree in forestry) in Uganda, for the organization, administration and technical management of forest operation in a district of Uganda.—The Appointments Officer, Room 301, Ministry of Overseas Development, Bland House, Stag Place, London, S.W.1, quoting RO 324/183/01.

ASSISTANT LECTURER IN INORGANIC CHEMISTRY.—The Secretary, Royal Holloway College (University of London), Englefield Green, Surrey.

CHIEF TECHNICIAN IN THE DEPARTMENT OF CHEMISTRY for duties concerned with the purchase of materials and supervision of laboratories.—Prof. J. N. Bradley, Department of Chemistry, University of Essex, Wivenhoe Park, Colchester, Essex.

RESEARCH ASSISTANT or PHYSICIST to work as assistant to the Head of the Electronics Unit of the Physics Department.—The Assistant Secretary, St. George's Hospital, London, S.W.1.

FELLOW IN THE CHEMISTRY DEPARTMENT, for work which will involve the application of far infra-red spectroscopy to problems in inorganic and physical chemistry, under the direction of Dr. A. Finch.—The Secretary, Royal Holloway College (University of London), Englefield Green, Surrey.

FIELD GEOLOGIST (national of the United Kingdom or the Republic of Ireland, with an honours degree in geology and five-ten years field experience in modern prospecting techniques including diamond drilling, geochemical and geophysical methods) in Jordan, to train a Jordanian field geologist in the principles of mineral research in the field, and in the running of a Mobile Prospecting Unit.—The Appointments Officer, Room 301, Ministry of Overseas Development, Bland House, Stag Place, London, S.W.1, quoting RO 318/94/03.

GRADUATE ASSISTANT IN BIOCHEMISTRY to assist with the investigation of the metabolism of surgical patients in infancy and early childhood.—The Secretary, Institute of Child Health (University of London), Great Ormond Street, London, W.C.1, quoting Ref. 8/GAB.

GRADUATE RESEARCH ASSISTANT in the field of mammalian neuro-anatomy in the Zoology Department. The work will entail the use of histological, histochemical and experimental techniques.—The Secretary, Royal Holloway College (University of London), Englefield Green, Surrey.

HOLT FELLOW (graduate in medicine or science) IN PHYSIOLOGY to collaborate in research into the electrophysiology of mammalian muscle spindle receptors and assist in the development of a new research unit for investigating the pharmacology of the muscle spindle.—The Registrar, The University, Liverpool, 3, quoting Ref. OV/168.

LECTURER IN THE DEPARTMENT OF ZOOLOGY at Fourth Bay College (The University College of Sierra Leone).—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1.

LECTURER or ASSISTANT LECTURER IN THEORETICAL PHYSICS.—The Secretary, Royal Holloway College (University of London), Englefield Green, Surrey.

LECTURER (well qualified academically with postgraduate experience in industry and/or teaching) IN PHYSICAL CHEMISTRY to teach to B.Sc. and Grad.R.I.C. Part II level.—The Principal, Midway College of Technology, Horsted, Maidstone Road, Chatham, Kent.

OFFICER (with a degree in horticulture, agriculture or agricultural botany, or other equivalent qualifications) FOR VEGETABLE TRIALS, to be stationed at Cambridge and will be responsible under the Head of the Trials Branch for the organization and supervision of the vegetable variety testing programme of the Institute.—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.



**RESEARCH ASSISTANT** (preferably with some competence in quantitative chemical analysis) to participate in physiological work with a biochemical bias—The Administrator, University Laboratory of Physiology, Oxford.

**RESEARCH BURSARY IN THE CHEMICAL ENGINEERING AND CHEMICAL TECHNOLOGY DEPARTMENT** for studies on the mechanism whereby certain additives render plastics non-inflammable—Dr. C. F. Collins, Imperial College of Science and Technology, London, S.W. 7.

**RESEARCH FELLOW IN THE DEPARTMENT OF CHEMISTRY** to work under the direction of Dr. R. Foster on studies of organic charge-transfer complexes—The Secretary, University of St. Andrews, Queen's College, Dundee.

**RESEARCH STUDENTS AND POST-DOCTORAL WORKERS IN THE DEPARTMENT OF MICROBIOLOGY**, for studies of microbial chemistry in relation to pathogenicity and immunity—Prof. H. Smith, The University, Edgbaston, Birmingham, 15.

**RESEARCH STUDENTS** (men or women) in the Department of Physics—The Secretary, Royal Holloway College (University of London), Egham, Surrey.

**RICE AGRONOMIST** (national of the United Kingdom or the Republic of Ireland, with an honours degree in agriculture or horticulture, one or two years postgraduate training, and preferably field experience with tropical crops) in Sarawak, to design and conduct experiments on wet rice and, to a lesser degree, on hill (dry) rice and off-season crops—The Appointments Officer, Room 801, Ministry of Overseas Development, Maud House, Stag Place, London, S.W.1, quoting ref. RO 213/155/03.

**SPECIALIST TECHNICIAN** for an instrument workshop concerned with design, construction and maintenance of specialised laboratory equipment—The Secretary, Institute of Psychiatry, The Maudsley Hospital, Denmark Hill, London, S.E.5.

**TECHNICAL ASSISTANT** (preferably graduate with some knowledge of biochemistry) for a research team working in the Department of Biochemistry—The Secretary, The University, Edinburgh.

**TECHNICAL OFFICERS** (with at least H.N.C. metallurgy) for basic and applied work in the field of making and properties of coatings—G. F. Waters, Department of Industrial Metallurgy, University of Birmingham, Edgbaston, Birmingham, 15.

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## PUBLIC AND PRIVATE ENTERPRISE IN DEVELOPING COUNTRIES

THE debate on the Commonwealth in the House of Lords on May 5, opened by Lord Casey, was notable for the warm tributes to the work of the Commonwealth Development Corporation by Lord Casey himself, Lord Wade, Lord Fraser of Lonsdale, and Lord Howick of Glendale, who took up the suggestion that the Corporation should attempt to bring together modern scientific ideas on tropical agriculture on a large scale with a social system of small holders, and said that this had been done in Malaya, Swaziland and Kenya, and that the Corporation hoped to do it in Nigeria. For the Government the Lord Chancellor and Lord Taylor particularly emphasized the extent of the work of the British Council in the Commonwealth, while the Lord Chancellor referred to the initiatives for a Commonwealth Secretariat, a Commonwealth Foundation and a Commonwealth Medical Conference which had come from the Prime Minister's Conference in 1964. In that year Britain's total bilateral aid, including technical assistance, was £174 million, of which £108 million was to the independent territories of the Commonwealth, including 72 per cent of our technical assistance programme. Under the present Government about £20 million of Britain's capital aid had been pledged to developing Commonwealth countries. Apart from this reference to financial aid the Lord Chancellor's main emphasis was on the encouragement of personal contacts, and this theme was taken further by Lord Taylor in relation to the interchange of students. He also mentioned family planning and said that, under the auspices of the United Nations, a five-man team led by Sir Colville Devereil had been sent to India in February 1965, at the invitation of the Indian Government, to advise on the course of action to make small families more acceptable, to encourage the practice of family planning and to reduce the national birth-rate. The team's report was now being prepared. Lord Taylor added that the Consultative Committee of the Colombo Plan had chosen for their topic for their next winter meeting the relation between population and economic development.

The debate on overseas investment on the following day was intended to call attention to the importance of British private investment, and in opening the debate Lord Aldington was particularly concerned with the possible adverse effects on overseas investment, and in consequence on Britain's future exports, of the clauses in the Finance Bill relating to corporation tax on overseas investment. Lord Aldington discussed fairly enough the advantages which accrue from overseas investments and also the disadvantages, and his concern was shared by other speakers. For the Government, Lord Shepherd welcomed the debate but maintained the Government's view that, for a relatively short period, Britain should curb her investments overseas, both on account of the balance of payments situation and to ensure sufficient investment for her own industries to strengthen her economy. None the less, his speech did not satisfactorily cover the points raised subsequently by Lord Chandos, Lord Kilmuir and Lord

Amory, nor were they fully met by the Earl of Longford in replying on the debate for the Government.

This debate, notably objective and free from party politics, gives the greater interest to a recent publication of the Overseas Development Institute, *Investment and Development*, in which the role of private investment in developing countries is discussed\*. Following an introduction by Sir Leslie Rowan, Mr. W. Clark considers the limitations attached to Government effort, Sir Jock Campbell discusses the role of big business in the new nations, Mr. J. H. Loudon the place of international enterprise in the development decade, and Mr. A. Gaitekall writes on how to make the best of capital risk in the developing countries. The uncertainty about the future of official aid, which appears to be levelling off partly because of balance of payments problems and partly because of disillusionment as to the effectiveness with which the aid has been used, encourages a re-appraisal of private investment which at one time was considerably circumscribed by political uncertainties. Sir Leslie makes the sound point that if private investors in Britain are to accept such a responsibility they must be provided with the wide knowledge on which the deep study leading to a conscientious and informed discussion can be based. There must also be frank public discussion of every aspect of the situation, including both profit and the use of trained manpower, and it is as a contribution to such discussion and the subsequent framing of continuous policy that the booklet is published.

Mr. Clark begins by noting that while aid in the form of public investment in the underdeveloped countries has been steadily increasing, private investment has been falling off. This is dangerous primarily because it may retard the whole process of development so that it falls below the increase of population or only just keeps pace with it. It is unlikely, moreover, that the Government will increase aid from public funds to fill any such gap, nor is it certain that Government aid alone would be adequate. Private investment retains certain advantages which public aid cannot easily match, and the Clay Committee in 1963 affirmed its conviction that it is the private sector, operating with the co-operation of a vital and democratic labour movement and enlightened management, on the basis of essential government services and sensible policies, which will make the greatest contribution to rapid economic growth and overall development.

Much the same point is made by Mr. Loudon, who comments that the ultimate solution to the world's economic problems is a huge increase in trade, and that State aid is limited by what taxpayers will accept. He pinpoints the significance of this whole discussion of overseas aid in the light of the proposals of the Finance Bill now before Parliament. He points out, moreover,

\* *Investment and Development: The Role of Private Investment in Developing Countries.* Sir Leslie Rowan, J. H. Loudon, Sir Jock Campbell, Arthur Gaitekall, and William Clark. Pp. 60 (London: Overseas Development Institute, 1965.) 7s. 6d.

that industrial investment tends to promote the local creation and sharing of expertise, divorced from political motives or ideology, though he recognizes that the attitude of some Governments to the whole private sector is sometimes a serious difficulty. A Government's role, Mr. Loudon believes, is to direct and liberate the energies of the people. Governments of developing countries must reconcile economic growth with reasonable financial stability, and they must also promote structural changes far more sweeping than are needed in the industrialized countries.

Mr. Loudon refers in passing to fears of the power of big business which he regards as ill-founded, but this question is discussed more fully by Sir Jock Campbell, who reminds us that modern big business is an economic grouping of men and women existing to produce and manufacture goods, to provide services and to distribute goods. To do this successfully its managers must recognize and fulfil responsibilities to shareholders, to employees and to customers, as well as to the community within which the business operates and in which it is rooted. Profits, and with them efficiency and productivity, are not ends in themselves but criteria—incisive instrumental means towards intelligible ends.

From this Sir Jock develops his view that there are projects necessary for the development of a country which, while not losing money and inappropriate for public subsidy, do not offer a commercial return on investment. They are appropriate either for State enterprise through public corporations, or for partnership between State and private enterprise on special terms, though he sees no reason why the State should not participate actively also in undertakings which promise to be profitable. It has to be remembered, moreover, that in establishing a great industry in an underdeveloped country a new way of life and a new series of motives, activities and disciplines are imposed on the people. Private property, punctuality, financial honesty, financial incentive, productivity, efficiency, technical invention and discipline, regularity, regimentation; all these may be new and strange as well as necessary. In practice a synthesis is required between the inevitable demands of industrialization, the inherent values and standards and culture of the new nations, and the aspirations and associations of nationalist leaders. Probably the critical factor, though Sir Jock does not mention this, is how long we can allow for this synthesis.

This situation is examined in greater length by Mr. Gaitskell in the final paper in the booklet, considering more especially the objectives of the African countries and the role of the West in achieving them. He emphasizes the need for a joint capital structure and suggests that there are four main requirements if we are to build a superstructure which can turn the inert base or infrastructure, which overseas aid has already done so much to provide, into higher standards of living, a superstructure which Africans can call their own. These requirements are: modernization of agriculture; education of personnel, particularly middle-group technical personnel, to execute such a programme; capital investment in agriculture and industry; and market openings for the products. The scope for private investment lies in both agriculture and industry. The market openings depend on a real drive to encourage world trade.

Mr. Gaitskell suggests that national policy might well evolve as a result of several convergent steps. First, more emphasis that the ultimate purpose of Western world

policy is to enable developing countries to stand on their own feet, and less on persuasion that we have the best answer to civilization. A second and new approach to the problem of mutual co-operation in investment is called for, with a general objective of promoting local control of the economy but on a broad basis, avoiding alliance between foreign private enterprise and local industrial oligarchies. It would include such remedies as joint capital structure and it would seek to harness private capital as an ally in development. Third, with this approach stronger pressure could be brought to bear on developing countries to accept and set up national plans of development which could integrate and control competing and conflicting external offers of aid. Fourth, clear co-operation between those who assist and plan expenditure on the infrastructure, whether internal or national agencies, and those who supply the public or private enterprise which must follow. Fifth, national discouragement of balkanization and encouragement of inter-African co-operation are particularly important; and sixth, mutual endeavour to avoid lop-sided development which appears to be one of the greatest present problems in the developing world. Seventh, Mr. Gaitskell puts active measures to increase the ability of developing countries to sell their products remuneratively in the developed world. Eighth, the terms of aid need overhaul to remove any impression that the developed countries' interests are dominant. Ninth, dispassionate enquiry into the necessity and feasibility of birth control; tenth, a forum for frank discussion of all these problems with African leaders instead of the separate conferences of developed and developing countries; and last, much more publicity in the Western world as to the complexity and importance of a new look at its relations with the developing world.

It is apparent from the two debates in the House of Lords that to some extent action is already being taken on some of these lines. The debates themselves, like the booklet, should assist the process of education and publicity. It is no less clear, however, that changes in Britain's own national policy require careful consideration from the point of view of their effects on world trade and on the developing world and not solely on our domestic situation. That makes it the more desirable for such discussion to be as free as possible from the heat of party politics. The debate in the House of Lords on May 6 demonstrated that this is practicable, given goodwill.

The programme "Second Thoughts on Aid" which was broadcast on the B.B.C. Third Programme in April and May is an important contribution to public discussion of the whole subject and covered many of the points raised in the papers in the Overseas Development Institute booklet and in the House of Lords. The role of private investment was the subject of the fourth discussion, for example, and the special problem of agriculture was considered in the sixth programme in the series. Technical assistance and priorities of education formed the subjects of the next two, and in the fifth the economic role of international institutes was discussed. The political aspects were considered in the opening two discussions which dealt with the politics and philosophy of donor countries and with some political realities of recipient countries, respectively. The whole series is well designed to stimulate interest and to promote the informal discussion and public understanding which are essential if the necessary support is to be forthcoming for the effort which Britain must make in this field.

## GENETICS AND THE BIOLOGICAL FRONT

### Advances In Genetics

Vol. 12. Edited by E. W. Caspar and J. M. Thoday. Pp. viii+388. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 100s.

VOLUME 12 of *Advances in Genetics* exhibits very well both the strengths and also the weaknesses of this type of annual publication. In a total of nearly 400 pages it contains only four articles, of which the two longer concern general problems and the two shorter quite specialized and restricted special fields. The coverage is very wide, from the stringently intellectual molecular biology to the most 'biological' plant systematics and genetics. There is probably no biologist alive who will be equally interested in all four articles. It is worth considering what various types of readers can get out of them.

The first is by Frank Lanni on "The Biological Coding Problem". It is not more than about four years ago that the discovery by Nirenberg and Matthaei of cell-free protein synthesis in response to synthetic ribonucleotide polymers opened up any hopeful-looking avenues towards its solution. Now, Lanni's bibliography runs to about 400 titles, of which approximately 85 per cent are dated 1960 or later. This is in fact one of the big break-through salients on the biological front. The question that arises is: can any biologist not personally engaged in this particular foray find the time to follow, blow for blow, the course of the engagement? Moreover, will those who are engaged in it find here anything they did not know already? The immediate tactical situation is altering so rapidly that there are certainly some things which they will not find. For example, anyone who attended the recent Royal Society Mendel Centenary Celebrations in London will have heard Brenner argue that there must be a DNA codon for chain termination. I spent about half an hour looking for a reference to this in Lanni's article and failed to find it.

The next article is by D. H. Davidson on differentiation in monolayer tissue culture cells. This is again a fashionable field but of a rather different kind. There has been no major theoretical break-through. It has been known for a very long time that cells in tissue culture tend "to lose their differentiation". Davidson's review contains at least 500 references, but of the 100 which I sampled, at least 60 were published before 1960 and as many as 50 before 1950, quite a number of them going back before 1930. His article, in fact, gives a convincingly inclusive review of the present state of a complex and slowly advancing field.

The other two articles are of a quite different kind, both being devoted to authoritative summaries of specialized investigations in circumscribed fields. Verne Grant gives an account of the studies that have been made of a group of sibling species in the genus *Gilia*—small herbaceous plants common in western North America and South America. This is a valuable addition to the general body of knowledge about plant evolution. Oelkers summarizes the results of species crosses in the plant genus *Streptocarpus*, in which there have been many tantalizing indications of the importance of cytoplasmic factors. Our understanding of at least some types of cytoplasmic inheritance is being so rapidly advanced by studies on more genetically amenable forms, such as those of Sager on *Ochlomyxdomonas*, that we are now faced with the challenge of passing well beyond the mere recognition of the existence of cytoplasmic factors; but this seems to be all that we can expect from the study of the more recalcitrant higher plants.

In sum, the new volume of *Advances in Genetics* well earns its place on the shelf of any well-stocked library. The first article will be extremely useful to a few people

for a short time before it becomes out of date. The last three will be valuable to perhaps fewer people at any one time, but for a longer period. C. H. WADDINGTON

## TETRAPYRROLE BIOSYNTHETICS

### Tetrapyrrole Biosynthesis and Its Regulation

By Dr. June Lascelles. (Microbial and Molecular Biology Series.) Pp. xii+132. (New York and Amsterdam: W. A. Benjamin, Inc., 1964.) 7.70 dollars.

DR. JUNE LASCELLES has made important contributions over the past ten years to our knowledge of the synthesis of porphyrins and bacteriochlorophyll in photosynthetic micro-organisms and, in particular, of the factors concerned with the regulation of this process.

The most important part of *Tetrapyrrole Biosynthesis and Its Regulation* is a critical and informative discussion of the control of this chain of reactions. However, this discussion, which forms chapter five of the book, is preceded by an account of the main structural features of tetrapyrroles, their physical properties and the methods used for the isolation and identification of both porphyrins and chlorophylls. A second chapter deals in a somewhat brief but concise manner with the distribution of haemoglobins in animal tissues. Dr. Lascelles considers, in more detail, the presence of the haemoglobins and especially of cytochromes in micro-organisms and in strictly anaerobic bacteria. She describes in a helpful manner the work which has been done, chiefly in the past few years, on the occurrence and probable function of the various cytochromes present in photosynthetic organelles and organisms.

Another section deals with the accumulation of porphyrins in micro-organisms, both of the photosynthetic and non-photosynthetic type; here it is of special interest that in most of these organisms it is coproporphyrin III which is present in relatively large amounts. A third chapter deals with the biosynthesis of tetrapyrroles, but this subject has been covered recently in a number of reviews. However, special emphasis is given here to work in micro-organisms, and the relationship between the biosynthesis of porphyrins and the more general metabolic reactions in the cell is emphasized.

Dr. Lascelles then deals with the chain of reactions leading from protoporphyrin to chlorophyll or bacteriochlorophyll. Many steps in this reaction sequence have been established, but there are many more for which no detailed information is available and for which enzymes have so far only been postulated. The structure of the chloroplast and that of the chromatophore is discussed in detail, and the important finding, which was mainly established by Dr. Lascelles and her colleagues, that the synthesis of bacteriochlorophyll and of protein is closely related, is rightly emphasized. However, as has already been stated, the most important part of the book is undoubtedly the chapter dealing with the control mechanisms, and this topic can be more easily investigated in micro-organisms than in animals.

The effect of light and oxygen tension has been known for some time, mainly due to the work of van Niel. Dr. Lascelles has herself contributed much to our understanding of the role of iron and she has also shown the repression of ALA synthetase by small amounts of haemin. Amino-laevulinic acid itself appears to repress ALA synthetase, but it is likely that other reactions may also play a part in the overall regulation. Thus we still know very little about the availability of glycine or of succinyl co-enzyme A or the importance of the methylation step in the overall regulation of the biosynthesis of chlorophylls by the cell.

There is obviously much more work to be done in this field, but the book by Dr. Lascelles not only reviews the present position fully but is also likely to stimulate many readers to do further investigations in this important field. A. NEUBERGER

## BIOLOGICAL ORGANIZATION

### Homeostasis and Feedback Mechanisms

Edited by G. M. Hughes. (Symposia of the Society for Experimental Biology, No. 18.) Pp. viii+460. (Cambridge: At the University Press, 1964. Published for the Company of Biologists on behalf of the Society for Experimental Biology.) 70s. net.

PREVIOUS symposia of the Society for Experimental Biology have covered a wide range of topics which fall within, between or across the accepted subdivisions of the science of life, and it has been clearly established that no aspect of biology is beyond the Society's terms of reference. This eighteenth symposium on *Homeostasis and Feedback Mechanisms* is concerned with every aspect of biological organization from subcellular to ecological levels, and beyond, and we are soon reminded that organismal homeostasis is only possible within quite small limits of change in the physical environment. Both the aqueous and gaseous environments in which life is found are themselves maintained in remarkably stable states of dynamic equilibrium which, though still little understood, are essential for life as we know it.

To encompass a theme of such wide biological application, it was necessary to choose between attempting a comprehensive but somewhat superficial coverage, or to seek a more profound understanding at a number of representative points. By tradition, and perhaps also by judgment, the latter course was followed, but at the risk that only the few or the very few would grasp fully the meaning of every contribution. Generally speaking, the editor has succeeded, and this whole series of wide-ranging papers is both pertinent and comprehensible.

There are twenty papers in the volume. After a brief discussion of homeostasis and the environment (Pantin), there are treatises on behavioural and physiological adaptation to both desert (Bartholomew) and arctic (Hart) conditions; on the control of temperature in man (Benzinger), respiration in fishes (Hughes), salt balance in crustaceans (Shaw) and growth in insects (Wigglesworth), while others consider the special adaptations in diving vertebrates (Anderson), the control of mammalian metabolism (Randle), the homeostatic significance of the hypothalamus (Cross) and of the adrenal cortex (Chester-Jones and Bellamy). Papers on genetic aspects of homeostasis (Robertson) and of circadian rhythms (Harker) complete the representation of biological systems in which the processes of control have been investigated.

There is not, because there cannot be, any sharp division between the presentation of experimental and theoretical aspects of biological control processes, but the latter part of the volume becomes increasingly concerned with the application of physical concepts to the study of these processes. Some biologists may feel somewhat humbled by the paper on the use of statistical mechanics in a theoretical approach to cellular organization (Goodwin), but subsequent contributors help to restore self-confidence. The discussion on the concept of feedback mechanisms in mental processes (Frank) is followed by a fascinating analysis of the wing control of insects (Weis-Fogh), of orientation control in flies, birds and man (Mittelstaedt) and of position sense and effort sense in man (Merton). In the paper on the techniques of system-analysis applied to feedback pathways in the control of eye movement (Fender), we are reminded of the need of the biologist to consider this whole subject of the application of control theory at some depth if he is to begin to understand the processes of physiological control: "... simple devices of linear feedback are not sufficient; we need the whole armamentarium of non-linear analysis, adaptive controls and sampled-data theory if we are to make any appreciable headway with the problem—it may be that new forms of mathematics must be developed especially to deal with the complexities of biological

problems". In the final paper (Machin) the whole complex subject of feedback theory is drawn together in about as near as one can hope to get to plain words.

A brief historical introduction to the theme of the symposium might have been included. Neither Virchow, who showed an early appreciation of the importance of the organizational levels of biological material, nor Claude Bernard, who gave birth to the concept of internal regulatory mechanisms of living material, is mentioned. But omissions were inevitable. When 'homeostasis' is in danger of being taken for granted, and 'feedback' has become a piece of popular jargon, this volume deserves high commendation as a brave effort to provide biologists with a faithful and readable guide to the level at which their thoughts must be projected when they seek to consider or express the processes of life in the language of the engineer, or to analyse them with the aid of his mathematical tools. It is also a reminder of the limitations of such expressions. The most complex machines yet devised are but simple things compared with the integrated functions of even a single cell. The engineer's concepts and formulae may be adequate for the construction of simple analogies, but may not serve to describe the processes of living organisms for many aeons.

This pleasantly laid-out and well-produced volume is not a handbook, and not every student of biology will read it with relish or absorb its contents with ease, but it is a thought-provoking introduction to a subject of increasing interest and importance and deserves wide attention.

J. BLIGH

## DEADLY PRAIRIE PLANTS

### Poisonous Plants of the United States and Canada

By Prof. John M. Kingsbury. (Prentice-Hall Biological Science Series.) Pp. xiii+626. (Englewood Cliffs, N.J., and London: Prentice-Hall, Inc., 1964.) 78s.

INFORMATION on specific poisonous plants is commonly required by workers in a number of disciplines. Frequently, however, such information is difficult to extract from the literature and even then may not be first-hand. A service in respect of both these deficiencies is provided in *Poisonous Plants of the United States and Canada*, a comprehensive but not exhaustive survey of the relevant literature marked by a successful attempt to "identify original sources of information for the reader". A well-chosen and extensive bibliography allows access to more detailed material.

As a prologue there is a compact and useful synopsis of the literature on poisonous plants up to 1900, accompanied by its own set of references. The next section deals with poisonous principles. This is necessary in order to treat certain groups such as alkaloids, various types of glycosides and some inorganic salts in a general manner so that the monographs are not repeatedly interrupted to describe properties common to all the members of each group. In our present state of knowledge, this must have been the most difficult chapter to write. Systematization is difficult as a chemical classification tends to overlap and, where the chemistry is still uncertain, terms in common use are often descriptive rather than scientific. In the circumstances the account is straightforward and comprehensible. There are some useful charts—of basic configurations of alkaloids and poisonous plants which contain them, of plants with cyanogenetic potential, of plants in which toxic concentrations of 'nitrates' have been found and of plants which may accumulate selenium. Other sub-sections deal with goitrogenic substances of botanical origin and plants causing photosensitization. There is a short but lucid account of the cardiac glycosides.

The main body of the book is arranged on a botanical classification and consists of a series of monographs interspersed with subsidiary information on related genera

and species. Each monograph is essentially divided into botanical data (description, distribution and habitat) for identification of the plant source and toxicological data (poisonous principles, toxicity, symptoms and lesions, and conditions of poisoning) for recognition of the effects produced. This design makes it most useful to the veterinarian and physician for the detection of plant poisoning. The author is aware that the bias is veterinary rather than human but, quite rightly, argues that this is "a true reflection of emphasis in the literature". The physician will nevertheless find much that is of interest, as also will the botanist, pathologist and pharmacologist. The monographs are well presented, provide accurate information and are suitably annotated. References to plant poisoning occurring outside North America are introduced, where relevant, with profit. The illustrations are clear and well placed in relation to the text; the few colour photographs make one wish that they were not so costly and could be used more extensively. The treatment of plant poisoning is not the concern of this book, though some specific antidotes are mentioned.

This is an excellent book. The author throughout strikes a good balance between essential facts and trivial, albeit interesting, information. His style is easy to read and his presentation shows a wide understanding of the subject. The book is well produced and typographical errors are minimal. Here are a few trifling criticisms. The formula of hypericin (page 173) is unnecessary as it has already been given on page 54. Coniine (page 381) and lobeline (page 392) are piperidine rather than pyridine derivatives. It is not clearly stated that atropine (pages 277, 280 and 282) is racemic hyoscyamine, does not occur naturally and is produced by racemisation during the processes of drug extraction. The titles of two formulae are incorrect, 5-vinyl-2-thioxazolidone (page 27) and mimosine (page 332) which should end "alpha-amino-propionic acid". None of this detracts from the enjoyment of reading this most valuable and interesting work. H. F. GRUNDY

## NUCLEAR PROPULSION AND POWER IN SPACE

### Nuclear Energy in Space

By Erik S. Pedersen. (Prentice-Hall Space Technology Series.) Pp. xi + 516. (Englewood Cliffs, N.J., and London: Prentice-Hall, 1964.) 160s.

"THIS book attempts to mould the various topics (of nuclear energy in space) together and, at the same time, cover each of them in great detail with theories, equations, pictures, tables and practical examples." So says Dr. E. S. Pedersen in his introduction to *Nuclear Energy in Space*. This, in itself, is a prodigious task, but also to include a wide range of peripheral subject-matter makes it all the more formidable.

The book opens with a brief account of the history of nuclear energy and a survey of nuclear aerospace programmes in the United States. The second chapter lays down some of the more basic satellite theory, deriving basic equations for propulsion nozzle design, the relation between mass ratio and burnout velocity for a rocket, the dynamics of injection into orbit and orbital theory. Finally, possible trajectories for Moon and Mars landings are considered, with some reference to rocket staging and the use of nuclear rockets. The foundation of the basic theory continues in Chapter 3, with a treatment of heat transfer and fluid flow, with particular reference to reactor cooling. Equations for heat transfer by conduction and forced convection are derived and empirical results presented. The theory is used to derive expressions for the transfer of heat into a fluid flowing in a pipe with axial symmetry and to show how the heat transfer characteristics play an important part in determining reactor

size and operating pressures. Chapter 4 discusses the principles of fission with particular reference to  $^{235}\text{U}$ , defining the basic parameters, such as cross-section, and outlining the way the fission processes may be controlled in reactors. Methods of reactor analysis are mentioned. The foundations of the basic knowledge required for nuclear rocket design are completed in Chapter 5 with a catalogue of a considerable number of structural, heat-resistant, moderator, shielding and other materials, giving their mechanical and a few chemical properties and including their resistance to radiation.

Chapter 6 is devoted to the design of a nuclear rocket engine; nozzle design is reconsidered, giving more attention to detail, and some theory is given for optimizing nozzle mass. Some of the problems of engine start-up and shut-down are described and the system dynamics are worked out for the design of a nuclear rocket giving expressions for the time constants of the reactor and turbo machinery: a block diagram for a possible control system is given. Finally, the chapter deals with a specific reactor design and some of the results are given of the calculations to determine power and temperature distributions for a 60-MW reactor.

Consideration is given in Chapter 7 to 'advanced propulsion methods'. These seem to be systems which are primarily in the research stage and include liquid fuel reactors for use with venturi nozzles, thermo-nuclear and photon propulsion, electric propulsion and a form of nuclear propulsion where it is proposed to explode small nuclear bombs ( $\sim 0.01$  kton) about 1,000 ft. behind a spacecraft: propellant emanates from the bomb and "propagates . . . to the vehicle where it hits a pusher plate that propels the vehicle".

Considerable attention is devoted, in Chapter 8, to direct conversion of energy into electricity. Thermo-electric and thermionic conversion are discussed with theory and sketches, and fuel cells, chemical batteries and solar cells are alluded to more briefly. Details are given of the SNAP series of power supplies, with both reactor and isotopic energy generation. The STAR systems are also mentioned.

Chapter 9 discusses the effects of the explosion of nuclear devices both in and above the atmosphere. It describes the effects of the release of vast quantities of energy, the blast, the thermal and nuclear radiations, and gives the energy yield of fission and fusion reactions. The discussion is accompanied by graphs and calculations.

The final chapter gives a brief account of the types of nuclear radiation encountered in space, their flux and energy; and describes the effect of radiation on man and on the components of satellites. Finally, shielding methods are described, including the possibility of using magnetic fields for charged particle deflection.

To write a comprehensive book on such a disjointed subject as nuclear engineering is not easy, to say the least. It is scarcely surprising, therefore, that one which also includes many additional topics seems to be a series of almost unrelated papers rather than a complete entity. While this does not detract from the value of the book as a wealth of information, it does make the information more difficult to extract.

With regard to notation, efforts have clearly been made to conform with accepted practice but sometimes this has led to confusion; for example, the symbol ' $n$ ' appears in the nomenclature for Chapter 8 as a number of fragments/sec, while in that same chapter it is also used for number of thermoelectric couples per module, and in the same calculation as total number of thermoelectric couples. In this particular case the latter two ' $n$ 's differ by a factor of 10, thus misleading an unwary reader. The use of several systems of units in the book causes little difficulty because of the admirable set of conversion factors the author has included. Even a viscosity expressed in one chapter in lb.<sub>m</sub>/ft. hr. can readily be converted into the more humble poise.

This book represents a considerable achievement. It has knitted together every conceivable application of nuclear energy in space and will be of enormous help, particularly to those who are just embarking in this field, but also to the student and established space engineer. Priced at 160s., it will probably not adorn many private shelves, but, considering that it contains enough material to fill several more modest books, the price would not seem unreasonable.

B. P. DAY

## CULT AND CULTURE

### The Temple and the House

By Lord Raglan. Pp. xi+218. (London: Routledge and Kegan Paul, 1964.) 30s. net.

THE recent death of Lord Raglan robbed ethnology of one of its most penetrating and independent minds. His work, however, has still, one feels, to be given the attention and esteem it merits. This comparative neglect he shares with A. M. Hocart, surely one of the most brilliant of British cultural anthropologists, whose friend he was, whose literary executor he became and with whose general standpoint in social studies he agreed. The central theory underlying their work is that ritual, a term under which they subsumed all the phenomena variously classified elsewhere as magical or religious, forms the seed ground of civilization; that, in tracing back to their origins features of contemporary cultures, even those which now and for some time have had purely secular and commonplace roles, we arrive at a matrix which is a fusion of cult and culture. This thesis is enormously powerful and fruitful, but obviously finds resistance in minds attuned by the ruling influences of our existing intellectual climate to thinking about human activities as pre-eminently representing an adaptation to needs, biologically or psychologically defined, and, consequently, finding plausible explanations of human cultural characteristics couched in functional, naturalistic or economic terms and dominated by the widely accepted concept of 'adjustment'. Hocart's work was concentrated mainly in the field of social organization. He produced in *Kingship and Kings and Councilors* a great weight of evidence in support of the idea that in a neolithic and pre-neolithic ritual matrix are found theocratic forms of society and government which, by diffusion from a few centres, have been greatly influential in shaping the social forms of peoples over large parts of the world. Raglan's work was supplementary to this and dovetails with it.

In *The Temple and the House*, his last work, Raglan argues that the house, far from being as with us a purely secular building, a close approximation in these days to "a machine to live in", has in a great many cultures a whole series of characteristics which attest to its sacredness; that, even in the modern West, there still remain associated with the house a number of practices which are explicable only as survivals of this same sanctity. The house is, or has been, the locus of the cult of domestic gods and deified ancestors. Indications that its site has been divinely chosen are sought before proceeding with a ritual demarcation which will establish it as a sacred island in a secular world. Sacrifice forms part of the foundation of house walls or pillars, and further sacrifices are required after completion of the structure and before occupation. The threshold, as leading to a divine abode and as itself the abode of a number of protecting deities, remains especially sacred: precautions have to be taken in crossing it, especially by a bride newly consecrated by a marriage ceremony that has made her a house mistress with a number of distinctly priestly attributes. The sanctity of the dwelling, henceforth pre-eminently in her charge, is even more clearly seen in a whole series of specifications for its preservation, for keeping it free from ritual pollution. It is impossible on utilitarian grounds to

account for these—for the taboos on cooking in the house, on childbirth, on raising children, on death in the house. They derive rather from a religious complex, the central notion of which was that the cosmos had originally emerged from a preceding undifferentiated potentiality—the 'Chaos' of Genesis—through the operation of a constructive dualistic principle that resulted in a world of paired entities and attributes, complementary but in tension, a world of sky and earth, land and water, right and left, good and evil, light and dark, life and death. This belief was taken so seriously that it gave rise to a prolonged series of endeavours to build the world of man's own making—his culture—on the same foundational principle, and this work was marked by a most extraordinary scope and consistency. The central rite associated with this doctrine was the marriage of sky and earth represented first by selected individuals who later developed into the divine king and queen. The generative, or regenerative, efficacy of this ritual union required its location in a building which symbolized the cosmos and which formed the prototype of palace, tomb and temple. These, in consequence, reflected in their shape and design the dominating cosmographic ideas; at first round or spherical, they later became square, their corners carefully orientated with the "Earth's imagin'd corners". The history of the house shows, apart from emulation, the control of the same notions, for it too was sacred because it was the dwelling of a man and wife consecrated by a sacramental union derived from the sacred marriage, a derivation made plain by the widespread treatment of bride and groom as queen and king and by the cosmic associations of their marriage bed.

The book presents its thesis and its supporting evidence with Raglan's customary economy of exposition and an engagingly succinct but sufficient rebuttal, *en route*, of various alternative explanations. It opens the gate on a very rich field. Questions of cultural origins are patently difficult, but a continuing enquiry into them, guided by the evidence, not by our preconceptions, is necessary. We would thus safeguard ourselves against the 'ennui' and trivialization that frequently attend social studies out off by a too functionalist approach from history and from that discovery of meaning which is a discovery of beginnings.

BRYNMOB THOMAS

## PROGRESS IN PETROLEUM CHEMISTRY AND REFINING

### Advances in Petroleum Chemistry and Refining

Vol. 9. Edited by John J. McKetta, jun. Pp. xv+439. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1964.) 150s.

IN 1958 a literary project was inaugurated with the ambitious objective of presenting in book form, at annual intervals, progress reports by leading authorities in the fast-growing disciplines of petroleum chemistry and refining. It was initially argued, with some justification, that: "The industry is so diversified that men in one phase have little knowledge of the progress made in other segments. The research scientist does not know what the engineer is accomplishing, nor does the engineer know what may come from the basic research of the scientist". To the extent that this dictum is sound, the appearance of the ninth consecutive volume of the rather expensive *Advances in Petroleum Chemistry and Refining* would seem ample justification. The only doubt in my mind is to what extent can the 'pure' petroleum chemist and the chemical engineer concerned with oil refining speak a common language, and thus understand the intricacies of the complex researches on which each is engaged? When one considers that oil refining, as originally understood, no longer consists of processing crude oil for fuels and



lubricants and end-products, but now embraces complex chemical processes in the industrial fields of fertilizers, petrochemicals, plastics, rubber, to name only a few branches, then this question would appear to have some substance in fact.

The problem is by no means resolved by appeal to the titles of the eight contributions contained in the present volume. These are: "Advances in Large-scale Oxygen Production", by R. L. Shaner and L. C. Matsch; "Hydrodealkylation", by G. F. Asselin; "Formulation and Structure of Lubricating Greases", by B. W. Hotten; "Thermal Cracking and Pure Saturated Hydrocarbons", by B. M. Fabuss, J. O. Smith and C. N. Satterfield; "The New Elastomers", by W. W. Crouch and R. S. Hammer; "Mechanisms of Carbonium Ion, Carbene and Carbanion Reactions", by G. M. Cramer and T. J. Wallace; "Synthetic Ammonia", by S. Strelzoff and L. C. Pan; "The Chemistry of Fuel Instability", by T. J. Wallace. A glance at the cumulative index of the specialized subjects dealt with in the nine volumes published so far spreads the net very wide indeed, but this presumably was the purpose of the series in the first place.

The series as yet does not add up to a text-book which the student can read to learn more of his chosen subject. Neither is it a cohesive dictionary of either petroleum chemistry or refining. It is, in fact, a reference series written by specialists for specialists and, as such, a valuable source of information on many subjects to those who wish to keep abreast of modern developments in the sciences and technologies wherein their main interests lie. Refresher courses in the sciences in which professional men and others have long since qualified are constantly in the minds of practising chemists and chemical engineers; those engaged in the petroleum industry, including its widest ramifications, are by no means behind members of other faculties in their recognition of the importance of revitalizing their work, but time and circumstances do not always permit such seminars. This series of volumes does, within obvious limitations, at least provide a useful means of leisure-study in the specialized subjects so far treated; to this extent it undoubtedly fills a gap in contemporary technical literature of scientific progress of petroleum technology which future volumes may well gradually narrow.

H. B. MILLER

## ADVANCES IN CHEMICAL ENGINEERING

### Advances in Chemical Engineering

Vol. 5. Edited by Thomas B. Drew, John W. Hoopes, jun., Theodore Vermeulen and Giles R. Cokelet. Pp. x+317. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 100s.

THE five topics in Volume 5 of the Academic Press series, *Advances in Chemical Engineering*, are all processes involving reaction kinetics or transport processes. Each represents a valuable contribution to the literature in the field concerned. Three of the authors are American, one is British and the fifth is Dutch.

The first article, on flame processes, by J. F. Wehner, is an excellent review of the fields of combustion and detonation. The author has approached the subject by acquainting the reader with the general theories of flame processes and shows how some useful approximations can be made to the equations for mass and heat balances within a flame. Experimental techniques for measuring temperature and concentration profiles are discussed and the importance of a prior knowledge of the magnitude of the reaction rates is emphasized. This is illustrated by investigating the probable chemical stages in the combustion of methane and showing the difficulties of estimating the concentration profiles of all the molecular species present. The hydrodynamic stab-

ility of stationary and propagation flames is approached with special reference to ignition and quenching. The transition from the laminar combustion wave to the shock wave is discussed in terms of the hydrodynamic conditions preceding the detonation. The burning of solid propellants is shown to be a particularly difficult problem because of the intermediate mixing problems involved. The article concludes with the possible applications of flame processes in chemical engineering. The flame is regarded as a form of chemical reactor from which normally short-lived molecular species might be isolated by quenching. An interesting possibility is the condensation of finely divided solid particles from flames.

The use of bifunctional catalysts which are discussed in the second article, by J. H. Sinfelt, represents an important application of heterogeneous catalysis within the chemical industry. These materials have been developed during the past ten years, particularly in petroleum refining, and there are now many opportunities for their more extended use. In the article, the general principles of functioning of such catalysts and the nature of the reactions occurring have been described. Hydrogenation and isomerization are taken as illustrative examples. Some of the outstanding problems to be solved before the catalysts can be widely used have not been presented, however. Thus, heat and mass transfer receive a rather perfunctory treatment even though they may be determining factors. Methods of preparation of bifunctional catalysts could well have been discussed in this article, particularly whether it is advantageous to include two components in the same catalyst pellet.

Heat conduction or diffusion with change of phase is the subject of the article by S. G. Bankoff. A number of diffusional processes, with both constant and variable diffusion coefficients, are discussed, and it is concluded that the subject is well developed in cases where the boundary conditions are simple. There are, however, many problems involving, for example, the melting of solids or the condensation of material on nuclei where the phase boundary is continually moving. Analytical solutions are given to a number of problems and numerical methods are also developed. On the whole the article would have been improved by a clearer statement of the physical problems presented and by a less piecemeal approach.

The article on flow of liquids in thin films by G. D. Fulford is a useful review of the hydrodynamics of such systems. There does not appear to have been a previous review of this important field, but the long list of references shows the amount of work carried out in recent years. The author is to be commended on providing a list, at the end of his review, giving a brief résumé of each of the major contributions in the field. The only criticism is that the treatment of the mechanical energy equations for unsteady systems is inadequate.

The volume concludes with an article by K. Rietema on segregation in liquid-liquid dispersions and its effect on chemical reactions. He reviews the concepts of flow patterns in reactors suggested initially by Danckwerts and then goes on to examine the effect of segregation on conversion rate for reactions of various orders. The effects of limitation due to mass transfer are considered with reactions proceeding both in the continuous and disperse phases. In many cases the behaviour of a process is difficult to interpret because of a lack of basic knowledge of the kinetics of the reaction in question. In stirred vessels, the effect of dead corners at baffles and the effect of increase in mean drop size with distance from the stirrer are examined. This article is particularly useful, as the field is one attracting an increasing number of investigations at present.

The volume is attractively produced, in the same style as its predecessors. It is, overall, a work of a high standard, but is expensive for its length.

J. F. RICHARDSON



### The International Zoo Yearbook

Vol. 5. Edited by Caroline Jarvis. Pp. ix+430+72 plates. (London: The Zoological Society of London, 1965.) 105s. net.

**THE International Zoo Yearbook** has already shown itself to be indispensable, and the publication is clearly going from strength to strength. The 1965 volume, edited as before by Caroline Jarvis, with the help of a strong advisory committee, exceeds that of 1964 by nearly 100 pages and includes more than 80 photographic plates and a large number of text illustrations. The reference section follows the accustomed lines and provides ready information about the technical developments and various lines of progress at the zoos and aquaria of the world during the past twelve months. This section alone makes it indispensable to directors of zoos and their staffs. In addition to this purely factual data, Section 2, comprising 150 pages, deals with new developments in the zoo world. The sub-headings of this section include "Breeding" (17 separate articles, contributed by 19 authors); "Stock" (25 articles by 30 authors); together with smaller groups of papers on "Aquarium Management"; "Veterinary Work"; "Zoological Research"; "Educational Activities"; and "Architecture and Construction". The veterinary papers range from accounts of new surgical operations and new hypnotic and anaesthetic agents for birds and fish, to tooth trimming in vicious lions and "false teeth for an old donkey".

In each issue of the *Yearbook*, it has also become customary to include one or two special features. Last year we had a symposium on aquatic mammals in captivity and a survey of animal milk analyses and hand-rearing techniques. This year the special emphasis is on "Ungulates in Captivity"—the whole of Section 1 (90 pages) being devoted to this subject. Conservation is again an important topic in the form of the report of the symposium on "Zoos and Conservation", held in 1964 at the London Zoo. A valuable section, arranged more or less after the manner of the zoological record, entitled "Veterinary Work and Zoological Research Undertaken at Zoos and Aquaria in 1963", is an important innovation in this volume.

Finally, there is a closely-printed 10-page section describing new buildings in zoos and aquaria during 1963-64. It is a sobering thought that this includes 30 zoos which are either entirely new or so extensively reconstructed or extended as to be virtually new. With this extraordinary development in the increase of interest in animals, and the desire to see them in captivity throughout the world, we must emphasize again the imperative need for world-wide and effective control of capture, transport, export and import, of the rarer wild animals. If this is not done, and done thoroughly, the demands of the world zoos could easily outstrip supply (indeed, they are already doing so), leading to many cases of extermination among those species which cannot as yet be readily bred in captivity.

W. H. THORPE

### The Action of Neuroleptic Drugs

A Psychiatric, Neurologic and Pharmacological Investigation. By Prof. Hans-J. Haase and Paul A. J. Janssen. Pp. viii+174. (Amsterdam: North Holland Publishing Company, 1965.) 40s.

**THE** drugs which are used in the treatment of psychiatric disorders produce a wide variety of effects. *The Action of Neuroleptic Drugs* is an attempt to define the psychic and somatic actions of the major tranquillizing or neuroleptic drugs. Part 1 of the monograph, by Prof. Hans-J. Haase, describes the neuroleptic effect and shows how this came to be regarded by him as a reduction of extrapyramidal conation. This interpretation is illustrated by the author's clinical observations. Several neuroleptic drugs are compared with chlorpromazine with

regard to dosage and equivalence of therapeutic effect and some subjective observations on the effect of neuroleptic drugs are given. Prof. Haase has found that fine motor extrapyramidal signs are associated with the neuroleptic effect and can be used as an indicator of this effect. He describes how handwriting tests can be used to observe the degree of fine motor hypokinesia which occurs and gives several illustrations. It is thought that most of the coarse motor extrapyramidal disturbances, which can occur when these drugs are used, are not related directly to the neuroleptic effect. Chapter 3 gives details of clinical schemes for the investigation of the neuroleptic action of drugs. This is followed by a review of the therapeutically undesirable effects of neuroleptic drugs, contributed by J. Wagensommer.

The final part of the book, by P. A. J. Janssen, is a short pharmacology of neuroleptic drugs. These can be divided into three main groups: the drugs which are structurally related to reserpine; the derivatives of phenothiazine and their isosteric compounds; and the drugs derived from butyrophenone. The neuroleptic, anti-emetic and adrenolytic activities of thirty-six drugs, as determined in tests on laboratory animals, are compared. The book contains much information about this group of drugs, but its use as a source of reference is limited by the absence of an index. It is, however, relatively cheap, and worth reading, and will no doubt stimulate further investigations into the action of neuroleptic drugs.

D. F. SHARMAN

Survey of the Limba People of Northern Sierra Leone By R. H. Finnegan. (Department of Technical Co-operation. Overseas Research Publication, No. 8.) Pp. 151. (London: H.M.S.O., 1965.) 25s. net.

**PRIOR** to the research of Miss Ruth Finnegan (now Mrs. Ruth Murray, of the University of Ibadan) the Limba people of the northern province of Sierra Leone had never been the object of anthropological investigation. This modest and useful book seeks primarily to provide basic information about political structure, social institutions, economy, religion and migratory labour. One of its virtues is the way the author has attended to what the Limba say about their own society and to its distinctive features, instead of trying to present the material in the categories found useful in one of the more celebrated investigations of some other African people.

A Limba chief is expected to be continually accessible to his people, from whom he is not separated by intermediary officials. Traditionally, his fundamental task has been to reconcile litigants and "cool their hearts". The setting up of the 'Native Authority' system of administration—useful as a check on unprogressive and autocratic chiefs—has, among the Limba, robbed chiefship of its traditional justification by establishing independent courts. At the same time, the chief, being a salaried official, no longer needs to cultivate the confidence of his people. Literate chiefs find the office 'boring' and now tend to look towards a career outside the chieftdom. Economic development is affecting many institutions—a man is no longer dependent on his kinsfolk to raise bride-price for his wife, and a woman who dislikes her husband may earn enough to repay this sum herself—but traditional ideas of shame, witchcraft, ownership, etc., remain important to any understanding of the contemporary position. The book concludes with a chapter showing that in spite of the new sense of economic individualism, many of the incentives to labour migration are still founded in the traditional Limba order. The farming year is still, for the Limba, an absorbing and God-given cycle; their work, disputes, ceremonies and entertainments are enjoyed with pride and skill. The peasant workers may sometimes be tired or hungry, but they, at least, are never bored.

MICHAEL BANTON

## TIME'S ARROW AND ENTROPY

By Sir KARL POPPER

University of London

SEVERAL years ago<sup>1</sup> I suggested that we should distinguish between two essentially different ways in which energy can be degraded or dissipated: "Dissipation in the form of increasing disorder (entropy increase) is one of them, and dissipation by expansion without increase of disorder is the other. For an increase of disorder, walls of some kind are essential: a sufficiently thin gas expanding in a 'vessel without walls' (that is, the universe) does not increase its disorder." Reasons for this view were given in the place cited.

In order to explain this a little more precisely, I shall here introduce, following Prigogine<sup>2</sup>, the term 'system' to denote the (energy and material) "contents of a well-defined geometrical volume of macroscopic dimensions" (so that, for example, an organism enclosed by its skin, or our solar system as enclosed by a sphere round the Sun with a radius of  $10^4$  light seconds, would be a 'system'); and I shall speak of the 'exterior' of a system  $X$  as a region of space (leaving it open whether or not this is in its turn a geometrically well-defined 'system') of which  $X$  forms a part.

Following Prigogine, I shall distinguish between (materially or at least energetically) 'open' and 'closed' systems. (An energetically closed system is called 'isolated'.) Moreover, I shall call a system  $X$  'essentially open' if it is part of a system  $Y$  such that all geometrically convex systems of which  $Y$  is a part are (at least energetically) open. (This definition makes it possible even for an isolated system to be essentially open.)

I further call  $X$  'essentially open towards a cooler exterior' if  $X$  is enclosed by some convex system  $Y$  such that: (a) all elements of any sequence  $Z_i$  of convex systems of which  $Y$  is a part are essentially open and of a lower average temperature than  $Y$ , and that (b) for every such system  $Z_i$  there is a system  $Z_j$  which encloses  $Z_i$  and which is not of a higher average temperature than  $Z_i$ .

The terminology here introduced makes it possible to clarify a number of points in connexion with the second law of thermodynamics which seem in urgent need of clarification.

Again following Prigogine<sup>2</sup>, we can split the change of entropy  $dS_X$  in any system  $X$  into two parts:  $dS_{X_e}$ , or the flow of entropy due to interaction with the exterior of  $X$ , and  $dS_{X_i}$ , the contribution to the change of entropy due to changes inside the system  $X$ . We have, of course:

$$dS_X = dS_{X_e} + dS_{X_i} \quad (1)$$

and we can express the second law by:

$$dS_{X_i} \geq 0 \quad (2)$$

For an energetically closed (or 'isolated') system  $X$ , for which by definition  $dS_{X_e} = 0$ , expression (2) formulates the classical statement that entropy never decreases. But if  $X$  is open towards a cooler exterior:

$$dS_{X_e} < 0 \quad (3)$$

holds, and the question whether its total entropy increases or decreases depends, of course, on both its entropy production  $dS_{X_i}$  and its entropy loss  $dS_{X_e}$ .

The fact that entropy can decrease in an open system  $X$  does not, of course, conflict with the second law as given by expression (2). But the second law is often formulated in a different way; for example, it is said that "if we . . . expand our system to include all the energy exchange, it would be found that in the larger system the entropy had increased. For example, to measure the entropy change

taking place in living organisms as a whole, it would be necessary to include in our system the Sun and some additional portion of the universe, as well as the Earth itself"<sup>4</sup>. Thus it is suggested that for sufficiently large systems  $X$  of our universe,  $dS_X \geq 0$ , so that the entropy always increases.

Yet, so far as our knowledge of the Universe goes, the precise opposite appears to be the case. With very few and short-lived exceptions, the entropy in almost all known regions (of sufficient size) of our universe either remains constant or decreases, although energy is dissipated (by escaping from the system in question). This is so, at any rate, if we assume that the law of conservation of energy is valid; and it is also so if we assume the 'steady state' theory of the expanding universe. (It is not so on the assumption of a finite and non-expanding universe with non-zero energy density.)

In order to see this, all that is needed is to be clear about the empirical fact that in our universe we know only essentially open systems, and only systems  $X$  which, so far as they produce entropy at all, are essentially open towards a cooler exterior. (This is true even of all so-called 'closed' or 'isolated' systems.) But for all such systems, one of the following cases must hold: (a) they are (practically) stationary, like the solar system and most stars known to us, in which case their entropy production (practically) equals their entropy loss, at least temporarily; or (b) they are losing temperature, and thereby entropy; or (c) they are producing more entropy than they lose, in which case they are in process of getting hotter, a process which, whether energy conservation is assumed or the steady state theory, can be only a comparatively rare and short-lived temporary process. (Even if the system in question should be one that collects matter from its environment until its gravitational field becomes so strong as to encapsulate and separate off the system from the rest of the universe, it would thereby presumably become stationary.) All we know about the universe points to (a) and (b) as being by far the most frequent and important cases: in almost all sufficiently large systems known to us, entropy production seems to be equalled, or even exceeded, by entropy loss through heat radiation.

This may be explained by the conjecture that every entropy-producing region is open towards some large (perhaps infinite) sinks of energy—regions the energy capacity or heat capacity of which, at least for heat in the form of radiation, is infinite (or approximately so for all practical purposes). The existence of such sinks seems to be strongly indicated by the darkness of the night sky. (We might represent this conjecture by the model of an infinite universe with zero energy density; or by that of an energy-conserving expanding—and therefore cooling and entropy-destroying—universe which tends towards zero energy density; or by that of an expanding steady-state universe with constant temperature, and entropy production equalled by entropy escape.)

So there do not seem to be theoretical or empirical reasons to attribute to expression (2) any cosmic significance or to connect 'time's arrow' with that expression; especially since the equality sign in expression (2) may hold for almost all cosmical regions (and especially for regions empty of matter). Moreover, we have good reason to interpret expression (2) as a statistical law; while the 'arrow' of time, or the 'flow' of time, does not seem to be of a stochastic character: nothing suggests that it is subject to statistical fluctuation, or connected with a law of large numbers.

As for the evolution of life, this seems to be connected, if at all, with a cooling rather than a heating process on Earth (or perhaps with periodic temperature fluctuations); that is, with increasing order and decreasing entropy. Yet it does not seem that 'feeding on neg-entropy' has much to do with the preservation of life, as has been suggested, for example, by Schroedinger<sup>4</sup>. For during the incubation of birds' eggs entropy rather than neg-entropy is supplied to them, though they are in a period of increasing organization; and while in an organism dying of heat

or of fever entropy may increase, if it dies of cold—say, by deep-freezing—its entropy certainly decreases.

<sup>1</sup> Popper, K. R., *Nature*, 176, 331 (1956); 177, 538 (1956); 179, 1296 (1957); 181, 402 (1958); *Brit. J. Phil. Sci.*, 8, 151 (1957).

<sup>2</sup> Prigogine, I., *Introduction to Thermodynamics of Irreversible Processes*, 3 (1955).

<sup>3</sup> Prigogine, I., *Introduction to Thermodynamics of Irreversible Processes*, 16 (1955).

<sup>4</sup> Blum, Harold F., *Time's Arrow and Evolution*, 15 (1933). (Similar statements are to be found, for example, on pages 16, 24, 23, 201.) Compare also Planck, M., *A Survey of Physics*, 17, 27 (1935).

<sup>5</sup> Schroedinger, E., *What is Life?* 72 (1944).

## THE OFFICE FOR SCIENTIFIC AND TECHNICAL INFORMATION

By DR. H. T. HOOKWAY

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BRITISH scientists and technologists are becoming increasingly aware of the rapid growth in the amount of scientific and technical information which is becoming available for dissemination and retrieval. The impact of this so-called 'information explosion' varies from subject to subject, and there are corresponding differences in the attitudes of individual scientists to it. But common to all is the realization that the problem of handling information will become steadily worse as the years go by and that it must be tackled effectively before it gets out of hand.

A new Office for Scientific and Technical Information (OSTI) has been set up within the Department of Education and Science to promote more activity on information problems and to co-ordinate the work of the various interested bodies. It is being built round a nucleus of staff transferred from the Department of Scientific and Industrial Research (DSIR), which was giving increasing attention to information problems during its last years. The functions of OSTI have been made very wide so that within the resources at its command it may stimulate or, if necessary, undertake almost any activity that can contribute to a better handling or utilization of information in both the natural and social sciences and their related technologies.

### Research

It has become clear in recent years that the problem of handling information by machine methods is much more difficult than was once thought. The application of computers to information problems has raised tantalizing possibilities—of mechanical translation, of storing whole texts on magnetic tape and of retrieving information selectively in packages of convenient size—but experience has shown that these goals cannot be reached by computer technology alone. It is necessary to conduct more fundamental research into the nature of the language in which scientific information is expressed, in order that satisfactory means can be found of manipulating data and ideas by machine with the minimum of human intervention. Thus, for example, machine translation cannot progress far beyond its present crude achievements until much more is understood about the grammar and semantics of language. Mechanical analysis of stored abstracts or texts for retrieval purposes can only be of limited value until more is understood about the linguistic and psychological relationships between indexing terms.

This is complex, long-term, multi-disciplinary research of a kind which does not easily attract research workers in its early days. Until recently the bulk of this work has been carried out in the United States, but teams now exist in the U.S.S.R. and several West European countries. In Great Britain the National Physical Laboratory has three such research projects within the programme of its Autonomics Division, and OSTI has taken over from

the DSIR support of five projects in the field of structural linguistics, each involving collaboration between linguists, computer specialists and mathematicians, and five more projects which in one way or another are aimed directly at better retrieval of information. One project in this second group, in the University of Sheffield, forms part of a planned British contribution to the vast programme of research and development at Chemical Abstracts Service.

### Development of New Techniques and Systems

It is impossible, of course, to foresee if and when research of this kind will lead to a radical solution of the information problem and, if so, how widely applicable the solution will be. Therefore, since much of research is long-term in character, a proportion of total resources must be devoted to the development and application of ideas that already exist, because some of them show promise of being better than, and as acceptable as, existing methods which they would supplement or replace. The bulk of these ideas fall into the category of reference retrieval systems; that is to say, they store titles and bibliographical citations on magnetic tape which can then be used for: (1) general publication; (2) selective dissemination to individuals or groups; (3) retrieval; or (4) a combination of these applications. The other approaches concern not bibliographical information but selected quantitative data, which can be more easily handled by machine than information expressed in words.

In the United States much thought is being given to the possibility of establishing a mechanized information service for each scientific discipline and for each major area of application—space, atomic energy, medicine, agriculture and engineering. In general the American institutions concerned recognize their problem as an international problem and are prepared—in many instances keen—to co-operate with institutions outside the United States that have something positive to offer. From the outset, then, OSTI has accepted the need for co-operation with the United States, and indeed with other countries, and to help international organizations to promote co-operation, where possible, on an even wider basis.

**Medicine.** The character and state of development work vary from one subject to another, and the nature of British participation must vary accordingly. At one extreme, in medicine, a mechanized search service known as 'MEDLARS' (Medical Literature Analysis and Retrieval Service) has existed in the United States since July 1964. Run by the National Library of Medicine, it enables the announcement journal *Index Medicus* to be printed by computer-controlled type-setting and provides a facility for machine searching of the indexed material that has already been put into the system. The National Library of Medicine is prepared to supply copies of the MEDLARS tapes to organizations in other countries which will provide national searching services for medical literature on the

lines of those already provided in the United States, and OSTI has launched a project for the establishment of a MEDLARS 'station' in Britain. It has placed a three-year contract with the Computing Laboratory of the University of Newcastle upon Tyne for the preparation of the necessary search programmes and the experimental operation of the service; and the National Lending Library for Science and Technology (for which OSTI is now responsible) has acquired the necessary expertise in search strategy and will back up the search service with the supply of literature.

I must emphasize that any new service such as this will be experimental at first. Inherent in the project is a programme of tests designed to indicate the potential value of the service to the medical profession and the pharmaceutical and related industries and to evaluate the indexing procedures used. The result of these tests will determine whether the service is worth establishing on a permanent basis and should be of use in the design of similar systems for other subject areas.

**Engineering.** In engineering the situation is rather different. The Engineers Joint Council, a body which brings together the main engineering institutions in the United States and has close ties with the abstracts publication *Engineering Index*, has produced an elaborate thesaurus of major engineering terms, which it hopes will become the basis of a mechanized bibliographical service. It has also been promoting experiments in the selective publication of abstracts taken from *Engineering Index*, the first two subject areas being plastics engineering and electrical/electronics engineering. In Great Britain the Institution of Electrical Engineers has been experimenting with new journals of a different kind—a list of current titles, which has already established itself, and a letters journal; while the new National Electronics Research Council, with support from OSTI, has embarked on a preliminary design study for a project concerned with the evaluation of a mechanized system for selective dissemination of information to electronics research workers. This Council is already discussing co-operation with the Engineers Joint Council (EJC) on the development of the EJC thesaurus as part of the design study, and this, it is hoped, will be only the first stage in a period of fruitful co-operation.

**Chemistry.** Although there are chemical thesauri which could be used as the basis of a chemical information service, interest in this field centres on the massive research and development programme of Chemical Abstracts Service (CAS), which is now supported by several American Government agencies. The aim of this programme is to develop a mechanized information service based on the structures of known compounds, and it is hoped that eventually the system will be able to handle information on physical and chemical properties and applications associated with particular structures. As I have said earlier, a contribution is already being made from Britain on the research side, and the Chemical Society, in consultation with OSTI, is working out plans for a British contribution to the CAS programme involving both long-term research and the testing of new services as they are developed, in order to relate these services as closely as possible to the needs of chemists.

**Physics.** This subject is of particular interest in Britain because responsibility for the major English-language abstracting system lies here. Recent discussions have brought the two British organisations concerned—the Institution of Electrical Engineers and the Institute of Physics and the Physical Society—into close relationship with the American Institute of Physics, and together they are working out a programme of research and development affecting both *Physics Abstracts* and *Electrical Engineering Abstracts*. It will be one of OSTI's functions to ensure that this work is fully co-ordinated with related work elsewhere, including work that OSTI is already supporting at the National Electronics Research Council.

### Critical Data

There is a quickly growing recognition that the task of data compilation, for too long left to enthusiastic individuals or groups, now needs proper stimulation and co-ordination. The U.S. National Bureau of Standards has launched a large National Standard Reference Data Program designed to identify and fill gaps and to ensure a uniformly good quality of output. The International Council of Scientific Unions has recently considered how best this problem, like many others in the information field, can be tackled on an international basis, and it is encouraging the individual unions to consider what is being done in their own subjects and what further work might be stimulated.

It is clear that Britain must mobilize her own contribution to the total effort and must consider, with other interested countries, how the growing quantity of data should be organized for ease of use. An *ad hoc* committee, with representatives of the Royal Society of London and DSIR (before its dissolution) as well as independent scientists, has compiled a short list of subject areas in which a clear British interest already exists and the total British effort could be stimulated; and OSTI is now supporting, or about to support, three 'urgent' items on this list. It is also considering what experiments should be made in Britain with specialized data centres, combining a measurement service for scientists, and perhaps research on methods of measurement, with the collection, evaluation, extrapolation, dissemination and retrieval of data. A feature of such centres would be the development and use of mechanized handling methods.

### Other Activities

The promotion of research, development and data compilation will be the frontier-pushing activities of OSTI. There are, however, two other important tasks which will demand increasing attention—help to existing information organizations to improve their services and stimulation of educational activities.

**Existing services.** It is not possible to say much about the future development of existing services at this early stage in the history of OSTI. However, the past decade has been one of considerable growth and experiment, with the establishment of the National Lending Library for Science and Technology, the preparatory work on a National Reference Library for Science and Invention, the expansion of Aalib's activities with the help of a large grant from DSIR (now from OSTI), the extension of specialized services in research associations and elsewhere, and the strengthening of personal liaison services both in the research associations and through the network of industrial liaison officers based on technical colleges—a network financed by the Ministry of Technology and the education departments.

At the same time the place of each type of organization in the total information system is the subject of much interest and investigation. For example, the University Grants Committee is examining the arrangements for meeting the needs of university libraries, while the Department of Education and Science is setting up two Library Advisory Councils (for England and Wales) under the Public Libraries and Museums Act, 1964. The Library Association has been examining the problems of library services in general and has recently published proposals for a national bibliographical service. The Ministry of Technology is giving urgent attention to developments in industrial information services that may secure more rapid application of science in industry. Aalib has been re-examining its research programme with a view to accumulating useful knowledge on modern information systems and techniques and offering an efficient technical service to members on information problems. It will be OSTI's task to bring together these various growing points and see how far the ideas that are

emerging can best be fused into a national policy for the development of scientific library and information services. It will also promote new services wherever they are proved to be necessary. But it will not seek to run such services itself unless no other suitable organisation exists inside or outside the Government.

**Education.** So far as educational work is concerned, OSTI will try to accomplish two fundamental tasks—systematic training for information work and systematic education of scientists and engineers in the use of literature and information services.

The increasing volume of scientific and technical information is leading to the establishment of more information departments and special libraries. Consequently more staff are required to operate them, and it is important that employers should be able to recruit a sufficient number with appropriate training. However, until recently there were no academic courses for information scientists. Information officers could receive no formal training except in library schools, which cover only part of the field, however, and so had to do much of their learning 'on the job' with the valuable help of short specialized courses run by the Association of Special Libraries and Information Bureaux and other bodies.

The first attempt to provide systematic training was a part-time course in information science which was started at the Northampton College of Advanced Technology, in 1962, mainly for scientists already engaged on information work. This proved popular, and was supplemented in the following year by a full-time postgraduate course. In October 1964 a full-time course for graduates in science and technology was started in the Postgraduate School of Librarianship in the University of Sheffield. Both these full-time courses have been accepted by the Science Research Council for the tenure of its studentships. OSTI is about to launch a national survey of the employment of information officers, hoping to discover, among other things, the distribution of officers in different kinds of work and the likely demand for trained officers in years to come. The results should help to determine the scale and character of training provision that are required. In addition OSTI is anxious to encourage research training in order to relieve the present acute shortages of both teachers for the training programme and research workers for the kind of research projects which I have described earlier.

Systematic education in the use of scientific literature and information services is so important to the success

of research, development and application that it seems incredible that so few scientists and engineers have received formal training in the use of information and that so many remain unable to make effective use of it throughout their working life. However, the National Lending Library, which as I said earlier is administered by OSTI, has in recent years been experimenting with short courses for postgraduate research students, university and academic staffs and library school tutors with the object of arousing interest in providing this sort of instruction as part of the normal university and college training. The response to these courses has been very encouraging and some universities and colleges are now providing such instruction themselves. But much more needs to be done, and OSTI, through the National Lending Library, intends to give it all possible encouragement.

### Conclusion

This description of OSTI's field of interest should be sufficient to show that the new organization has a difficult and complex task to perform. In addition to promoting a wide range of activities, it must endeavour to co-ordinate the efforts of all kinds of organisations—Government and independent—and to link these efforts with what is happening in other countries and in international organizations. Indeed, co-ordination should be regarded as perhaps the most important part of OSTI's total function.

The creation of OSTI has been widely recognized as timely, and it starts life with a large number of well-wishers. How successful it will be must depend to a large extent on its resources—human and financial. For the present financial year it has been allocated the sum of £180,000 for grants and contracts—considerably more than was available to DSIR for this work in former years. But I must emphasize the fact that it would be very easy to allocate the entire sum to worth-while projects which are already under discussion. We shall have, therefore, to exercise great care in the selection of priorities. Staffing is likely to present more serious problems, because relatively few scientists and technologists have both interest in the subject and the administrative capacity which is necessary for promotional and co-ordinating work of this kind. The period of recruitment, which starts this summer, will thus be crucial to the long-term success of OSTI.

## CIBA IN BRITAIN

### NEW FUNDAMENTAL RESEARCH UNIT AT HORSHAM

CIBA's new Horsham fundamental research unit was opened on May 12 by His Excellency M. Béat de Fischer, the Swiss Ambassador, and an inaugural address was given by Lord Snow, Parliamentary Secretary of the Ministry of Technology. The new £250,000 unit consolidates the Company's pharmaceutical research plans which first took shape in Great Britain in 1939. During the Second World War, CIBA Laboratories, Ltd., supplied drugs to the armed Services, hospitals and medical practitioners, despite shortage of staff and materials. The subsequent growth of the Company's business has been such that to-day sales are twenty times as great as they were in 1946.

In 1962, CIBA decided to establish the fundamental research division at Horsham, adjoining the existing laboratories, for long-term research study of drug action with emphasis on biochemical pharmacology—how drugs achieve their effect in the body. This was a concrete expression of the Company's appreciation that in recent

years the whole field of biochemistry has opened up at an accelerating pace. As the total knowledge derived from research all over the world has increased, even greater precision has to be applied in the testing of medications. With techniques for gauging the action and reaction of chemicals in the body becoming more and more accurate, the pharmacologist to-day aims towards more precise, and therefore safer, drug effects.

Work on drugs in human medicine will be paralleled in the veterinary field, in which CIBA has already contributed several therapeutic advances.

CIBA has an experimental farm at Monks Gate (four miles south of Horsham), and, as its work develops, collaboration with the new research unit on biochemical problems is envisaged.

Present techniques help research scientists to find the answers to such questions as how drugs are distributed through the body, drug metabolism (changes which may occur in a drug through the body's enzyme action) and



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(A930) July 1965, 748 pp., 192s. ; Subscription price 156s.\*

\* Subscription price valid on orders for the entire work received by the Publisher before publication of last volume.

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This volume is intended to provide an integrated account of the state-of-art in telemetry as it affects medical and biological applications in both research and routine practice. The book will prove useful to engineers, biologists, and physicians who are concerned with planning systems for research or for investigative studies of physiologic signals, and who face problems of monitoring patients and subjects.

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so on. Knowledge of the action of a drug on cellular metabolic pathways may also help research scientists to envisage possible undesirable long-term side-effects.

These, and many other investigations, are the subjects of research in the new unit, which is equipped with some of the most modern and comprehensive facilities in Great Britain. The main working laboratories are housed on the first floor of the new building and comprise three biochemical, one isotope, two pharmacological and two analytical laboratories. The latter constitute the analytical department, which assesses the purity of all products which are produced at Horsham and also provides consultative and collaborative facilities for the research workers. Here, too, are the chromatography room, the optical instrument room and a balance room.

The ground floor houses such general-purpose sections as special accommodation for the animals used in research, a mechanic's workshop, a centrifuge room, glass-washing and cold rooms, large-scale laboratory and a flame and flood-proof room for hazardous experiments. There are photographic facilities and a library which contains more than ninety scientific journals and several hundred reference works.

The equipment reflects the complexity and cost of biological research to-day. There are radiometer autotitrators and gas chromatography equipment for analysis, an Associated Electrical Industries EM6B electron microscope specially designed for biological work such as examining changes in cellular structure under the influence of drugs with a magnification of 250,000, a scintillation counter for isotope measurement, a preparative ultracentrifuge with a rotor speed of 50,000 r.p.m., a polygraph recorder for pharmaceutical work, self-recording spectrophotometers and a spectrophotofluorimeter.

Work of the kind carried out at Horsham is slow and painstaking. Although modern research is a team endeavour, progress is still made by the individual research scientist, who must be backed up by skilled assistants and resources. The Horsham research team of ten graduate scientists—who work under the overall direction of Dr. D. F. Elliott, head of the research unit—have such support in the persons of thirty technicians and ancillary staff, for the most part locally recruited.

Close liaison is also maintained with the Clinical Research Division and the CIBA research laboratories in Switzerland and the United States, from where selected compounds are sent to be investigated at Horsham. Constant exchanges of ideas, too, are made with academic and clinical institutions and similar bodies in Great Britain.

CIBA (Chemical Industry in Basle) was formed in Basle, Switzerland, in 1884. To-day, it comprises 58 associated companies throughout the world, employing more than 28,000 people. In Great Britain itself there are seven associated companies, of which CIBA Laboratories at Horsham manufacture and sell ethical pharmaceuticals and agrochemical products; CIBA Chemicals, Ltd., at Grimsby, which manufactures bulk pharmaceuticals and chemicals; the Clayton Analine Co., Ltd., in Manchester, which manufactures dyestuffs; CIBA Clayton, Ltd., also in Manchester, which sells dyestuffs, pigments, and textile application products (finishing agents used in textiles, plastics and many other industries); and CIBA (A.R.L.), Ltd., and Bonded Structures, Ltd., both at Duxford, which manufacture and sell synthetic resins and adhesives and deal with their application in construction materials. The holding company in London is CIBA United Kingdom, Ltd.

Through CIBA's international research programmes, the Company is able to make available to the National Health Service drugs which have been significant advances in many fields of medicine. After earlier contributions in the fields of anaesthetics, stimulants and sedatives have come major new developments in treating cardiovascular disease, especially hypertension. The broad fields of dermatology and allergy have benefited from several CIBA drugs, as have much more specialized problems such as iron-poisoning and the diagnosis of rare conditions.

CIBA also believes that pharmaceutical research should be international. Research is at present being carried out not only at the Company's headquarters in Basle, but also at CIBA Corporation, Summit's establishment in New Jersey, United States, and also at the CIBA of India, Ltd., Goregaon Research Centre.

#### Agrochemical and Veterinary Developments

**Veterinary.** Although the veterinary surgeon has been using CIBA medical products for many years, it was not until 1959 that the first CIBA preparation was fully tested in veterinary medicine. Since then, a large staff of veterinary surgeons has been built up at the three chief centres of CIBA's veterinary activity, that is, Switzerland, the United States and Great Britain.

The large-scale research with many types of drugs enabled CIBA to market several new compounds for the veterinary surgeon. The Company's work over many years in the field of corticosteroids allowed it to introduce 'Vecortenol' (prednisolone trimethylacetate), the

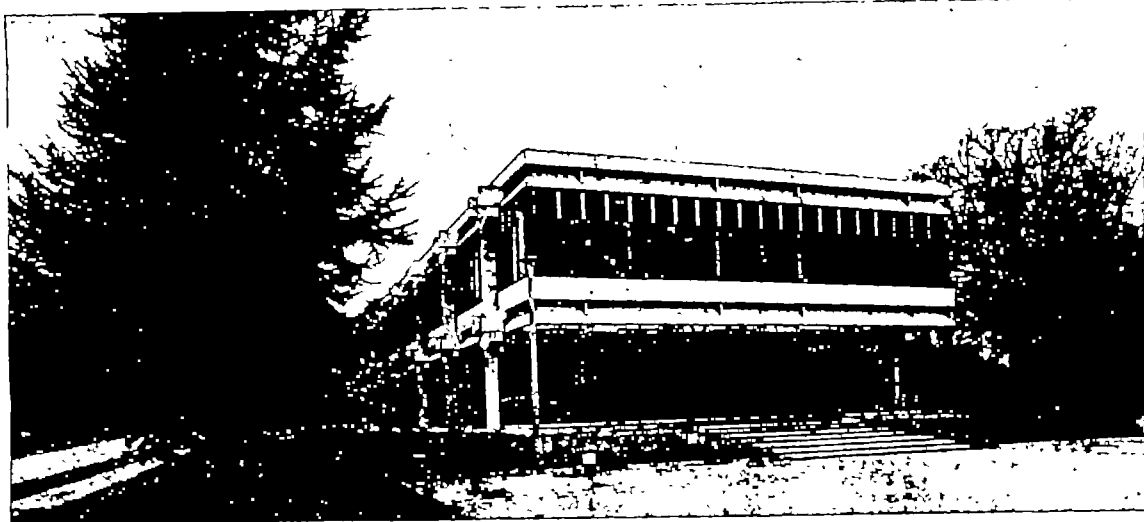


Fig. 1. New CIBA fundamental research unit at Horsham



Fig. 2. Base works of CIBA, headquarters of the organization.

longest-acting corticosteroid available in veterinary medicine. Other corticosteroids such as hydrocortisone were marketed in dermatological preparations.

CIBA was one of the first companies to manufacture sulphonamide preparations, and many new sulphonamides have come from the CIBA research laboratories. Three such compounds not previously seen in Great Britain have all been marketed exclusively for veterinary medicine.

The Company's experience of diuretics has enabled Horsham to introduce the first non-toxic injectable diuretic for veterinary medicine, and it has given the veterinary surgeon the first antihistamine with a stimulant action. CIBA is rapidly building up an interesting range of veterinary specialties.

**Agricultural.** In the course of research conducted by CIBA, the problem of chlorine utilization provided the impetus for research in pesticides. The substantial amount of chlorine produced as a by-product at the organization's Monthey plant was turned to use in the manufacture of copper oxychloride, then replacing the traditional Bordeaux mixture as a protectant fungicide.

The development of pesticides and their formulations, and of detergents, also had an important prerequisite in the scientific experience gained in textile application products and plastics. Similarly, experience gained in the pharmaceutical field gave a knowledge of bactericidal agents.

It was with this background that CIBA started its agricultural activities. When, in 1963, Horsham entered the field, it was with a dairy detergent/sterilizer that sales activities were first started. This was quickly followed by the introduction of a new selective herbicide, mainly used to kill weeds on strawberry beds. Since then other products have been developed for such diverse uses as the control of insects in glasshouses, and for the prevention and control of scour in calves and pigs.

Of prime importance in the development of both agricultural and veterinary products is the work of the CIBA Farm near Horsham, which was in fact purchased and in operation on research projects by 1960. This farm works in close co-operation with other CIBA research stations all over the world in developing and investigating both new and existing chemicals for use in connexion with agriculture and veterinary medicine.

### AIMS AND OBJECTIVES OF CIBA

During his speech at the opening of the New CIBA fundamental research unit at Horsham, Dr. Robert Kappeli, chairman of the board of directors, stressed that it was an international company. That does not merely mean that it manufactures and sells its products in many different parts of the world. CIBA as a living organization has taken root in the soil of many countries, and flourishing offshoots have grown up round the parent tree. Each of these affiliates is native to the country where it operates, and shares its fortunes. The British Company is an example. After small beginnings in a London office, it moved—still small—to Horsham shortly before the outbreak of war in 1939. During the war years some of its members served in the Armed Forces, and the others maintained the output of essential drugs under very difficult conditions.

When the post-war expansion came, CIBA, Horsham, also grew—in the volume of business, the size of its establishment, and in the number of its personnel.

From the very beginning down to the present day the Company has always had British personnel—from the senior management down to the youngest recruit. At the same time CIBA, Horsham, is part of a world-wide organization. This means that it shares in the wealth of scientific experience and industrial knowledge accumulated over a period of many decades, and in many parts of the globe.

However, the main centre of CIBA research is in Basel, Switzerland (Fig. 2), where a new pharmacological building equipped with the most modern facilities is being erected at a cost of about six and a half million pounds. This figure may serve to indicate the importance which is attached to research—and also the burden which it imposes on the industry.

The Horsham research unit is, of course, much smaller in size than that in Basel. However, with its strong accent on biochemistry, it will make a very important contribution to CIBA's research effort. One looks forward to a lively exchange of ideas and experience between the Horsham Research Division and its counterparts in Switzerland, the United States and India (in the last-mentioned a modern research centre at Goregaon, near Bombay, has recently been established).

A great deal more might be said about the importance of international co-operation in industrial pharmaceutical

research as it is practised in an organization like CIBA, and also about the fruitful interchange which goes on daily between academic and industrial research laboratories.

It is an established fact that many, if not the majority, of the most effective therapeutic agents available to-day for the treatment of bacterial infections, hypertension, diabetes and many other formerly intractable diseases could not have been developed and made available at reasonable cost and in abundant quantity without the pharmaceutical industry. Yet, Dr. Käppeli claims, this industry is at the present time and in many countries the object of the most violent attacks and accusations of profiteering and reckless exploitation. The cry for increased supervision, Government control, and even for nationalization is constantly being raised. In view of the indisputably substantial contributions of the pharmaceutical industry to the health and well-being of the individual and the community at large, this attitude seems highly illogical. How is it to be explained?

The *Journal of the Royal Society of Arts* of October 1963 and *Nature* (200, 441; 1963) printed the text of the Truman Wood Lecture, delivered by Prof. Ernst Chain, entitled "Academic and Industrial Contributions to Drug Research". In his lecture Prof. Chain, a Nobel Laureate and a biochemist of world-wide reputation, pays eloquent tribute to the co-operation between academic and industrial research, and gives a large number of examples, drawn from many fields of clinical medicine, which demonstrate the important part played by the research-minded industry in the development of new drugs. It would be impossible to overstate the importance of this Lecture as an impartial appraisal of a situation which is in danger of being completely clouded by sentiment and ignorance of the facts. Nobody unfamiliar with the development of drug research during the past half-century should attempt to pass an opinion on the merits or shortcomings of the pharmaceutical industry without having read Prof. Chain's lecture very carefully.

It is not necessary here to list the many examples given by Prof. Chain to demonstrate the value of co-operation between academic and industrial research. Yet attention

should be directed to some of Prof. Chain's remarks on the place which the pharmaceutical industry holds in the world to-day and on the effect which any tampering with its basic structure would be likely to have. He says: "The layman must understand that the pharmaceutical industry is life-saving and as such fulfils a public function of very great importance. Let it be clearly understood that I refer here, of course, only to those industrial organizations which are actively concerned in drug research and production. This type of industry is essentially productive, and not parasitic in nature, and is one of the most positive assets to our form of society".

Prof. Chain thus makes a clear distinction between firms which engage in research and those which merely thrive on the labours of others. This is a distinction which is highly important. All the leading companies of the industry maintain large research laboratories at great cost in money and manpower, because it is certain that without them progress would be impossible. Then come the copyists, who are domiciled in countries where the law allows them to operate with impunity. They seize on substances developed by others at great cost and often after years of laborious research, manufacture them at little expense beyond the bare production costs, and offer them for sale at cut-throat prices, sometimes even with the active support of Government authorities. Immediately, the Company which developed the original product is branded as a profiteer and an exploiter of the public, and soon the cry for control or nationalization of the drug industry is raised.

It is a commonplace which scarcely bears repeating that virtually all the progress in the development of new drugs during recent years has been in those countries where the principle of free enterprise still operates. The reason for this is not far to seek: the development of a new drug, to say nothing of breaking ground in an entirely new direction of research, is a highly speculative venture requiring very large sums of money and a willingness to take the calculated risk that all the effort and expenditure may yield no tangible result. Except possibly in war-time, these pre-requisites to success are not usually found in State-owned organizations.

## ROYAL BOTANIC GARDENS, KEW

### NEW JODRELL LABORATORY

By DR. C. R. METCALFE

Keeper

ON May 7, Lord Florey, president of the Royal Society of London, opened the new Jodrell Laboratory at Kew. The ceremony was attended by the Minister of Agriculture and other distinguished guests.

This bald statement of fact covers an event of very great significance in the history of the Royal Botanic Gardens, Kew, as a centre for botanical research. The new building (Fig. 1) stands on the same site as the modest laboratory, some 40 ft. long by 20 ft. wide, which was presented to Kew by Mr. T. J. Phillips Jodrell and opened in 1876. It is well known to botanists that, despite its small size and unassuming appearance, the old laboratory was the scene of many classical investigations such as Dukinfield Henry Scott's work on coal measure plants, and the work by Horace T. Brown and F. Escombe on the diffusion of gases through minute, closely crowded holes in plates of celluloid so fashioned as to resemble stomata in the surfaces of leaves. Then, again, there was the work on plant diseases by George Massee, which led ultimately to the opening of a small plant pathological laboratory on Kew Green, where the work in due course expanded into the present-day activities of the Commonwealth Myco-

logical Institute and the Ministry of Agriculture's Laboratory of Plant Pathology at Harpenden. We can thus see that these two important departments are, historically speaking, 'descendants' from the old Jodrell Laboratory. In the old building we can also picture Arthur W. Hill (who later became director of Kew) and Walter Gardiner studying the continuity of protoplasm, F. O. Bower working at Pteridophytes, W. C. Worsdell studying plant anatomy and teratology, all of which investigations were, in their day, of first-class importance. Later on we think of L. A. Boodle and F. E. Fritsch translating Solereder's *Systematic Anatomy of the Dicotyledons* into English, and of Boodle with his well-known care and caution establishing the botanical identity of a great number of archaeological specimens from Ancient Egypt, notably those from the now world-famous tomb of Tutankhamen.

These events, important as they are in the history of British botany, belong to the past and we may well ask ourselves why Kew now requires a new laboratory, what sort of accommodation and equipment it contains and for what purposes it will be used. In considering these

matters it must first be remembered that although many kinds of work are undertaken at Kew the foremost importance of the institution is as a centre for the study of plant classification. Furthermore, there are more species of living plants in cultivation at Kew than elsewhere and these have been assembled from many different parts of the world. Bearing these facts in mind, we must go on to recall that plant classification, while still based primarily on the external characters of plants, which are the special concern of herbarium workers, has now reached a stage where very little further progress in taxonomy can be achieved without the aid of ancillary investigations relating to the histology and cytology of the plants concerned. Indeed, we are now also approaching an era in which a knowledge of the chemistry of plants (chemotaxonomy) seems destined to assume a more important taxonomic role.

For some years the anatomists at Kew have been engaged in producing a series of reference books on systematic anatomy, volumes which are now widely used throughout the botanical world. It must clearly be a first duty to continue this work. But this fundamental work on systematic anatomy must be supplemented by further, more detailed, histological work and by parallel studies concerning the cytology of plants in relation to their classification. Karyological investigations not only produce additional taxonomic characters, but also deepen our knowledge of breeding mechanisms and so help us to understand the interrelationships of closely related species and to lay bare the probable course of their immediate past evolution. Besides all this, the successful cultivation of so many living species from so many diverse localities within the 300 acres of Kew poses many physiological problems that are scarcely likely to come to the notice of physiologists at other centres in Britain. Seeds are sometimes hard to germinate, cuttings are difficult to strike, other species can be kept alive successfully but will not flower, and there are the still more difficult problems presented by species which cannot at present be maintained in cultivation at all. With the passage of time the old Jodrell Laboratory became inadequate to enable us to deal with all these subjects, and the accommodation and equipment were insufficient.

With this background in mind, it was a great day for Kew when the members of the visiting group under the chairmanship of Sir Eric Ashby recommended that one of

our most pressing needs was an up-to-date laboratory to enable us to face the challenge of modern developments.

The research work in the new Jodrell Laboratory will be conducted in three departments concerned with (i) morphology and anatomy, (ii) cytology, and (iii) physiology, respectively. This division of subjects is, however, somewhat artificial and has been adopted only as a matter of administrative convenience. It is clearly evident that many of our problems will call for collaboration between specialists in each of the departments. The really important point is that specialists in all these subjects will be working together in the same building where they can help each other.

In these days of extreme specialization it is becoming increasingly important to maintain an adequate sense of perspective and to review the aims and objects that we have in mind when we undertake our investigations. There is always a danger that a specialized branch of research may become fashionable and be continued for that very reason rather than because of its intrinsic merits. In viewing the research prospects of the new Jodrell Laboratory we must remember that it has come into being in an age of almost unparalleled changes throughout the world. These changes are bound to have an impact on our botanical work. In particular, air travel is opening up possibilities that were not previously available to us. As we travel over deserts, oceans, mountain ranges and the dense forests of the tropics, we are reminded, for example, that only a small proportion of the world's species have been examined under the microscope; that soil erosion is active in many regions, and unless we press on with our task of microscopical examination there is a danger that many species will have become extinct before we have even taken a really close look at them. Meanwhile, in our universities the interests of botanical students are largely determined by the relative mark-earning values of different disciplines. Among these a knowledge of the world's vegetation and of the sheer diversity of form exhibited by the plants of which it is composed does not appear to rank very high. There is, therefore, a danger that modern students will not be taught to view the excitements of ultra-structure against the broad background of the tropical forest. This lack of sense of perspective can lead to a wrong sense of values and we may even forget what botany is all about. It is, therefore,



Fig. 1. The new Jodrell Laboratory at the Royal Botanic Gardens, Kew.

particularly appropriate that there is a widespread hope that the opening of the new Jodrell Laboratory will mark the beginning of an era of closer co-operation between Kew and the universities, a form of symbiosis which could, in favourable circumstances, be of great mutual advantage to all concerned. The incorporation in the new laboratory of a fine, modern lecture theatre, with accommodation for audiences of up to 200, should also facilitate collaboration between Kew and the universities, since it will provide an appropriate setting for meetings of learned societies as well as for groups of students. It will also enable our many visiting research workers, who come from all over the world, to make the nature of their investigations more widely known. The lecture theatre will also provide more adequate opportunities to make the scientific work of Kew more widely known among the general public.

It would be wrong to conclude this article without referring to the fact that the new building contains two much-needed teaching laboratories, intended primarily for

giving instruction to our student gardeners. To meet the occasion, the training scheme for these young people is being revised and modernized. If all goes well they should indeed be fortunate, for there can be few students who have both horticulturists and botanists of high standing as their mentors and are at the same time privileged to work in the midst of the wealth of living plants that are in cultivation at Kew.

In conclusion, it may be said that the new Jodrell Laboratory is a well-balanced unit that will serve alike as a research centre and for teaching and discussion, and which will also provide a means of communication between Kew and other botanical institutions as well as with the general public. The staff inherit a strong tradition for hard work and tenacity of purpose. They have better facilities and equipment than their predecessors and, if these are rightly used, there is every expectation that our knowledge of plant life will be both widened and deepened. The new Jodrell Laboratory provides both a challenge and a wonderful opportunity.

## NEWS and VIEWS

### Universities Federation for Animal Welfare:

Major C. W. Hume, O.B.E.

THE retirement of Major Hume from the post of secretary-general of the Universities Federation for Animal Welfare ends nearly forty years service on behalf of the welfare of animals, especially those used in laboratory experiments. He graduated in physics at Birkbeck College, and then joined the Patents Office, becoming senior examiner. He was editor to the Physical Society during 1919-40, and as honorary secretary of the British Science Guild organized the campaign which resulted in the Patents Act, 1932. He served in the Royal Engineers (Signal Service) during the First World War and afterwards in the 47th Divisional Signals (Territorial Army). During the Second World War he also served in the headquarters staff for Army Operational Research, and took part in various work in connexion with Signals Development. Hume will be remembered especially, however, for his life-long devotion to the welfare of animals. He was the founder in 1926 of the University of London Animal Welfare Society (ULAWS), followed in 1939 by the Universities Federation for Animal Welfare (UFAW). In addition to the administration of these organizations, first as honorary secretary and later as secretary-general, he has taken a very active part in the promotion of many Bills concerning the welfare of animals. His publications include *The Status of Animals in the Christian Religion* (1926), *Man and Beast* (1962), and numerous articles on animal welfare, statistical analysis, the strategy and tactics of experimentation, patent law, rabbit control, and other topics.

### Metallurgy in the University College of Swansea:

Prof. H. O'Neill

In September 1964, when he retired, Prof. H. O'Neill concluded a period of seventeen years at the University College of Swansea as head of the Department of Metallurgy. Thus he concluded a professional career which, commencing in 1920, has embraced all aspects of metallurgy. Hugh O'Neill was born at Sheffield in 1899 and, after two years National Service in the First World War, graduated with honours in the University of Sheffield. The next fourteen years were spent in teaching metallurgy, and during this period he published his well-known book, *The Hardness of Metals and its Measurement*. In 1929 he was awarded a D.Sc. degree by the University of Manchester. In 1935 he resigned his post as senior

lecturer in the University of Manchester and joined the Research Department of the London, Midland and Scottish Railway at Derby, where he served as chief metallurgist until 1947. During this period he was much concerned with welding and helped to initiate the Abington Research Station. His publications ranged from bearing metals to the welding of steels and the study of rails, and the paper on this last subject to the Institution of Civil Engineers was awarded the Trevithick Premium. After his appointment as professor of metallurgy at Swansea, he continued research on the deformation and hardness of metals, including transformation effects in electrodeposited alloys and deformed austenite and, in 1950, a Nuffield travelling fellowship enabled him to visit metallurgical plants in Canada and the United States. Prof. O'Neill has been a member of council of the British Iron and Steel Research Association, a visitor for the Department of Scientific and Industrial Research and an honorary member of council of the Iron and Steel Institute. A founder member of the Institution of Metallurgists, he was elected president in 1952. In 1961 he was elected president of the Institute of Metals. Other activities have included presidency of the Newman Association and chairmanship of the Newman International Foundation, besides work for World Student Relief and World University Service. As a prison visitor for many years, he still takes a part in the Discharged Prisoners' Aid Society. He played an important part in the inauguration of the Industrial Museum of South Wales, which he serves as secretary, and has been president of the Royal Institution of South Wales. He remains very active in metallurgy, and at present is engaged in writing a further book.

### Mechanical Engineering in the Battersea College of Technology:

Prof. J. M. Zarek

DR. J. M. ZAREK, whose appointment to the chair of Mechanical Engineering and the headship of the Mechanical Engineering Department at Battersea College of Technology has recently been announced, was born in 1912 and received his engineering training at the Technical University of Warsaw. When Germany invaded Poland in 1939 he succeeded in making his way to Britain by a circuitous route and joined the Free Polish Army in Britain. Thereafter he studied at the University of St. Andrews, the Imperial College of Science and Technology and King's College, London. After three years as a

lecturer at the University of St. Andrews he joined the staff of King's College, London, in 1950, and, since then, has occupied posts as lecturer, senior lecturer (1956), and reader in engineering science (1961). Dr. Zarek's researches fall mainly into two fields. First, he was one of the first workers in Britain in the field of biomechanics, the application of the principles of engineering to problems in surgery and medicine. His work in this area of research has done much to establish and advance the science. Secondly, he has sustained an interest in the metering of pulsating flow and has served as the chairman of the International Standards Organization Working Group for Pulsating Flow Measurement.

### Voluntary Service Overseas

IN a written answer in the House of Commons on June 28, Mr. A. E. Oram, the Parliamentary Secretary to the Ministry of Overseas Development, stated that after consultations with the voluntary bodies and discussion in the Lookwood Committee, it had been agreed that the target for the graduate and qualified volunteer programme for 1966-67 should be 1,500. Voluntary Service Overseas intended to send out about 500 cadet volunteers (school-leavers and young volunteers from industry), and the Government would meet 75 per cent of the British costs of 400 of these cadets and of the 1,300 graduates. The programme of the graduate volunteers was only 100 more than this year's programme, which was proving difficult to complete. The voluntary bodies and the Government were agreed that quality should not be sacrificed for quantity and that in 1966 Britain should concentrate on strengthening the selection of projects. For this purpose the voluntary societies intended to arrange more frequent visits to the developing countries by members of their staff to discuss, at first hand, the selection of projects and to evaluate the work their volunteers were doing. Extra staff would be recruited where necessary and there would be fresh publicity to stimulate recruitment, including a film about volunteers produced by the Central Office of Information.

### European Organization for Nuclear Research

IN reply to a question in the House of Commons on July 1, the Secretary of State for Education and Science, Mr. A. Croxall, stated that the Government had agreed in principle to participate in the project for adding intersecting storage rings to the 28-GeV proton synchrotron of the European Organization for Nuclear Research at a cost of about £28 million, of which the United Kingdom's share would be rather less than £7 million over about 5-5 years. The formal decision might be taken by the participating States at a meeting of the Council in December. The intersecting storage rings would provide European high-energy physicists with a unique facility for maintaining a programme of advanced research.

### The United Steel Companies

STEEL is very much in the news at the present time and it is therefore not without pertinent interest to read the *Review of Progress 1964* recently published by the United Steel Companies, Ltd. (Pp. 52. Sheffield: The United Steel Companies, Ltd., 1965.) This report covers the period October 1, 1963, to September 30, 1964, and is specifically dedicated to all employees of the organization and their families; it thus makes available in easily understood language, accompanied by generous illustrations in black-and-white and colour, much inside information, technical and economic, which would otherwise perhaps be excluded from a more formal statement of affairs. 1964 was a record year for steel production in the United Kingdom, as indeed it was for the United Steel group. The total output of steel in Britain as a whole was 26.2 million ingot tons in 1964, nearly 2 million tons more than was produced in 1960, the best previous year. A

message from the chairman, Mr. A. J. Peech, in this report states: "The expectations of a prosperous outcome of the past year have been fulfilled and our works will continue to be fully occupied for, at any rate, the first part of 1965. It is, however, particularly difficult to forecast the effect on the longer-term outlook of the present balance of payments problem and the steps which are being taken to deal with it". The impressions to be gained from perusal of this report are those of a thriving and efficiently managed industry, an appreciation of the economic difficulties of the times, ability to live with and ultimately to surmount them, and a welcome basis of mutual understanding between executive and personnel from which alone future progress in this organization can be assured, whatever the ultimate political circumstances of the steel industry in Britain as a whole.

### Museums Journal

THE March issue of the *Museums Journal* (64, No. 4; 1965), in accordance with recent policy, is devoted to one subject. In this case, the work of the International Council of Museums is dealt with from several angles, in papers read at a one-day conference in London of the British members of the International Council. Present at the meeting was the president of the International Council, Sir Philip Hendy, who in his contribution to the conference did not minimize the difficulties facing those who want to make the International Council work well. The president of the British National Committee, Sir Frank Francis, spoke of the needs yet unmet by the International Council of Museums. Other speakers gave details of schemes of museum development that they had carried out in the United States, Poland, Russia, Tanganyika and Ghana, under the aegis of the International Council.

### Chemical-Biological Activities

A new journal, *Chemical-Biological Activities*, issued by the American Chemical Society, reports on the activity of organic compounds on biological systems exclusive of botanical. The digests indicate the host (if known), system (organ, etc.), function or characteristic of the system being modified, the condition created, the significant concomitant diseases or other conditions of the host or system, and the comparative drug activity and route of administration. Each bi-weekly issue contains three indexes, which will be combined and issued semi-annually. Further information can be obtained from Chemical Abstracts Service, the Ohio State University, Columbus, Ohio 43210.

### New Icelandic Islands: Surtsey and Surtling

THE new Icelandic island of Surtsey, born on November 15, 1963, about 32 km off the south coast of Iceland and 19 km south of the Vestmann Islands, has grown steadily since it first appeared above the waves, and by the spring of this year it had reached a height of 170 m and an area of 2.3 sq. km. Initially the volcano erupted ashes and steam and the island seemed likely to perish rapidly from marine erosion, as has been the fate of several new islands which have been recorded from time to time in the sea off south-west and north-east Iceland; but in April 1964, as soon as a sufficiently strong reef of tuffs was created to exclude sea-water from the vent, lavas began to be erupted, so ensuring a greater degree of permanence. In view of the widespread interest which the event aroused, the University of Reykjavik, the Museum of Natural History, and other Icelandic institutions set up a Surtsey Research Committee to co-ordinate and strengthen geophysical, geochemical, and biological studies of the island, and a first mimeographed report of the work of the Committee appeared in February 1965. Shortly after this another new volcano, now named Surtling, appeared about 0.5 km to the east of Surtsey.

Remarkable photographs of this eruption published in *The Times* on July 8, 1965, show that Surtling is already 250 m long and 20 m high. Prior to these outbursts the last volcanic island to be created off Iceland was that named Nyð south-west of Reykjanæs, on which the Danish flag was ceremoniously raised in 1788, the year of the disastrous fissure eruption of Laki; but Nyð was soon eroded to below sea-level. Surtsey is apparently made of sterner stuff and already there have been many aeroplane landings on its tuffaceous foreshore.

### The Wyeth Institute of Medical Research

THE Wyeth Institute of Medical Research at Taplow, near Maidenhead (Fig. 1), officially opened on June 22 by Lord Todd, is a 40,000 sq. ft., two-storeyed steel and concrete structure representing a £500,000 investment in research and development facilities for the future. It adjoins the Company's existing administrative headquarters, now two years old, while all production remains centred at the eight-year-old manufacturing plant at Havant, Hants. On the ground floor of the new research block are housed the two main Chemistry Departments, Medicinal Chemistry and Natural Products Chemistry. Here, too, are the physical chemistry, large-scale chemistry and radioisotope laboratories. Centrally placed on this floor are the laboratories, sterile suites and constant temperature rooms of the future Microbiological Department, at present based at Havant, but due to move into these premises in the near future.

Pharmacology, together with toxicology and the small-animal colony, occupy most of the first floor of the Institute. The animal colony, serviced from a central corridor running the length of the building, has been carefully designed to minimize contact between clean and soiled materials and to prevent the development of animal odours. Located in this unit also are an animal behaviour observation room and a fully-equipped operating theatre. The pharmacy laboratories of the Product Development Department are on the ground floor. The final sections of Wyeth research, chemical development, chemical manufacturing and accommodation for large animals, remain in Havant. The Taplow research building, with five separate air-treatment systems, hot and cold water supplies, high- and low-pressure steam, compressed air, distilled water, vacuum and gas services and an emergency electric generator in addition to the mains supply, contains in excess of £100,000 worth of equipment. Total research personnel strength, including administration and maintenance staff in Taplow, is 106.

### Ashmolean Museum, Oxford

THE report of the Ashmolean Museum for 1964 records that the rearrangement of the valuable Cypriote Collection is virtually complete and has enabled the pattern of cultures and settlement in the island to be illustrated with a clarity and interest not possible in its previously crowded condition (Report of the Visitors, 1964. Supplement No. 6 to the *University Gazette*, March 1965. Pp. 87. Oxford: The University, 1965. 2s. 6d.). New features include a display of material from the late Bronze Age sanctuary at Pigadhes-Myrtou, excavated by a joint expedition to Cyprus of the Universities of Oxford and Sydney in 1950-51. A major rearrangement and re-shelving of the Randolph basement has made it possible to move the Arundel Marbles in reserve, and also the Latin and Greek inscribed stones, to more accessible positions.

### Transvaal Museum, Pretoria

IN the report of the Transvaal Museum for the year ended March 31, 1964, it is recorded that the Namib Desert Research Station is now firmly established, and throughout the year workers in various fields of interest appertaining to conditions of extreme aridity have been carrying out research at the Station (Pp. 40. Pretoria: Transvaal Museum, 1965). Under the general title of *Scientific Papers of the Namib Desert Research Station*, some twenty papers have already been published. The buildings of the Station are well furnished, and the laboratory in particular is especially equipped with modern and valuable research instruments which, for the greater part, were given by the South African Council for Scientific and Industrial Research, and other research institutions.

### Science Museum, London

THE new Agricultural Implements and Machinery Gallery at the Science Museum, London, conveys in a simply understood way the technological complexity of modern farming methods. History has been used to emphasize the differences that have so greatly improved the agricultural workers' way of life. So far as possible, the exhibits themselves are so grouped that their message is more or less independent of the explanatory labels, but even these are set up in easily readable positions. The arable farming section is historically much as before in content. Modern arable farming is shown chiefly by a series of fifty new tractor and implement models set in new backgrounds and representing the four seasons of the year. The dairy farming section is entirely new.

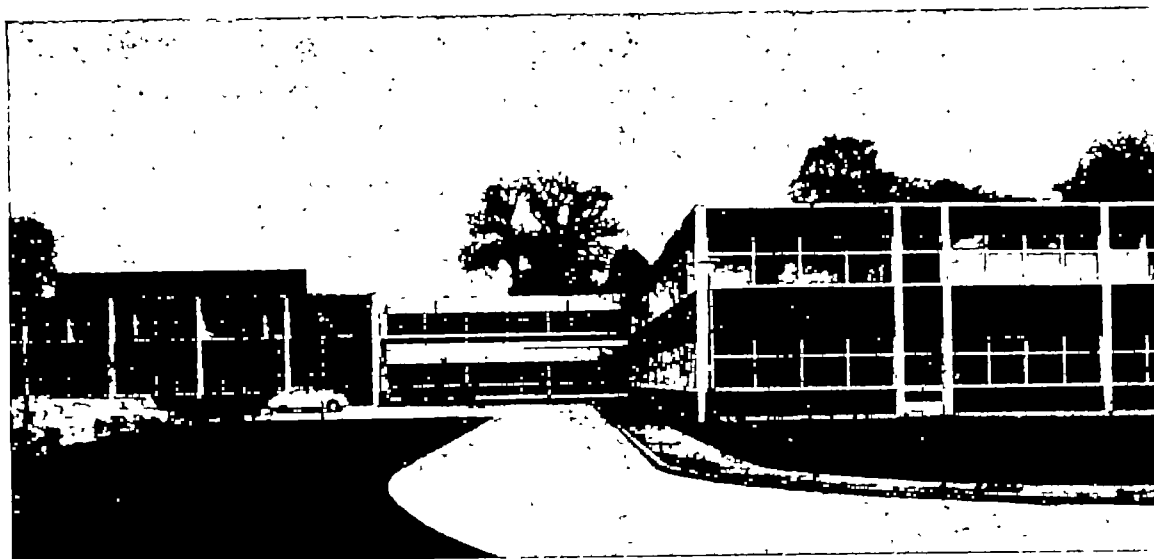


Fig. 1. The new Wyeth Institute of Medical Research at Taplow



### New Sports Stadium at Rotorua, New Zealand

THE construction of a new indoor sports centre at Rotorua in New Zealand, built at a cost of £90,000 and recently opened, embodies some rather unusual features worthy of brief record. The total floor area is 41,000 sq. ft. and consists of three main sections: stadium, gymnasium and foyer. The stadium has a centre court playing area of 16,500 sq. ft. and provides tiered seating along two sides for 1,000 spectators. One of the important features of the design was the use of 120-ft. laminated timber arches to support the roof. The timber was local *Radiata* pine and an 'Aerolite' melamine-modified urea-formaldehyde glue was used for all laminating. Illustrated details of this construction are given in *CIBA Technical Notes* 264 (December, 1964. Pp. 7. Duxford: Ciba (A.R.L.), Ltd., 1964). Rotorua is situated in an area of thermal springs and geysers in North Island, and, because of the hazard in such an area of infiltration of hydrogen sulphide through the foundations, special treatment of the site was necessary and a 3-ft. raft of suitable filling material was made to provide a firm base. The laminated timber work alone consumed 50,000 super feet; the total quantity of timber used was 250,000 super feet, all *Radiata* pine except 50,000 ft. of heart matai used for the flooring. "To combat corrosion from sulphurous gases, galvanized nails were used in the floor, bronze rather than chromium-plated fittings installed and extensive use made of aluminium piping. Nearly two tons of galvanized nails were used and almost five miles of electrical wiring installed." In view of the insidious and far-reaching effects of hydrogen sulphide emanations in situations such as this 'sportsdrome' it is to be hoped that the filling material used (it is not named) to seal the site and ensure a firm base for the foundations proves adequate and permanent.

### University News:

#### Edinburgh

PROF. F. F. BONBALL, at present professor of pure mathematics in the University of Newcastle upon Tyne, has been appointed to the McLaurin chair of mathematics as from October 1.

#### Cambridge

THE following elections to fellowships at Churchill College have been made: *Title A*, Dr. J. D. Eshelby, on appointment as College lecturer in physics. *Title F (Overseas Fellowships)*, Prof. M. Gell-Mann, professor of theoretical physics at the California Institute of Technology (April-July 1966); Prof. J. A. Barnes, professor of anthropology at the Research School of Pacific Studies, Australian National University, Canberra (October 1965-February 1966).

#### London

PROF. G. W. A. DIJK, at present professor of microbiology at the Queen's University of Belfast, has been appointed to the Bland-Sutton chair of pathology tenable at Middlesex Hospital Medical School. The following readers have also been appointed: Dr. H. Stern (virology, tenable at St. George's Hospital Medical School); Dr. C. J. M. Stirling (organic chemistry, tenable at King's College); Dr. M. Spiro (physical chemistry, tenable at the Imperial College of Science and Technology). The following titles have been conferred: *Professor*, Dr. H. C. K. Henderson (geography, in respect of his post at Birkbeck College); Dr. D. Lacey (zoology, in respect of his post at St. Bartholomew's Hospital Medical School); *Reader*, Dr. A. G. J. MacFarlane (electrical engineering, in respect of his post at Queen Mary College); *Miss A. M. Coleman* (geography, in respect of her post at King's College); Dr. P. J. Lindop (radiobiology, in respect of her post at St. Bartholomew's Hospital Medical College).

### Proposed U.S. University Research Association, Inc.

THIRTY-FOUR universities in the United States have been invited to participate in Universities Research

Association, Inc., a new corporation formed as a result of a meeting of university presidents at the National Academy of Sciences on June 20, 1965. The corporation will offer its services to the Federal Government as manager of a proposed high-energy proton accelerator, should Congress approve its construction. Articles of incorporation, filed in the District of Columbia on June 21, list the following university presidents as temporary trustees: Detlev W. Bronk, Rockefeller University; Robert F. Goheen, Princeton University; Fred H. Harrington, University of Wisconsin; Grayson Kirk, Columbia University; Joseph R. Smiley, University of Colorado; Elvis J. Stahr, jun., Indiana University; H. Guyford Stever, Carnegie Institute of Technology; and John C. Warner, former president of Carnegie, who has headed the organizing committee.

Other institutions invited to participate in the corporation are: California Institute of Technology; University of California (Berkeley); University of California at Los Angeles; University of Chicago; Cornell University; Duke University; Harvard University; University of Illinois; University of Iowa (Iowa City); Johns Hopkins University; University of Maryland; Massachusetts Institute of Technology; University of Michigan; University of Minnesota; University of North Carolina; Northwestern University; University of Notre Dame; University of Pennsylvania; Purdue University; Rice University; University of Rochester; Stanford University; University of Texas; Tulane University; Washington University (St. Louis); University of Washington; and Yale University. The list is subject to later extension by the member institutions. The corporation will function under the authority of a council of presidents, in which each member institution will be represented by its chief executive. Its operations will be managed by a board of trustees, composed of six trustees-at-large to represent a broad section of public interest, and fifteen distinguished scientists and senior university administrators elected by the member institutions. The universities will form fifteen regional groups for the purpose of making these nominations.

### Announcements

PROF. S. PETERSEN, professor of meteorology in the University of Chicago from 1962 until 1963, has been awarded the International Meteorological Prize. The Prize is awarded for outstanding work in meteorology and international collaboration. It was established in 1955 by the World Meteorological Organization in honour of the former non-Governmental Organization which had initiated international collaboration in 1878 and which was replaced in 1951 by the World Meteorological Organization.

THE eighth European Congress on Molecular Spectroscopy will be held in Copenhagen during August 14-20. Further information can be obtained from the Eighth European Congress on Molecular Spectroscopy, Universitetsparken 5, Copenhagen Ø, Denmark.

A NATO advanced study institute on "The Biochemistry of Chloroplasts" will be held in the University College of Wales, Aberystwyth, during August 19-28. Further information can be obtained from Prof. T. W. Goodwin, Department of Biochemistry, Penglans, Aberystwyth, Cardiganshire.

THE 1966 congress of the European Organization for Research on Fluorine and Dental Caries Prevention will be held in Perugia during June 6-8, 1966. The main theme of the congress will be "Carbohydrates in Relation to Nutrition, Economic Standards and Dental Caries". Further information can be obtained from Prof. A. Seppilli, Istituto d'Igiene della Università, Casella Postale No. 150, Perugia.

## METHODS IN TAXONOMY

ON March 31 the Systematics Association held a one-day demonstration meeting at the British Museum (Natural History). This meeting attracted forty-five exhibits concerned with botanical, zoological and palaeontological taxonomy. The exhibitors were drawn from the British Museum (Natural History), University of Cambridge, the Imperial College of Science and Technology, the National Institute of Oceanography, the Nature Conservancy, New York State College of Agriculture, University of Oxford, Rothamsted Experimental Station, the Royal Botanic Gardens, Kew, the University of Leeds and the University of Manchester.

After a welcome had been extended by the Director of the Museum, the proceedings were opened by Prof. P. C. Sylvester-Bradley, president of the Association. Prof. Bradley heralded the meeting as a contribution to "the biological dialogue". He suggested that the controversy that had sprung up a few years ago between cell biologists (who stressed the unity of life) and systematists (who investigated the diversity of life) had now entered a constructive phase. In most universities and most schools of biology a debate was in progress which promised to initiate research in the fruitful field that lies in the region of overlap between cell biology and comparative biology. In investigating the diversity of life, taxonomy answers 'the what' of comparative biology, and systematics 'the how'. New methods in taxonomy supply an essential vitality to this biological dialogue.

The exhibits at the meeting were organized under five headings ("Collecting", "Preparation and Maintenance", "Examination and Extraction of Information", "Analysis and Synthesis of Information", and "Presentation and Dissemination of Results") and were laid out so that visitors were guided around the exhibition in this sequence. As the catalogue of exhibits suggested, this sequential classification—while representing an ideal picture of orderly progress through a programme of taxonomic research—cannot be assumed to represent the usual pattern of taxonomic work; nor are the five stages necessarily clearly recognizable.

Collecting is the process of sorting organisms from their environment, and, in so doing, concentrating them. The eight exhibits in the section on collecting illustrated the variety of ways in which this can be achieved. In most groups mechanical devices can increase the efficiency of collecting. These devices either exploit the behaviour of the organism or else overcome behaviour and the speed of movement. To the first group of aids belong equipment for the extraction of microarthropods from soil and the attraction of insects to light; to the second group belong the great variety of stationary and moving traps and nets.

In many instances neither the organisms nor the nets or traps are visible to the collector, and in these cases control of sampling is difficult to achieve; but it is interesting to note that only where collecting takes place in large water-masses, such as lakes or the sea, is it feasible to monitor the equipment and thus know exactly how it is behaving. Monitoring apparatus such as that used at sea for tracking the behaviour of nets are scarcely worth devising for collecting in other environments such as streams or dense herbage. A particular advantage of collecting devices lies in the fact that if the behaviour of the equipment is known accurately it becomes easy to sample quantitatively. For the most sophisticated taxonomy, giving the most highly predictive results, quantitative sampling is essential.

Sorting organisms from their environment does not itself produce useful collections, and without careful labelling and annotation even the finest collections are of limited use to the taxonomist. Great care and effort have gone into the preparation of labels which can carry, in standardized forms, information essential to the taxonomist. Even the lay-out and type-face of printed labels are important, having significance for the rapid sorting and selection of prepared specimens. Probably in no branch of biology has this been more clearly recognized than in botany. But, however clearly and neatly labels may be designed and printed, it is still necessary to ensure that the collector completes them intelligibly and permanently in the field. Collection labels are best accompanied by sets of hard-and-fast rules for their use.

While even quantitative sampling of the larger plants may require no elaborate apparatus, botanists face far greater difficulties in the preservation of their material in the field than do most zoologists and palaeontologists. Skinning or the rapid chemical fixation of animals do not compare with the lengthy and elaborate processes of drying, mounting and pressing of fragile plants—all processes which must be completed perfectly in the field if specimens are to be of value later. In contrast, the field preservation of fossils—while sometimes essential—is not always necessary. More often the palaeontologist is faced in the laboratory with the greatest difficulty in separating the fossils from their environment. Because of this the chemical development and mechanical separation of fossils are fields of highly developed technology.

The second section of the exhibition, concerned with the preparation and maintenance of organisms, was necessarily mostly concerned with methods which come within the domain of museum curation. Of particular interest were the exhibits which demonstrated the advantages to biology of the recent rapid advances in the fields of plastics and polymer resin technology, which allow impregnation and imbedding of such diverse structures as fossil and recent bones, plants and invertebrates. These newly available compounds and their properties have brought almost to the level of mass-production standards of mounting, dissecting, sectioning and display which were hitherto reserved for special exhibits, and which could be achieved only with the greatest labour. While some of these plastics and resins are useful both as preservative and preparatory media, other new compounds have been developed for the bulk preservation and storage of organisms. Perhaps the most important of these is phenoxetol, which, with its good properties as a preservative, its low price, absence of smell, and avoidance of fire risks, promises to be a useful substitute for alcohol and formalin in large collections. Another significant innovation, increasingly used in museums, is the practice of freeze-drying whole specimens, when colour, shape, texture and posture of even quite large vertebrates can be most amazingly retained.

A specimen preserved for several decades by any technique is of little use if the labels that accompany it have deteriorated. In recent years much research has been done to determine the qualities of paper and ink which will remain intact and unfaded for many years in both herbarium and spirit store, despite wide fluctuations in temperature, humidity, and other features of the environment. Furthermore, not only must labels not deteriorate, but in design and layout they must be as clear in the store as they are to the collector in the field.

Surprisingly, perhaps, few exhibits in this section dealt in detail with the administration of large collections. All the more interesting therefore were the exhibits of the curation of extensive insect and mammal collections, which demonstrated the importance to taxonomic research of type specimens, named series, and the most effective physical arrangements of animals of widely varying shapes and sizes. The proper curation of large collections requires speedy and accurate storage and retrieval, not only of objects but also of information about the objects. This presents a problem that is further complicated by the difficulty of deciding how much information the objects themselves display and how much should be documented. As exhibits in the sections of the demonstration on analysis and synthesis and presentation and dissemination revealed, this complication is becoming increasingly troublesome. Small collections made for special research purposes appear to be readily suitable for documentation with punched cards, special maps and other recent innovations.

The exhibits displayed in the section dealing with examination and extraction of information were all concerned with methods of obtaining information not immediately detectable without the help of instrumentation, and this section, perhaps more than any other in the demonstration, showed how very many developments in the physical sciences are rapidly turned to their advantage by taxonomists. Two exhibits of striking electron-micrographs complemented nicely a demonstration of the range of light microscope techniques. X-ray illustrations of skeletal structures, moulds of leaf surfaces, and electronic recordings of insect sounds were the themes of other exhibits showing exploitation of techniques which might be thought to belong solely within the fields of the physical sciences.

Demonstrations of the uses to taxonomists of paper chromatography, electrophoretic separation of proteins, and a variety of serological techniques indicated the increasing significance to taxonomy—especially of  $\gamma$ -taxonomy—of advances in biochemistry.

Many techniques and instruments can provide the taxonomist with assessable information, and a point which the exhibits in this section underlined is that no one technique, whether simple or the ultimate in sophistication, can be held to be the most useful. It was perhaps unfortunate that there was no exhibit which demonstrated the usefulness in taxonomy of the unaided human senses and such simple instruments as callipers.

Just as the significance to taxonomy of the human senses is not easy to display, so the vital importance of human mental processes of induction and deduction did not lend themselves readily to demonstration in the section on analysis and synthesis. The ever-increasing use of mathematical and geometrical methods of analysis and synthesis in taxonomy was demonstrated by the use of geometrical transformations of structure and a wide array of methods of multivariate analysis. The development of these last techniques, which is closely linked with advances in computer technology, allows taxonomists to take on research problems which, in the past, would have required many years for completion—if indeed they could

have been contemplated—but which can nowadays be completed in a few weeks or months. Numerical treatment of taxonomic data is still a subject of much controversy; but there can be no doubt that its use encourages taxonomists to regard the organisms with which they deal as parts of populations. This is essential for progress in some areas of taxonomy; but what is urgently needed, if numerical taxonomy is to become an accepted tool of routine identification and classification, is proof that the labour of collecting the vast quantities of data required for the statistics is rewarded by greatly increased usefulness of the results.

Numerical methods of analysis and synthesis may allow taxonomists to handle rapidly large amounts of data; but, as the section concerned with presentation and dissemination of results showed all too clearly, biological data are not the only items which are accumulating faster than they can be handled by traditional methods. Collections of organisms are growing rapidly all the time, and will continue to grow at a high rate for many years to come. Every specimen is a source of useful information, and so, ideally, every specimen should be easily retrieved from the collection, while the information which accompanies it should be readily available. Unfortunately, because of the continued growth of collections, and because the theories and practice of information storage and retrieval are still in an early stage of development, probably all institutions which maintain large collections are faced with apparently insuperable problems of management. While collections the items of which can be counted in hundreds can be adequately controlled and used by means of written guides and indices, these time-honoured methods cannot be efficient where collections must be numbered in tens or hundreds of thousands. Considerable thought is now going into the design of machinery and procedures which can deal efficiently and rapidly with the documentation and control of large collections. If these efforts are successful the exchange of information and material between taxonomists could be greatly improved.

Not only are collections of organisms expanding at a tremendous rate but the volume of published work which each taxonomist must review grows every year. Collection management and the control of published results have many problems in common, and many of the same principles are employed to devise information storage and retrieval aids in both fields. However, some problems of bibliography control can be solved more easily than can their counterparts in collection management. Uniform high standards of illustration, typography and layout can be demanded, as can condensation of text, but organisms to be stored will always be highly heterogeneous in the amount of information which they reveal on casual inspection and in their storage requirements. It was clear from several exhibits in this section that much taxonomic publication could be standardized to allow efficient storage and easy perusal. Ideas differ as to the best ways of achieving these aims, and it is to be hoped that some measure of agreement will be reached soon. Otherwise the taxonomist will find himself forced to read yet more journals with different and conflicting methods of presentation.

WILLIAM G. FRY

## TELEVISION AND RADIO IN BRITAIN

IN opening a debate on broadcasting in the House of Commons on May 13, Sir Peter Rawlinson pressed for information as to the Government's plans for television and advocated that the new fourth channel should be devoted to commercial television. As regards sound broadcasting, he thought that this was the appropriate time to encourage local broadcasting and the right

solution was to be found in low-range local broadcasting stations. In replying for the Government, the Postmaster-General, Mr. Wedgwood Benn, said that it was essential before taking a decision on the allocation of the fourth channel that we should be clear as to what purposes the fourth channel should be required to meet. Educational broadcasting was very closely tied to the question of the

fourth channel and this was being urgently considered, as well as the alternative possibilities, including the utilization of unused hours; decision would only be reached after most careful consideration of the alternatives.

On colour television, he said that the failure of the Vienna Conference to reach agreement on an acceptable uniform colour system in Europe had set back our hopes of European standardization. For Great Britain, the choice, if one had to be made, lay between American and European systems and would be a particularly difficult one, which would be even harder if there were two different systems on the Continent of Europe and not just one. We would also have to consider the question of payment for colour television, and it might not be right to ask the main body of licence holders to pay for a colour service which they may never wish or be able to afford to receive themselves.

Pirate ships had no part whatever in the future development of broadcasting in Britain or in Europe. They were interfering with reception in other countries, and the development of a really serious pattern of local sound stations in Britain depended on the use of very high frequency which made a great number of channels available.

Mr. Benn restated the traditional doctrine which had been accepted by all governments and was designed as a safeguard against political interference. There was a great danger in going beyond this limit with regard to ministerial interference. Mr. Benn insisted that the increase of the licence fee was fully justified and would only hold the present position and keep the British Broadcasting Corporation within its present borrowing limits. We had reached a point where the Corporation could no longer rely in anything like the previous extent on increased revenue arising from new licence holders. The Government was satisfied that all reasonable economy was

exercised within the Corporation, and the review in which the Corporation was co-operating was instituted in view of the changed position for increased revenue.

The problem of evasion was also being examined. In reaching its decisions, Mr. Benn said the Government would be guided by its profound belief in the growing importance of radio and television to the community and to the world, by its desire to see television and radio expand as rapidly as national resources permitted, to meet important national needs and to develop still further their potential for education, information and entertainment. It also recognized that technological changes had opened up new possibilities which had hitherto been entirely excluded. It was determined to uphold and entrench the principle of public service in all further development in television and radio, and it also wished to allow the greatest possible freedom and scope for creative talents to express themselves through radio and television. It was prepared to consider fully the various methods by which broadcasting could be financed, including a combination of broadcasting licences, grants-in-aid, local government grants and advertising revenue under proper supervision.

Mr. W. Shephard, who followed, agreed that a decision on the fourth channel should not be rushed, but he did not wish to see existing contractors given any more scope, or contractors obtained who had made fortunes elsewhere. He and subsequent speakers were somewhat critical of standards maintained by the British Broadcasting Corporation, and in the debate there was strong support for the development of local broadcasting. Mr. H. Bowden, replying for the Government, added little to what Mr. Benn had said, but reiterated the need for increased educational facilities and the dependence of a decision on the fourth channel on a careful review of the whole situation.

## EXPERIMENTAL EVIDENCE OF A TWINKLING LAYER IN THE EARTH'S ATMOSPHERE

By CRAIG C. HUDSON

Sandia Corporation, Sandia Base, Albuquerque, New Mexico

THE scintillation of stars is that part of astronomical seeing which deals with intensity fluctuations of the image, more commonly called twinkling. A large part of twinkling occurs at frequencies too high to be observable to the human eye, that is, greater than 15 c/s. For many years the cause has been thought to reside in the atmosphere, in the form of fluctuations in the index of refraction caused by air turbulence.

Other optical effects, such as blurring and dancing of the image, often accompany twinkling, but can be distinguished from it both by the method of observation and by layer of origin in the atmosphere.

Reviews of the various experiments and theories of astronomical seeing (especially twinkling) are included in Tatarski's well-known monograph<sup>1</sup>, in the report by Keller *et al.*<sup>2</sup>, and in the report by Wimbush<sup>3</sup>. The present discussion will be limited to twinkling.

The observational properties of star twinkling have been reported by many<sup>4-7</sup>. It is least in the zenith direction and has the broadest frequency spectrum there. For low angles of observation, the amplitude increases but the high frequency end of the spectrum fades away. The spectrum near zenith is approximately flat below 100 c/s but falls off rapidly at higher frequencies. Twinkling is enhanced by the use of a small aperture, and tends to a minimum for apertures greater than 20 in. diameter. For small apertures, the modulation (fluctuation relative to mean light level) can exceed 100 per cent even at the

zenith, while for large apertures 10-20 per cent is more typical. Twinkling also depends on the source size; the larger planets, for example, showing very little effect. Finally, twinkling depends on meteorological conditions; in particular, there is correlation between the intensity of the effect and the speed of winds above 20,000 ft.

Contemporary theories of twinkling, according to Keller<sup>2</sup>, can be classified into three groups: (a) those governed by the refraction of light by turbulent elements of large size not too far from the observer (also contributing to blurring and dancing); (b) those governed by the diffraction of light with the zeroth order predominating (thin layer); and (c) those governed by the diffraction of light with the higher orders predominating (thick layer). Using a theory of type (a), Chandrasekhar<sup>8</sup> deduced that twinkling could be explained on the basis of a hypothetical layer 100 m thick, of homogeneous, isotropic turbulence at 4 km altitude. Keller suggests a thinner layer at a higher altitude. Wimbush argues against a discrete layer, citing evidence of several other workers in favour of a continuous distribution of turbulence.

Keller<sup>2</sup>, using Protheroe's autocorrelation data<sup>9</sup>, made a parameter study on the basis of the theory of scintillation<sup>10,11</sup> which is of interest in what follows. The scale of turbulence is  $L_m$ ;  $\Delta z$  is the layer thickness; and  $\delta^2 n$  is the mean square fluctuation of the index of refraction. By assuming no pressure fluctuations,  $\delta^2 n$  may be transformed into the mean square temperature fluctuation  $\delta^2 T$ . Estim-

ates of  $L_m$  range over factors of two while  $\Delta z$  and  $\delta T$  are uncertain by orders of magnitude. The primary variables in the theory of scintillation, therefore, are the thickness of the turbulent layer and the intensity of its turbulence. Keller's parameter study is partially reproduced in Table 1. In each case, the experimental data are satisfied by a different set of parameters (assuming isotropic turbulence with a Gaussian distribution of element sizes).

Table 1					
Parameters	March 1, 1964			March 16, 1964	
Altitude $z$ (km)	6.1	9.2	15.3	9.2	15.3
$L_m$ (cm)	6.7	5.9	6.3	6.7	6.7
Thickness $\Delta z$ (km), ( $\sigma^2 T$ ) <sup>1/2</sup> = 0.1° C	0.361	0.043	0.14	0.021	0.037
Thickness $\Delta z$ (km), ( $\sigma^2 T$ ) <sup>1/2</sup> = 0.01° C	6.1	4.3	14	2.1	3.7

Keller rejected the second set of thicknesses on the grounds that adiabatic mixing through such a thick layer would produce r.m.s. temperature fluctuations greatly in excess of 0.01° C.

Light beacons suspended from balloons which drifted with the wind have been used to study scintillation by Gardiner *et al.*<sup>12</sup> and Mikecell<sup>6</sup>. The twinkling due to the lights was compared with that due to nearby stars. The amplitude twinkling in the range 1–10 c/s of the beacons was 50–75 per cent less than that of stars, and in the range 147–153 c/s was more than a factor of two less than that of the stars.

They also studied scintillation as a function of the correlation of light pulses due to double stars. In the frequency range 1–10 c/s, the twinkling appeared to arise from altitudes of 100,000 ft. or higher. For higher frequencies, about 150 c/s, the twinkling appeared to be caused near 30,000 ft. To account for the high-altitude low-frequency variations, they suggest that there exist large density variations in the atmosphere at these levels, essentially at rest relative to the Earth, which are swept through the line of sight to the star by the Earth's rotation.

Some new experiments are now being made which may shed additional light on the structure of the atmosphere and the causes of stellar twinkling. The first of these is reported here.

The idea of the experiment was to allow a point source light beacon to fall through the twinkling region, observing it continuously with a telescope fitted with a recording photometer. The beacon was carried to 65,000 ft. as the pay-load of a 6-in *Apache* rocket motor. To restrain the free fall somewhat, to keep the light on the bottom of the body, and to allow the beacon to sink more nearly vertically, a small parachute was deployed which caused the sink rate to be about 300 ft./sec at 30,000 ft. altitude. A 16-in. tracking telescope fitted with an RCA '7265' photomultiplier cooled with carbon dioxide was used to observe scintillations. No filters were used, so white light was observed.

The experiments were performed at the U.S. Atomic Energy Commission's rocket range at Tonopah, Nevada, at an elevation of 5,300 ft. A strong cold wind was blowing on the night of December 11, 1963, the air was dry, and visibility was nearly unlimited although a small amount of fine powder snow was being blown around at low levels. On March 10, 1964, the air was again clear, cold and dry, but the wind was calm, a front having passed the night before. Meteorological data were taken by radiosonde as often as practicable (every 2–3 h). Position of the beacon was obtained both by radar and by an optical triangulation system. Altitude accuracy is about  $\pm 200$  ft. Altitude accuracy of the radiosonde is believed to be no worse than  $\pm 300$  ft.

The scintillations were recorded on magnetic tape after being telemetered from the remote telescope site. The telemetry used a 70-kc/s channel on a 220-mc carrier. The frequency response of the system was limited by the telemetry to the range DC to 10 kc. The tapes were then played back to give (a) a measure of the twinkling

amplitude as a function of time and (b) a measure of the frequency spectrum.

In addition to observations of the falling beacon, scintillation measurements were also made on ground lights at various distances, on a number of stars and planets, and on the satellite *Echo I*. The purpose of these measurements was mainly to check and calibrate an untried system, but also to make available a more complete set of twinkle measurements.

The data from the beacons are shown in Fig. 1 in the form of percentage modulation of the mean light intensity. The values are weighted in favour of frequencies between 10 and 100 c/s. A sample of the frequency spectrum of the

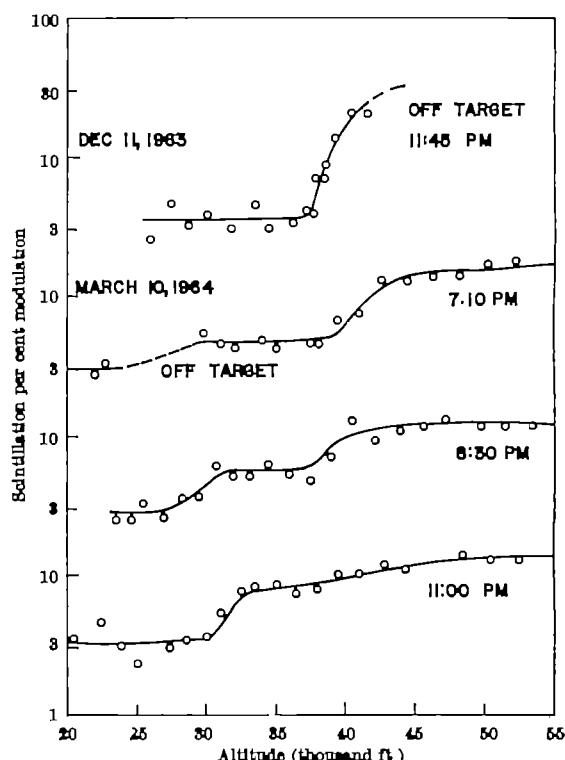


Fig. 1. Evidence of twinkling layer. Scatter in the data is partially due to hand reduction

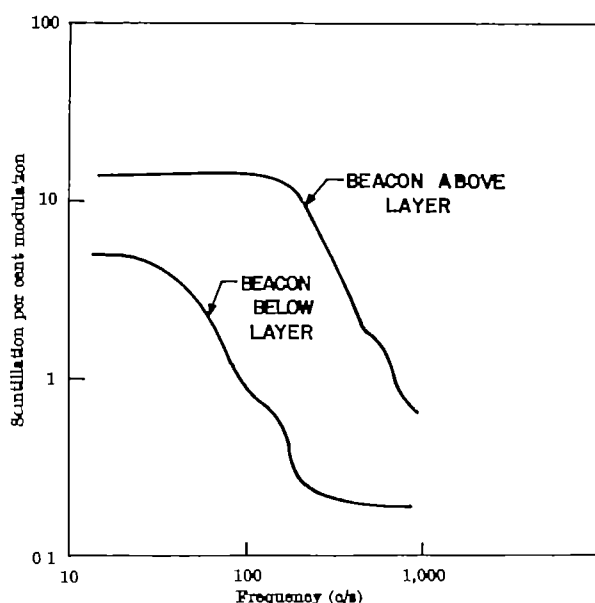


Fig. 2. Frequency spectrum of twinkling signals. System capability and data actually recorded were good to 10,000 c/s, but reduction technique was not reliable above 1,000 c/s

twinkling of the beacon above the layer and below the layer is shown in Fig. 2.

The meteorological data are shown in Fig. 3. Data points are about 400 ft. apart. A tentative assignment of the tropopause has been made. The horizontal bars indicate approximately the twinkling layers.

Referring to Table 1, the set of parameters which best corresponds to the present measurements comprises those of the altitude 9.2 km which imply r.m.s. temperature fluctuations of the order of  $0.01^\circ\text{C}$ , appreciably smaller than the estimates typically seen. Of course, if the observed layer actually consists of several thin layers, the temperature fluctuations need not be so small.

From Fig. 3 one gets the impression that the twinkling layer is not associated either with the wind speed structure or with the tropopause; but since it seems typically to occur in that neighbourhood, a re-check of the wind vectors was made to see if strong shears or wind gradients could be found in the layer; but the shears in those regions were no greater than in nearby regions where a change in twinkling was not observed. The U.S. Weather

Bureau maps for the days in question showed no jet stream in the near vicinity.

The peak intensity of scintillation on December 11 was probably not more than 30 per cent modulation, and on March 10 scarcely more than 15 per cent modulation. Unfortunately, no measurements were made on stars at the zenith, but for Capella ( $30^\circ$  from the zenith on December 11) and Pollux ( $10^\circ$  on March 10) the approximate intensity can be found by making a sec<sup>2</sup> $\theta$  correction. The values obtained in this way were 33 per cent (December 11) and 18 per cent (March 10). These data indicate that only a small fraction of the total scintillation above 10 c/s occurs above the measured twinkle zone.

By the experiments already performed, the existence of a twinkle layer, long suspected, has been confirmed. The height of the layer corresponds roughly to the tropopause, although no unique relationship seems to exist between the layer and either the tropopause or wind structures. In terms of Keller's theory of twinkling, the layer thickness corresponds to temperature fluctuations of the order of  $0.01^\circ\text{C}$ , somewhat smaller than has been conjectured. In the higher frequency portion of the spectrum (above 10 c/s), it seems that about 80 per cent of the total twinkling occurs in this layer, about 10 per cent below it, and 10 per cent above.

A second experiment now in progress will attempt to measure temperature fluctuations directly and to relate them to the observed twinkle layer. The mean square temperature fluctuation  $\delta^2 T$  will be determined at the frequency that seems to be most prominent, and this frequency (corrected for the rocket's motion) will also establish the scale of turbulence. As a precaution, microphones will also be used to determine the level of pressure fluctuations, and the instruments are so mounted that correlations between pressure and temperature fluctuations can be made.

This work was supported by the United States Atomic Energy Commission.

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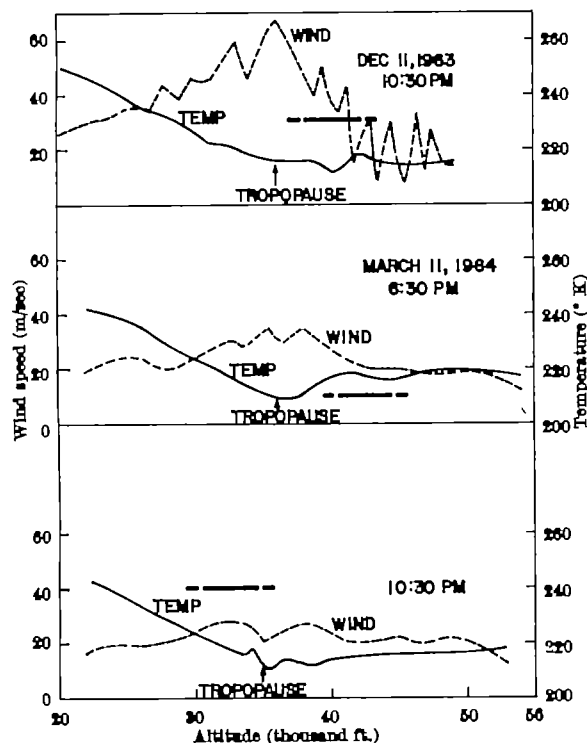


Fig. 3. Meteorological conditions. Heavy bars indicate position of principal twinkle layers.

## VEMA SEAMOUNT

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THE Vema Seamount is situated in latitude  $31^\circ 38' \text{S}$ , longitude  $8^\circ 20' \text{E}$ , near the centre of the Cape Basin, South Atlantic Ocean, approximately midway between the Walvis Ridge and the South African mainland. It was discovered in 1959 during cruise 16 of the Lamont Geological Observatory research vessel *Vema*. The seamount base is approximately 35 miles in diameter at ocean floor-level (2,500 fathoms) and the overall slope inclination is 1 in 4, or  $15^\circ$ , terminated upwards by a plateau of some 5 miles diameter and 40 fathoms depth from which the highest peak rises to within 14 fathoms of sea-level.

With the co-operation and generous assistance of the Marine Diamond Corporation a fairly detailed investigation of the seamount summit zone was carried out in November 1964, using scuba divers and the special air-lift dredging equipment in the diamond prospecting vessel *Emerson K* which enabled bottom samples up to 4 in. in diameter to be recovered in great quantity to depths of 45 fathoms.

Using radar fixes from a centrally positioned reflector buoy, bathymetric survey traverses totalling approximately 170 miles were run along radial lines at  $30^\circ$  intervals and along a number of tangential lines to fill in detail.

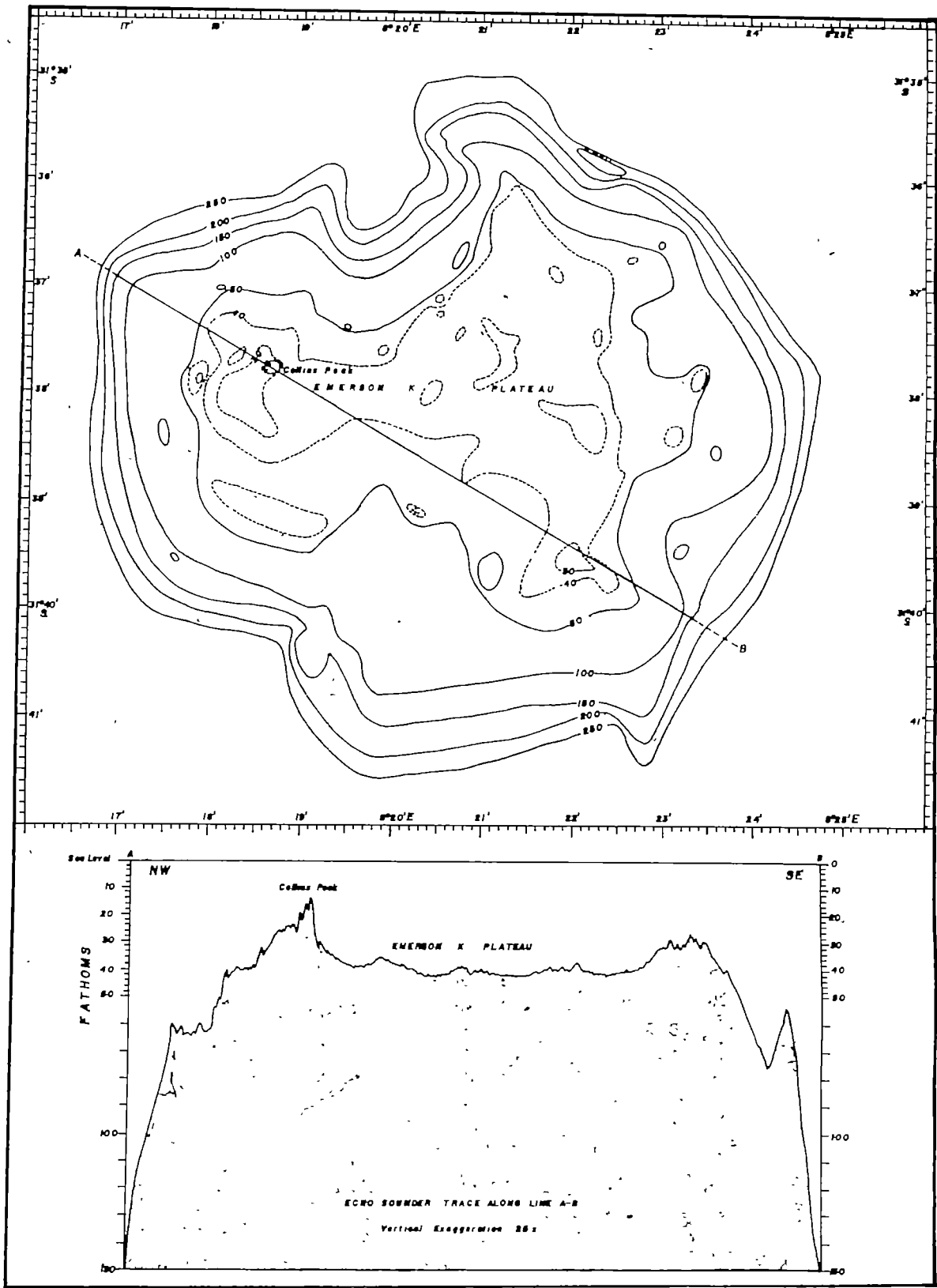


Fig. 1. Provisional bathymetric chart and profile of the Vema Seamount summit zone. Depths in fathoms



The plotted data were used to compile the accompanying provisional bathymetric chart and profile of the summit zone (Fig. 1). At the 250-fathom isobath the seamount is roughly circular in plan with a diameter of some 7 miles. Slope gradients are generally low above the 70-fathom isobath, but below this level the flanks are steep ( $\pm 15^\circ$ ) and broken in places by occasional subsidiary peaks.

The seamount is capped by a well-defined shelf (Emerson K Plateau) of mean depth 40 fathoms from which rise several eminences, particularly near the margins, of which the highest is the steep and rugged Collins Peak. The Emerson K Plateau occupies an area of approximately 5 square miles. The consistent depth of the Plateau and the frequent recovery from it of rounded boulders and pebbles suggest strongly that it is a wave-cut platform which probably corresponds to a eustatically lowered sea-level during the Pleistocene. Another persistent bevel, probably also wave-cut, was noted on most traverses at a mean depth of 60 fathoms (see Fig. 1, profile).

Approximately a ton of bottom samples was recovered by the air-lift dredge at six different localities and several specimens of bedrock *in situ* were collected by scuba divers.

The rugged submarine outcrops on Collins Peak are composed of a dense green phonolite which comprises small nepheline phenocrysts set in an aphanitic ground-mass of nepheline, aegirine-augite, a little alkali feldspar and iron ore, with much clear isotropic glass. A  $^{40}\text{K}/^{40}\text{Ar}$  determination made on a sample of the phonolite by Dr. Ian McDougall in the Department of Geophysics, Australian National University, has yielded an age of  $11.0 \pm 0.3$  million years. Owing to the possibility of loss of argon, this value must be regarded as a minimum age.

All rock samples taken on the Emerson K Plateau consisted of medium to dark brown tuffaceous agglomerate or volcanic ash of basaltic composition, many of them rounded. A few rounded boulders of black amygdaloidal olivine basalt were recovered only at one station, south of Collins Peak.

Geomagnetic measurements were made over approximately 60 traverse miles with an Elsec proton precession magnetometer by Mr. K. Biehevel of the Anglo-American Corporation. Preliminary results indicate a regional magnetic field intensity of  $29,700 \pm 100 \gamma$  at some distance from the seamount. An area of low magnetic values ( $29,000 \gamma$ ) is centred on Collins Peak, with values of 29,400 and 29,850  $\gamma$ , respectively, to the north and over the south-eastern edge of the Emerson K Plateau. Several local anomalies on the plateau show 'lows' to the north accompanied by 'highs' to the south, which suggests that the causative bodies are reversely magnetized.

As an isolated peak in the ocean, reaching within the zone of sunlight penetration but separated from the neighbouring mainland and islands by barriers of depth as well as distance, the Vema Seamount harbours an exceptionally interesting and abundant biological community hitherto unspoiled by commercial exploitation.

At all dredging stations on the Emerson K Plateau great quantities of rounded calcareous accretions up to 3 in. diameter were recovered. Many of these have a botryoidal outer surface with a thin pink layer coating, beneath which are concentric calcareous layers which in many examples surround a small rounded pebble of volcanic ash. After a cursory examination, Prof. J. H. Day reports that the encrusting material consists mainly of coralline or lithothamnian algae but includes also non-calcareous algae, Foraminifera of at least two species, sponges, serpulid and other worm tubes, and Bryozoa.

Steep-sided rock gullies on the bottom contain accumulations of finely comminuted calcareous debris which include arenaceous and calcareous Foraminifera, several species of gastropod, pelecypod and bryozoan fragments, some echinoderms and worms.

Collections of sessile benthic fauna and flora were made from depths ranging from 14 to 40 fathoms by scuba divers and the air-lift dredge. The rocky bottom is covered by a prolific and varied growth of sponges, hydroids, ascidians, a large species of holothurian, and seaweeds with abundant kelp (*Ecklonia* sp.). The fresh remains of three small oysters were recovered at one locality. It is of interest to note that the composition of the benthic fauna is not typically South African and appears rather to show zoogeographic affinities with Tristan da Cunha.

Rock lobsters (*Jaanus tristani*) were observed in great numbers by scuba divers and 111 were recovered for examination. The sample comprised 88 males and 23 females, with very few juveniles. The most abundant size (cephalothoracic length) of the males was 5.5-5.9 in. and that of the females 3.9-4.3 in. Of particular interest is the similarity to the rock lobsters of Tristan da Cunha. Intensive commercial exploitation of this community is now in progress, and examination of a large sample of the initial catches has confirmed the size frequency distributions as observed during the expedition. Further catches will be examined at regular intervals, as the intensive exploitation of the restricted and geographically isolated population on this seamount should afford a unique opportunity of observing the effect of fishing on a virgin resource.

An abundance of fish was observed by scuba divers and confirmed by hand-line fishing with the following results:

Species	No. caught	Size range (in.)
<i>Seriola lalandi</i>	58	27-48
<i>Acanthaluteres monodactylus</i>	42	15-25
<i>Poliprion americanus</i>	3	30-40
<i>Decapterus longimanus</i>	4	10-12
Undetermined species	3	$\pm 10$

The similarity to fish from Tristan da Cunha is again striking. No sharks or other elasmobranchs were observed (sea surface temperatures varied between  $18.3^\circ \text{C}$  and  $18.8^\circ \text{C}$ ) although many have appeared on the scene since the onset of commercial fishing operations.

Over the seamount random currents of variable direction, strength and duration were very evident during the course of the survey, which lasted four days. As the Emerson K was not equipped with hydrographic winches or davits, observations with regard to hydrology and planktology were necessarily restricted and will have to be augmented by further observations during a proposed visit by an oceanographic research vessel to the seamount in the near future. According to Mr. M. J. Orren and Dr. A. de Decker of the Division of Sea Fisheries, who analysed the hydrological and planktological samples respectively, the temperature and salinity values on and around the seamount are characteristic of Central South Atlantic Ocean waters and, further, suggest local sub-surface upwelling of cold, nutrient-rich Antarctic Intermediate water, which has a strong northerly component of flow in this region. This view is strengthened by the presence, in a single vertical plankton haul which was taken above the plateau, of the copepods *Calanoides carinatus* and *Calanus tonsus* (typical of Sub-Antarctic water) among a great majority of South Atlantic warm-water forms. The sample as a whole revealed a rich plankton community with a high volume for an oceanic station. More than 40 adult euphausiids (*Euphausia recurva* and *Thysanoessa gregaria*) indicate a high organic production.

All the data and material collected from the seamount are being processed by specialists who will in due course publish the results in greater detail. Only the uppermost one-tenth of the seamount was examined during the 1964 visit and much material remains to be collected in the course of future cruises.

# STRONTIUM ISOTOPE COMPOSITION AND POTASSIUM-RUBIDIUM RATIOS IN SOME ROCKS FROM RÉUNION AND RODRIGUEZ, INDIAN OCEAN

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THE isotopic composition of strontium and the potassium/rubidium (K/Rb) ratio are useful geochemical parameters in the understanding of the genesis of history of rocks<sup>1-4</sup>. Because most volcanic rocks in oceanic regions must be derived from the upper mantle with the minimum possibility of contamination from crustal materials, the examination of such rocks is of value in placing restrictions on the composition and chemical evolution of the upper mantle.

In this article we present results obtained on a series of rocks covering a wide range of composition from the volcanic island of Réunion, Indian Ocean. Two samples also were analysed from Rodriguez, an island which lies about 800 km east of Réunion. The aims of the work recorded here were first, to contribute to the question of the geographic homogeneity of the isotopic composition of strontium and the K/Rb ratio in rocks derived from the upper mantle, to which end much current work has been directed<sup>1-4,7-11</sup>. Secondly, we wished to use these two parameters to examine the relationship between the various rock types, and to see whether a correlation exists between the values found and the bulk chemical composition of the rocks.

Réunion lies near the south-west limit of the Mauritius-Seychelles Ridge, some 700 km east of Madagascar. The island is elliptical in outline with major diameter of 75 km and minor diameter of 55 km; it is built of two volcanoes. Piton des Neiges, an extinct volcano, occupies the north-western two-thirds of Réunion, and rises to an altitude of 3,069 m. Much of the original form of the volcano is preserved, although the central parts are deeply eroded into spectacular 'cirques', in which volcanic breccias are of common occurrence. Rivals<sup>12</sup> and Upton and Wadsworth<sup>13</sup> showed that Piton des Neiges is built of basaltic lavas covered by an extensive veneer of later lavas consisting mainly of alkali andesites. Piton de la Fournaise, which forms the south-eastern part of Réunion, is an active volcano rising to an altitude of 2,631 m. It is constructed entirely of basaltic lavas. Most of the rocks that have been analysed from Piton de la Fournaise are normatively slightly undersaturated with respect to silica, but rarely show nepheline in the norm<sup>14</sup>. Tilley<sup>15</sup> noted that these basalts are tholeiitic, and the few analyses available from Piton des Neiges suggest that this volcano also may be built of tholeiites. Petrographic examination of a number of samples lends support to this conclusion. The volcanoes of Réunion appear to be very similar as regards composition and evolution to those of the Hawaiian Islands, although the potassium content in Réunion tholeiites is considerably higher than in tholeiites from Hawaii. Most volcanoes exposed in oceanic basins appear to be composed of alkali lavas; however, based on the evidence from Hawaiian volcanoes, Tilley<sup>15</sup> suggested that the primary magma in oceanic regions may be tholeiitic basalt, from which the alkali lavas are derived by fractionation. This conclusion is strongly supported by recent investigations of samples dredged from the ocean floors<sup>16</sup>. In 1964 samples were collected from Réunion by the senior author for K-Ar dating, palaeomagnetic and geochemical, including isotopic, investigations. Preliminary dating on these rocks shows that the older basalts of Piton des Neiges are about 1 m.y. old, with some later basalt flows as young as 0.5 m.y., and that the veneer of alkali andesites is younger than 0.5 m.y. Piton de la Fournaise has been active over at least 0.19 m.y.

The rocks chosen for analysis in the work recorded here cover a wide range of composition. From Piton des Neiges three basalts, three alkali andesites and a syenite were analysed. From petrographic examination the basalts appear to be tholeiites, and the alkali andesites (1257, 1264) resemble hawaiites, whereas 1260 is similar to mugearite. The syenite sample (1266) is from an irregular transgressive sheet more than 100 m thick intrusive into agglomeratic material in Cirque de Cilaos. This rock, described and analysed by Lacroix<sup>14</sup>, is a peralkaline syenite consisting mainly of perthitic feldspar with aegirine and soda amphibole. Of the two samples analysed from Piton de la Fournaise 1263 is an olivine basalt (tholeiitic?) from a series of thick lava flows, possibly filling an old caldera in the older parts of the volcano. Sample 1267 is a tholeiitic picrite basalt (oceanite) from the 1961 lava flow.

Rodriguez is an extinct volcano, the exposed lavas of which are alkali basalts<sup>17</sup>. Two basalts, dated at 1.3-1.5 m.y. old, were analysed in this investigation.

For the determination of <sup>87</sup>Sr/<sup>86</sup>Sr, strontium from representative aliquots of 50-g whole rock samples was concentrated by cation exchange, and isotopically analysed as the chlorides on a 12-in. radius of curvature 60° sector mass spectrometer using a triple filament ion source and a single plate collector. Isotope ratios were measured by switching the accelerating voltage; details of this technique will be given elsewhere<sup>18</sup>. Measurements were made with the collected <sup>86</sup>Sr beam equal to 2.5 × 10<sup>-11</sup> amperes or greater and the digitized output system described earlier<sup>18</sup> was used to obtain sensitivity and linearity at the 0.05 per cent level. Corrections to the measured <sup>87</sup>Sr/<sup>86</sup>Sr were not more than 0.5 per cent for <sup>87</sup>Rb and 0.05 per cent for the <sup>86</sup>Sr tail, and averaged a few tenths of 1 per cent for normalization to a standard <sup>87</sup>Sr/<sup>86</sup>Sr value of 0.1194.

The observed variance in normalized <sup>87</sup>Sr/<sup>86</sup>Sr under these conditions is about 6.5 × 10<sup>-6</sup> from eight comparisons with magnetically switched ratios measured on identical samples with a 6-in. 90° sector mass spectrometer. This indicates that a single <sup>87</sup>Sr/<sup>86</sup>Sr measurement on an unknown sample will fall within ± 0.0006 of the true value at the 95 per cent confidence level. Voltage-switched measurements on the Eimer and Amend standard SrCO<sub>3</sub>, which averages 0.7082 for <sup>87</sup>Sr/<sup>86</sup>Sr, give a comparable estimate of variance based on a small number of independent analyses; additional measurements are now in progress. Because the rocks analysed are so young no age correction has to be made to the measured <sup>87</sup>Sr/<sup>86</sup>Sr values.

Rubidium was determined by isotope dilution and potassium was measured by flame photometry after the method of Cooper<sup>19</sup>. The precision of both rubidium and potassium measurements is better than ± 1 per cent at the 95 per cent confidence level, and the accuracy is regarded as better than 2 per cent. Data are given in Table 1, and localities of the samples are given in the Appendix.

The measured <sup>87</sup>Sr/<sup>86</sup>Sr values for the samples from Réunion fall within the extremely narrow range 0.7040-0.7046, and average 0.7044. These values are experimentally indistinguishable from one another; there is no difference between the <sup>87</sup>Sr/<sup>86</sup>Sr for rocks from Piton des Neiges compared with Piton de la Fournaise. This remarkably low range in <sup>87</sup>Sr/<sup>86</sup>Sr indicates that the

Table 1. POTASSIUM AND RUBIDIUM CONCENTRATIONS AND THE ISOTOPIC COMPOSITION OF STRONTIUM IN ROCKS FROM RÉUNION AND RODRIGUEZ

Sample No. (GA)	<sup>87</sup> Sr/ <sup>86</sup> Sr	K (%)	Rb (p.p.m.)	K/Rb	Rock type and locality
1255	0.7044	0.675	14.6	462	Olivine basalt (tholeiite) Grande Montagne
1256	0.7041				
1256	0.7046	0.614	19.9	443	Olivine basalt (tholeiite) Grande Montagne
1261	0.7044	0.641	16.4	391	Olivine basalt (tholeiite) St. Denis
1257	0.7042	1.445	41.3	350	Alkali andesite, Le Bois de Neiges
1260	0.7044	1.943	59.6	326	Alkali andesite, Ravine de la Fontaine
1264	0.7044	1.559	49.7	320	Alkali andesite, Belouve
1266	0.7046	3.909	106.6	370	Syenite, la Chapelle
1266	0.7046	0.785	23.5	334	Olivine basalt, Riv. des Remparts
1263	0.7040	0.389	9.0	343	Picrite basalt, Grand Brûlé
1267	0.7046	1.205	35.2	343	Alkali basalt
1263	0.7039	1.096	36.8	298	Alkali basalt
1264	0.7040				

source region of all these rocks was very homogeneous in its <sup>87</sup>Sr abundance. It implies a close genetic relationship between the basalts and alkali andesites, and it is consistent with the view that the later alkali lavas were produced by fractionation of tholeiitic magma.

The samples analysed show a wide range in bulk chemical composition; for example, silica is less than 50 per cent and probably about 47 per cent for the basalts, and even lower for the picrite basalt, whereas the syenite has 64 per cent silica<sup>14</sup>. Thus, at least for the range of samples analysed, there is no evidence in Réunion for a change in the <sup>87</sup>Sr/<sup>86</sup>Sr with bulk composition as found by Leeming and Catanzaro<sup>8</sup> and Hamilton<sup>10</sup> in Hawaii and by Gast *et al.*<sup>9</sup> for volcanic rocks from Gough and Ascension Islands.

The two alkali basalts from Rodriguez have <sup>87</sup>Sr/<sup>86</sup>Sr values of 0.7039 and 0.7040, and have measured silica contents of about 47 per cent.

The <sup>87</sup>Sr/<sup>86</sup>Sr values for the Réunion and Rodriguez rocks lie in the middle of the observed range of 0.702–0.706 for this parameter for essentially all oceanic volcanic rocks that have been analysed. That there are real local and regional variations in <sup>87</sup>Sr/<sup>86</sup>Sr within this narrow range of values, and rarely beyond, has been demonstrated<sup>8</sup>, and this provides evidence for inhomogeneity in the strontium isotope composition of the oceanic upper mantle. In the context of the restricted area sampled for the present study there is no evidence for local variations in the strontium isotope composition. Gast *et al.*<sup>9</sup> showed that the isotopic composition of lead is a much more sensitive indicator of variation in the source regions, and it is planned to undertake such measurements on the rocks used in this investigation in the near future.

Because of the similarity in ionic charge and electro-negativity of potassium and rubidium these elements form a coherent geochemical pair. Some enrichment in rubidium relative to potassium may occur with magmatic differentiation owing to the larger size of the rubidium ion<sup>21</sup>.

In the Réunion samples there is a tendency for the K/Rb ratio to decrease with increasing potassium content. The two older basalts from Piton des Neiges have ratios of 450, and the younger basalt (1261), of comparable potassium content, gives a somewhat lower value of 390. The basalt and picrite basalt from Piton de la Fournaise, which respectively have higher and lower potassium contents than the Piton des Neiges basalts, yield K/Rb values of about 340. Hence, there appears to be an indication of a real difference between the lavas from the two volcanoes. The three alkali andesites from Piton des Neiges show K/Rb ratios of between 350 and 320, which are considerably lower than those found for the basalts from this volcano. The two alkali basalts from Rodriguez have K/Rb values of 300–340.

Except for the two older basalts from Piton des Neiges the measured K/Rb ratios on samples from Réunion are all lower than those reported<sup>8</sup> for rather similar lavas from Hawaii, where an average of about 510 was found for rocks with a potassium content of less than 2 per cent. However, the values of between 300 and 350 are similar to the K/Rb ratios reported for average crustal and oceanic basaltic rocks<sup>1,19</sup>.

The K/Rb ratio of 370 for the syenite from Piton des Neiges is high compared with the alkali andesites from this volcano, even though the potassium content is over a factor of two greater. This indicates impoverishment of rubidium relative to potassium; such behaviour seems to be characteristic of syenites and may be related to restricted entry of rubidium into the sodium-rich alkali feldspar of these rocks<sup>21,22</sup>.

We thank Dr. B. G. J. Upton and Dr. W. J. Wadsworth for their advice and R. Fannucci and E. Hugot for assistance while one of us was in Réunion. M. J. Vernon carried out the rubidium and strontium chemistry and J. A. Cooper made the potassium measurements. We also thank Dr. K. S. Heier for his advice.

## APPENDIX

## Rodriguez

GA 1253: Alkali olivine basalt, south side of Bale Lascar 19° 44' 10" S, 63° 19' 50" E.

GA 1254: Alkali olivine basalt, Pointe Monnier. 19° 40' 45", 65° 24' 45".

## Réunion

GA 1255: Olivine basalt (tholeiite), la Montagne road at Ravine Laffeur. Grande Montagne massif, Piton des Neiges. 20° 54' 52" S, 55° 22' 11" E; altitude 590 m.

GA 1256: Olivine basalt (tholeiite), la Montagne road 0.5 km south of le Ddt Huilleme. Grande Montagne massif, Piton des Neiges. 20° 54' 46", 55° 25' 52"; altitude 620 m.

GA 1257: Alkali andesite (hawaiite ?), western flanks of Piton des Neiges. Road OD 4, 1 km south of le Bois de Neiges at Ravine Tete Dure. 20° 59' 58", 55° 19' 42"; altitude 460 m.

GA 1260: Alkali andesite (mugearite ?), western flanks of Piton des Neiges. Road OD 18 at Ravine de la Fontaine. 21° 8' 56", 55° 19' 50"; altitude 440 m.

GA 1261: Olivine basalt (tholeiite), le Brûlé road (OD 42) 3 km south of St. Denis; Piton des Neiges. 20° 53' 43", 55° 37' 2"; altitude 440 m.

GA 1263: Olivine basalt on track from Piton dans Bout into the gorge of Rivière des Remparts, Piton de la Fournaise. 21° 11' 13", 55° 38' 15"; altitude 1,730 m.

GA 1264: Alkali andesite (hawaiite ?) on track from Hell Bourg to Plateau de Belouve, Cirque de Salade, Piton des Neiges. 21° 2' 47", 55° 32' 32"; altitude 1,430 m.

GA 1266: Syenite at la Chapelle on Bras Rouge, Cirque de Chicaos, Piton des Neiges. 21° 7' 17", 55° 27' 37"; altitude 820 m.

GA 1267: Picrite basalt (oceanite), 1961 lava flow from Piton de la Fournaise. Grand Brûlé on road N 2, 2 km south of Rempart de Bou Blanc. 21° 18' 22", 55° 48' 20"; altitude about 100 m.

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## STRENGTH OF ALUMINIUM NITRIDE WHISKERS

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ANALYSIS of the strength of materials, from an atomistic point of view, predicts that the ratio,  $\alpha$ , of fracture stress ( $\sigma$ ) to initial strain ( $\epsilon$ ) in atomic bonds will be of the order of 10 per cent  $E$  ( $E$  = Young's modulus). A value of  $\alpha$  of this magnitude should be obtainable for perfect crystals. Whereas in practice yielding occurs in high-strength structural materials when  $\alpha$  is about 1 per cent  $E$ , it has been known for a long time that whiskers or fibres of almost all materials, with diameters of  $\sim 10^{-4}$  cm, and large length/diameter ratios show values of  $\alpha$  near to the ideal value. Non-metallic whiskers having mixed ionic/covalent bonding with low specific densities and high melting-points have been examined as strengthening media for fibre-reinforced materials<sup>1</sup>. Analysis of fibre-reinforced systems have recently been made by Cottrell<sup>2</sup> and Kelly<sup>3</sup>.

Aluminium nitride is a refractory material which may be considered suitable as a fibre-reinforcing material. Whiskers of this material were prepared by heating aluminium nitride powder (contained in an alumina crucible), at temperatures up to 1,820°C in an alumina tube in a flowing atmosphere of high-purity nitrogen diluted with high-purity argon. Chemical and X-ray analysis of the whisker product confirmed that the whiskers were aluminium nitride. The whiskers formed on cooler sections of the container. It is suggested that the whiskers grow by a process of dissociation of aluminium nitride powder at the operating temperature with subsequent growth of whiskers, from a vapour phase, at a cooler substrate. Straight whiskers, about 18–20 mm long, were formed after 15 h at temperature, giving an average growth rate of about 1.5 mm/h. Some of the whiskers with good morphological symmetry had 'kinks' and 'branches' (Fig. 1) and it was also observed that a whisker changed its axial growth direction by about 2° (minimum) to 20° (maximum) over its length. Platelets formed at slightly higher temperatures showed surface striations (Fig. 2); under oblique illumination these striations appeared to be

growth steps and not slip planes perpendicular to the major growth axis. The results of bend and tensile strength determinations on whiskers are given in Tables 1 and 2.

**Bend-strength tests.** The whiskers were subjected to bending on a Reichart microscope stage. Fig. 3 shows a typical bend in a whisker before fracture. All these tests were conducted with the whisker lying in a film of oil.

For perfectly elastic bending, the tensile stress in the outer surface of a fibre can be expressed as:

$$\sigma = \frac{Er}{p}$$

where  $\sigma$  = tensile stress in outer fibre;  $E$  = Young's modulus;  $r$  = radius of fibre;  $p$  = radius of curvature. It was observed that fracture occurred most frequently in whiskers containing common types of structural imperfections, that is, low-angle kinks, whiskers with twists of about 10° along their length and whiskers with surface growth steps.

The majority of whiskers were extremely flexible and it was sometimes difficult to obtain a sufficiently small



Fig. 1

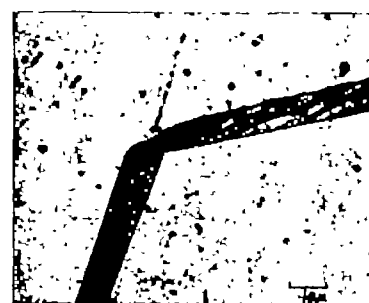


Fig. 2



Fig. 3

Table 1. BEND STRENGTH OF ALUMINIUM NITRIDE WHISKERS ( $E = 50 \times 10^4$  lb./in.<sup>2</sup>)

No.	Length ( $\mu \times 10^3$ )	Cross-section ( $\mu$ )	$p$ ( $\mu$ )	$\sigma = \frac{Er}{p}$ (lb./in. <sup>2</sup> )	$\frac{\sigma}{E}$ (%)
1	7.3	2.5 × 4.0	60	1.04 × 10 <sup>4</sup>	2.08
2	4.2	2.5 × 3.0	58	1.08 × 10 <sup>4</sup>	2.16
3	5.0	2.8 × 3.5	72	0.97 × 10 <sup>4</sup>	1.93
4	7.5	7.2 (hex)	1,820	9.9 × 10 <sup>4</sup>	0.02
5	5.2	6.5 (hex)	1,720	9.5 × 10 <sup>4</sup>	0.19
6	5.0	5.5 (hex)	1,900	7.25 × 10 <sup>4</sup>	0.03
7	8.2	3.0 × 8.0	75	1.0 × 10 <sup>4</sup>	2.00
8	8.3	8.0 × 10 <sup>3</sup> × 2.5 × 10 <sup>3</sup> (platelet)	10 <sup>4</sup>	1.25 × 10 <sup>4</sup>	—
9	4.7	3.5 × 2.5	78	0.8 × 10 <sup>4</sup>	1.60
10	4.2	3.7 × 2.3	82	0.88 × 10 <sup>4</sup>	1.75
11	5.0	4.0 × 2.2	2,500	2.2 × 10 <sup>4</sup>	—
12	5.2	8.0 × 2.5	10 <sup>4</sup>	6.7 × 10 <sup>4</sup>	—

All specimens except No. 6 were immersed in oil during testing. All specimens except Nos. 11 and 12 were bent about an axis parallel to the longest side of the cross-section; Nos. 11 and 12 were bent about the shorter cross-sectional axis. Specimens 4, 5 and 6 were produced in the image furnace<sup>4</sup>; for these specimens with a hexagonal cross-section the mean diameter is given.

Table 2. TENSILE MEASUREMENTS (GAUGE LENGTH 1.0 cm)

No.	Whisker section ( $\mu$ )	Load at fracture (g)	Fracture stress ( $\sigma$ ) (per lb./in. <sup>2</sup> )
1	10 × 38	272	1.02 × 10 <sup>4</sup>
2	17 × 22	188	0.72 × 10 <sup>4</sup>
3	9 × 42	260	0.68 × 10 <sup>4</sup>
4	11 × 37	268	0.94 × 10 <sup>4</sup>
5	10 × 39	272	0.99 × 10 <sup>4</sup>
6	8 × 45	268	1.06 × 10 <sup>4</sup>
7	26.3 mean diam. (hex)	52	0.14 × 10 <sup>4</sup>
8	28.6 mean diam. (hex)	53	0.18 × 10 <sup>4</sup>

radius of curvature to induce fracture. Where fracture did not occur the bent whiskers reverted to the initial shape when the constraint was removed. The results of bend tests on whiskers of different cross-section are given in Table 1.

From direct observations of the whiskers during bending and from the results of Table 1 it was concluded that:

(a) The maximum strength and flexibility were associated with whiskers of small cross-section and large length:diameter ratios. These whiskers also possessed smooth and apparently defect-free surfaces.

(b) Whiskers with an hexagonal cross-section had poor strength.

(c) When the bending moment was applied on the shortest side of a whisker section the whisker showed low strength.

(d) The presence of an oil film appeared to improve the strength of the whiskers, possibly by reducing the chance of surface damage.

(e) All whiskers fractured within the elastic limit.

**Tensile testing.** The tensile strength of whiskers was measured with an 'Instron' tensile testing machine with a load cell giving full-scale deflection for 400 g. The whiskers were mounted on a reinforced cardboard holder with an accurately punched gauge length of 1 cm. Some of the

whiskers tested had a taper of about  $2^\circ$  over the gauge-length; for these the cross-sectional area was taken as the average of the minimum and maximum measured. Although more than 50 specimens were mounted and tested, only 8 of these fractured within the gauge-length; a large percentage of fractures occurred at the base of the mounting resin. The results are given in Table 2.

All fractures occurred without plastic deformation taking place. The fracture surfaces showed that all the fractures were conchoidal. The lower strength of the whiskers of hexagonal cross-section is not completely understood and, although these may contain axial voids, examination of the fracture surfaces did not reveal this phenomenon. Excluding specimen number 2, the mean value of the experimental tensile fracture stress of the first six specimens is  $1.0 \times 10^6$  lb./in.<sup>2</sup>, that is, about 2 per cent  $E$ . Transmission electron microscope examination of a large number of thin whiskers and platelets has not, so far, provided conclusive evidence of the presence of dislocation.

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## OPENING ELECTRICAL CONTACT: BOILING METAL OR HIGH-DENSITY PLASMA?

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THE processes occurring at the opening of a low-voltage ( $\sim 4$  V) electrical contact have considerable fundamental physical interest as well as having practical importance in the field of electronic and communication engineering. It is well known<sup>1</sup> that, starting with the electrodes closely pressed together in the fully closed position, the opening process leads to a constriction of the current stream lines, which can produce intense local heating and melting of the penultimate microscopic region of contact. The maximum temperature in the contact is related to the potential difference by the  $\psi$ ,  $\theta$  theorem:

$$\psi = \left[ 2 \int_0^{\theta_m} \frac{\lambda}{x} d\theta \right]^{1/2} \quad (1)$$

where  $\psi$  = a generalized potential equal to the electrical potential in the absence of thermo-electric effects,  $\theta$  = temperature,  $\lambda$  = thermal conductivity and  $x$  = electrical conductivity. Thus, on gradual separation of the electrodes the constriction resistance increases and the temperature rises up to and past the melting-point of the metal. On continuing the withdrawal the molten volume thus increases and gets drawn out into a microscopic bridge of molten metal joining the solid electrodes; the contacts finally separate and the circuit opens only when this bridge is broken. The rupture process, however, can be very complicated and lead to transfer of metal from one electrode to the other, a process which, when continually repeated, can lead to the 'pip' and 'crater' formation which renders the contacts useless after some time. There is evidence<sup>1,2</sup> to show that the matter transferred per operation ( $\sim 10^{-13}$  cm<sup>3</sup> in a 5-amp circuit) is related to the size of the molten metal bridge (width  $\sim 10^{-4}$  cm/amp), so that the stability, growth and final rupture of the bridge are a matter of importance,

not only from practical considerations, but also from the point of view of the physical properties of metals in the molten state and at high temperatures.

In the first place, an important condition of equilibrium, at least in the earlier stages, is that which depends on the application of surface tension forces. The shapes of the bridges would then be surfaces of revolution satisfying the equation:

$$\Delta p = T \left( \frac{1}{R_1} + \frac{1}{R_2} \right) \quad (2)$$

and these are unduloids, catenoids or nodoids according as  $\Delta p$  is positive, zero or negative respectively<sup>3</sup>. Photographs of static microscopic bridges have indeed confirmed that these stable shapes can be attained<sup>1</sup>. In the later stages of opening  $\Delta p$  will be negative, and experiment has established that the final stable shape is usually the nodoid. The  $\psi$ ,  $\theta$  theorem shows that the hottest region of the microscopic molten metal bridge between like electrodes will probably be the narrow neck and, at first sight, it might appear that this is the region at which the bridge is most likely to break. However, detailed investigation of this final process raises some important problems in the physics of metals at high temperatures, and, in particular, near their boiling points.

### Mechanisms of Break

It can be seen at once from the  $\psi$ ,  $\theta$  theorem that the mechanism of rupture of the molten metal bridge involves the physical properties of the metal, not at any one temperature, but over a wide range of temperatures up to boiling-point, and a number of different processes of rupture are possible.

In the first place, continued separation of the electrodes and the drawing out of the bridge increases the contact resistance  $R_c$ ; consequently, the contact voltage  $V_c$  ( $= R_c I_c$ ) for a given circuit current  $I_c$  continually rises.

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Inspection of the  $\psi$ ,  $\theta$  theorem shows that the maximum temperature  $\theta_m$  correspondingly increases; in fact, it is readily seen by using the Wiedemann-Franz law that, when  $V_s$  exceeds about 1.5 V, the corresponding value of  $\theta_m$  from (1) exceeds the boiling-point of any known metal. Thus, this process of rise of maximum temperature may well continue until  $\theta_m$  reaches the boiling point  $\theta_b$  so breaking the molten metal bridge. The voltage  $V_s$  at which this occurs is called the boiling voltage, and is related to  $\theta_b$  by equation (1). Further analysis of the  $\psi$ ,  $\theta$  theorem shows that, since the relationship between  $\psi$  and  $\theta$  depends on the variation of  $\lambda/\kappa$  with  $\theta$ , thermal instability can occur for certain functional relationships of  $\lambda/\kappa$  with  $\theta$ , in which case a sudden rise of  $\theta_m$  up to the boiling point can, in certain circumstances, take place. Measurements of the contact current, potential difference and maximum temperature, and its location, enable a determination to be made of the transport properties of metals such as their thermal and electrical conductivities and Thomson coefficients and their dependence on temperature to be determined for the molten state at high temperatures<sup>1</sup>.

These thermal effects, however, are not the only processes which may sever the bridge. For example, the electromagnetic pinch effect might well, with large currents, so constrict the bridge at the narrowest, hottest and therefore weakest point as to rupture it there. Again, the known variation of surface tension with temperature is an important factor influencing the stability of the bridge, and the consequences of this can only be neutralized by the influence of surface impurities or compensating internal viscous motions in the bridge. Restriction due to the size and geometry of the actual electrodes in a given practical contact might well prevent the continued formation of the stable nodoid form, and instability could result.

The shape and volume of the final bridge and its mechanism of rupture are very important from the practical point of view on account of their relation to the rate of matter transfer on rupture. For example, suppose that a thermo-electric effect displaced the hottest section of the molten metal bridge towards one electrode, then it follows that the rupture at that particular section could have the effect of producing net transfer from one electrode to the other. The amount transferred may then only be that of the hottest region in the neck. On the other hand, if, in a different process of rupture, the molten metal bridge disintegrated as a whole and was transferred to one or other electrode (say, by mechanical splashing of minute droplets), then in this case the matter transfer could be relatively high and this, of course, could have serious practical effects.

Thus, the precise processes of the actual opening of the circuit are a matter of considerable practical and theoretical importance, and these phenomena have been under investigation at Swansea for some time. The relationships of the size of the molten metal bridge to the current and to the matter transferred per operation and the local self-inductance have been investigated<sup>1,2-10</sup>.

Accurate measurement of the amount of metal of a given electrode ( $\sim 10^{-12}$  g) actually transferred on the rupture of the molten metal bridge was found possible using the radioactive tracer technique, and a very rapid variation with local inductance, particularly in the range  $10^{-8}$  to  $10^{-3}$  H, was found for a number of metals and, particularly so, for platinum<sup>1,2-4</sup>. Further, optical examination of the crater formed on bridge rupture indicated that in some metals the whole volume of the molten metal bridge took part in the transfer. The shape and volume of the microscopic bridge were determined from the geometry of the melting isothermals in each electrode after rupture.

Facts such as these are difficult to reconcile with the picture of matter transfer occurring as the result of a mechanical splashing of small droplets formed from the disintegrating molten metal bridge. On the contrary,

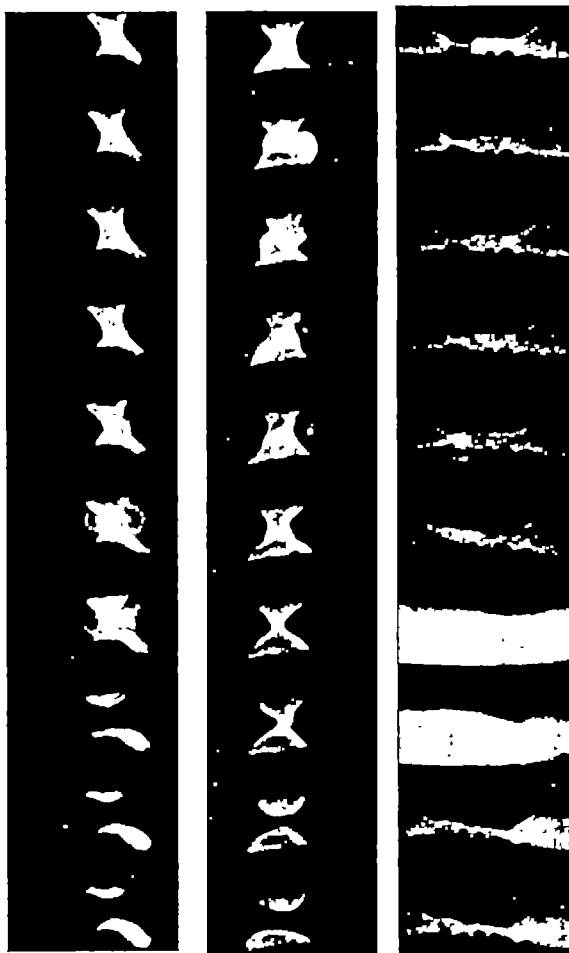


Fig. 1

Fig. 2

Fig. 3

Fig. 1. Material, iron; atmosphere, air; current, 30 amp; circuit R.M.F., 6 V; polarity, top electrode negative; magnification,  $\times 6$ ; framing rate, 6,000 f.p.s.

Fig. 2. Material, iron; atmosphere, air; current, 30 amp; circuit R.M.F., 6 V; polarity, top electrode negative; magnification,  $\times 6$ ; framing rate, 2,000 f.p.s.

Fig. 3. Material, iron; atmosphere, vacuum; current, 60 amp; circuit R.M.F., 6 V; polarity, top electrode positive; magnification,  $\times 19.5$ ; framing rate, 7,000 f.p.s.

they are more consistent with the view that the transfer may well be ionic, the motions of the ions being determined by the oscillatory electric field between the electrodes after the bridge has broken. For reasons such as these an alternative view was put forward based on the production of a micro-plasma, possibly initially formed at the broken neck of the molten metal bridge<sup>11</sup>. Consequently, in recent years effort has been directed to finding direct evidence for the existence of such a plasma. It will be appreciated that, owing to the speed of events in the final stages of the development of the molten metal bridge, extensive expansion of the plasma to a size which can readily be seen may not take place. In fact, particularly in the presence of a high-pressure ambient atmosphere, a plasma of metal vapour might well be severely restricted in size throughout its short life.

#### Photography of the Microscopic Molten Metal Bridge

Early attempts to photograph the development of the exploding bridge were confined to cases in which it would be expected that surface tension would be the dominant controlling force and large stable bridges obtainable. Such photographs have been previously obtained for large iron bridges in air<sup>1</sup>. In appropriate circumstances the oxide film on the surface would enable a constant surface

tension to be set up over the whole surface, and thus produce stability in accordance with (2).

A number of standard ciné films (25 f.p.s.) were taken of the formation, development and final rupture of the bridge, and many thousands of frames were examined in the hope of finding an illustration of the actual rupture. One or two frames were found which showed that the actual rupture process might not be a simple parting of the nodoid, and this indicated that it was necessary to use high-speed photography if rupture was to be examined in more detail. There were considerable difficulties in the high-speed photography of the molten bridge mainly on account of the small area to be photographed ( $\leq 10^{-4}$  cm<sup>2</sup>), the low luminosity for metals other than platinum and tungsten, and the difficulty associated with the synchronization of the camera and the phenomenon to be photographed. A certain degree of elusiveness of the bridge at all stages of its life also made photography difficult. However, an optical system incorporating a high-speed camera was designed and constructed to examine the development of a molten metal bridge for time-intervals down to about 10  $\mu$ sec. In this way a large number of metals were investigated under varying conditions of ambient atmosphere and pressure. Some preliminary photographs thus obtained were shown at conferences at Oklahoma<sup>1</sup>, at Graz<sup>2</sup> and at Berlin<sup>3</sup>. Sets of later photographs giving a succession of frames extending over a total time of a few milliseconds covering in some detail various phases of the rupture of the microscopic molten metal bridge are given here. The results for iron are of particular interest in that they illustrate the three different aspects of the rupture process discussed here, and these are given in Figs. 1, 2 and 3.

Fig. 1 shows a series of photographs at a rate of 10<sup>4</sup> frames per sec and deals with iron in air. Doubtless on account of consequent oxidation affecting the surface tension, well-shaped stable bridges were formed after a number of operations, and these are in accordance with the theoretical prediction of the stable nodoid. Stability could be controlled by surface tension forces, and the fact that the bridge ruptured when the hottest section boiled is clearly indicated by the photographs, which show the two white-hot separate parts of the bridge after rupture.

Fig. 2 illustrates a less stable condition in which the degree of oxidation was such that the surface tension could not be maintained constant over the surface. In such cases stability can only be produced by internal viscous motion, and this is consistent with the effect illustrated

by the rapid change from frame to frame in the location of the hottest region of the bridge surface.

In order to minimize the effect of surface tension forces in establishing stability, the iron surfaces were cleaned by a glow-discharge treatment in hydrogen and the contact was also operated in a vacuum in order to avoid further oxidation. The development of the bridge in this case is illustrated by the series in Fig. 3. This is dramatically different from the two series in Figs. 1 and 2 in that the molten metal bridge no longer severs over a very small section while the bridge remains in the liquid form. On the contrary, in this case an electric micro-discharge appears to have been formed from the completely exploded bridge, producing a high-density plasma of great brightness the duration of which is less than 100  $\mu$ sec and probably less than 10  $\mu$ sec. On account of the inability to expand appreciably in these conditions, the particle density in the micro-plasma must be extremely high.

Similar effects have been found for other metals, and the radio-tracer technique enables the directions of the metallic transfer occurring during this short existence of the plasma to be measured.

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## BASAL PLANE CONTRACTION IN GRAPHITE DUE TO MONO-VACANCIES

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IT has been known for a number of years that crystals of graphite expand along the c-axis direction and contract along the a-axis direction under fast neutron irradiation. The dimensional changes of the c- and a-axis lattice parameters have been observed by X-ray diffraction<sup>1,2</sup> and it is found that the fractional change  $\Delta c/c$  in the c-axis lattice parameter is positive and the fractional change  $\Delta a/a$  in the a-axis lattice parameter is negative. Direct measurements<sup>3,4</sup> of the dimensional changes of graphite crystals and deductions from irradiation data on polycrystalline graphite have shown that the strains  $\epsilon_c$  and  $\epsilon_a$  in the c-axis and a-axis direction of a graphite crystal either exceed or are equal to the lattice parameter changes.

The growth of the crystals in the c-axis direction is explained by the presence of interstitial atoms between the crystal layer planes. The inequality between  $\epsilon_c$  and  $\Delta c/c$  has been explained by Simmons and Reynolds<sup>5</sup> as due to

the aggregation of these interstitial atoms into small new layer planes which contribute to crystal growth but not, of course, to lattice parameter changes. The c-axis growth of the crystal may be expressed in terms of the interstitial atom concentration  $C_i$  as:

$$\epsilon_c = KC_i \quad (1)$$

The constant  $K$  is of order unity, tending to decrease to a limit of unity with increasing aggregation of the interstitial atoms into areas of new layer plane.

The contraction in the a-axis lattice parameter and the basal plane contraction  $\epsilon_a$  have been the subject of some speculation. It has at various times been proposed that the decrease in the a-axis lattice parameter may be explained as follows:

1. Elastic relaxation around the vacant lattice site; however, the extremely tight covalent binding of the layer suggests that this would be a small effect.



2. A Poisson's ratio effect accompanying the *c*-axis lattice parameter change. This follows from the negative thermal expansion coefficient along the basal planes observed by Nelson and Riley<sup>4</sup> and attributed to the same mechanism. The magnitude of this effect is easily shown to be:

$$\frac{\Delta a}{a} = \frac{S_{12}}{S_{33}} \left( \frac{\Delta c}{c} \right) \quad (2)$$

It is shown later that this alone is not adequate to explain experimental data.

3. A buckling of the lattice by interstitial clusters, this being observed as an apparent decrease in lattice parameter.

It is proposed in this note that the major contribution to basal plane contraction is due to the modification of the interatomic linkages in the layer by the presence of the vacancy.

The lengths of the basic C—C and C=C bonds are known to be 1.542 Å and 1.334 Å respectively<sup>5</sup>, whereas the bond length in graphite lies between these values (1.416 Å). This is interpreted by assuming that a partial bond is formed between nearest neighbours by the  $\pi$ -electrons in graphite, as well as the bond formed by the  $\sigma$ -electrons. Coulson<sup>6</sup> has shown that a bond order may be defined in terms of molecular orbital calculations of the electronic structure of molecules which may be used to predict bond lengths accurately. In particular, the interatomic distance in graphite has been predicted by Bradburn, Coulson and Rushbrooke<sup>6</sup>. Table 1 shows the bond-length change relative to graphite as a function of bond order. Coulson<sup>6</sup> shows that the bond length *a* as a function of bond order *P* is given by:

$$a = 1.542 - \left[ \frac{0.298}{1 + 0.765 \left( \frac{-P}{P-1} \right)} \right] \text{Å} \quad (3)$$

The molecular orbital method has recently been applied by Coulson and co-workers<sup>10</sup> to the calculation of the change in  $\pi$ -electron energy of graphite layers of various sizes on removal of one atom (including its  $\pi$ -electron). If the layer contains *n* atoms, then the change  $E$  in the  $\pi$ -electron energy of the molecule is given by:

$$\Delta E_{\pi} = \left[ \frac{n}{0.363n - 0.548} \right] \beta \quad (4)$$

where  $\beta$  is the resonance integral<sup>6</sup>.

It is now postulated that the basal plane contraction is due to the changes in the bond order of the layer due to the formation of a vacancy. The magnitude of this effect may be estimated as follows:

Consider a uniformly distributed concentration of vacancies  $C_v$  in an infinite graphite layer, then each vacancy has associated with it *n* atoms where  $n = 1/C_v$ . If a 'molecule' of the type considered by Coulson *et al.* is drawn around each vacancy containing *n* atoms, then the change in  $\pi$ -electron energy of the molecule is:

$$\Delta E_{\pi} = \left[ \frac{1}{0.363 - 0.548 C_v} \right] \beta \quad (5)$$

The bond order is given by Coulson *et al.* for the graphite layer as:

$$P = 1 + \frac{E_{\pi}}{2B\beta} \quad (6)$$

Table 1. BOND LENGTH CHANGES RELATIVE TO GRAPHITE AS A FUNCTION OF BOND ORDER

Bond	Bond order	Bond length	Length change relative to graphite %
Ethane C—C	1	1.542	+ 8.9
O—O*	1	1.504	+ 6.2
Ethylene C=C	2	1.334	- 5.8
Acetylene C≡C	3	1.206	-14.8

\* Value given by Pauling<sup>5</sup> for use in conjugated and aromatic systems.

where *B* is the number of bonds in the molecule. The change in bond order assuming all unbroken bonds to be equivalent is:

$$\Delta P = \frac{1}{2\beta} \left[ \frac{E_{\pi}^1}{B^1} - \frac{E_{\pi}}{B} \right] \quad (7)$$

where  $B^1 = B - 3$  is the number of unbroken bonds in the 'molecule',  $E_{\pi}^1$  is the  $\pi$ -electron energy of the molecule. Provided  $C_v$  is not too large we may set  $B = B^1$  and thus:

$$\Delta P = \frac{\Delta E_{\pi}}{2\beta B} \quad (8)$$

The 'average' bond-order of the remaining bonds is now given by:

$$P = 1.525 + \Delta P = 1.525 + \frac{1}{2} \left[ \frac{C_v}{(0.363 - 0.548 C_v) 1.500} \right] \quad (9)$$

(1.525 is the calculated bond order of the normal C—C bond in the layer plane).

The change in average bond length of the layer may now be estimated using equation (3) and equation (9). It is found that for a vacancy concentration of up to 5 per cent the length change, which we assume equal to the observed lattice parameter changes,  $\Delta a/a$ , can be represented by:

$$\frac{\Delta a}{a} = -0.14 C_v \quad (10)$$

The presence of an increase  $\Delta c/c$  in *c*-spacing gives a further contraction due to the Poisson effect, thus:

$$\frac{\Delta a}{a} = \frac{S_{12}}{S_{33}} \frac{\Delta c}{c} - 0.14 C_v \quad (11)$$

The values of  $S_{12}$  and  $S_{33}$  given by Davidson and Losty<sup>11</sup> lead to  $S_{12}/S_{33} = -0.07$ .

The principal characteristics of the mechanisms of basal plane contraction discussed here are: (a) The X-ray lattice parameter change  $\Delta a/a$  should agree with the directly measured basal plane contraction, that is  $\epsilon_a \equiv \Delta a/a$ . The same considerations apply to clusters of a few vacancies. (b) The effect of the same total number of vacancies on both lattice parameter and  $\epsilon_a$  will decrease as they aggregate into larger and larger clusters. In the extreme case a large disk-like hole in a layer plane produces no basal plane contraction and no lattice parameter changes. The collapse of this type of cluster gives a *c*-axis contraction of the crystal.

Equation (11) may be compared with direct measurements of basal plane contraction if independent estimates of  $C_v$  and  $\Delta a/a$  are available.

Pluchery<sup>12</sup> has irradiated graphite with reactor neutrons at  $-196^\circ\text{C}$  at which temperature both interstitial atoms and vacancies are immobile. In these conditions no defect aggregation should have occurred at low doses and the interstitial and vacancy concentrations should be equal; it is thus reasonable to assume that  $\epsilon_c \equiv \Delta c/c$ , and  $\epsilon_a \equiv \Delta a/a$ . Using equation (11) for this case we can write:

$$\begin{aligned} \frac{\Delta a/a}{\Delta c/c} &= \frac{S_{12}}{S_{33}} - \frac{0.14}{K} \\ &= -0.07 - \frac{0.14}{K} \end{aligned} \quad (12)$$

where *K* is the value for single interstitial atoms and is expected to be greater than unity. The experimental value taken from Pluchery's data is  $\frac{\Delta a/a}{\Delta c/c} = -0.08$ , thus suggesting that  $K \sim 10$ . The *c*-spacing change due to interstitial atoms has been calculated by Agranovich and Semenov<sup>13</sup> using interatomic potential functions of the spherically symmetric Lennard-Jones type with the constants adjusted to give the interlayer spacing correctly. This force function gives good agreement between theory and experimental determinations of compressibility and

surface energy. Their results yield  $\Delta c/c = 5.3 C_i$  in very reasonable agreement considering the approximations in the present calculation and the theoretical result.

A better comparison with experiment is obtained under conditions where  $K$  is close to unity—that is, where interstitial atoms, but not vacancies, have been allowed to cluster. This condition is realized for small doses where the vacancies are randomly distributed and essentially single at temperatures from about 150° to 350° C. However, although  $\epsilon_s \equiv \Delta a/a$  in these circumstances  $\epsilon_s \neq \Delta c/c$ . The homogeneous nucleation theory<sup>14</sup> suggests that the numbers of interstitial atoms and vacancies are still equal and thus equation (11) and equation (1) can be combined to give:

$$\frac{\Delta a/a}{\epsilon_s} = \frac{S_{11}}{S_{11} - \epsilon_s} \frac{\Delta c/c}{\epsilon_s} - \frac{0.14 C_i}{\epsilon_s}$$

$$= -0.07 \frac{\Delta c/c}{\epsilon_s} - 0.14 \text{ since } C_i = C_i \text{ and } \epsilon_s = C_i$$

In neutron irradiations at 200° C these conditions are fulfilled in Pile Grade A and pyrolytic graphites<sup>15</sup>, and  $\Delta c/c \sim 0.5$ , thus  $\frac{\Delta a/a}{\epsilon_s}$  is expected to be  $-0.17$ , while the observed value is  $-0.17$  in very good agreement. In irradiation experiments at 350° C for doses below  $5 \times 10^{18}$  neutrons per sq. cm (ref. 16),  $\epsilon_s \equiv \Delta a/a$  and an initial value of  $\frac{\Delta a/a}{\epsilon_s}$  of approximately  $-0.17$  is observed. In this case  $K \sim 1$  and the agreement is good.

It is clear from these considerations that the presence of interstitial atoms and single vacancies cannot if they are in equal numbers cause a value of  $-\epsilon_s/\epsilon_s$  in excess of about 0.2. The aggregation of vacancies into the expected type of cluster<sup>17</sup> would reduce  $\epsilon_s$ , and thus reduce the ratio  $-\epsilon_s/\epsilon_s$ , since  $K$  cannot be less than unity. A change of crystal shape, but not volume, corresponds to  $-\epsilon_s/\epsilon_s = 0.5$ , but for this to occur vacancies must be able to annihilate in some way without recombining with an

interstitial atom. It is interesting to note that this condition is approached under some irradiation conditions<sup>18</sup> where vacancies are comparatively immobile and where loss of interstitial atoms and vacancies to sinks can be ruled out. It is thus necessary to postulate new types of vacancy configuration to explain high dose results.

The results given here may be used to estimate the vacancy concentration after neutron irradiation and enable comparison to be made with determinations from other physical property changes. The accuracy of the factor 0.14 in equation (11) is expected to be quite good since the theoretical calculations of bond order are used with empirical information and the results do not rely on any uncertain parameter such as the resonance energy. This is discussed further by Coulson *et al.* in ref. 8.

I thank Prof. C. A. Coulson for his advice.

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## ABNORMAL HAEMOGLOBINS AND THE GENETIC CODE

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DR. F. H. C. CRICK has directed our attention to the latest work of Nirenberg *et al.*<sup>1</sup> which has considerably advanced the definition of the code whereby messenger RNA determines which amino-acid is incorporated into a protein. The code is 'degenerate' and more than one arrangement of three bases or a codon can spell the same amino-acid. The first two bases are, however, somewhat more specific than the third. For example, for glutamic acid (Glu) they are guanine adenine (GA), but the third can be either A or G. However, the first two bases in the codon spelling aspartic acid (Asp) are also GA, but the third may be cytosine or uracil (C or U). Thus the coding for Glu is GAA or GAG. Some amino-acids are coded by any of the four possible bases in the third place of the codon. For example, the coding for threonine (Thr) is ACA or G or C or U. For a few amino-acids only, alternatives have also been found for the first two bases of the codon: leucine (Leu), for example, may be coded UUA or G or as CUG or C or U. From the considerable evidence for A being an alternative third part of the codon when G is one, one might write the second coding for leucine as CUA or G or C or U. Table 1 gives the codons for amino-acids involved in substitutions of human haemoglobin; those surmised because A and G, and C and U, respectively, are alternatives in the third position, are in italics. Table 2 summarizes the amino-acid

substitutions which have been reported in human haemoglobin. They include the abnormal haemoglobins fully described and those for which an amino-acid substitution is known but has not yet been finally allocated to a definite part in one of the polypeptide chains. Furthermore, as the  $\beta$ - and  $\delta$ -polypeptide chains differ in only a very few amino-acid residues, likely to be point mutations, differences between these two chains have also been listed.

Fig. 1 illustrates the possible one-step mutations for the amino-acids listed in Table 2. This scheme covers all substitutions with one exception. As Dr. Crick has pointed out to us, haemoglobin I  $\alpha 16$  Lys  $\rightarrow$  Asp cannot have been the outcome of a single mutation but requires two muta-

Table 1. Messenger Ribonucleic-acid Codons for Amino-acids Involved in Substitutions in Human Haemoglobin

Lysine	AAA or G	Proline	CCA or G or C or U
Asparagine	AA	Leucine	GUA or G or C or U
Threonine	ACA or G or C or U		UUA or G
	AGA or G	Glutamic acid	GAA or G
Arginine	OGA or G or C or U	Aspartic acid	GA
	AG		C or U
Serine	UCA or G or C or U	Alanine	GCA or G or C or U
	AUA or G	Glycine	GGA or G or C or U
Methionine	AUA or G	Valine	GUA or G or C or U
Glutamine	GAA or G	Tyrosine	UA
Histidine	CA		C or U

(Surmised bases in italics)

A, Adenine; C, cytosine; G, guanine; U, uracil

Table 2. AMINO-ACID SUBSTITUTIONS IN HUMAN HAEMOGLOBINS

Substitution	Examples	Substitution	Examples
Lys→Asn	β61, Hikari <sup>1</sup>	Val→Glu	α67, M Milwaukee <sup>22</sup>
Lys→Asp	α16, I <sup>3</sup>	Gly→Asp	α15, J Oxford <sup>24</sup> (also described as "T" Interlaken <sup>25</sup> ); α23, J Medellin <sup>26</sup> ; α57 Norfolk <sup>27</sup> ; β16, Baltimore <sup>28</sup>
Lys→Glu	β98, N <sup>4</sup>	Asp→Gly	α47, L Ferrara <sup>29</sup>
Thr→Lys	β87, D Ibadan <sup>3</sup>	Asp→Asn	β79, G Accra <sup>30</sup>
Asn→Lys	α68, G Philadelphia <sup>4</sup>	His→Tyr	α58, M Boston <sup>31</sup> ; α87 M Iwato <sup>32</sup> ; β2, Tokuch <sup>33</sup> ; α63 M Saskatoon <sup>34</sup>
Glu→Lys	α116, O Indonesia <sup>7</sup> ; β6, O <sup>5</sup> ; β7, Siriraj <sup>6</sup> ; β96, B <sup>12</sup> ; β21 O Arab <sup>7</sup> ; γ6, F Galveston <sup>11</sup>	His→Asp	β143, Kenwood (or His-Glu) <sup>35</sup>
Glu→Gln	α30, G Chicago <sup>13</sup>	His→Asn	β117, A <sub>1</sub> <sup>19,36</sup>
Gln→Glu	α54, Mexico <sup>13</sup>	His→Arg	β68, Zurich <sup>37</sup> ; β117, A <sub>1</sub> <sup>19,36</sup>
Gln→Arg	α54, Shimonoseki <sup>14</sup>	Leu→Arg	β14, A <sub>1</sub> <sup>19</sup> (or B <sub>2</sub> ) <sup>38</sup>
Glu→Gly	β7, San Jose <sup>15</sup>	Ser→Thr	β9, A <sub>1</sub> <sup>19,39</sup>
Gly→Glu	β48 or β56, K Ibadan <sup>16</sup>	Thr→Asn	β13, A <sub>1</sub> <sup>19,36</sup>
Glu→Ala	β23 or β26, G Coonahatta <sup>17</sup> ; β43, G Galveston <sup>18</sup> ; β230, A <sub>1</sub> <sup>19,36</sup>	Thr→Ser	β60, A <sub>1</sub> <sup>19,36</sup>
Ala→Glu	β70 or β76, Seattle <sup>21</sup>	Pro→Gln	β120, A <sub>1</sub> <sup>19,36</sup>
Glu→Val	β6, S <sup>38</sup>		

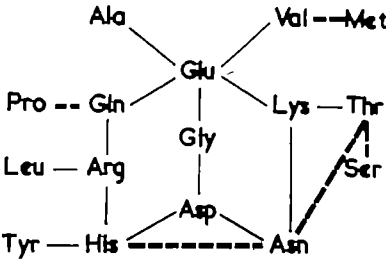


Fig. 1. Possible one-step mutations for amino-acids involved in substitutions in human haemoglobin. The dotted lines indicate differences between  $\beta$ - and  $\delta$ -chains

tions within one codon. As this is a point of considerable theoretical interest we have re-investigated haemoglobin I and have found that, in fact, the substitution is  $\alpha 16$  Lys  $\rightarrow$  Glu. Thus, at present, all amino-acid substitutions in human haemoglobin can be the outcome of single mutations. Degeneracy of the code must be assumed because the last base in the codon for glycine cannot be the same for the mutations Glu  $\rightarrow$  Gly<sup>13</sup> and Gly  $\rightarrow$  Asp<sup>14-16</sup>; similarly, it cannot be the same for arginine in His  $\rightarrow$  Arg<sup>14</sup> and Gln  $\rightarrow$  Arg<sup>14</sup>. The exclusion of the step Lys  $\rightarrow$  Asp or Asp  $\rightarrow$  Lys as a single mutation strongly supports the formula involving  $\alpha 116$  Glu  $\rightarrow$  Lys for haemoglobin O Indonesia<sup>7</sup>. The electrophoretic mobility suggests a double charge change from an acidic to a basic residue. The peptide involved ( $\alpha T p X I I$ ) contains both, one Glu ( $\alpha 116$ ) and one Asp ( $\alpha 120$ ), but as Glu  $\rightarrow$  Arg, Asp  $\rightarrow$  Arg, and Asp  $\rightarrow$  Lys would require a double mutation the likely formula is  $\alpha_1 116$  Glu  $\rightarrow$  Lys<sup>39</sup>. By similar reasoning one can assume that haemoglobin Kenwood<sup>35</sup>, for which two alternative formulae involving  $\beta 143$  His  $\rightarrow$  Glu and  $\beta 143$  His  $\rightarrow$  Asp have been proposed, is likely to be  $\alpha_2 \beta_2 143$  His  $\rightarrow$  Asp.

So far as human haemoglobin is concerned, there are four possible charge changes detectable by electrophoresis at pH 8.6 (that is, a pH at which charge changes do not include the influence of histidine) (Fig. 2). A loss of a negative charge can arise from the replacement of Glu or Asp by a neutral residue, or from replacing a neutral residue by Lys or Arg. The variant of haemoglobin A will then move in the position of haemoglobins S or G. A positive charge can be lost by substitution of a Lys or Arg by a neutral residue or of a neutral residue by Glu or Asp. The resulting variants will then have the mobility of haemoglobins J or K. A double charge change involving either the replacement of a negative by a positive or of a positive by a negative charge can arise by a single mutation only when Glu is substituted by Lys, or Lys by Glu. Thus the haemoglobins C, E, O are all Glu  $\rightarrow$  Lys mutations, and the haemoglo-

bins of the I and N type must be expected to involve a change of Lys  $\rightarrow$  Glu.

It seems remarkable that of eleven glutamic acid residues, nine and possibly ten have been involved in mutations, three of them twice, yet of seventeen aspartic acid residues only one<sup>39</sup>, or possibly two<sup>39</sup>, have been subject to substitutions. On the other hand, changes to Asp and Glu occur with similar frequency, six and five times respectively. The genetic code would permit Glu and Asp to mutate equally well to Val, Ala, or Gly. In addition, whereas Glu can mutate to Lys and Gln, Asp can mutate to Asn, His or Tyr. All such mutations would involve charge changes in the molecule which would be detected by electrophoresis. The frequency of mutations from Glu to other residues and the rarity of replacements of Asp could suggest that Asp may be more important for the structure and function of the haemoglobin A molecule

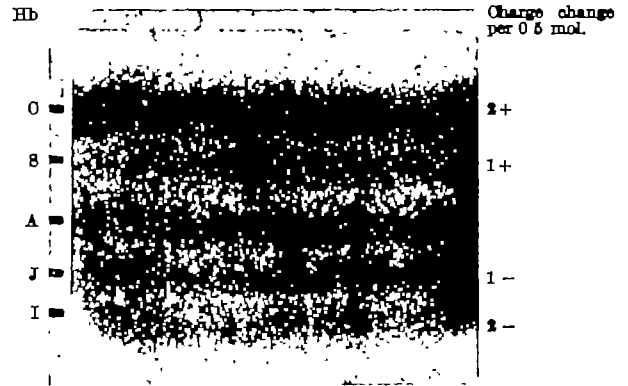


Fig. 2. Paper electrophoresis at pH 8.6 of haemoglobin A, and four variants showing respectively one and two additional positive or negative charges per half molecule

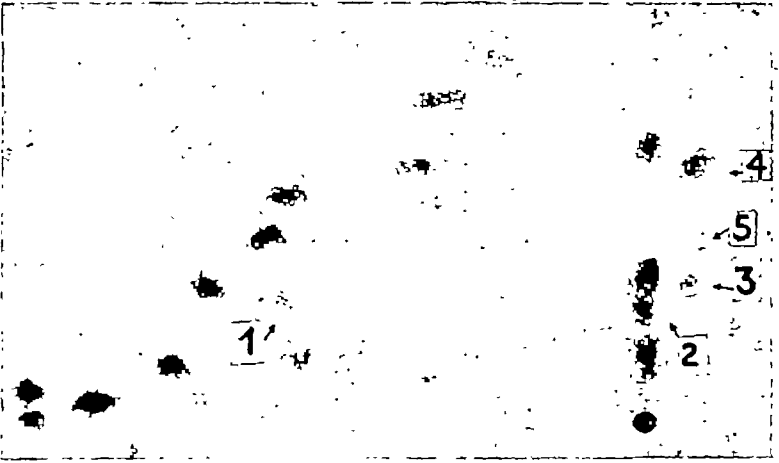


Fig. 3. The 'fingerprint' of haemoglobin I. 1,  $\alpha 2 T p I I I$  missing; 2,  $\alpha 2 T p I V$  missing; 3, new peptide ( $\alpha 2 T p I I I - I V$ ); 4,  $\beta 2 T p V$ ; 5,  $\beta 2 T p V$  (oxidized). Note that the  $\beta 2 T p V$  peptides have the same electrophoretic mobility (horizontal) as the abnormal peptides from haemoglobin I, but they are well separated by chromatography (vertical)



# DECOMPOSITION OF CRYSTALLINE AMMONIUM NITRATE BY ULTRA-VIOLET LIGHT

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**AMMONIUM** nitrate is an important industrial chemical, widely used in explosives and agricultural fertilizers. It decomposes exothermally in moderate heat, yielding mainly nitrous oxide and water, and it therefore presents some hazards during storage and transportation<sup>1</sup>, since it may explode as a result of a local overheating; this partly explains the continued recent interest in the mechanism of its thermal decomposition<sup>2-4</sup>. At the same time, both  $\text{NO}_2$  and  $\text{NH}_4^+$ , and hence ammonium nitrate, account for a substantial part of the atmospheric aerosol<sup>5,6</sup>, which is probably formed predominantly from atmospheric nitric acid and ammonia.

Decomposition of solid, inorganic, nitrates by ultra-violet light, with consequent formation of nitrite and oxygen, was qualitatively investigated in 1935 by Narayanaswamy<sup>7</sup> and, more recently, by Doigan and Davis<sup>8</sup>. Since no analyses of gaseous products resulting from photolysis of  $\text{NH}_4\text{NO}_3$  were carried out at that time the reaction was given further attention in this laboratory, and it was found that exposure of the pure, crystalline, salt to the light obtained from a high-pressure mercury ultra-violet lamp resulted in its superficial decomposition with the yield of  $\text{N}_2\text{O}$  and  $\text{H}_2\text{O}$ , as well as of nitrogen and oxygen.

Experiments were carried out in a tubular, horizontal quartz cell, 3.0 cm inside diameter and 14.0 cm effective length, which was heat-shielded by a quartz jacket cooled by an electric fan. The cell was half-filled with  $\text{NH}_4\text{NO}_3$  and was connected on one side to a high-vacuum and gas-volumetric assembly. The connexion was done through two traps, one of which was removable; interchangeable stoppered glass bulbs could be connected to the volumetric system, in order to take samples for mass-spectrographic analyses of the gaseous products. The quartz cell was provided with a stoppered inlet in order to permit experimentation at atmospheric pressure and in a current of nitrogen or air. The nitrogen gas used was of 99.99 per cent purity: both nitrogen and air were made to pass through a liquid oxygen trap before being admitted to the quartz cell. The flow of gas was constant and of about 20 l./h, as monitored by a wet-test meter (Precision Scientific Co.). A stabilized high-pressure mercury 550-W. 'Hanovia' ultra-violet lamp, model 673 A, was mounted on a rigid support above the cell and illuminated the surface of the salt with about  $16 \times 10^{11}$  quanta/cm<sup>2</sup>/h in the 2540-4350 Å region, as estimated by uranyl-oxalate actinometry<sup>9</sup> which was carried out in the same cell at the beginning and end of the project. Dry, reagent grade ammonium nitrate (Merck, 'as received') was used for the experimentation: the salt was desiccated in the experimental assembly for 48 h, in a vacuum obtained by a fast diffusion pump, before each photo-decomposition was initiated. Experiments were carried out under conditions described in Fig. 1; the oxygen and nitrogen evolved were volumetrically determined after condensing  $\text{H}_2\text{O}$ ,  $\text{N}_2\text{O}$ , etc., in the first trap, which was cooled with liquid nitrogen. This coolant was then replaced by a dry-ice and acetone bath, and the nitrous-oxide fraction (which contained some NO-impurities) was distilled under evacuation into the next trap which, at that time, was cooled with liquid nitrogen. The gases condensed in the second trap were then volumetrically determined and sampled for mass-spectrometry; this was followed by gravimetric determination of the liquid residue in the first trap and a semi-quantitative estimation of its content. All volumetric measurements were greatly simplified by maintain-

ing the room in which the experimental assembly was mounted at  $20.0^\circ \pm 0.5^\circ \text{C}$ .

Fig. 1 gives the total amounts of  $\text{N}_2\text{O}$  evolved with time under different conditions, while Fig. 2 gives a plot of nitrogen-oxygen mixtures evolved in a closed circuit assembly against the amount of nitrous oxide collected. Mass spectrographic analyses, carried out on six different samples of non-condensable gases evolved during this latter series of experiments, indicated a constant ratio of nitrogen to oxygen of  $5.1 \pm 0.1$ ; the NO-content of the nitrous-oxide fraction was found to be of the order of 4.0

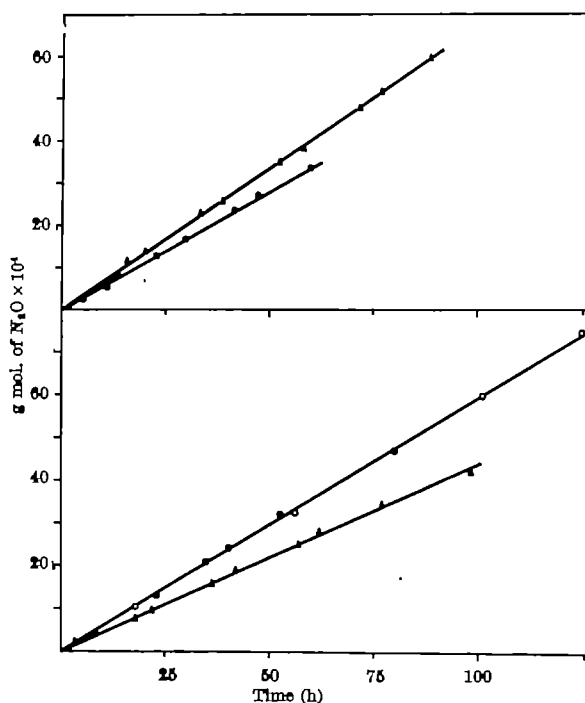


Fig. 1. Plots of nitrous oxide evolved, with time, under the experimental conditions specified, and under irradiation of 48 cm<sup>2</sup> of ammonium nitrate surface with  $6.7 \times 10^{11}$  quanta in the range of 2540-4350 Å, per h. Top: ●, continuous evacuation; ▲, flow of dry air at atmospheric pressure. Bottom: ○, apparatus evacuated and closed; ▲, flow of nitrogen at atmospheric pressure.

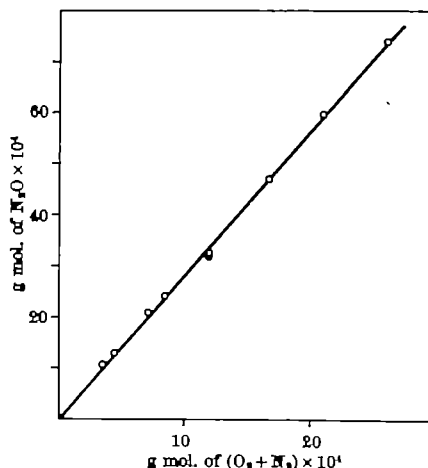


Fig. 2. Ratio of nitrous oxide and NO impurities, to oxygen-nitrogen mixture evolved, with time, in a closed vessel.

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Table 1. SUMMARY OF RESULTS OBTAINED UNDER DIFFERENT CONDITIONS OF PHOTOLYSIS OF SOLID AMMONIUM NITRATE

Conditions of experiment	Total time of irradiation (h)	$\frac{dN_2O}{dt}$ (moles/h $\times 10^4$ )	$\frac{N_2O}{N_2 + O_2}$ (moles)	Molar ratio, $H_2O : N_2O$ , developed during total irradiation	Analysed content of liquid residue, in moles $\times 10^4$		
					$NH_4^+$	$NO_2^-$	$NO_3^-$
Continuous evacuation	60.17	$5.6 \pm 0.06$	—	6.25	7.2	0.8	4.1
Assembly evacuated and closed	134.75	$5.9 \pm 0.03$	$2.8 \pm 0.02$	5.2	3.0	0.1	0
Flow of $N_2$ , atmospheric pressure	98.50	$4.4 \pm 0.04$	—	4.90	3.0	0.8	9.1
Flow of air, atmospheric pressure	89.18	$6.6 \pm 0.08$	—	4.96	6.6	0.1	4.6

Ten g of salt used, when dissolved in water, gave 1 l of solution at pH = 5.50; ultra-violet-irradiation of salt did not appear to change acidities of solutions prepared, appreciably. Liquid residues were at all times alkalinised with NaOH immediately after trap was opened. The experiment, which was carried out in a closed assembly, yielded, however, 0.38 g of liquid which gave, with water, 100 ml. of solution at pH = 3.0.

$\pm 2.0$  per cent. Water was estimated gravimetrically as total liquid residue;  $NO_2^-$ ,  $NH_4^+$  and  $NO_3^-$  were determined colorimetrically with brucin, Neesler and Griess reagents, respectively. Since, under our experimental conditions and during 3 days of continuous evacuation, we were unable to transfer, in a blank run, from the quartz cell into the trap at temperatures of liquid nitrogen, amounts of  $NH_4NO_3$  which could be colorimetrically detected by the Neesler reagent, it was assumed that the vapour pressure of this salt was low<sup>18</sup>, and that the bulk of the reaction occurred in the solid phase.

The differences between the rates obtained under the experimental conditions described, are probably due to products of reaction which contaminate the surface photolysed (compare with ref. 2). 'Contaminations' of the frozen water fraction are at their lowest in the series of experiments which were carried out in a closed circuit, possibly due to higher retention times of the gaseous products in the quartz cell. This may indicate that the impurities quoted and found in the water fraction originate from reactions occurring beyond the irradiation zone between free-radical species, with possible contribution of vapours of such compounds as nitramide and nitrosamine, which are thought to be precursors of  $N_2O$  and  $N_2$  in the thermal decomposition of  $NH_4NO_3$  (refs. 3, 11). It may, in fact, be of interest that since no hydrogen-peroxide could be found by the titanium sulphate test in the liquid fraction, the introduction of  $NH_2-NO$ , as one of the main products of the reaction, together with  $N_2O$ ,  $H_2O$ ,  $N_2$  and  $O_2$ , is convenient here in order to account for the material balance of the process expressed along the lines of the results contained in Table 1; although it is evident that some of the 'impurities' condensed in the water-fraction may originate directly from photolysis of  $N_2O$  in the near ultra-violet (compare with ref. 12).

Nitrous oxide is known to be present in the Earth's atmosphere<sup>13,14</sup>; and it was hitherto thought to originate

either from  $N_2$  and oxygen atoms derived from ozone, as shown by Harteck and Dondes<sup>15</sup>, or through the action of short ultra-violet light on nitrogen and oxygen mixtures, as discovered by Groth and Schierholz<sup>16</sup>, or else, apparently, by bacteria of the soil<sup>17</sup>. The present contribution strongly indicates that photolysis of atmospheric ammonium nitrate aerosol may contribute to the formation of atmospheric  $N_2O$ , and that ultra-violet light may partly account for the decomposition of the  $NH_4NO_3$ -component of the natural aerosol, and hence reduce its power as 'atmospheric fertilizer'.

We thank Dr. G. G. Volpi, and his collaborators at the University of Rome, for the gas analyses, which were carried out on an analytic Ital-Elettronica 'SP 21 F' mass spectrometer; we thank Prof. V. Caglioti for his advice and Mr. C. Luttazzi for his help.

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## DEMONSTRATION OF AN AVIAN LEUCOSIS GROUP ANTIGEN BY IMMUNODIFFUSION

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A COMPLEMENT-FIXING antibody response has been demonstrated in tumour-bearing mammals in the adenovirus<sup>1</sup>, polyoma<sup>2</sup>, SV40 (ref. 3) and Rous sarcoma<sup>4,5</sup> systems. This response has been shown to be directed against viral antigens in some cases, and in other cases against antigens present in tumours but not in standard viral preparations. In the case of the Rous system, the antigen in question was found in various tumour and viral preparations of all members of the

avian leucosis group, and was specific for that group of viruses<sup>4-7</sup>.

It was our purpose to determine whether the Schmidt-Ruppin (S-R) Rous hamster antibody not only fixes complement but also gives precipitation in agar gel. The basic technique consisted of testing various preparations of avian leucosis antigens against S-R tumour-bearing hamster serum with the Ouchterlony double diffusion technique.

The test used was a modification of the micro-method described by Crowle<sup>8</sup>. The agar was 1 per cent 'Ionagar' with 1 per cent sodium azide. Plates were routinely

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Table 1. SUMMARY OF ANTIGENS TESTED IN GEL DIFFUSION AGAINST SCHMIDT-RUPPIN HAMSTER SERUM  
CF and gel reactions by type of antigen

	Tissue culture CEF	OAM	Serum	Organ extracts (spleen, muscle, liver and kidney)	Chicken tumour	Ham- ster tumour
Bryan Rous virus	> 64/± *				64/+	
Schmidt-Ruppin- Rous virus	8/-	> 64/+			64/+	32/+
Purdy strain, Rous virus					32/-	
RIF (field isolate)	64/-					
Lymphomatosis (field strain)				64/+		
RPL 12	32/+			4/-		
Myeloblastosis (strain A)	< 8/-		40++	> 256/+		
Erythroblastosis (strain R)	> 16/-		40/+	8/-		
Warrenton agent					64/+	
OT 10					2/-	
Fuginami					64/-	
Normal controls†	0/-	0/-		0/-		

\* CF antigen titre to S-R hamster pool 11/gel diffusion reaction. +, Antigen preparations giving positive reactions; —, antigens tested that did not give positive reactions; blanks indicate no test performed.

† 40, anti-complementary.

‡ Normal chicken muscle, chorio-allantoic membrane and chick embryo fibroblast.

incubated for 48–72 h in a moist chamber, examined, dried, stained with a protein stain\* and examined again. Complement-fixation tests were carried out by the method previously described\*.

The following leucosis antigens were tested: Rous sarcoma (Bryan, Purdy and Schmidt-Ruppin strains), resistance-inducing factor (RIF) (a naturally infected chicken embryo fibroblast culture), lymphomatosis (organ extracts of a field case of visceral lymphomatosis), RPL 12 strain, myeloblastosis (strain A), erythroblastosis (strain R), and Warrenton agent (naturally occurring avian sarcoma)<sup>10</sup>. Fuginami and OT 10 avian sarcomata were also tested.

Antigen preparations included clarified extracts of chicken and hamster tumours, clarified chicken organ suspensions, chorioallantoic membrane preparations, infected chick fibroblast tissue, culture harvests\* and serum of viraemic birds. Most of these have been more fully described elsewhere\*. Hamster sera consisted of several lots of pooled Schmidt-Ruppin tumour-bearing hamster sera from animals with large transplanted tumours. Most tests were done with pool 11, which was derived from 15 hamsters and had a complement fixation titre of 1:160 against four units of hamster tumour extract antigen.

Results are summarized in Table 1. Both the Bryan and S-R strains of Rous sarcoma virus gave positive reactions. Both hamster and chicken tumours reacted with the latter strain. With the exception of Fuginami, OT 10, and RIF antigens, positive gel-diffusion reactions were obtained with at least one preparation of all the leucosis viruses tested. Although the Fuginami, OT 10 and RIF preparations gave positive complement-fixation reactions, the antigen may have been poorly precipitating.

A positive reaction usually consisted of a single precipitation line between the antigen in question and the hamster serum. Rarely this appeared as a double line. The occurrence of single or double reactions was not consistent in successive tests, and no definite pattern could be established. In all cases, however, the lines of the various antigens tested (whether single or double) were continuous, showing a reaction of identity. This was true regardless of the source of the preparation. No spurs or crosses were observed with the hamster serum. Fig. 1 is a pattern illustrating these findings. The hamster serum gives a continuous precipitation line with Bryan wing web tumour extract, myeloblastosis and lymphomatosis kidney extracts. The S-R hamster tumour extract at the top of the field did not react in this test. Two control antigens also failed to react.

Several attempts to use avian sera in the Ouchterlony system were unsuccessful. Despite the presence of high salt (8 per cent sodium chloride), the lines have either

been very faint or absent. The results of one pattern, however, did indicate at least partial identity, with spurring between the reactions of the avian hyperimmune and hamster sera. This indicated a possible one-way cross.

Immunoelectrophoresis (using Scheidegger's technique\*) was attempted in the hope of distinguishing any separate components in the system. Only one precipitation line, migrating toward the negative pole at pH 8.2, was found. However, the possibility that other components were lost cannot be excluded.

Attempts were made to determine whether the precipitating antigen was identical to the soluble complement-fixation antigen previously described\*. Gel-diffusion tests were performed on soluble and sedimentable fractions (pellet and supernatant of clarified tumour extract spun at 105,000g for 2 h) of S-R and Bryan chicken tumour extracts. Soluble and sedimentable fractions of a Bryan wing web tumour preparation having titres of 1:64 in complement fixation against S-R hamster serum pool 11 (refs. 4, 5) both gave positive reactions (Fig. 2). Similar results were also obtained with S-R wing web antigen. Precipitates were obtained with both ether-tested and non-treated material. In general, reactions with the sedimentable fractions tended to be somewhat more pronounced than those with the soluble fractions.

To show the specificity of the gel diffusion reaction, the S-R hamster serum was tested against many control antigens, all of which were negative. These included normal chick muscle, chorioallantoic membrane, and

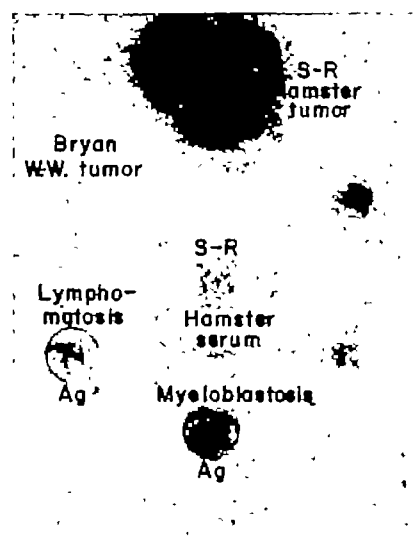


Fig. 1. The S-R hamster serum undergoes a common reaction with the myeloblastosis, lymphomatosis and Bryan wing-web antigens. The S-R hamster antigen does not react in this test.

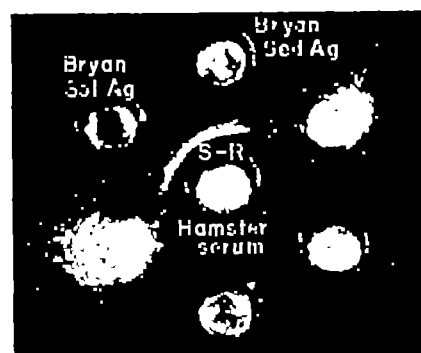


Fig. 2. Soluble and sedimentable fractions of Bryan wing-web tumour share a common reaction with the hamster serum.



Table 2. COMPLEMENT-FIXATION TITRES OF ROUS LEUCOSIS ANTIGENS IN RELATION TO REACTIVITY IN GEL DIFFUSION AGAINST S-R HAMSTER SERUM

Ouchterlony reaction	OF titres			Total
	High (64+)	Medium (16-32)	Low (0-8)	
Positive	18	9	1	28
Negative	5	11	16	32
Total	23	20	17	60

$$\chi^2 = 2.15, P < 0.05$$

chicken embryo tissue culture cells. Other chicken and human viruses which also failed to react included mumps, *ORLO* (ref. 11), vesicular stomatitis, a chicken enteric virus<sup>14</sup> and measles. Oncogenic viruses such as adenovirus 12 (virus and tumour extracts), *SV40* and Moloney mouse leukaemia virus were also negative when tested against the S-R hamster serum. In addition, tumour-bearing hamster sera of the adenovirus 12 and *SV40* systems and Yaba monkey tumour serum were all negative when tested against the S-R antigen (chorioallantoic membrane grown virus that reacted in gel with the S-R hamster serum pool 11).

Two other S-R hamster serum pools<sup>9,12</sup> and one individual hamster serum (24338) all gave identical reactions to that described here for hamster serum pool 11, with which most of the work was done.

Efforts were made to show statistical correlation between the complement fixation<sup>8</sup> and Ouchterlony results. These are summarized in Table 2, where all the antigens tested were arbitrarily divided into high (64+), medium (16-32) and low (0-8) reactors and compared with the Ouchterlony results. High complement-fixation titre and positive gel reactions correlate well, as evidenced by the  $P$  of  $< 0.01$  on  $\chi^2$  testing.

These results confirm the findings of Huebner *et al.*<sup>4</sup>, Sarma *et al.*<sup>5</sup>, and Armstrong *et al.*<sup>7</sup> regarding the presence

of a common group-specific antigen shared by all members of the avian leucosis group of viruses and reactive with the sera of hamsters bearing Schmidt-Ruppin tumours. The specificity of this gel-diffusion reaction for avian leucosis viruses has been confirmed by the negative control reactions with normal chicken antigens, unrelated avian viruses and non-avian tumour virus preparations. None of the tests provided unambiguous evidence for the presence of more than one antigen.

Gusev<sup>15</sup> has used the gel-diffusion test with hyper-immunized rabbit anti-Rous sera to demonstrate a specific Rous sarcoma antigen, not present in normal chick tissue or chemically induced tumours. It remains to be ascertained whether this antigen is the same as the one we have demonstrated.

We thank Dr. Wallace P. Rowe for his advice, and Dr. Robert J. Huebner and William T. Lane for providing tumour-bearing hamster antigens and antisera.

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## PROLIXIN-S AND PROLIXIN-G; TWO ANTICOAGULANTS FROM *Rhodnius prolixus* STÅL

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WE have previously described an anticoagulant<sup>1</sup> and a fibrinolysin<sup>2,3</sup> in the gut of the blood-sucking hemipteran *Rhodnius prolixus*, and another anticoagulant in the salivary glands of this insect. The two anticoagulant activities appeared to be due to two distinct substances, and experiments confirming this are described here. We have called these two activities prolixin-S and prolixin-G to indicate the anticoagulants from the salivary gland and gut respectively.

Saline extracts were prepared and tested as described earlier<sup>1</sup>. The salivary glands and the entire gut were dissected out and placed into ice-cold 0.9 per cent saline allowing 0.1 ml. for each insect. All extracts were homogenized and centrifuged and the supernatant, which contained the anticoagulant activity, was decanted and tested on recalcified horse plasma.

Neither extract could be diluted to any great extent without considerable loss of demonstrable activity. It seemed a possibility, however, that if their mechanisms of action could be established and if the crude extracts could be purified, more specific test systems and greater dilutions of the active materials might be used.

The actions of prolixin-G and -S were examined on isolated stages of the clotting system. The last stage, the conversion of fibrinogen to fibrin by thrombin, was examined first. If inhibition of this reaction was insufficient to account for the anticoagulant activity, the preceding stages of the clotting system were examined by

means of the one-stage prothrombin time<sup>4</sup> and the kaolin-cephalin clotting time<sup>5</sup>.

The action of thrombin (Leo Laboratories Ltd., 5 units/ml.) on bovine fibrinogen (0.5 per cent Armour Pharmaceutical Co. Ltd.) was inhibited by prolixin-G even at dilutions of 1 in 1,000. The antithrombin activity of prolixin-S was too weak, however, to account for its anticoagulant activity and it did not retard the one-stage prothrombin time. On the other hand, the kaolin-cephalin time was greatly prolonged, which suggested that prolixin-S inhibited any or all of the clotting factors VIII, IX, XI and XII. The increase in kaolin-cephalin clotting time was unchanged irrespective of whether prolixin-S was added before or after activation of the contact factors XI and XII. The implication, therefore, was that prolixin-S must interfere with the activation of factors VIII or IX. Reconstitution of factor VIII or IX deficient plasmas with their respective factors, which had previously been incubated with prolixin-S, showed that the main activity of this substance was on factor VIII (the anti-haemophilic factor).

Purification of prolixin-G was attempted by running extracts of gut in saline through 'Sephadex G 100' (1 g in saline; column width 1 cm; length 35 cm; fraction volume 2 ml.) and eluting with 0.9 per cent saline. When elution with distilled water was tried the activity precipitated out on the column. Antithrombin activity began to appear when the elution volume had reached 14 ml. and attained

its peak at 18 ml. The molecular weight of prolixin-*G* thus seemed to be greater than the exclusion limit (100,000 for 'Sephadex *G* 100'). When extracts were run through 'Sephadex *G* 200' (1 g in saline; column width 1 cm; length 35 cm; fraction vol. 2 ml.) the appearance of antithrombin activity was retarded and did not appear until fractions 42 and 43 (84–86 ml.). These results would seem to give prolixin-*G* a molecular weight of between 100,000 and 200,000. They also suggest that prolixin-*G* is a protein of high molecular weight and possibly a euglobulin; a suggestion supported by its solubility in saline and insolubility in distilled water.

Purification of the crude material from gut and salivary glands was also attempted by partial precipitation with alcohol, ammonium sulphate and acetone. Of these, acetone proved to be the most useful. Both prolixin-*G* and -*S* were precipitated by addition of 3 vol. of acetone to 1 vol. of extract, and prolixin-*G* could be extracted from the precipitate with 1 vol. of saline, but not with distilled water. On the other hand, with prolixin-*S* the reverse was the case. In both instances a large amount of inactive material remained in the precipitate.

Extraction of the acetone precipitate of gut extract with distilled water resulted in a clear solution, active in inhibiting the clotting of recalcified plasma though not the thrombin-fibrinogen reaction. This suggests that the activity recovered by distilled water extraction was not the gut antithrombin prolixin-*G*. If saline was used to extract the precipitate rather than distilled water, then the resultant activity had both a greater inhibition on the clotting of recalcified plasma, and an antithrombin activity. It is, therefore, reasonable to assume that the gut extract consists of the two activities prolixin-*G* and prolixin-*S*, though in greatly different proportions, and that prolixin-*S* is found in the gut extract because it is carried back with the blood meal.

Acetone precipitation of gut extract and saline extraction of the precipitate achieved an approximately ten-fold purification of prolixin-*G* when calculated on the nitrogen content of the crude and extracted material (Table 1).

Table 1. NITROGEN CONTENT OF EXTRACT BEFORE AND AFTER PURIFICATION

µg Nitrogen per ml. extract		
Estimation	Crude	Purified
I	8,100	800
II	1,060	137

The inhibition of thrombin by gut extract was found to be a time-consuming reaction. When gut extract and thrombin (5 units/ml.) were incubated together at 37° C and 0.1 ml. aliquots added to fibrinogen every minute, the length of the clotting time increased with length of incubation. After 10 min a maximum of approximately three times the original inhibition was reached and on further incubation the clotting time decreased again. When, however, partially purified prolixin-*G* was incubated with thrombin, the clotting time increased over a period of 1 h, at which time it was five times that of the initial value. It is probable, therefore, that in the crude extracts, gut enzymes destroy prolixin-*G*. Furthermore, it appears that the reaction between prolixin-*G* and thrombin is not stoichiometric.

It seemed possible that prolixin-*G* might be derived from prolixin-*S* by digestion in the gut of the insect. In order to test this possibility, gut extracts were incubated with prolixin-*S*. If the hypothesis was correct, then an increase of prolixin-*G* might be expected to develop in the incubation mixture, but no such increase occurred. It is unlikely, therefore, that although both substances are found in the gut any conversion of prolixin-*S* to prolixin-*G* occurs.

Tests using the thrombin-fibrinogen reaction confirmed earlier findings reported on plasma<sup>1</sup> that loss of activity was slight when gut extracts were heated to 80° C for 5 min, but considerable after 5 min at 100° C. The clotting inhibition of prolixin-*S* and prolixin-*G* remained largely unaffected at 4° C, but prolixin-*G* increased with

standing freezing and immediate thawing could not be stored at -20° C without loss of activity during the first 24 h, after which period no further loss occurred. This is somewhat difficult to explain, though the effect of freeze-drying the gut extract which always resulted in a moderate loss of activity is more readily understandable. In contrast to this, prolixin-*S* could be stored at -20° C, and freeze-dried, without loss of anticoagulant action.

Attempts to stabilize prolixin-*G* by adjusting the pH showed that alkalinization did not prevent the loss of activity which occurs on standing for 24 h or longer at room temperature while acidification to pH 3 did so in 3 out of 5 experiments.

Better results were obtained when the crude extract prepared in the usual way was dried at room temperature in a desiccator under reduced pressure and over calcium chloride. It would then store at 4° C without appreciable loss of anticoagulant activity.

Dialysis of salivary gland extract at 4° C against saline caused no appreciable loss of activity of prolixin-*S*, but gut extracts lost about half their prolixin-*G* activity.

Gut extract incubated for 10 and 30 min with a concentration of trypsin which failed to affect the test reactions (final concentration of 10<sup>-3</sup> mg/ml.) resulted in a slight reduction of anticoagulant activity on recalcified plasma, but did not affect the increase of clotting time of the thrombin-fibrinogen reaction. On the other hand, with salivary gland extracts, a considerable loss of activity occurred after incubation for 10 min.

It is clear from the foregoing experiments that the plasma test system is not sufficiently critical to distinguish between the anticoagulants prolixin-*S* and prolixin-*G* in gut extracts, and that only with a more specific test system, such as the thrombin-fibrinogen reaction, can these activities be separated. A summary demonstrating this point is given in Table 2.

A quantitative assay of the antithrombin activity of partially purified gut extract was made using the thrombin-fibrinogen reaction. 1 mg of this extract had an antithrombin activity of 100 antithrombin units (ATU), where 1 ATU is equivalent to that amount of heparin which blocked one National Institutes of Health Unit of thrombin. Heparin under the same experimental conditions has an activity of 500 ATU/mg and 1 mg of hirudin is reported to neutralize 8,500 units of thrombin<sup>4</sup>.

Because of the relative stability of the prolixin-*G* to acidification it seemed unlikely, however, that it was either a heparinoid substance or heparin itself<sup>4</sup>. This assumption was strengthened by lack of inhibition of prolixin-*G* by protamine sulphate and lack of meta-chromatic reaction with toluidine blue<sup>3</sup>. It would also suggest that prolixin-*G* is not a highly sulphated compound. Prolixin-*G* and heparin do, however, give similar dilution curves when tested on plasma (Figs. 1 and 2).

The differences in molecular weight (hirudin mol. wt. equals 18,000 (ref. 5); prolixin-*G* mol. wt. equals 100,000–200,000) rule out the possibility that the gut antithrombin activity of *Rhodnius prolixus* is due to hirudin.

No anticoagulant activity could be demonstrated in gut extracts prepared immediately after a blood meal (although this developed 4 h later (Fig. 3)). However, the

Table 2

Treatment	Salivary gland extract (Prolixin- <i>S</i> )	Gut extract (Prolixin- <i>G</i> + prolixin- <i>S</i> ) on plasma	(Prolixin- <i>G</i> ) on thrombin
Heating 80° C (5 min)	+	+	+
100° C (5 min)	+++	+++	+++
Cooling -20° C (10 min) (24 h)	-	++	++
N/10 HCl	+	-	-
N/10 NaOH	-	-	-
Freeze-drying	-	++	++
Dialysis	-	++	++
Centrifugation (100,000g)	-	-	-
Trypsin	+++	+	-
Protamine sulphate	Not done	-	-
Toluidine blue	Not done	-	-

Loss of activity, nil, -; slight, +, moderate, ++; considerable, +++.

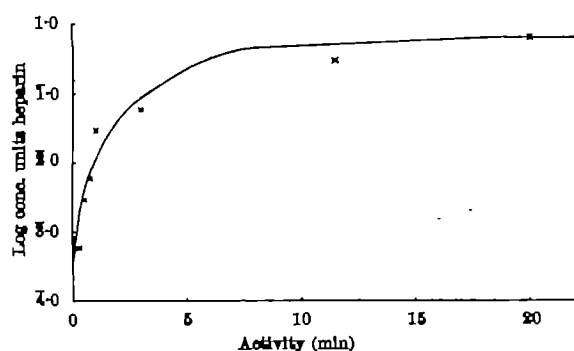


Fig. 1. Dilution curve of heparin on plasma

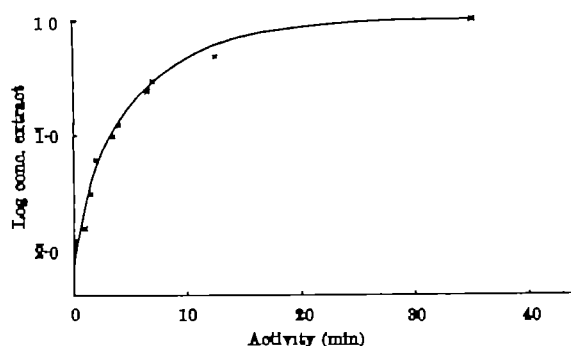


Fig. 2. Dilution curve of gut extract on plasma

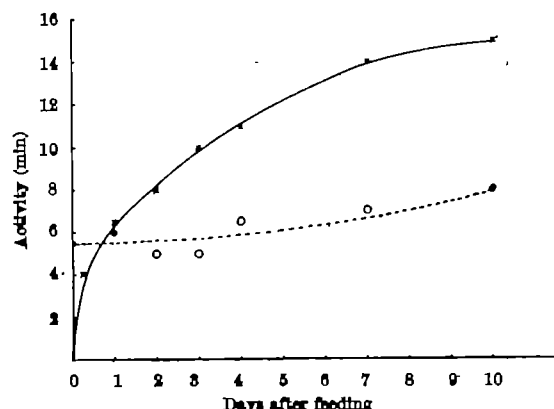


Fig. 3. Effect of starvation on prolixin-G (x) and prolixin-S (O) activity

salivary gland activity did not disappear after feeding and, therefore, gut extracts might have been expected to show some prolixin-S activity.

It seemed possible that anticoagulant action in these extracts was masked by inhibitors, an assumption which was the more likely when acetone precipitation or heating to 60° C for 3 min revealed anticoagulant activity (Table 3).

Table 3. ANTICOAGULANT ACTIVITY PRESENT IN GUT EXTRACT IMMEDIATELY AFTER BLOOD MEAL

Extract	Activity (min) on recalcified horse plasma
Crude	0'
Extract of acetone precipitate	12'
60° C for 3 min	19'

Markwardt and Schulz<sup>7</sup> have obtained an anticoagulant, reduviin, by puncturing the abdomen of *Rhodnius prolixus* fed on saline. This anticoagulant, which was claimed to be largely purified, was found to react stoichiometrically with thrombin, with an activity of 10 ATU/insect. This compares with 50 ATU/insect found with our partially purified material which does not appear to enter into a stoichiometric reaction with thrombin. Artificial diets such as saline are known to alter drastically enzyme activities in the insect gut<sup>8</sup>, and it is difficult to know whether in this particular instance secretions are stimulated when the insect is fed this artificial diet. Because the gut anticoagulant in the normally fed insect is a mixture of prolixin-S and prolixin-G, it is also difficult to compare these two anticoagulants with that described by these authors. It is furthermore conceivable that other members of the reduviid family may possess anticoagulants with different characteristics from those of *Rhodnius prolixus*<sup>9</sup>. For all these reasons and whatever may be the character of the anticoagulant described by Markwardt and Schulz, we regard the name reduviin as inappropriate.

In contrast to the 'anticoagulin' present in certain species of mosquitoes, prolixin-S and prolixin-G are found in both sexes of the adult insect and in the pre-adult nymph stages. Furthermore, no evidence has been obtained of the presence of any 'coagulin' or agglutinin such as have been reported for some mosquitoes<sup>10</sup> and blood-sucking muscids<sup>11</sup>.

Naturally occurring antithrombins are known in blood-suckers, but the presence of an antifactor VIII has not previously been reported, and the presence of two anticoagulants with different mechanisms of action in one such insect also appears not to have been previously recorded.

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## EFFECT OF OXYGEN PRESSURE ON RADIOSENSITIVITY OF EHRLICH ASCITES TUMOURS AT VARIOUS INTERVALS AFTER INOCULATION

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THESE experiments were designed to compare the radiosensitivity of the hyperdiploid Ehrlich ascites tumour ELD Lettré (EAT) cell X-irradiated *in vitro* in the presence of either nitrogen at 1 atmosphere or oxygen at 5 atmospheres absolute pressure (HPO). The method was essentially that described by Dechener

and Gray<sup>1</sup>. The ascites tumour was removed after 5 days growth in O3H mice. The fluid from 3 or 4 mice was rapidly pooled and diluted to 4 ml. with normal saline solution at 35° C. This sample was introduced into a flat polystyrene dish, which was placed in a special pressure vessel consisting of an insulated water-jacketed steel

chamber, thermostatically controlled to  $\pm 0.5^\circ\text{C}$ , with a 'Perspex' irradiation portal sealed into the flat upper surface. The latter was removable and sealed by an O-ring pressure seal. The gas circulated through a metal coil in the water-jacket and entered the chamber through 3 angled microjets directed to the surface of the fluid as described by Gray<sup>2</sup>. The circular stirring action gave rapid equilibration. Using pure nitrogen ( $\text{O}_2$  content  $< 20$  p.p.m.) as the gas phase at atmospheric pressure at  $20^\circ\text{--}35^\circ\text{C}$ ,  $p\text{O}_2$  was measured with open-ended gold electrodes<sup>3</sup> and showed zero current in the fluid within 5 min of commencing gassing. 20–25 min equilibration time preceded all irradiations. The plastic dish rested on bolus material in the chamber and was surrounded by similar bolus to provide back scatter. The X-ray factors used were 200 kV, 15 mA half-value layer 1.0 mm Cu, giving a dose rate in the fluid of 52.8 rads/min. The dose variation, measured within the 2-mm depth of ascitic suspension, was  $\pm 3$  per cent. The time taken between aspiration of the donor cells and re-inoculation of recipient mice with 0.3-ml. aliquots of the suspension after irradiation or mock irradiation was less than  $1\frac{1}{2}$  h. 20–25 min was allowed for gas equilibration and 10–20 min for radiation times. For a further 20–25 min, the same gas phase was maintained as a post-irradiation treatment. Thus about 30 min was largely occupied in preparing the suspension, etc., before gassing and re-inoculation of mice. At intervals of 6–24 h after inoculation, recipient mice were killed by cervical fracture and peritoneal fluid was removed and added to acetic-methanol (1:3) for fixation. Smears were prepared and stained in acetic-orcein, and all telophase abnormalities (acentrics and dicentrics), classed as bridges, fragments or both, were scored and pooled as aberrations. 100 (including normal) telophases were scored per slide. Percentage aberrations for 4–6 slides per mouse were averaged. The statistical analysis was based on 8–48 mice per post-inoculation time. Doses used were 20–250 rads in HPO, and 40–875 rads in  $\text{N}_2$ . However, samples irradiated with lower doses and showing  $< 10$  per cent aberrations after correction for the spontaneous aberration rate, which averaged 4 per cent for parallel gassed but unirradiated controls, were excluded to restrict calculation of aberration coefficients to the exponential portion of the dose-response curves (see below). The aberration coefficients ( $\alpha$ ) were calculated as described by Gray<sup>2</sup> from:

$$\alpha = \frac{\log_e F_i - \log_e F_R}{D}$$

where  $F_R$  and  $F_i$  represent the percentage abnormal telophases in irradiated and unirradiated control samples respectively and  $D$  is the dose in rads.

In our irradiated material, a peak in the induction of abnormal telophases at 15 h post-inoculation time, described by Deschner and Gray<sup>1</sup> for both  $\text{O}_2$  and  $\text{N}_2$  irradiations, did not occur. In similar studies of Hornsey *et al.*<sup>4</sup>, radiosensitivity parameters were calculated 18 h after inoculation, but their reported data did not establish a significant maximum effect for this time. Gray *et al.* have recently reported a "shift" in the peak effect for "aged" cells to a shorter post-inoculation time<sup>5</sup>. In our material, percentage aberrations for all radiation doses in HPO,  $\text{O}_2$  and  $\text{N}_2$ , respectively were consistently higher at shorter post-inoculation times (6–8 h), and thereafter progressively decreased (Fig. 1). However, aberration rates were high and mitotic indices low for early post-inoculation times (6–10 h) for HPO,  $\text{O}_2$  and  $\text{N}_2$  exposures respectively.

A striking difference between irradiations in HPO and  $\text{N}_2$  was that the mitotic index at 6 h in HPO was too low to score the incidence of aberrations. This applied also to the unirradiated HPO control samples. However, the spontaneous aberration rates in unirradiated HPO-exposed cells were not significantly greater than those in  $\text{N}_2$  controls irrespective of post-inoculation time and never

exceeded 5 per cent. Apparently HPO *per se* induced a period of arrest in cell division. Oxygen at 1 atmosphere caused less mitotic delay. The arrest in HPO could not reasonably be attributed to the medium (normal saline) used to suspend cells, to temperature differences ( $35^\circ\text{C}$  throughout) or to 'ageing' or 'metabolic damage' resulting from different pre- or post-irradiation manipulation times. The latter were kept to a minimum despite the report<sup>1</sup> that when cells stood for 30 min after irradiation at room temperature the results were not significantly affected and, in experiments lasting 6–8 h, spontaneous aberration rates did not increase.

The results illustrated by Fig. 1 showed that aberration rates for several post-inoculation times should be measured to study the oxygen effect. However, a further complication arose in the calculation of aberration coefficients. Log response-dose curves for irradiations in HPO and  $\text{N}_2$  were not strictly linear for higher doses in HPO which yielded  $< 40$  per cent normal telophases; the percentage telophase aberrations exceeded the value based on linearity, particularly for longer (16–24 h) post-inoculation

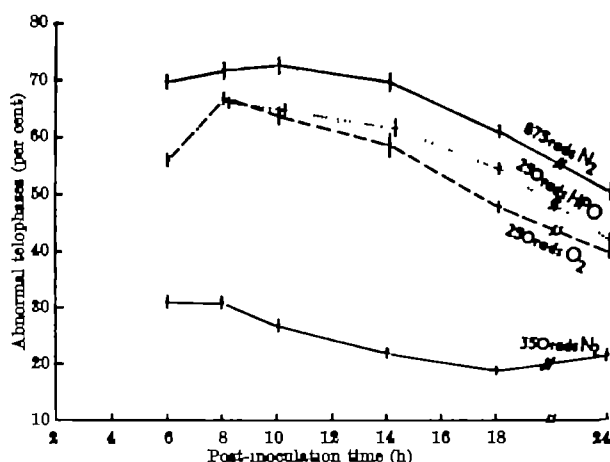


Fig. 1. Telophase aberrations induced in BMT cells by X-irradiation *in vitro* for various post-inoculation times in recipient mice

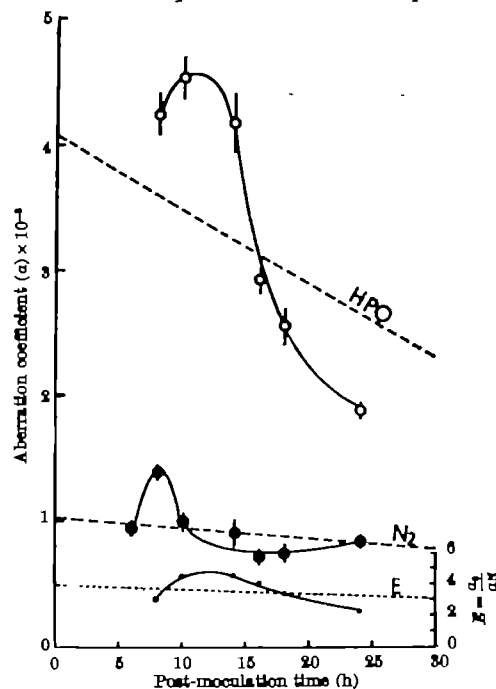


Fig. 2. Relation of radiation-induced aberration coefficients ( $\alpha$ ) to post-inoculation time. Interrupted lines represent regression for HPO and  $\text{N}_2$  radiations, respectively, calculated from data. Dotted line  $H$  represents the mean oxygen effect ratio based on linear regressions, and the lower (●) curve the values for  $H$  based on the experimental data. (○) represents  $\alpha$  for HPO, and (●)  $\alpha$  for  $\text{N}_2$  radiations, respectively

times. Moreover, responses for radiation doses in the range 40–875 rads in  $N_2$  and for post-inoculation times 6–24 h did not depart significantly from linearity.

Values of  $\alpha_{HPO}$  and  $\alpha_{N_2}$  for different post-inoculation times ( $T$ ) are plotted in Fig. 2, for radiation doses in HPO and  $N_2$  respectively, yielding 10–50 per cent aberrations at an exponential rate with increasing dose. The results show that the value of  $\alpha$  for both HPO and  $N_2$  varies with  $T$ . The data were fitted with regression lines, using the least-squares method on the assumption that  $\alpha$  decreased linearly with  $T$ . The regression equations:

$$\alpha_{HPO} = 4.08 - 0.058 T$$

$$\alpha_{N_2} = 1.03 - 0.008 T$$

were obtained and suggest that the oxygen effect factor  $E$

( $E_{HPO} = \frac{\alpha_{HPO}}{\alpha_{N_2}}$ ) decreases linearly from  $\sim 3.7$  ( $T=8$  h)

to  $\sim 3.2$  ( $T=24$  h). This result agrees with the value ( $E_{HPO} = 3.2$ ) obtained in this laboratory<sup>8</sup> for regression of this tumour growing in the leg of mice and irradiated *in vivo* in HPO or with tourniquet occlusion. However, a statistical analysis of the present data showed that the relation of  $\alpha$  to  $T$  was not linear. A variance ratio ( $F$ ) test gave a probability of  $P < 0.001$  that both regressions were non-linear. It appears that the relations of  $\alpha_{N_2}$  and  $\alpha_{HPO}$  to  $T$ , respectively, are curvilinear and that each regression may be better fitted by a polynomial cubic equation or a more complex regression of the general form:

$$\alpha = \beta + \beta_1 T + \alpha_2 T^2 + \beta_3 T^3$$

The data show that  $\alpha$  rises to a peak value ( $\alpha_p$ ) at time  $T_p$ , and afterwards rapidly decreased for a period of several hours. In Table 1 mean values of  $\alpha$  and the corresponding values of  $T$  are tabulated, and the values of  $E_{HPO}$  calculated.

Table 1

	6	8	10	14	16	18	24
$\alpha_{HPO}$	—	4.25	4.55	4.19	3.94	3.57	1.90
$\alpha_{N_2}$	0.95	1.40	1.00	0.91	0.72	0.75	0.84
$E_{HPO} = \frac{\alpha_{HPO}}{\alpha_{N_2}}$	—	3.03	4.55	4.60	4.08	3.43	2.26

Table 1 shows that calculated oxygen effect ratios vary considerably with post-inoculation time. The mean peak aberration coefficient ( $\alpha_p$ ) values give a ratio  $E_p$ —

$$E_p(HPO) = \frac{\alpha_p(HPO)}{\alpha_p(N_2)} = \frac{4.6}{1.4} = 3.3$$

This 2 h delay in  $T_p(HPO)$  ( $\approx 11$  h) after  $T_p(N_2)$  ( $\approx 9$  h) seems related to the delay in onset of mitosis due to HPO treatment *per se*. Assuming that ascites cells aspirated on the fifth day are in asynchronous logarithmic growth, parasynchrony possibly results from a temporary arrest in one or more selected phases of the cell replication cycle as a result of metabolic stresses produced by experimental manipulations. Evidence, somewhat conflicting, exists that cellular radiosensitivity may be decreased in the presynthetic ( $G_1$ ) 'resting' phase, but is greater in certain parts of other ( $S$ ,  $G_2$ ,  $M$ ) phases<sup>9</sup>. However, in certain hypotetraploid sublines of EAT, cell cycle times of about 18–20 h have been reported, the  $S$ -phase lasting 11–12 h and the post-synthetic ( $G_2$ ) period plus mitosis ( $M$ ) period 6–7 h, whereas  $G_1$  is very short or absent<sup>8</sup>. Thus, in 3–5 day EAT 54 per cent of interphase nuclei were labelled 1 h after <sup>3</sup>H-thymidine administration, and the mitotic index (including early prophase) was about 25 per cent<sup>8</sup>. In our hyperdiploid EAT, the corresponding 5-day cell cycle time was about 21 h,  $17 \pm 2.4$  per cent of interphase nuclei were labelled at 1 h and there were 4 per cent mitoses. This suggests that the  $S$  period is much shorter in the hyperdiploid tumour. Data based on labelling and mitotic indices after colchicine arrest indicated that the  $S$  plus  $G_2$  period is 6–7 h, and that

mitosis lasts about 1 h. A long prophase period (4 h) is reported for the hypotetraploid tumour<sup>8</sup>, but is much shorter in the hyperdiploid tumour. These differences in the growth cycles of hyperdiploid and hypotetraploid sublines appear important in evaluating radiosensitivity parameters.

Assuming  $\alpha_p$  to correspond to radiosensitivity being maximum in  $S$  phase, the values  $T_p(N_2) \approx 9$  h and  $T_p(HPO) \approx 11$  h seem to fit this hypothesis, since HPO caused more prolonged arrest of cells in all stages of division. Arrest of growth and division by HPO have been described in various biological systems<sup>9</sup>. Possibly HPO causes changes in endogenous SH — SS redox systems. The latter have been associated with various biosynthetic events in cell replication<sup>10</sup>, and shown to alter in HPO poisoning<sup>11</sup>.

Assuming  $\alpha_p$  values in our material to correspond to higher radiosensitivity of those cells in DNA synthesis at the time of irradiation, a calculation has been made of this increase in radiosensitivity ( $\theta$ ) for HPO and  $N_2$ , respectively (Table 2).

Table 2

Radiosensitivity ratio ( $\theta$ ) for peak value ( $\alpha_p$ ) relative to mean value ( $\alpha_m$ ) calculated from linear regression curve	$\theta_{HPO} \left( \frac{\alpha_p}{\alpha_m} \right)$	$= \frac{4.60}{3.45}$	$= 1.30$
	$\theta_{N_2} \left( \frac{\alpha_p}{\alpha_m} \right)$	$= \frac{1.40}{0.95}$	$= 1.45$
Extreme radiosensitivity ratio ( $\theta^1$ ) for peak value ( $\alpha_p$ ) relative to minimum value ( $\alpha_{min}$ ) at 24 h	$\theta^1_{HPO} \left( \frac{\alpha_p}{\alpha_{min}} \right)$	$= \frac{4.60}{1.90}$	$= 2.40$
	$\theta^1_{N_2} \left( \frac{\alpha_p}{\alpha_{min}} \right)$	$= \frac{1.40}{0.84}$	$= 1.67$

If  $\alpha_{max}$  in HPO and  $N_2$ , respectively, corresponds to cells irradiated in  $G_1$  phase, and seen in telophase at 24 h, the oxygen effect factor for  $S$  phase (for HPO) may be

$$\text{higher (by an additional factor } E_s = \frac{\theta^1_{HPO}}{\theta^1_{N_2}} = \frac{2.40}{1.67} \approx 1.4)$$

than for  $G_1$  phase. At present DNA labelling techniques in conjunction with measurement of aberration rates to identify phases of cell replication for HPO and  $N_2$  irradiations, respectively, are being used to verify this hypothesis.

In conclusion, the results obtained show that the incidence of chromosome aberrations in the hyperdiploid Ehrlich ascites tumour, induced by ionizing radiation *in vitro* and measured after incubation of the cells in recipient mice for varying times, is highly dependent on the post-inoculation period ( $T$ ). In both HPO (5 atmospheres absolute) and in  $N_2$ , respectively, the aberration coefficient ( $\alpha$ ) rises to a peak value ( $\alpha_p$ ) and then rapidly decreases as a curvilinear effect ( $\alpha = f(T)$ ). HPO treatments delay division by an additional  $\sim 2$  h and this effect corresponds to the HPO regression curve and  $\alpha_p(HPO)$  value being displaced to the right. Calculation of the oxygen effect factor ( $E$ ) must be based on a range of values of post-inoculation time ( $T$ ). Peak  $\alpha$  values observed are considered to represent a higher radiosensitivity of cells in DNA synthesis (or possibly  $G_2$ ) at the time of irradiation. The mean oxygen effect factor  $E_{HPO}$  ranged from 3.7 to 3.2 for  $T = 8$  to  $T = 24$  h, respectively. The value  $E_p = 3.3$  (for corresponding peak values of  $\alpha$ ) was similar. However, values of  $E_{HPO}$  corresponding to the various values of  $T$  after radiation in HPO and  $N_2$ , respectively, ranged from as high as 4.60 to as low as 2.26—an anomaly partly due to an additional mitotic delay caused by HPO, but also attributed to a higher oxygen effect factor for cells irradiated during DNA synthesis ( $S$  phase). An estimate of the ratio ( $E_s$ ) of oxygen effect factors for cells in DNA synthesis to that for  $G_1$  cells is 1.4. The oxygen effect factor for X-irradiation in HPO was  $\sim 2.3$  minimum for cells considered to be mostly in the presynthetic  $G_1$  phase, and  $\sim 3.3$  for cells in DNA synthesis ( $S$  phase). The practice adopted of calculating oxygen effect ratios from peak aberration rates, irrespective of post-irradiation incubation time, seems unacceptable if radiosensitivity varies during the replication cycle, and if experimental factors produce selective effects on

timing of the replication cycle or differential sensitizing, protective or additive effects related to phases of replication.

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## SILURIAN MARINE COMMUNITIES AND THEIR ENVIRONMENTAL SIGNIFICANCE

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PETERSEN<sup>1</sup>, in a classic investigation in marine ecology, demonstrated that the benthonic animals of Danish waters tend to occur in several distinctive communities, and he produced maps showing the distribution of these communities. Other workers<sup>2,3</sup> have followed Petersen's lead in treating the animal community as the basic ecological unit, and the general implication is that communities are ultimately controlled by environmental factors. If this is true, then maps showing the distribution of co-existing communities in remote geological times should reflect the different environments of those times; furthermore, the relationships of the communities to such geologically verifiable features as shorelines should provide a clue to the type of environmental factors responsible. Although some palaeontologists<sup>4,5</sup> have defined specific fossil communities in Palaeozoic rocks, few have been able to map coexisting communities, probably because of the difficulties of stratigraphical control.

The early Silurian deposits of Wales and the Welsh Borderland contain fossil assemblages which fall naturally into several groups. The distinctions between some of the assemblages were noticed by earlier workers who invoked proximity to volcanoes<sup>6</sup> or submarine ridges<sup>7</sup> to account for the differences. At least five assemblages or communities may now be recognized, and these are briefly defined in Table 1. In the column headed 'Characteristic Species' are the animals which normally occur abundantly in the community; the 'Associated Species' usually occur in the community, but are not as abundant or regular in their appearance. The communities are probably completely intergrading, and it should be emphasized that their recognition depends as much on relative abundances of species as it does on occurrences of particular species; thus *Ecocelia* is very abundant in its own community, but it frequently occurs in the *Pentamerus* community and is found occasionally in the other communities. The communities are widely distributed and, in fact, the same associations occur in collections known to me from as far afield as Alabama and Hudson's Bay.

The five communities: (1) *Lingula*; (2) *Ecocelia*; (3) *Pentamerus*; (4) *Stricklandia*; (5) *Olorinda*, are related to each other in a linear fashion; adjacent communities, for example 1 and 2, or 4 and 5, have many species in common, while 1 and 4, or 2 and 5, are mutually exclusive. The communities defined, with the exception of the

Table 1		
Community name	Characteristic species	Associated species
1. <i>Lingula</i> community	<i>Lingula pseudoparallela</i> <i>Cameroecocelia decemphloia</i> <i>Nucula eschscheri</i>	<i>Hormotoma</i> sp. <i>Pterinea</i> sp. <i>Cornulites</i> sp.
2. <i>Ecocelia</i> community	<i>Ecocelia</i> spp.* <i>Leptocryptus</i> sp. <i>compositus</i> <i>Delavensis eschscheri</i>	<i>Hemellia crispata</i> <i>Salopina</i> sp. <i>Pterinea</i> sp.
3. <i>Pentamerus</i> community ( <i>Pentameroides</i> community)*	<i>Pentamerus</i> spp.* <i>Atrypa reticularis</i> <i>Dalmanella</i> sp.	<i>Ecocelia</i> spp.* <i>Hemellia crispata</i>
4. <i>Stricklandia</i> community ( <i>Coelotrichianella</i> community)*	<i>Stricklandia</i> spp.* <i>Ecypirifer radiatus</i> <i>Atrypa reticularis</i>	<i>Renssela</i> sp.
5. <i>Olorinda</i> community	<i>Olorinda</i> spp.* <i>Dicelotris blobs</i> <i>Cyrtus asperatus</i> <i>Stenidiolites laevi</i>	<i>Plectodonta willmerensis</i> <i>Oolonia applanata</i> <i>Plectrotrypa marginata</i>

\* Chronological species or genera succeed one another in the same community.

*Lingula* community, are dominated by articulate brachiopods which typically constitute more than 85 per cent of the fossil remains, so the distinctions between the communities are based largely on the brachiopods. In the case of the *Lingula* community, only one species of

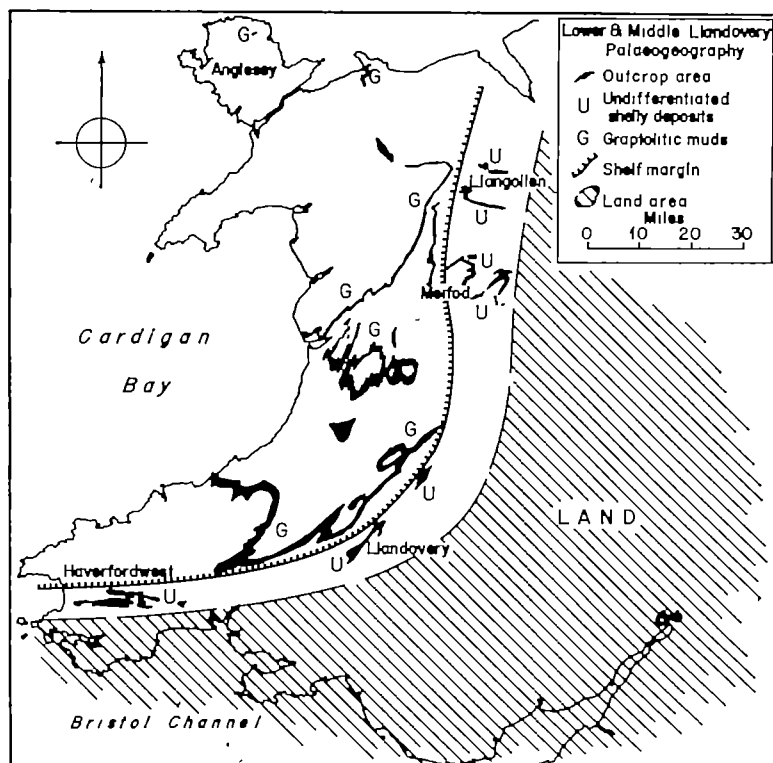


Fig. 1

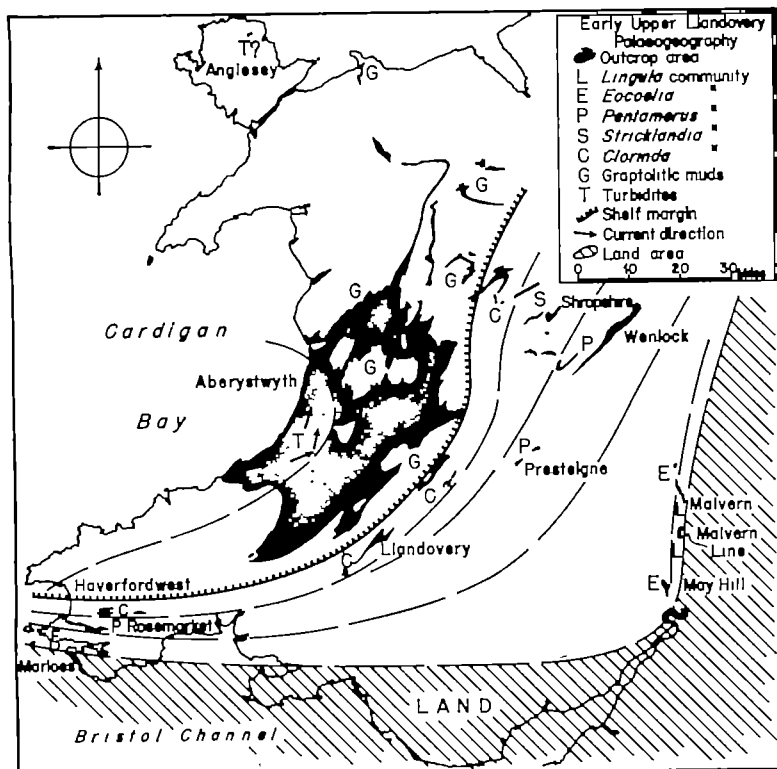


Fig. 2

articulate brachiopod, a rhynchonellid, is present, and many groups such as the corals, crinoids, and trilobites are completely unrepresented. Because of the restricted nature of this community and because of its occurrence at the base of transgressive sequences, such as the Kenley Grit of Shropshire, or the Cowleigh Park Beds of the Malvern Hills, a coastal environment is postulated, similar perhaps to the environment inhabited by many modern lingulids<sup>4</sup>.

Sediment type does not seem to have been a controlling factor in community distribution, as each community has been found in a wide range of sedimentary rocks. But there is a general tendency for communities 1 and 2 to occur in sandstones, communities 3 and 4 in sequences of varying proportions of sandstone and shale beds, and community 5 in shale.

It is evident that a transgressing sea, such as the Upper Llandovery sea of the Welsh Borderland, would deposit stratigraphical sequences representing environments progressively farther from the shore. The Damery Beds of Tortworth show the stratigraphic sequence of communities 2, 3, and 4, whereas at May Hill the community sequence is 2 (Huntley Hill Beds), 3, 4 and 5 (Yartleton Beds). In the Malvern District the community sequence is at Eastnor 1 (Cowleigh Park Beds), 3, and 4 (Wyche Beds); at Gulley quarry it is 3 and 4 (Wyche Beds); and at Old Storridge Common it is 2 (Cowleigh Park Beds), 3, 4, and 5 (Wyche Beds). In Shropshire the general sequence is 1, 3 and 5, representing the Kenley Grit, *Pentamerus* Beds and Purple Shales respectively. Thus the communities, as numbered, represent progressively offshore environments, though the complete sequence of communities is not present in any one area.

The off-shore sequence of the communities may be clearly demonstrated with palaeogeographical maps showing two phases of the Upper Llandovery transgression (Figs. 2 and 3). The communities of the Lower and Middle Llandovery have not yet been studied in detail, but a map of this time period has been included (Fig. 1) to show the extent of the land area later flooded by the Upper Llandovery transgression, and also to contrast the widths of the shelf area during different times. The accuracy of these maps depends largely on the correlation of the various sections, and this has been accomplished by studying evolutionary trends in various brachiopod lineages<sup>5</sup>. The graywackes and the graptolitic muds are included on the maps; these deposits typically do not contain benthonic animals. The graptolites are largely restricted to these deposits, only because the environment of deposition was such that their delicate remains were preserved; they probably lived in the surface waters<sup>10</sup> and drifted wherever seas existed.

A first approximation of the depths at which the various communities existed may be derived from volcanic flows which occur both at Marloes, Pembrokeshire, and Tortworth, Gloucestershire<sup>11</sup>. At Renny Slip, near Marloes, a 20-ft. thick pillow basalt flowed out on deposits containing an *Eocoelia* community. The water was apparently shallowed by an amount equal to the thickness of the flow and this was enough to displace the *Eocoelia* community by its neighbour, the *Lingula* community which occurs in the beds just above the flow. However, the duration of the *Lingula* community at this locality was short as the *Eocoelia* community occurs about 40 ft. higher in the succession, its return being due to the

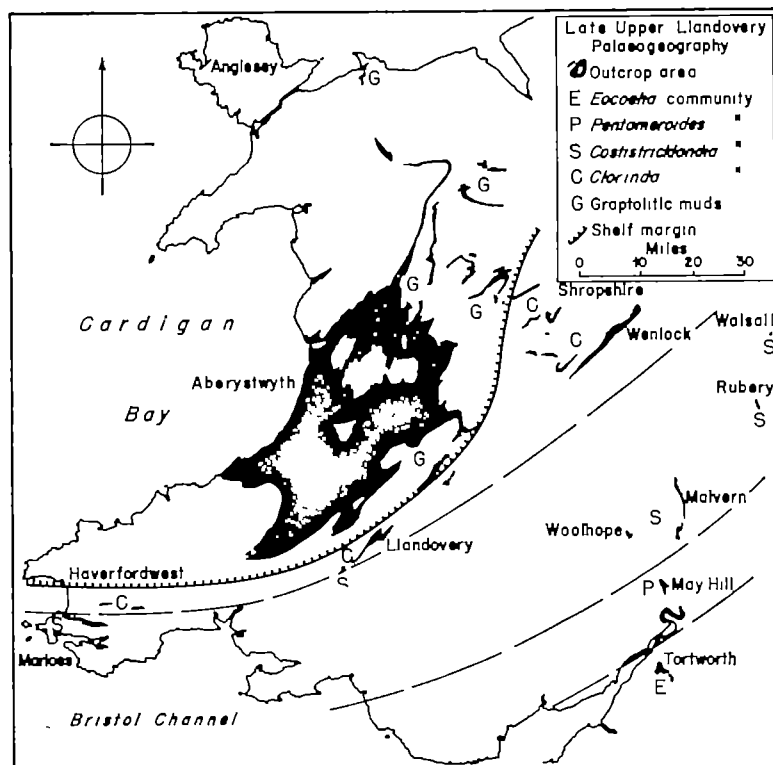


Fig. 3



generally transgressive nature of the sea at this time. Two miles away, however, at Marloes Bay, the basalt is much thicker (130 ft.) and therefore had a greater effect. Two flows occurred and the lower flow has a red-dened surface<sup>11</sup>, suggesting that deposition of the lower flow was at least in part subaerial. The *Lingula* community did not return until several hundred feet of conglomerates and sandstones had been deposited on top of the basalts. The relationships of these volcanic flows suggest that the depth ranges of these communities were of the order of tens of feet rather than hundreds of feet.

In the Tortworth inlier, an andesite flow of variable thickness occurs on top of deposits containing the *Costistricklandia* community. At Woodford, its thickness is small, perhaps 15 ft., and the *Costistricklandia* community is developed on top of the flow. However, less than two miles to the south, the flow is 150 ft. thick and the *Ecocelia* community is developed in the sediments above the flow; here, both the *Costistricklandia* and *Pentameroides* communities were displaced. Relief of 135 ft., then, seems likely between the levels of the *Ecocelia* and *Costistricklandia* communities, and the *Pentameroides* community must have occupied some intermediate range.

In summary, the various lines of evidence on the community environments are: (1) comparison with modern communities in the case of *Lingula*; (2) sediment type; (3) stratigraphical succession; (4) areal relationships; (5) volcanic displacement. All these considerations point to an off-shore sequence of the communities, the *Lingula* community being the closest to the strand and the *Clorinda* community furthest from the strand. If this interpretation is correct, then several conclusions regarding the geological history follow.

The transgression of the Upper Llandovery sea occurred in two pulses. In early Upper Llandovery times the sea extended to cover much of South Wales and most of the Welsh Borderland. The record of its relatively rapid transgression is preserved in the *Ecocelia* community low in the succession of Presteigne and the *Lingula* community of the Kenley Grits of Shropshire. This was just a transient phase, however, and Fig. 2 shows the relationships during much of early Upper Llandovery time, with the shoreline existing at the 'Malvern Line'<sup>12</sup>. About this time, turbidity currents began to deposit the Aberystwyth Grits in the geosyncline<sup>14</sup>. These turbidites are known to have extended further to the east and north with time, eventually covering much of central Wales<sup>15</sup>. It is a singular fact that there is a gap in the sequences of the shelf area which corresponds quite well with the deposition

of the turbidities in the geosyncline; for example, north-west of Malvern at Old Storridge Common, Wyche Beds of *O*<sub>1</sub> age rest directly on Cowleigh Park Beds of *O*<sub>1</sub> or *O*<sub>2</sub> age. Possibly there was a slight regression causing much of the sediment that had accumulated on the shelf to be transported to the shelf margin where it was carried to the depths of the geosyncline by turbidity currents. Confirmation of this regression is found at Llandovery, about the only place where the sequence is continuous, where the *Stricklandia* community of *O*<sub>4</sub> beds succeeds the *Clorinda* community of *O*<sub>1</sub>-*O*<sub>3</sub> beds.

The second pulse of the transgression occurred about *O*<sub>5</sub> time. It is perhaps significant that a transgression occurred on the north side of the geosyncline as well, in County Galway, Ireland<sup>16</sup>, suggesting that the change of sea-level was eustatic. Much of south-east England became submerged, to judge by bore-hole information<sup>17</sup>, turbidite deposition stopped abruptly, and fine-grained shales accumulated over much of the shelf and deeper area. By the end of Upper Llandovery times, the *Clorinda* community existed as far east as May Hill and Old Storridge Common, showing that subsidence continued at a faster pace than the accumulation of sediment.

To conclude, it is clear that the animal community technique of the ecologists is applicable to fossil assemblages and can provide the basis for interpreting past environments. This article reports some preliminary results and is intended only as an announcement of a much more complete treatise, which would define the communities quantitatively, describe the various stratigraphical successions, and present a detailed geological history of the Llandovery of the British Isles.

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## PARTICIPATION OF ADRENOCORTICAL HYPERACTIVITY IN THE SUPPRESSIVE EFFECT OF SYSTEMIC ACTINOMYCIN D ON UTERINE STIMULATION BY OESTROGEN

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INVESTIGATIONS in several laboratories have been directed toward localization of the oestrogen-sensitive step(s)<sup>1-3</sup> in uterine metabolism. In a recent series of reports<sup>4-7</sup> reviewed elsewhere<sup>8</sup>, evidence has been provided that augmentation of RNA synthesis is an early correlate of oestrogen action in the uterus. Thus, actinomycin D, which inhibits DNA-dependent RNA synthesis in isolated systems<sup>9,10</sup> and depresses *in vivo* the incorporation of labelled orthophosphate into all forms of RNA in rat liver<sup>11</sup>, also inhibits some characteristic early actions of oestrogen on the rat uterus including imbibition of water

and accentuation of phospholipid and protein synthesis<sup>4,12</sup>. Moreover, augmentation of uterine RNA polymerase activity has been noted within 2 h of oestrogen pretreatment<sup>4,13</sup>. Puromycin administration abolished this response<sup>4</sup>, as well as the anabolic effects of the hormone<sup>4,14</sup>.

Enhancement of RNA synthesis is undoubtedly an early indicator of the uterine stimulatory action of oestrogen. This would be anticipated from the generalized accentuation by oestrogen of anabolic responses including that of protein elaboration<sup>1</sup>. What is less clear, however, is whether the hormone influences RNA synthesis directly as a primary step on which the metabolic stimulatory pattern depends<sup>4,15,16,17</sup>, or whether the accentuated rate

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of formation of RNA is but one of the secondary consequences of increased availability of substrates, co-factors and energy sources resulting from oestrogenic stimulation of localized transport<sup>8</sup>.

The work recorded here demonstrates that a significant portion of the inhibitory influence of actinomycin pretreatment on oestrogen stimulation of uterine metabolism is due to activation of adrenocortical hypersecretion by this highly toxic<sup>17</sup> antibiotic. This inhibitory effect can be prevented or partially overcome by prior adrenalectomy or by swamping doses of oestrogen. The counteractive influence on oestrogen action of adequate concentrations of exogenous 0-11 steroids<sup>1</sup> and of endogenous adrenocortical hypersecretion<sup>18</sup> has long been known.

Female Sprague-Dawley rats, 2 months old and weighing about 200 g were used. They were ovariectomized 14-16 days earlier. Adrenalectomy, where indicated, was performed 24 h prior to experiment. Post-operatively, adrenalectomized animals were given 0.9 per cent saline as the drinking fluid. Actinomycin D, 375 or 500 µg in 0.5 ml. of 0.154 M neutral sodium chloride containing 1 per cent ethanol, or the saline control solution, was injected intraperitoneally 30 min before oestrogen treatment. Oestradiol-17β (1 µg in 1 ml. of saline)<sup>1</sup>, or the appropriate control solution, was administered via the saphenous vein under light ether anaesthesia. Four hours later the animals were bled under light sodium pentobarbital ('Nembutal') anaesthesia. Uterine horns were incubated at 37.5°-38° C in Krebs-Ringer-bicarbonate buffer under a constant stream of 95 per cent oxygen-5 per cent carbon dioxide in the presence of radioactive substrates for 2 h (ref. 8). Substrates added to 2 ml. medium were either 0.133 µmole glycine-U-<sup>14</sup>C (Nuclear-Chicago) or 0.175 µmole adenine-8-<sup>14</sup>C (Schwarz), in 0.1 or 0.25 ml. water, respectively. After incubation, the contents of each beaker were rapidly brought to 5 per cent trichloroacetic acid (TCA) and frozen at once. Protein isolation, plating and gas-flow counting procedures were essentially as earlier described<sup>8</sup>. The final weight of the plated radioactive protein plus carrier was 30 mg. Isolation of nucleic acids and separation into RNA and DNA fractions were carried out by methods similar to those described by Wool<sup>19</sup>. RNA and DNA concentrations were determined by ultra-violet analysis, and by the orcinol and the diphenylamine reactions, respectively. Radioactivity in aliquots of uterine nucleic acid fractions was measured by liquid scintillation counting in the Bray<sup>20</sup> medium.

**Uterine water.** Fig. 1 reveals that the characteristic water imbibition response to 1 µg oestradiol-17β was abolished ( $P < 0.01$ ) by actinomycin D pretreatment (375 µg). In contrast, the effectiveness of the hormone was not diminished by actinomycin D if prior adrenalectomy had been carried out. Adrenalectomy alone had no influence ( $P > 0.06$ ) on oestrogen sensitivity 24 h following operation. Suppression of the effects of swamping doses of oestradiol required proportionately higher concentrations of actinomycin D. Thus, in the presence of 10 µg of oestradiol, little or no curtailment of the uterine water response was noted ( $83.91 \pm 0.26$  per cent versus  $83.11 \pm 0.22$  per cent), unless the antibiotic was increased to 750 µg. Moreover, when experiments were carried out with animals which had been ovariectomized only 7 days earlier and therefore were unusually sensitive to oestradiol, incomplete obliteration of the uterine response was achieved with 375 µg actinomycin D (data not shown). These observations appear to render untenable the hypothesis<sup>2-5,12,14,15</sup> that even the uterine permeability changes elicited by oestrogen require prior induction of specific proteins.

**Protein labelling *in vitro*.** Uterine segments taken from animals pretreated as described above were tested for their capacity to incorporate radioactivity from glycine-U-<sup>14</sup>C into protein *in vitro*. The increased specific activity of uterine protein due to oestrogen administration was

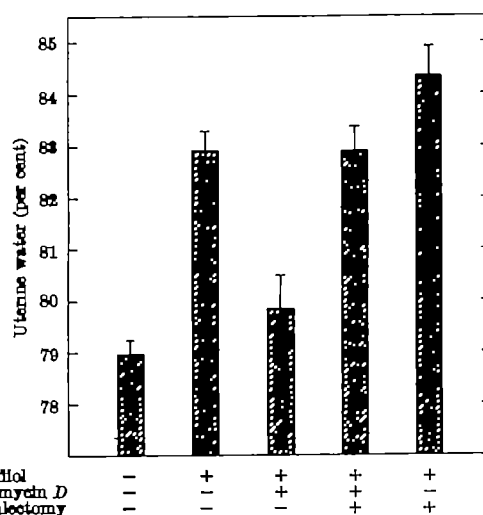


Fig. 1. Suppressive influence of actinomycin D pretreatment on oestrogen-stimulated uterine water imbibition and its reversal by prior adrenalectomy. The height of the bars represents the average uterine water content<sup>1</sup> of groups of 4-8 ovariectomized rats treated as indicated. The positive segments of the S.E.M. are shown.

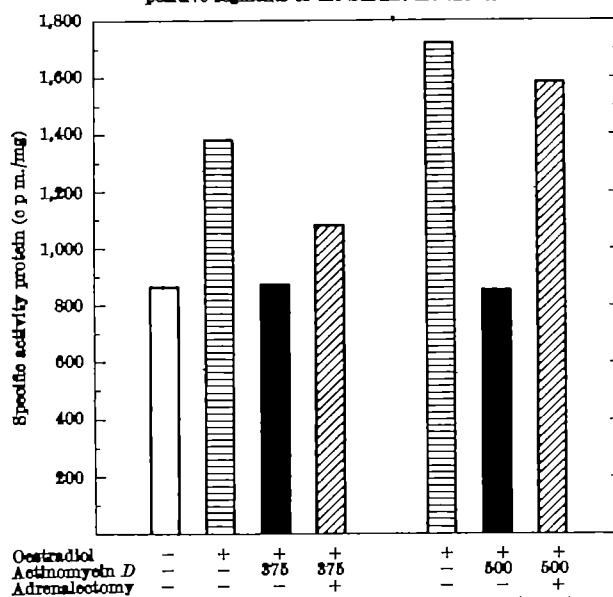


Fig. 2. Incorporation of radioactivity from glycine-U-<sup>14</sup>C into protein of surviving uterine segments: reversal by prior adrenalectomy of the suppressive influence of actinomycin D on oestrogen stimulation. The data (corrected for background and self-absorption as previously described<sup>8</sup>) indicate the results of successive experiments carried out at 2 levels of administered antibiotic. Each bar represents the mean of 3-4 observations, except for the last bar which represents 2 cases.

reduced to the control level by actinomycin D (Fig. 2). Prior adrenalectomy, however, restored a substantial portion of the hormonal sensitivity.

**Plasma corticosteroid levels.** Direct evidence for stimulation of adrenal cortical secretion by actinomycin D in ovariectomized, otherwise intact animals was seen (Fig. 3) in the sharply elevated levels of circulating corticosterone ( $P = 0.02$ ) with both doses of the antimetabolite (see also ref. 21). Levels of plasma corticosterone in adrenalectomized animals receiving actinomycin D were minimal as anticipated.

These dramatic effects of actinomycin D on circulating steroids were associated with intense adrenocortical hyperactivity as judged by extreme lipid depletion of all but the immediately subcapsular zone of representative frozen sections. A similar situation may occur with puromycin<sup>22</sup> and cycloheximide<sup>23</sup>, highly toxic inhibitors both of protein synthesis<sup>24</sup> and of uterine responsiveness to oestrogen<sup>2,24</sup>. Adequate levels of adrenocortical steroids are required for suppression of oestrogen-induced stimulation of uterine metabolic activity<sup>1</sup>. Where these

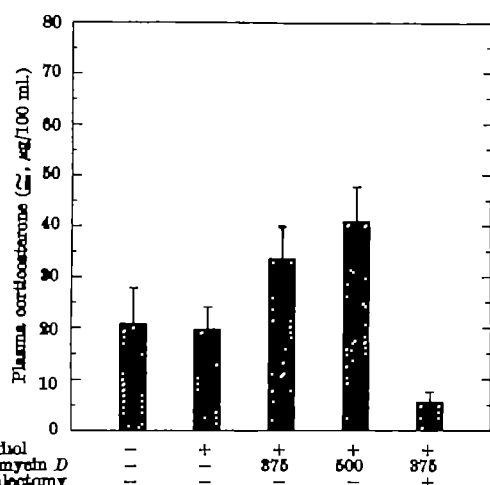


Fig. 3. Augmentation by actinomycin D of circulating corticosterone in ovariectomized rats. Blood was collected from the abdominal aorta in heparinized syringes and centrifuged at 3,000 r.p.m. for 10 min. The separated plasma was promptly frozen. Afterwards, the freshly thawed plasma was analysed for corticosterone by the method of Silber as modified by Guillemin, R., Clayton, G. W., Smith, J. D., and Lipscomb, H. B., *Endocrinology*, 63, 849 (1958). The numbers of observations contributing to the mean plasma corticosterone values represented by successive bars, reading left to right, are 5, 8, 7, 5, and 8.

dose requirements are not met<sup>22</sup>, little or no acute inhibition of oestrogen activity may be evident. Levels of actinomycin D which effectively suppress RNA synthesis in mammalian tissues can produce the generalized stressful state associated with massive endogenous adrenocortical hypersecretion which has been demonstrated in the present investigations.

**RNA labelling.** As noted above, prior adrenalectomy virtually eliminated the inhibitory effect of actinomycin D on the stimulation of protein labelling by oestrogen. In contrast, removal of the adrenals had no comparable influence in relieving the antibiotic suppression of hormonally induced incorporation of carbon-14 from glycine or adenine into RNA (Table 1). As anticipated, the incorporation of isotope into DNA was negligible (<1 c.p.m./μg) in these experiments in which oestrogen was given 4 h earlier (well before stimulation of mitosis is evident<sup>1</sup>). The results recorded here do not support the assumption<sup>4,12,16</sup> that actinomycin D inhibits uterine responses to oestrogen by selective disruption of messenger RNA elaboration and, thus, protein synthesis. Instead, the results indicate that depression of the early uterine responsiveness to oestrogen after systemic administration of antibiotics such as actinomycin, puromycin and cycloheximide is due primarily to adrenocortical hyperactivity.

It is evident that the uterine growth response to oestrogen is associated with an increased formation within a few hours of RNA components which sediment in density gradient centrifugation as template<sup>8</sup> and ribosomal<sup>4,12</sup> fractions. However, expansion of the microcirculation to the uterus is induced by oestrogen through selective liberation of locally sequestered histamine within 30–40 seconds<sup>27</sup>. This very early effect may, by increasing the

Table 1. INFLUENCE OF ACTINOMYCIN D AND OF ADRENALECTOMY ON THE OESTROGEN-STIMULATED INCORPORATION OF RADIOACTIVITY FROM LABELLED PRECURSORS INTO RNA OF SURVIVING UTERINE SEGMENTS *in vitro* RNA, c.p.m./μg\*

Treatment	Exp. I (Gly)	Exp. II (A)	Exp. III (A)
Oestra- diol†	—	—	—
Actino- mycin D‡	—	—	—
Adrx.	—	—	—
—	9.08 ± 0.76 (4)	19.48; 23.28	—
+	24.26 ± 2.59 (4)	25.53; 28.20	50.77 ± 0.25 (3)
+	16.79 ± 2.17 (4)	23.16; 18.52	20.43 ± 3.42 (2)
+	19.55 ± 0.64 (8)	13.97; 13.50	34.70 ± 13.19 (3)

\* Data shown are individual values (Exp. II) or means and S.E.M. (Exps. I and III). Numbers contributing to each average are shown in parentheses.

† Oestradiol-17β, 1 μg intravenous, 4 h prior to sampling and incubation. For details, see text.

‡ 375 μg (Exp. I, II) or 500 μg (Exp. III), intraperitoneal 30 min before oestrogen.

§ Glycine-U-<sup>14</sup>C was the radioactive substrate present during incubation.

¶ Adenine-8-<sup>14</sup>C was the radioactive substrate present during incubation.

availability of a variety of substrates and co-factors to the uterine cell, culminate in stimulation of synthetic reactions (including elaboration of RNA) and uterine growth<sup>4,28</sup>.

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## POTENTIAL EFFECT OF THE PLASMA PROTEINS ON DRUG DISTRIBUTION

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THE theoretical and practical aspects of the interaction of drugs with plasma proteins have been considered by Goldstein<sup>1</sup>, Edsall and Wyman<sup>2</sup> and others<sup>3–6</sup>, and it is generally accepted<sup>7</sup> that the 'protein-binding' of a drug in this manner can modify its distribution in the body and therefore influence both the dose-response relationship

and the rate of drug elimination. The work recorded here was undertaken to gain a more exact theoretical impression of the quantitative aspects.

The extent to which a drug is bound to a particular protein depends on the concentration of drug, on its affinity—which is quantitatively expressed by the asso-

ciation constant of the drug-protein complex ( $K_{sp}$ )—on the concentration of protein and its capacity—the number of sites ( $n$ ) available for drug binding on the protein molecule. When  $n = 1$ , then:

$$K_{sp} = \frac{[DP]}{[D_f] [P_f]} \quad (1)$$

where  $[D_f]$ ,  $[P_f]$  and  $[DP]$  represent respectively the molar equilibrium concentration of free (unbound) drug, free protein and bound drug. If  $\beta$  represents the fraction of the total drug in plasma which is bound to the protein, it

may be deduced<sup>1</sup> that:

$$\beta = \frac{[P_t]}{[P_t] + (1/K_{sp}) + [D_f]} \quad (2)$$

where  $[P_t]$  is the molar concentration of total protein.

In the present connexion, greater interest centres on that fraction of the drug which is free ( $\alpha$ ), for only the concentration of free drug contributes potential to its rate of transport across cell membranes. The rate of excretion of unchanged drug is related to the concentration of free drug rather than that of the total drug in the plasma. The

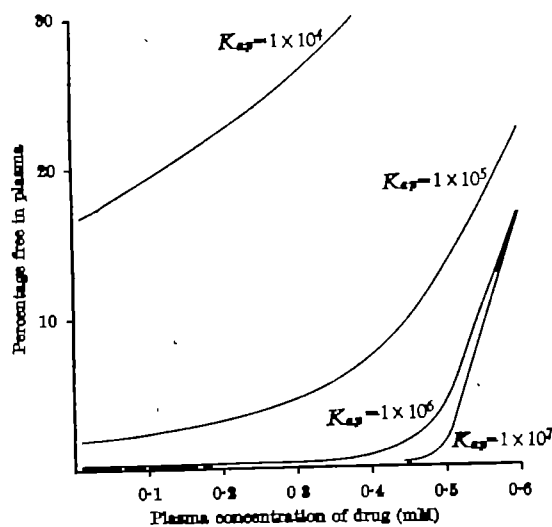


Fig. 1

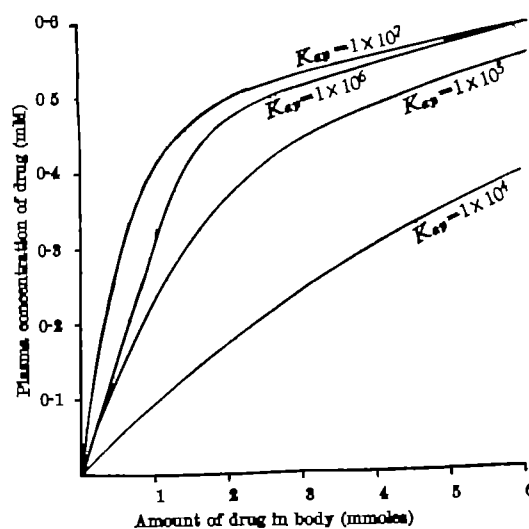


Fig. 2

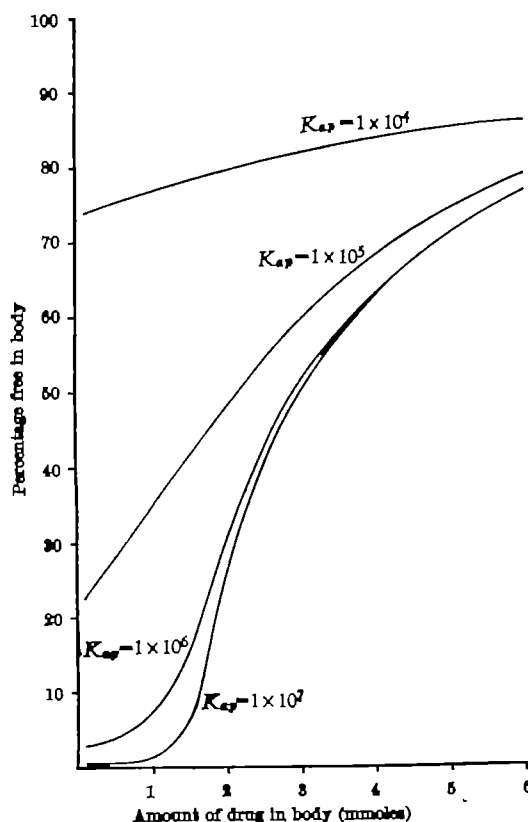


Fig. 3

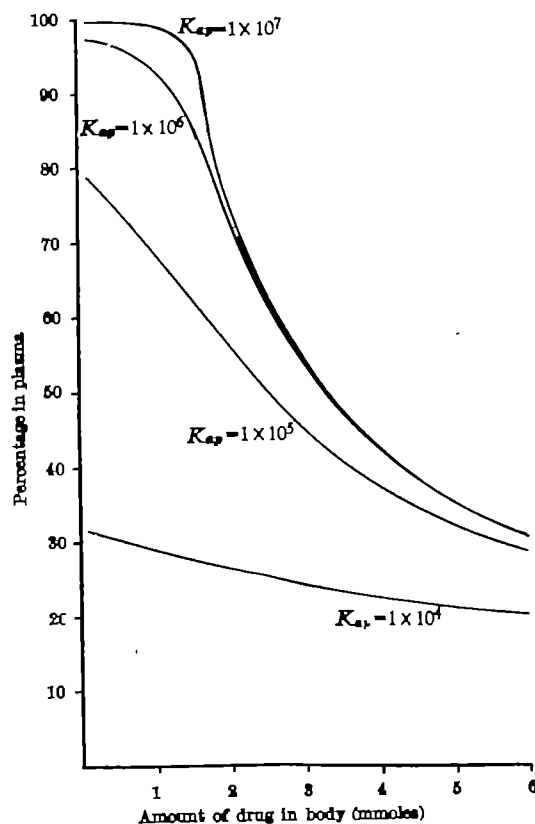


Fig. 4

Figs. 1-4. The graphs are constructed from data calculated for four model drugs which interact with a single plasma protein and which have association constants ( $K_{sp}$ ) of  $1 \times 10^4$ ,  $1 \times 10^5$ ,  $1 \times 10^6$  and  $1 \times 10^7$ . The protein (albumin) concentration was  $5 \times 10^{-4}$  M, each protein molecule having one available site for interaction with drug. Plasma data (Fig. 1) were calculated from equation (8). The extended data (Figs. 2-4) relate to a man weighing 70 kg with plasma volume 3 l. and total body water 43 l., the free drug being uniformly distributed in total body water, with no binding or localization of drug in the tissues. If  $D_f$  and  $D_b$  are the respective molar equilibrium concentrations of free drug and bound drug in the plasma: amount of drug in body =  $42D_f + 3D_b$ ; percentage free =  $\frac{42D_f}{42D_f + 3D_b} \times 100$ ; percentage in plasma =  $\frac{3D_b}{42D_f + 3D_b} \times 100$

concentration of free drug in the tissues is in equilibrium with the free drug in plasma and hence the latter also influences both the rate of drug elimination by biotransformation and the magnitude of the pharmacological response.

Equation (2) may accordingly be transposed in terms of  $\alpha$  and—for simplicity in subsequent calculations—in terms of the dissociation constant  $K_{sp}$  ( $K_{sp} = [1/K_{sp}]$ ).

$$\alpha = \frac{[D_f]}{[D_t]} = \frac{K_{sp} + [D_f]}{[P_t] + K_{sp} + [D_f]} \quad (3)$$

It is also desirable to relate  $\alpha$  to the concentration of total drug in plasma ( $D_t$ ) rather than that of the free drug ( $D_f$ ), for it is the former which is normally measured clinically.

If  $K_{sp}$  and  $[P_t]$  are specified,  $\alpha$  may be calculated for selected values of  $D_f$  and the corresponding values of  $D_t$  may then be derived. Calculations were made for a series of model drugs which react with a single protein with values of  $K_{sp}$  ranging from  $1 \times 10^4$  to  $1 \times 10^7$ , with  $P_t = 8 \times 10^{-4}$  M, this being the typical concentration of albumin in plasma.

The foregoing considerations apply to an isolated, single-compartment system, that is, the drug and the protein occupy a common volume. While they provide an account of the quantitative changes in plasma, they give no indication of the changes in drug distribution in the body as a whole, or of the dosage-level at which they occur. The body must be considered at least as a two-compartment system, the plasma proteins being confined to the plasma volume while the free drug is distributed in a volume which approaches that of total body water. The plasma volume and the total body water constitute typically 4.3 and 60 per cent, respectively, of body-weight, so that on this basis the free drug in plasma is also in equilibrium with an additional volume which is 13 times that of the plasma volume. In this treatment the effect of any binding of drug in the tissues, with the consequent creation of additional compartments, has not been considered. The amount of drug localized in tissues can, however, be substantial and deviations from idealized behaviour will arise whenever any tissue attains a concentration of drug which is either appreciably higher or lower than the free concentration of drug in the plasma.

The calculations were therefore extended on the basis of the foregoing considerations and, in order to gain insight on absolute dosage values, they were applied to a man weighing 70 kg with a plasma volume of 3 l. and total body water equal to 42 l. The results are plotted in Figs. 1-4; each illustrates a different facet of drug distribution. Consider, for example, the model drug with  $K_{sp} = 1 \times 10^7$ ; increasing the dose or the amount of drug in the body from 1.4 to 1.9 mmoles (an increase of one-third) produces a ten-fold increase in the concentration of free drug in the body (Fig. 3), but the concentration of total drug in the plasma rises by only 10 per cent (Fig. 2). Whereas only 3 per cent of the smaller dose exists in its free form in the body, 22 per cent of the larger dose is free (Fig. 3); whereas 97 per cent of the smaller dose is confined in the plasma, only 79 per cent of the larger dose is in the plasma (Fig. 4).

The extended calculations for the whole body indicate that the binding of a model drug to the plasma proteins only has an appreciable effect on drug distribution if the drug has a  $K_{sp}$  greater than  $1 \times 10^4$ . The plasma data reveal that for the model drug with  $K_{sp} = 1 \times 10^4$  as much as 83.4 per cent of the drug in plasma can be bound, whereas the extended data show that at least 78 per cent of the drug in the body is free. As a consequence, the effect on the rate of elimination will be small, relative to drugs with  $K_{sp} = 1 \times 10^4$  or  $1 \times 10^5$ , when the percentage of the drug which is free can be as low as 22 and 2 per cent, respectively.

Differences in drug distribution arising from differences in  $K_{sp}$  are greatest at low drug concentrations, when  $\alpha$  approaches a minimum limit. This limit may be calculated from equation (3), for as  $D_f \rightarrow 0$ ,  $\alpha \rightarrow (K_{sp})/([P_t] + K_{sp})$ . At higher dosage-levels, differences in  $K_{sp}$  have a much smaller effect on the distribution characteristics, which converge towards a common pattern. No constant relationship can therefore be expected between  $K_{sp}$  and its effect on drug elimination or dose-response, for this depends also on the amount of drug present in the body. Elimination will always become progressively slower as elimination proceeds. In respect of the dose-response, there is a dosage-range within which small increases in dose produce relatively large increases in the concentration of free drug and as a result the biological response may also show a pronounced increase at this level. Again, these effects will only be prominent in the instance of drugs possessing a high  $K_{sp}$ .

The classical method of establishing the rate of elimination of a drug is to examine the decline of the plasma level of the drug with time. This involves the assumption that the plasma always contains a constant proportion of the total drug in the body. If the drug has a high  $K_{sp}$  value, this is not so. A plot of log plasma drug concentration against time may, over a limited period, appear to be linear, but the calculation of an elimination rate 'constant' from its slope can appreciably underestimate the rate of elimination, for the decline in the plasma level as a result of drug elimination is partly off-set by an increase in the proportion of the remaining drug which is located in the plasma (Fig. 4). The detailed consideration of this aspect will be the subject of a separate communication.

The rate of elimination of a drug is frequently observed to be proportional to the amount present in the body ( $D$ ), that is,  $-(dD)/(dt) = KD$ , where  $K$  is the first-order elimination rate constant. If a drug has a high  $K_{sp}$  value, this will not be so,  $K$  will progressively diminish and the half-life of the drug will increase as elimination proceeds because an increasing proportion of the remaining drug is bound to the plasma proteins (Fig. 3). A constant relationship will only exist in respect of the amount of free drug ( $D_f$ ), that is,  $-(dD)/(dt) = K'D_f$  and  $D_f = f(D)$ .

The theoretical considerations correlate well with the studies by Burns *et al.*<sup>6</sup> on phenylbutazone, certain features of which they explained in terms of the binding of the drug to the plasma proteins. Calculation<sup>4</sup> from the data of Burns *et al.*<sup>6</sup> indicates a  $K_{sp}$  for phenylbutazone of about  $1 \times 10^4$  with  $n = 1$ , and the experimental observations may then be compared with those calculated for the corresponding model drug.

In general, agreement is sufficiently close to suggest that a knowledge of the affinity and the capacity of the plasma proteins to complex with a drug can provide a useful guide to those aspects of a drug's behaviour which are related to its distribution. The statement that a certain percentage of a drug is bound to the plasma proteins is of limited value, whereas the determination of  $K_{sp}$  and  $n$ , though they may for a number of reasons lack precision, would be far more informative. Similar determinations in respect of the plasma proteins of relevant animal species, which often exhibit major differences<sup>6\*</sup>, would also permit a better correlation of the effects observed in animals with those expected in man.

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## EFFECT OF 8-AZAGUANINE ON LEARNING OF A FIXED-INTERVAL SCHEDULE

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**M**ODIFICATION of RNA in neurones of the central nervous system has been suggested as a mechanism for the phenomenon of learning and memory<sup>1,2</sup>. This hypothesis has been tested by Hydén and Egyházi<sup>3</sup> by selective stimulation of neurones and analysis of RNA content and composition. These stimulated cells showed an increase in RNA content. Corning and John<sup>4</sup> have studied the effect of ribonuclease in blocking the retention of a conditioned response in regenerated planaria.

Drugs known to affect RNA metabolism have also been used to examine the hypothesis that RNA is modified in learning. Chamberlain *et al.*<sup>5</sup> have used 8-azaguanine to prolong the time for spinal fixation in rats; Dingman and Sporn<sup>6</sup> have used the same drug, injected intracerebrally, to modify water maze learning; and Flexner *et al.*<sup>7</sup> have studied the effects of intracerebral puromycin on learning of a Y-maze by adult mice. In general, the results of the experiments tend to support the concept that RNA is involved with learning and memory, and drugs capable of modifying RNA synthesis can adversely affect learning and memory.

The purpose of this investigation was to examine the effects of the drug, 8-azaguanine, in a conditioned discrimination study in rats using food rewards and two fixed intervals. Thirty female rats (140–150 g) of the *OPB* strain (Carworth Farms) were used in this experiment. Five animals were housed per cage (10 in. × 16 in. in dimension) and received food and water *ad libitum*. Food was withheld for 12 h before lever-pressing training.

Animals were trained to press a lever for a food reward in an operant conditioning apparatus twice weekly for 5 weeks. After the animals had learned to press for the food reward, continuous reinforcement for 15 min at each session was allowed. Twenty-five animals learned the lever-press food-reward association and were divided into control (6), placebo (7) and drug (12) groups, randomized for lever-pressing rate and body-weight.

After learning the lever press-food reward association, the following training schedule was started. In any one training session, called a trial, the programme started with rewarding the first lever press. Then a short interval (*SI*) was interposed before a second and then before a third reward could be obtained. The rewarded *SI*s were separated by a long interval (*LI*) before the sequence was repeated. Fourteen such sequences (*SI*, *SI*, *LI*) were presented in each trial. Training trials were conducted twice a week. Two trials were run at each of the following schedules: *SI* 1 sec, *SI* 1 sec, *LI* 30 sec; *SI* 2 sec, *SI* 2 sec, *LI* 60 sec; *SI* 3 sec, *SI* 3 sec, *LI* 90 sec; and *SI* 4 sec, *SI* 4 sec, *LI* 120 sec. These eight trials, during which the time intervals were progressively increased, will be referred to as Segment 1. Sixteen additional trials at the final schedule were given. The first eight of these trials will be referred to as Segment 2 and the last eight trials as Segment 3.

During Segment 1, the drug-treated animals received a suspension of 25 mg/kg of 8-azaguanine in polyethylene glycol 400 intraperitoneally in a volume equal to their weight in tenths of a ml. (that is 250 g  $\equiv$  0.25 ml.). Placebo animals received the vehicle alone in equal volume. All injections were given 30 min before the animals were tested in the conditioning apparatus. Animals were weighed immediately before injection.

Criteria were established for exclusion of rats from the study. If any animal (control or placebo) did not press at all, or did not complete two consecutive trials, no additional trials were given. If a drug-treated animal did not press at all, or did not complete two consecutive trials, no injections were made before subsequent trials. These animals (to be referred to as 8-azaguanine non-injected) were afterwards given trials which were equal in number to controls and carried on through the sequence.

Data were collected for the responses during all three segments. The equipment was programmed to record the number of lever presses during the short interval food reward period, the number of lever-presses during the 15-sec period after cessation of rewarded presses, and the total number of presses during the remainder of the long unrewarded interval (*LI*–15 sec). The number of presses for these individual parameters was averaged from the fourteen sets during each trial and converted to a response rate per minute.

The following parameters are included in this study: the response rate during the *SI*, the response rate during the 15-sec period, and the response rate during the *LI*–15 sec period.

All controls (6) completed the 24 trials. Two animals of the seven receiving placebo injections were excluded and had no additional training. Five of the original twelve 8-azaguanine rats completed the full 24 trials. Six of the seven animals excluded from injection were carried throughout the 24 trials. In order to evaluate toxicity, the weights of the animals were obtained throughout the trial period. No significant difference in weight gain between trials 1 and 24 was found for the various groups. Analysis by *t*-test did not demonstrate significant differences between control and placebo animals in any parameter of lever pressing or weight gain. Therefore, these groups were combined for analysis and will be referred to as controls.

**Comparison of controls with 8-azaguanine rats.** During the *SI*, the drug-treated animals showed a significantly slower rate of lever pressing during Segment 1 (Table 1). Drug-treated animals did not show significantly different response rates during the other segments. In the 15-sec interval, the 8-azaguanine-treated rats pressed significantly more slowly than controls during Segment 1. During Segment 2, no differences were found. However, in Segment 3, the response rates of the treated animals were significantly increased over control animals. The lever-pressing rate during *LI*–15 sec was also significantly slower during Segment 1. No significant differences were seen in this parameter during the final segments.

Table 1. LEVER-PRESSING RATE  $\pm$  S.D.

Controls (C) and 8-azaguanine (D) rats

	Segment 1	Segment 2	Segment 3
<i>SI</i>	C 45.4 $\pm$ 15.8 D 39.1 $\pm$ 14.4*	45.6 $\pm$ 12.8 42.2 $\pm$ 18.5	50.2 $\pm$ 14.2 48.3 $\pm$ 12.7
15 sec	C 15.0 $\pm$ 5.8 D 10.2 $\pm$ 4.7†	14.3 $\pm$ 4.3 15.2 $\pm$ 7.1	10.7 $\pm$ 4.2 13.6 $\pm$ 4.4†
<i>LI</i> –15 sec	C 9.1 $\pm$ 4.2 D 6.4 $\pm$ 3.2†	3.6 $\pm$ 1.9 4.0 $\pm$ 1.7	2.1 $\pm$ 1.3 1.8 $\pm$ 1.0
<i>N</i>	C 88 D 40	88 40	80 33

\*  $P = 0.05$ .

†  $P = 0.001$ .

*Comparison of control and 8-azaguanine (non-injected) rats.* These animals demonstrated interesting differences from both control and 8-azaguanine animals. The *SI* lever-pressing rate was significantly slower during Segment 1, but not Segment 2 (Table 2). In Segment 3 these animals pressed significantly more slowly than controls. In both the 15-sec period and *LI-15 sec*, these animals showed no differences in Segments 1 and 2, but were significantly slower than controls in Segment 3.

*Comparison of 8-azaguanine and 8-azaguanine (non-injected) rats.* No differences in *SI* pressing rate were seen except during Segment 3 where the non-injected animals pressed at a significantly slower rate (Table 3). In the 15-sec period and the *LI-15 sec* the non-injected group pressed faster than treated animals, but during Segment 3 pressed at a rate significantly slower than treated animals.

Table 2. LEVER-PRESSING RATE  $\pm$  S.D.

Controls (C) and 8-azaguanine non-injected (D-NI) rats

		Segment 1	Segment 2	Segment 3
<i>SI</i>	C	45.4 $\pm$ 15.3	45.6 $\pm$ 12.3	50.2 $\pm$ 14.2
	D-NI	40.0 $\pm$ 12.6*	44.0 $\pm$ 12.4	40.9 $\pm$ 8.3†
15 sec	C	15.0 $\pm$ 5.8	14.8 $\pm$ 4.3	10.7 $\pm$ 4.2
	D-NI	15.2 $\pm$ 5.4	14.2 $\pm$ 4.7	8.5 $\pm$ 2.7†
<i>LI-15 sec</i>	C	9.1 $\pm$ 4.3	3.6 $\pm$ 1.9	2.1 $\pm$ 1.3
	D-NI	9.1 $\pm$ 3.8	3.4 $\pm$ 2.3	1.2 $\pm$ 0.7†
<i>N</i>	C	88	86	80
	D-NI	42	48	48

\*  $P = 0.05$ .†  $P = 0.001$ .Table 3. LEVER-PRESSING RATE  $\pm$  S.D.

8-azaguanine (D) and 8-azaguanine non-injected (D-NI) rats

		Segment 1	Segment 2	Segment 3
<i>SI</i>	D	39.1 $\pm$ 14.4	42.2 $\pm$ 13.5	46.3 $\pm$ 12.7
	D-NI	40.0 $\pm$ 12.6	44.0 $\pm$ 12.4	40.9 $\pm$ 8.3*
15 sec	D	10.2 $\pm$ 4.7	15.2 $\pm$ 7.1	13.6 $\pm$ 4.4
	D-NI	15.2 $\pm$ 5.4†	14.2 $\pm$ 4.7	8.5 $\pm$ 2.7†
<i>LI-15 sec</i>	D	6.4 $\pm$ 3.2	4.0 $\pm$ 1.7	1.8 $\pm$ 1.0
	D-NI	9.1 $\pm$ 3.8†	3.4 $\pm$ 2.3	1.2 $\pm$ 0.7†
<i>N</i>	D	40	40	32
	D-NI	42	48	48

\*  $P = 0.05$ .†  $P = 0.005$ .‡  $P = 0.001$ .

*Potentiation of barbiturate sleeping time.* In view of the depressed rate of lever pressing observed when the treated animals were receiving drugs, two additional rats were given 50 and 200 mg/kg of 8-azaguanine intraperitoneally, and gross reduction in motor activity was observed.

In the light of these observations, potentiation of barbiturate sleeping time was studied. Twelve female rats of the same strain, age and weight as the experimental animals during Segment 1 were used. Thiopental (20 mg/kg) was injected intraperitoneally with either placebo (polyethylene glycol 400) or 25 mg/kg 8-azaguanine in polyethylene glycol 400. The animals receiving placebo did not show significant potentiation of sleeping time (time from loss to return of righting reflex), but those animals receiving 8-azaguanine showed significant potentiation over controls ( $P < 0.001$ ) and placebo ( $P < 0.025$ ).

The measure of learning of this discrimination procedure during the later trials may be considered to be a ratio of the lever-pressing rate during the *SI* food reward period to the rate during the 15-sec and *LI-15 sec* intervals. With repeated trials, the ratio would be expected to increase, that is, less lever pressing during the 15-sec and *LI-15 sec* periods compared with the *SI*.

The administration of 8-azaguanine during learning of the fixed-interval food-reward sequence appeared to decrease discrimination of the end of the *SI* as evidenced

by the significant elevation of the 15-sec lever-pressing rate over controls during Segment 3 (trials 17-24). The ratio of *SI* : 15 sec for the drug-treated group is 3.4 : 1 and for controls is 4.7 : 1. No significant disturbance of the rate during the *SI* or *LI-15 sec* was demonstrated at this time. The *SI* : *LI-15 sec* ratio was 25.7 : 1 for the drug group and 23.9 : 1 for the control group.

In contrast to the results of Dingman and Sporn<sup>6</sup>, who did not show depression of swimming ability in the water maze with 8-azaguanine, a significant depression of motor activity, as indicated by lever pressing, was demonstrated during the time of drug administration (Segment 1). This difference may be explained on the basis of motivation associated with the testing procedure, presumably less in our experiment, and on the basis that lever-pressing rate in a learned interval schedule is an easily disturbed measure of drug action<sup>6</sup>.

Chamberlain *et al.*<sup>5</sup> reported that no depression of activity or food and water intake was seen in rats receiving less than 50 mg/kg of 8-azaguanine intraperitoneally. In addition, these investigators showed a prolongation of the time for spinal fixation from 45 min to 70 min in rats receiving 8-azaguanine (50-200 mg/kg intraperitoneally, 1.75-8.75 h before postural asymmetry). These animals had received sodium pentobarbital 50 mg/kg for the surgery required to produce postural asymmetry<sup>5</sup>. This prolongation of the time required for spinal fixation may represent the direct depressant properties of 8-azaguanine as demonstrated in our experiments by depressed lever pressing rate and potentiation of barbiturate by 8-azaguanine. It has also been demonstrated that narcotic (barbiturate) sleep and hibernation reduce turnover rates of ribonucleic acids in rat brain and spinal cord<sup>10</sup>.

The 8-azaguanine non-injected animals showed significantly reduced responses in all parameters over control animals in Segment 3. The ratio of *SI* : 15 sec was 4.7 : 1 for the controls and 4.8 : 1 for the 8-azaguanine non-injected, indicating that learning of this parameter had not been changed. The *SI* : *LI-15 sec* ratio, however, demonstrated that the controls (23.9 : 1) pressed more than 8-azaguanine non-injected (34 : 1) indicating improved discrimination of this interval. Except for the *SI* in Segment 1, their pressing rates were the same as controls for all parameters during Segments 1 and 2. Since these animals had received 8-azaguanine during several trials before stopping injections, they may have had some degree of disturbed RNA metabolism. The possibility exists that enhanced RNA turnover, secondary to the reduction caused by drug, may account for the improved final performance. While these animals were originally excluded from additional injections by their refusal to press, they performed as well as controls with respect to the rate of pressing the lever. Their ratios compared with controls during Segment 2 were 3.1 : 1 and 3.2 : 1 respectively for *SI* : 15 sec and 12.9 : 1 and 12.7 : 1, respectively, for *SI* : *LI-15 sec*. These factors suggest that the observation of improved discrimination of the *LI-15 sec* interval during Segment 3 by this group is not due to lever-pressing rate alone.

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# LETTERS TO THE EDITOR

## COSMOLOGY

### Creation Rate of Matter and the Heisenberg Uncertainty Principle

ACCORDING to the steady-state theory of the universe, new matter is continuously being created out of nothing<sup>1,2</sup>. The Heisenberg uncertainty principle, however, precludes the possibility of proving 'nothing', that is, absence of energy, unless the time of observation is infinite. Now, whatever the explanation of the famous red-shift phenomenon in astronomy, the result is always a finite volume for the observable universe; any observer, irrespective of his position, finds himself in the centre of an analogous observable universe. On the Sandage scale<sup>3</sup>, and assuming a Euclidean space for simplicity, extrapolation of Hubble's law yields a value of about  $1.3 \times 10^{10}$  light years for the radius ( $R$ ) of the theoretically observable universe.

The lowest energy quantum possible in a finite observable universe is limited by the largest possible wave-length,  $\lambda = 2R\pi$ ; thus, the theoretically lowest energy quantum in our universe has a mass  $m = \frac{h}{2R\pi c}$  (about  $3 \times 10^{-66}$  g)

and its period of vibration is  $\frac{2R\pi}{c}$  (about  $2.5 \times 10^{18}$  sec).

This length of time—about  $8 \times 10^{10}$  years—is considerable even on a cosmological scale, yet it is not infinite. Making use of the uncertainty relation  $\Delta E \times \Delta t = h$ , one finds that in our universe the theoretically minimal quantum mechanical energy fluctuation of vacuum around zero is equal to  $\pm \frac{h}{2R\pi\tau_0}$  every  $2.5 \times 10^{18}$  sec. It should be noted that

this process does not violate the energy conservation law. Obviously, to make use of this value, one must know how many times the process occurs in our universe. One can assume that this number equals the number of centres of analogous observable universes contained in our universe and that the shortest distance between such neighbouring centres cannot be less than the quantum mechanical 'minimum length',  $\lambda_0$  ( $1.32 \times 10^{-13}$  cm); this space quantization yields about  $3 \times 10^{13}$  such centres in our universe. Since the subsequent calculations refer to positive energy, the positive half of fluctuations will be taken into account.

On the foregoing assumptions the amount of matter created in the observable universe in each vibration period ( $\frac{2R\pi}{c}$ ) is:

$$\frac{h R^2}{3c\lambda_0^3} \quad (1)$$

where  $h$  is the Planck constant,  $R$  the Hubble radius of the observable universe,  $c$  the velocity of light in vacuo, and  $\lambda_0$  the quantum mechanical minimal length. The resulting value for the creation rate of matter is  $2.5 \times 10^{-46}$  g cm<sup>3</sup> sec<sup>-1</sup>. It seems of some relevance that Bondi<sup>4</sup>, using a different approach, found practically the same value for the rate of creation of matter.

In a system with continuous creation of matter and disappearance of matter over the horizon, according to Hubble's law the two processes automatically tend towards a state of equilibrium at which the mean density of matter in the universe ( $\rho$ ) is constant; using (1),  $\rho$  has the value:

$$\rho = \frac{\text{creation rate} \times R}{3c} = \frac{h}{24\pi^2 R c \lambda_0^3} = 3.3 \times 10^{-30} \text{ g/cm}^3;$$

thus, more exact determination of  $\rho$  might represent an observational test of (1).

To sum up, according to the present suggestion all energy, including matter, in our universe is continually being created as virtual energy on loan to be repaid after some  $8 \times 10^{18}$  years; the continuous creation of matter *ex nihilo*, and its disappearance into the void again, is interpreted by the Heisenberg uncertainty principle applied to a finite observable universe.

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## GEOPHYSICS

### Infra-sonic Waves from Aurorae

DURING times of high geomagnetic activity, long-period acoustic waves have been observed at the ground<sup>1,2</sup>. The source of these atmospheric waves can be ascribed to auroral activity, particularly to the periodic heating around the  $E$ -layer in the polar upper atmosphere corresponding to pulsating types of aurorae<sup>3,4</sup>. Auroral pulsations, which are sometimes called auroral commutations, have periods that are quite similar to those of geomagnetic pulsations recorded simultaneously at the ground<sup>1,4</sup>. However, periods of auroral sonic waves are significantly longer than some of the auroral and geomagnetic pulsations recorded within the same period of geomagnetic activity.

The main purpose of this communication is first to point out the characteristics of auroral infra-sonics, and secondly to give some interpretation of the wave-form and periodicity of these atmospheric travelling auroral pressure waves which are observed at the ground.

Many kinds of atmospheric travelling pressure waves have been observed at the ground, particularly those produced by volcanic eruptions, large-scale explosions, earthquakes and meteorological disturbances such as tornadoes. These sounds from distant tropospheric sources are generally characterized by horizontal phase velocities near the local speed of sound, and by signal velocities (determined from the travel time from the source) of about the average sound speed of the atmosphere. These waves show irregular dispersion<sup>5</sup>. By contrast, sounds from aurorae have been shown to exhibit horizontal phase velocities usually greater<sup>1</sup> than 400 m/sec. They appear to radiate from the night side of the auroral zones<sup>1,2</sup>.

Examples of auroral pressure waves recorded at Fort Yukon, Alaska, and at the National Bureau of Standards in Washington, D.C., are shown in Figs. 1 and 2. Each line in the figures corresponds to a record from a microphone. The separate microphones for each figure were spaced 2–7 km from each other in a space array at ground-level. Fig. 1 shows clearly the apparent acoustic mode dispersion typical of auroral zone measurements<sup>6</sup> with short periods arriving first and the longer periods arriving later in time.

It is well known that there are two types of pressure waves in the atmosphere, which are called acoustic (sonic) and thermobaric (internal gravity) modes, respectively<sup>7,8</sup>. Periods of thermobaric waves are longer than  $\tau_B$ , which is called Brunt's period or Väisälä's period, while those of acoustic waves extend to short periods below  $\tau_A$ , which has been called the atmospheric acoustic resonance period<sup>9</sup> or modified acoustic stability period<sup>11</sup>.

18 AUG 62

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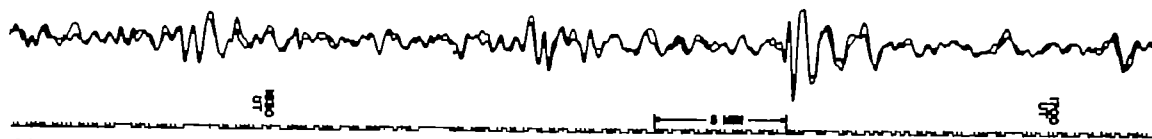
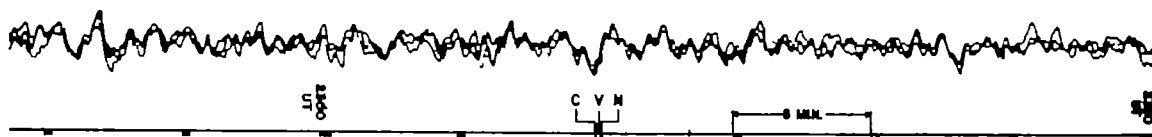


Fig. 1. Auroral zone records of infra-sonic waves during the period of auroral activity on August 18, 1962, recorded at Fort Yukon, Alaska

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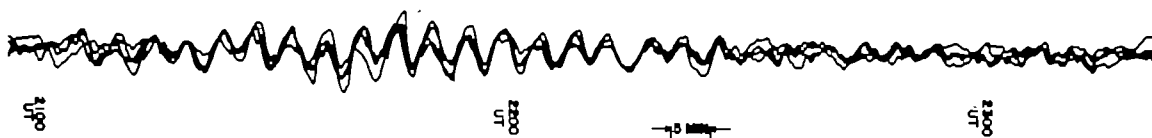


Fig. 2. Record of infra-sonic waves during the geomagnetic storm on July 14, 1960, recorded in Washington, D.C.

For simplicity,  $\tau_A$  may be called Martyn's or Hines's period<sup>13</sup>. Angular frequencies ( $\omega = 2\pi/\tau$ ), corresponding to  $\tau_A$  and  $\tau_B$ , are given by:

$$\omega_A = \frac{\gamma g}{2c} \text{ and } \omega_B = \left[ \frac{g^2}{c^2} (\gamma - 1) + \frac{g}{c^2} \cdot \frac{dc^2}{dz} \right]^{\frac{1}{2}} \quad (1)$$

respectively, where  $g$ ,  $c$  and  $\gamma$  are the gravitational acceleration, sound velocity in the air and the ratio of specific heats of air,  $c_p/c_v \approx 1.4$ . If we assume constant molecular constitution, sound velocity is a function of atmospheric temperature  $T(z)$  only, that is:

$$c^2 = \gamma R T(z) \quad (2)$$

where  $R$  is the gas constant of air ( $2.87 \times 10^{-4} \text{ km}^2/\text{sec}^2 \text{ } ^\circ\text{K}$ ).

It should be noticed that in the isothermal atmosphere  $\tau_A$  is smaller than  $\tau_B$  (that is,  $\omega_A > \omega_B$ ) and no atmospheric wave exists between these two resonance periods, except the so-called Lamb's wave with propagation strictly limited to horizontal directions<sup>14</sup>. As shown by equation (1), if the atmosphere is unstable, that is, the negative temperature gradient exceeds the adiabatic lapse rate  $-g/c_p$ , then thermobaric oscillations do not occur ( $\omega_B$  becomes imaginary). Acoustic oscillations can propagate through the layers so long as stratification is sustained without collapse. This occurs when the lapse rate exceeds that of autoconvection,  $-g/R$ . On the other hand, as can be seen from equation (1), the more stable the layer, the shorter will be  $\tau_B$  (larger  $\omega_B$ ). Therefore, when the atmosphere is very stable, as in the upper stratosphere and in the thermosphere,  $\tau_B$  becomes less than  $\tau_A$ .

In short, from equations (1) and (2) it can be written that:

$$\omega_A^2 = \omega_B^2 + \Delta\omega^2 \quad (3)$$

where:

$$\Delta\omega^2 = \frac{g}{T(z)} \left[ \frac{g}{R} \left( \frac{2-\gamma}{2} \right)^2 \cdot \frac{1}{\gamma} - \frac{dT}{dz} \right] \quad (4)$$

Thus if  $\frac{dT}{dz} > 2.2 \text{ } ^\circ\text{K}/\text{km}$ , then  $\omega_A < \omega_B$  ( $\tau_A >$

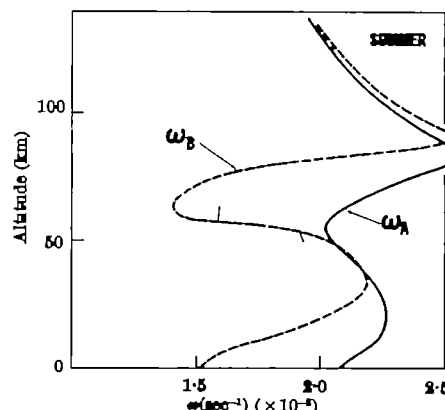


Fig. 3. Atmospheric resonance frequencies  $\omega_A$  and  $\omega_B$  for the summer-time polar upper atmosphere

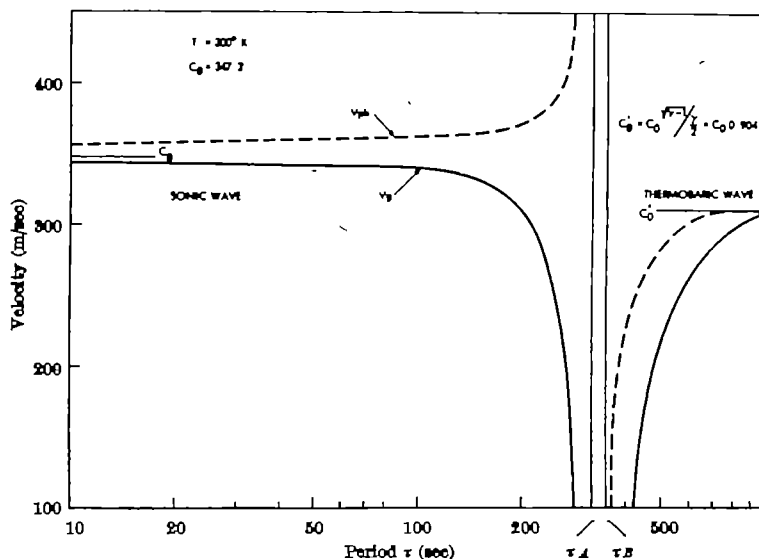


Fig. 4. Dispersion curve for atmospheric pressure-waves in the isothermal atmosphere ( $T = 800^\circ\text{K}$ ). Full and dashed lines indicate horizontal group and phase velocities

$\tau_B$ ), as is shown in Fig. 3 for a summer polar atmosphere<sup>13</sup>. Furthermore, in this case it can be shown that the waves with period between  $\tau_A$  and  $\tau_B$ , which are forbidden when  $\omega_A > \omega_B$ , can now propagate even in the vertical direction through these layers<sup>9,12,15</sup>.

On the other hand, as shown in Fig. 4, group velocities of acoustic waves and those of thermobaric waves decrease as the period of oscillation approaches  $\tau_A$  and  $\tau_B$ , respectively. Although in Fig. 4 these dispersions are shown only for an isothermal atmosphere ( $T = 300^\circ\text{K}$ ), one can present arguments for an increase of intensity of atmospheric pressure waves around these resonance periods because of the energy flux conservation. It has been shown that in the non-isothermal atmosphere there are several minima of group velocity in each mode of atmospheric waves<sup>16</sup>.

It should be emphasized that characteristic features of observed atmospheric waves indicate clearly that auroral pressure waves are essentially acoustic, while very-long-period pressure waves from other atmospheric disturbances are thermobaric. However, due to the existence of very stable layers in the actual atmosphere, ranges of the periods of the two types of atmospheric waves are overlapped at the minima of group velocity, one of which exists around periods of 3–5 min. Therefore, the occasional appearance of enhanced sinusoidal pressure waves from aurorae with periods around 5 min shown in Fig. 2 can be explained as follows: (1) During strong aurorae, not only the acoustic mode but also the thermobaric mode of atmospheric waves near the Brunt's period can be excited. (2) Short-period acoustic waves propagate through the wave duct, which is around the mesopause in summertime and more effectively around the tropopause in the winter-time<sup>17</sup>, with normal sound velocity and attenuate rather rapidly. Longer-period slower infra-sonic waves follow. (3) Thermally excited auroral thermobaric waves also propagate horizontally with inverse dispersion (that is, weak long wave first and strong short wave later). (4) At a certain distance the longest-period sonic waves can be overtaken by the shortest-period thermobaric waves. (5) Both waves then oscillate with the same period, at near Brunt's period.

The foregoing phenomenon is very similar to the so-called Airy phase, known in sound propagation under shallow water<sup>18</sup>. It should be noted that since the source of auroral excitation of atmospheric waves may sometimes have a wide horizontal area the two modes of atmospheric waves overlapping with opposite dispersion are not necessarily produced at the same point source. Details of this work<sup>19</sup> will be published in the *Proceedings of the Second Benedum Symposium, Pittsburgh*.

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## PHYSICS

### Transmission of High-power Laser Light through Tapered Dielectric Tubes and Rods

THE transmission of high-power laser light was investigated for dielectric tubes and rods of a geometry schematically shown in Fig. 1. The measurements were performed with a Q-switched ruby laser as radiation source and with a calibrated 'rat's nest' bolometer (type Westinghouse) as energy measuring device. The energy in the laser beam could be varied between 0.07 and 1.0 J. The characteristic values for the laser pulse were 15 nsec rise-time and 30 nsec half-width. For all values of output energy, the beam showed a typical two-mode pattern. The energy distribution in each mode was approximately Gaussian. The beam was focused by a 6.4-cm focal length lens on to the centre of the entrance surface of the light guide. In all experiments the angle between the mode axes and the optical axis of the system was  $2^\circ$ . In the focal plane 99 per cent of the total energy was concentrated within a circular area of 1.5 mm diameter. 75 per cent of the beam energy was within an area of 0.5 mm<sup>2</sup>. The average power density within the 75 per cent limit calculates to about 20 MW/mm<sup>2</sup> for 0.5 J laser output. The light guides were drawn by glass-blowing techniques from materials with equal optical properties. The measurements are shown in Table 1.

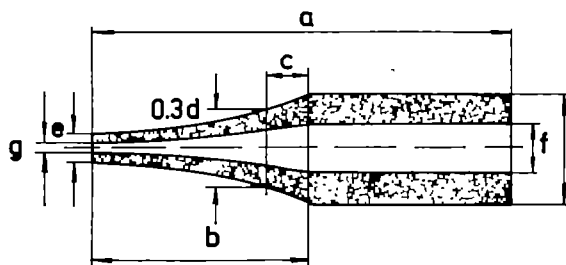


Fig. 1. Schematic diagram of a tapered light guide. The beam is propagated from the right to the left. The measurements are given in Table 1.

Table 1. MEASUREMENTS OF THE TAPERED GUIDES

No.	Type	a*	b	c	d	e	f	g
(mm)								
1	Glass rod	75	32	21	3.0	0.9	—	—
2		148	34	20	3.5	1.0	1.8	0.5
3		140	40	15	6.2	1.0	1.5	0.2
4	Glass tube	96	42	13	6.2	1.1	3.8	0.9
5		78	31	14	3.8	1.3	2.7	0.9

\* Symbols as defined in Fig. 1. Maximum deviation from given value 2 per cent.

The energy transmission properties of a light guide can be characterized by: (1) The energy transmission ratio  $T = E_{out}/E_{in}$ , where  $E_{in}$  and  $E_{out}$  are the total energy transmitted through the entrance and the exit surfaces; (2) the directivity  $D = E_Q/E$ , where  $E_Q$  is the energy within the space cone  $\Omega$  symmetrically with regard to the optical axis,  $E$  is the total energy transmitted through the reference surface in the origo of the unit sphere; (3) the density coefficient  $\delta = T/S$ , where  $T$  is the energy transmission ratio as defined here and  $S$  is the radiating end surface of the guide. For a given  $\delta$ , both the energy and the power density can be calculated when  $E_{in}$  and the time characteristic of the laser pulse are known.

Experimental results for  $T$  and  $D$ , obtained with 0.5-J laser output, are given in Table 2. These values were reproducible to within 10 per cent. Control measurements with a laser output from 0.07 to 1.0 J did not result in any significant change of  $T$  and  $D$ . In tubes at least 99 per cent of the transmitted energy was found to be emitted from the bore. For both tubes and rods, the energy distribution along a diameter of the exit surface was sufficiently uniform to allow  $\delta$  to be calculated according

Table 2. EXPERIMENTAL VALUES FOR ENERGY TRANSMISSION RATIO, DIELECTIVITY AND DENSITY COEFFICIENT

No. (1)	$T_{tot}$ (%)	$T_{cor}$	$\delta$ (mm <sup>-2</sup> )	$\delta_{cor}$ (2) (mm <sup>-2</sup> )	$D_{tot}$ (3) (4)	$D_{cor}$ (5)	$D_{tot}/D_m$ (4)	$D_{cor}/D_m$ (5)
1	0.11	0.22	0.17	0.24	0.13	0.30	0.54	0.70
2	0.10	0.21	0.51	1.1	0.20	0.35	0.58	0.92
3	0.05	0.11	1.6	3.5	0.15	0.32	0.67	0.84
4	0.13	0.17	0.30	0.27	0.09	0.21	0.38	0.65
5	0.08	0.09	0.13	0.14	0.05	0.11	0.21	0.29

(1) Refers to Table 1, col. 1.  
(2) Values corrected for measured energy loss in the straight section (a-b in Fig. 1)  
(3) Corresponding values for the input beam:  $D_m=0.24$  for  $\Omega=0.002$  sterad and  $D_m=0.88$  for  $\Omega=0.004$  sterad.  
(4)  $\Omega=0.002$  sterad.  
(5)  $\Omega=0.004$  sterad.

to the definition given here. A shift in the shape of the transmitted laser pulse was not recorded. Radiation damage in the guide material could not be observed. The power density that can be achieved by tapered guides seems to be sufficiently high to be useful in, for example, biological applications<sup>1</sup> when it is desired to irradiate small areas with a rather well-defined energy distribution and when it is of interest to replace conventional focusing systems by a more flexible light guide system.

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GEOLOGY

Radiometric Age of the Serra Geral Formation

THE Serra Geral Formation consists of lava flows and is associated with dolerite dykes. It covers an area of about one million square kilometres in Uruguay and southern Brazil and is one of the largest volcanic masses in the world. From stratigraphic evidence its age could lie in the range from Upper Triassic to Upper Cretaceous; quite possibly it may be uppermost Triassic (Rhaetic) to Jurassic in age<sup>1</sup>.

This communication reports and comments on some samples originally collected for palaeomagnetic study<sup>2</sup> that have now been dated by the whole-rock potassium-argon method (Table 1).

Petrographically the rocks consist of:

- (1) *S-482*: Medium-grained (0.6 mm) olivine basalt with fresh, zoned plagioclase laths, fresh pale brown augite and scattered, partially serpentinized subhedral olivine. Occasional corroded, zoned xenocrysts of basic plagioclase are present. Extensively chloritized interstitial biotite is developed.
- (2) *S-21a*: Medium-grained (0.6 mm) basalt with fresh, strongly zoned plagioclase laths; augite and pigeonite, minor hornblende all margined by black dust. Occasional corroded, zoned xenocrysts of basic plagioclase are present. No chlorite is present.
- (3) *S-9a*: Medium-grained (0.5 mm) andesite with strongly zoned plagioclase laths in stellate aggregates.

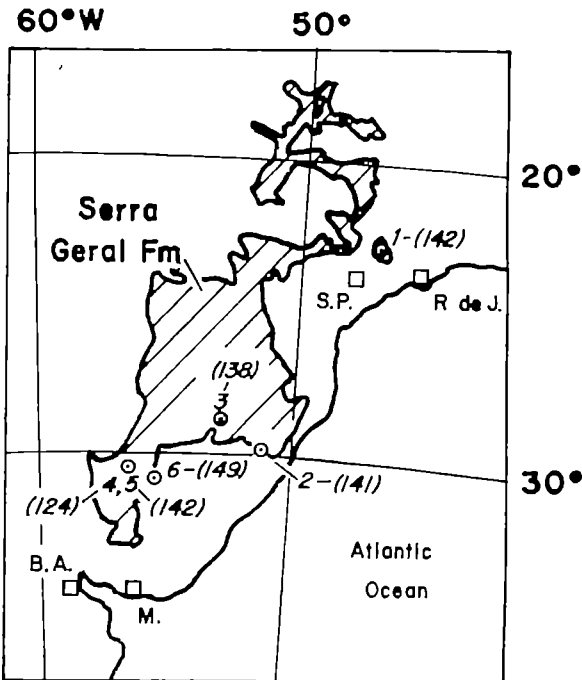


Fig. 1. Sample localities and radiometric ages.

Hornblende and (?) biotite have been partially resorbed and are margined by black dust; augite is present in the groundmass. Weathering may have affected the alkali feldspars. Chlorite is absent.

(4) *U-20b*: Fine-grained (0.15 mm) andesite with fresh, zoned plagioclase laths, augite and partially resorbed hornblende extensively margined by black dust. Chloritic patches are common in the interstitial matrix.

(5) *U-22-A1*: Medium-grained (0.2 mm) pyroxene andesite with fresh, zoned plagioclase laths, rounded anhedral augite aggregates and partially resorbed hornblende margined by black dust. The interstitial matrix contains many chloritic patches.

(6) *U-30-A1*: Medium-grained (0.5 mm) pyroxene andesite with fresh, markedly zoned plagioclase laths, resorbed (?) hornblende, small subhedral aggregates of augite. The interstitial matrix contains what are probably patches of devitrified glass. Chlorite is absent.

Most of the potassium is probably present in the very fine-grained interstitial matrix as potash feldspar, biotite, etc. If these potassium-bearing phases have been altered since magmatic crystallization the K-Ar ages may be low. Chlorite, probably formed by hydrothermal activity, is present in the interstitial matrix of samples 1, 4 and 5; and weathered alkali feldspars are probably present in 3. These observations suggest that the most reliable ages are provided by 2 and 6, where chlorite and weathering effects appear to be absent.

According to the most recent geological time-scale<sup>3</sup>, all six samples are Upper Jurassic to Lower Cretaceous in

Table 1

Sample	Site	Sense of magnetization	% K <sub>2</sub> O in total rock	Atmospheric contamination (%)	Vol radiogenic <sup>40</sup> Ar (mm <sup>3</sup> at N.T.P./g sample)	Age (m.y.)
(1) <i>S-482</i>	Chapodas Quarry Campinas, São Paulo State	Normal dyke	1.08 ± 0.016	29.7	0.00625	142 ± 14
(2) <i>S-21a</i>	Quarry, 30 km from Porto Alegre on the federal road to Rio. Rio Grande do Sul	Reversed sill	1.14 ± 0.014	65	0.00553	141 ± 16
(3) <i>S-9a</i>	From the escarpment near Santa Maria on the road to Julho de Castilhos. Rio Grande do Sul State	" flow	5.4 ± 0.049	14.2	0.02550	128 ± 6
(4) <i>U-20b</i>	4 km from Artigas on road to Rivera, Uruguay	" "	2.47 ± 0.046	16.0	0.01046	124 ± 3
(5) <i>U-22-A1</i>	" "	" "	2.47 ± 0.065	44.0	0.01208	142 ± 5
(6) <i>U-30-A1</i>	Cantera del Cerro del Maroó, Livramento	Normal flow	1.85 ± 0.064	36.1	0.00949	149 ± 8

$\lambda_1 = 4.72 \times 10^{-10} \text{ y}^{-1}$ ;  $\lambda_2 = 0.584 \times 10^{-10} \text{ y}^{-1}$

Table 2

Continent	Formation	Approx. age (m.y.)	Reference
Africa	Karoo lavas	154 (U. Jurassic)	4
		190 (U. Triassic-L. Jurassic)	
Antarctica	Ferrar dolerites	147-163 (U. Jurassic)	5
Australia	Tasmanian dolerites	170 (M. Jurassic)	6
S. America	Serra Geral	115 (L. Cretaceous)	4 and this communication
		140 (U. Jurassic)	

age. Five of the samples yield ages of about 140 m.y., and the sixth yields an age of 120 m.y. Although episodes of these ages have been confirmed by workers at the University of São Paulo<sup>4</sup>, it is not certain whether the determination on a chloritized sample at 124 m.y., reported here, represents a genuinely younger episode.

The Serra Geral Formation is one of several Mesozoic volcanic and hypabyssal episodes that are represented in the Gondwanic continents. When interpreting palaeomagnetic data these episodes have been assumed to be contemporaneous, but radiometric ages now show this assumption to be invalid (Table 2). Data for more refined interpretations are now being accumulated.

We thank Dr. I. D. Muir, Department of Mineralogy and Petrology, University of Cambridge, for his advice on the petrography.

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### Occurrence of Talc as a Clay Mineral in Sedimentary Rocks

TALC is a metamorphic and hydrothermal mineral which is found typically in the greenschist facies of metamorphic rocks, and in shear zones where it is an alteration product. Talc has recently been found to be widespread in limestones of many formations ranging in age from Devonian through Recent. Where present, it is almost invariably accompanied by chlorite; other clay minerals may or may not be present. The occurrence of talc has also been noted in sandstones and shales, but in these rocks it is extremely uncommon.

For identification, the clay minerals in the samples studied were separated from the rocks by routine methods. Limestones were treated with dilute hydrochloric or acetic acid and after washing were centrifuged. Sandstones and shales were disaggregated in an ultrasonic transducer. The 2-5 $\mu$  and < 2 $\mu$  fractions in the samples were obtained by standard settling techniques. X-ray diffraction patterns were taken with a Philips 'Norelco' unit, using copper radiation. Porous plates and glass slides were prepared. Table 1 gives X-ray diffraction spacings and intensity measurements for oriented talc samples from a metamorphic rock and from a limestone.

Ancient carbonate sediments in which talc was recognized are too many to be listed here, but include the following: Whiskey Creek Pass limestone (Deamoinean),

Table 1. X-RAY DIFFRACTION SPACINGS AND INTENSITY MEASUREMENTS FOR ORIENTED TALC SAMPLES FROM METAMORPHIC ROCK AND LIMESTONE (Copper radiation, 40 kV 20 mamp, 1°/min)

Talc from metamorphic rock Barton, Pennsylvania (20656)* (aluminium sample holder)			Talc from limestone Oolitic limestone, Pleistocene, Fresh Creek, Andros Island, Bahamas (glass slide)		
2 $\theta$	d (Å)	I <sub>rel</sub>	2 $\theta$	d (Å)	I <sub>rel</sub>
001	9.40	100	0.40		100
002	4.60	25	0.60		25
003	3.12	93	0.82		83
004	2.329	5.1	0.97		2.5
005	1.870	8.0	1.07		7.0
006	1.569	4.0	1.26		1.6
007	1.336	3.0			

\* The number in parenthesis is the American Museum of Natural History sample designation.

La Veta Pass, Colorado; Beaverhill Lake Formation (Devonian), Swan Hills area, Alberta, Canada; Bell Canyon limestone (Permian), Guadalupe Mountains, West Texas; Green River Formation (Eocene), Piceance Basin, Colorado; and Pleistocene limestones of the Bahamas and Bermuda. The range of environments is open marine, reef, shelf, and non-marine, and includes both dolomitic and non-dolomitic rocks.

Griffin<sup>1</sup> found talc as a clay mineral in Recent unconsolidated sands of some of the beaches along the Gulf Coast. In the present study talc was recognized in Recent sand sediments from many areas, including beaches of Long Island, New York, bays of the Atlantic coast (Barnegat Bay, New Jersey) and Gulf of Mexico (Laguna Madre, Texas), and the Continental Shelf of the Atlantic Coast. Recent carbonate sediments in which talc was found include reef apron sands of the Red Sea (Gulf of Aqaba). Talc was also recognized in Recent salt lakes of West Texas.

The occurrence of talc as a clay mineral in evaporite deposits has been reported by others (Stewart<sup>2</sup>, Langbein<sup>3</sup>, Raymond<sup>4</sup>, and Dreizler<sup>5</sup>). Stewart<sup>2</sup> and Raymond<sup>4</sup> suggested a diagenetic origin for talc in the evaporitic sediments which they described, and Dreizler<sup>5</sup> noted that talc and other minerals in the Zechstein (Upper Permian) sediments which he studied are syngenetic or diagenetic. In non-evaporitic rocks, Millot and Palasus<sup>6</sup> reported talc from a dolomitic limestone. These authors favour a sedimentary (diagenetic) origin for talc in the limestone which they studied.

An authigenic origin is proposed for talc which occurs in limestone. The following observations support this inference: 1, talc favours limestone association and is extremely uncommon in other lithologies, except in evaporites; 2, it prefers chlorite association in limestones and also in metamorphic rocks (greenschist facies); 3, the associated chlorite is a magnesium chlorite; 4, the talc-chlorite ratio tends to be essentially constant, approximately 3 or 4 to 1; and 5, the talc-chlorite clay mineral suite is found in Pleistocene carbonates, such as in the Bahamas and in Bermuda, where influx of terrigenous debris was very minor. A reaction between Mg ions and silica may be responsible for talc formation.

Another three-layer (2:1) silicate of the talc group, pyrophyllite, has been reported in sediments. Diagenetic pyrophyllite has been recognized in Devonian shaly sands by de Segonzac and Millot<sup>7</sup>, in the Zechstein (Upper Permian) sediments of Germany by Fuchtbauer and Goldschmidt<sup>8</sup>, and in the Gothlandien-Devonian sediments of the Sahara by Rouge, Kulbicki and Kubler (oral communication to de Segonzac and Millot 1962, p. 3440). Ehlmann and Sand<sup>9</sup> reported pyrophyllite as an important constituent of shales, but believed that a hydrothermal or pneumatolytic origin is most likely for this mineral. Pyrophyllite was not found in the sediments I have examined.

This work was carried out at the Research Center of Pan American Petroleum Corporation; Mr. Truman Robinson prepared most of the X-ray patterns. Samples of metamorphic and hydrothermal talc were provided by the

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## MINERALOGY

### Differential Thermal Analysis: Effect of Particle Size

SINCE differential thermal analysis involves the approach to equilibrium while the conditions are regularly changing (steady temperature increase), the recorded temperature of transformation depends on the reaction rate. This rate is affected by a catalyst. Rees<sup>1</sup> states that although the theory of catalytic action is only just being established, the dominant role of defects is generally accepted. More defects cause a decrease in the degree of crystallinity, which lowers the recorded temperature of transformation, as shown by the experimental results quoted by van der Marek<sup>2</sup>.

One of the many defects tabulated in the first category of a review on the significance of solid state defects by Rees<sup>1</sup> is the discontinuity at a perfect surface. This surface is similar to that of a mosaic, which is an arrangement of sub-parallel blocks less than one micron in size. The fact that nearly all perfect-looking crystals contain sufficient defects to be idealized as a mosaic was deduced from the reduced intensities of the observed X-ray diffraction reflexions compared with the expected intensities from a perfect crystal. These deductions agree with my earlier results<sup>3</sup>. I observed that any particle size range greater than one micron gave a similar recorded temperature of transformation for a particular reaction.

Since the surface area is inversely proportional to the particle size, the less than ten milli-micron fraction, which has a greater surface area than a mosaic, will produce a decrease in the recorded temperature of transformation. In order to check the reduction in the recorded temperature of transformation, it is necessary to obtain fractions possessing identical other defects. However, the only available methods of fractionation and grinding have been shown to yield unsuitable material<sup>4</sup>. It is expected, therefore, that other defects in this size range would make the plane surface defect appear to be insignificant.

In conclusion, the recorded temperature of transformation is not affected by any particle size range greater than one micron, and is unlikely to be materially affected by a sub-micron size range.

Another type of structure, or an impurity, may affect the reaction rate. The accelerating catalysts are called activators or promoters, while those that decrease the reaction rate are called poisoners or depressants. The technique of annealing will remove many other types of structure and their associated effects on the reaction rate.

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## OCEANOGRAPHY

### High Interstitial Water Chlorinity in Estuarine Mangrove Swamps, Florida

RECENT investigations have shown that in coastal mangrove swamps of south-western Florida the average chlorinity of interstitial water contained in 2-4 ft. of unconsolidated sediment overlying bedrock is 2-12 parts per thousand greater than the average value of the overlying bottom water. General considerations would suggest that the two averages should be the same if chloride ions move across the sediment-water interface by molecular diffusion only.

During the rainy summer and autumn months these paludic swamps receive run-off from the extensive mainland swamps of southern Florida (Everglades) and consequently are flooded by fresh and brackish water of low chlorinity (0-15 parts per thousand). Throughout the following winter and spring drought months, marine water of normal (19 parts per thousand) to hyper-chlorinity invades the mangrove swamps. Submerged areas of the coastal swamps (excluding the mangrove forest) are typically 4-5 ft. deep at low tide<sup>1</sup>.

The high interstitial water chlorinity observed in the sediments of these swamps was initially interpreted as a palaeochlorinity<sup>2</sup>. The results of this investigation, however, have established that the difference between the average sediment-water and bottom-water chlorinity is too large to be attributed to palaeochlorinities. In some back-swamp areas the difference is as much as 12 parts per thousand; the typical contrast is 6-7 parts per thousand. In general, the difference between the two averages increases with distance into the coastal swamps; the difference narrows as the mainland fresh-water swamps are approached. The large discrepancy rules out a palaeochlorinity interpretation because the sediment column is so thin (2-4 ft.) that any systematic decrease (or increase) in bottom-water chlorinity of the magnitude required would soon bring about similar changes in sediment-water chlorinity owing to ionic diffusion. Only if a dramatic decline in average bottom-water chlorinity had recently occurred could a palaeochlorinity interpretation be maintained. Chlorinity measurements during the past decade and the average bottom-water chlorinity estimated from the molluscan fauna of the surface sediment indicate that this instigating requirement has not occurred.

Anomalous high interstitial water chlorinity may also result from an *in situ* mechanism which retains chloride ions within the sediment. Such a mechanism, involving compactive dewatering of the sediment and the development of a clay-water system behaving as a semipermeable membrane, has been described by Siever *et al.*<sup>3</sup>. Deposits of the coastal mangrove swamps, however, are thin, essentially lack clay minerals, and, except for peat layers, have necessarily undergone little compaction. Many of the sediments are quartzose and shelly sands and coarse silts and accordingly are generally permeable; the necessary semipermeable membrane would therefore appear to be absent.

Digging animals may mechanically enrich surface sediment in relatively high-chlorinity water. Burrowing would in general be most active at times when the swamps are occupied by marine water. Biological turn-over of the sediment, or penetration of bottom water into the sediment mass along burrows, would therefore tend to cause the entrainment of water having a chlorinity considerably higher than the average value for the area. Arguing against this is the fact that the number of marine or euryhaline burrowing organisms found in areas of low average water chlorinity—the areas of maximum difference in average bottom-water chlorinity and sediment-water chlorinity—is quite small<sup>4</sup>. Thus digging organisms would appear to account for only localized enrichment of chloride ions in interstitial waters.

Laboratory experiments by Callame<sup>4</sup> have shown that convective interchange across the sediment-water interface may rapidly implant water of relative high chlorinity in sediment saturated with water of lower chlorinity. Convective interchange involves the sinking, penetration, or entrainment of relatively dense bottom water into sediment containing water of lower density. In the mangrove swamps the density of the water is largely a function of, and directly proportional to, its salt content, of which chlorinity is a measure. The depth to which interchange may displace interstitial waters, and the rate at which interchange proceeds, is largely a function of the density difference and the permeability of the sediment. In this regard, anomalously high interstitial-water chlorinities in the coastal swamps are restricted to regions affected by rapid and pronounced seasonal changes in bottom-water chlorinity, that is, density. Consequently, sediment water which has had its density appreciably lowered by ionic diffusion across the sediment-water interface during the time that low-chlorinity water floods the swamps, is rapidly (1-4 months) covered by water of relatively much higher density as the dry season commences. *In situ* permeabilities of the loosely compacted peaty and shelly surface sediment<sup>1,2</sup> of the swamps are probably high enough to permit some sort of convective interchange. In the laboratory, permeabilities as high as 1-6 darcys have been measured on these sediments.

Even though supporting work has yet to be done, it is suspected that a convective interchange mechanism may account for the high interstitial-water chlorinity measured in mangrove estuarine sediments of south-western Florida. If this suspicion proves to be correct, then the interchange probably occurs during just the initial phase of the seasonal incursion of marine water into the coastal swamps. Following the gravity-induced interchange of bottom water and sediment water in the upper foot or so of the sediment, additional exchange of chloride ions across the sediment-water interface probably proceeds only by molecular diffusion. The diffusion-only stage is illustrated in Fig. 1 by the vertical distribution of interstitial-water chlorinity at station 62-100. These data represent conditions prevailing several months after marine water had completed its seasonal flooding of the swamps. Station

K2 (Fig. 1) was occupied in an area near that of 62-100, but during the low-chlorinity season.

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## CRYSTALLOGRAPHY

### Preliminary X-Ray Data on Phenothiazine and Certain of Its Derivatives

Work is now in progress at the University of Cape Town to determine the complete molecular structures of phenothiazine and of certain of its derivatives, chlorpromazine base<sup>1</sup> and stelazine. The last-mentioned two are well-known drugs, used as tranquillizers and in the treatment of certain mental diseases, such as schizophrenia, and it is hoped that knowledge of their molecular structure will help in the understanding of their biochemical action in man.

**Phenothiazine.** Pale yellow needle-shaped crystals were prepared by evaporating slowly from a solution of carbon tetrachloride. Methyl alcohol was also a satisfactory solvent. X-ray rotation and Weissenberg photographs, using copper  $K\alpha$  radiation, have shown the unit cell to be orthorhombic with dimensions as given in Table 1. A previous report<sup>2</sup> gave cell dimensions in good agreement with those of this investigation.

The conditions for non-extinction were found to be  $h00, h = 2n$ ;  $0kl, k + l = 2n$ ;  $h00, h = 2n$ ;  $0k0, k = 2n$ ;  $00l, l = 2n$ . These lead to two possible space groups,  $Pna2_1$  and  $Pnma$ , the first of which is non-centrosymmetrical, and the second centrosymmetrical. The  $h00$  intensity data were measured on the microdensitometer, and the method of Howells, Phillips and Rogers<sup>3</sup> was applied. A statistical survey of the intensities of the reflexions gave an  $N(z)$  distribution very close indeed to the theoretical curve for a centrosymmetric projection, and the space group is therefore assumed to be  $Pnma$ .

The density of 1.35 implies 4 molecules per unit cell, in special positions.

**Chlorpromazine hydrochloride.** This substance was obtained in powder form from the May and Baker Laboratories, and crystals were formed by slow evaporation from a heated solution of benzene to which a few drops of ethanol had been added. The crystals were thin, transparent plates, forming in clusters, which were difficult to separate. They turned brown if exposed to light and were affected by exposure to X-rays when in air. Following a valuable suggestion made by the Société des Usines Chimiques, Rhône-Poulenc<sup>4</sup>, better crystals were obtained when the evaporation was carried out under nitrogen, and the deleterious action of the X-rays in air was considerably reduced by mounting the crystals in fine capillary tubes which were also filled with nitrogen and sealed.

X-ray rotation and Weissenberg photographs taken about the  $a$  and  $s$  axes have shown the unit cell to be monoclinic with the dimensions as given in Table 1.

The systematic absences were found to be  $0k0$  missing for  $k$  odd, and  $h0l$  missing for  $l$  odd, which lead uniquely to the space group  $P2_1/c$ .

X-ray powder data have been obtained for both phenothiazine and chlorpromazine hydrochloride, from powder photographs, and, in good agreement with these, from the automatic diffractometer.

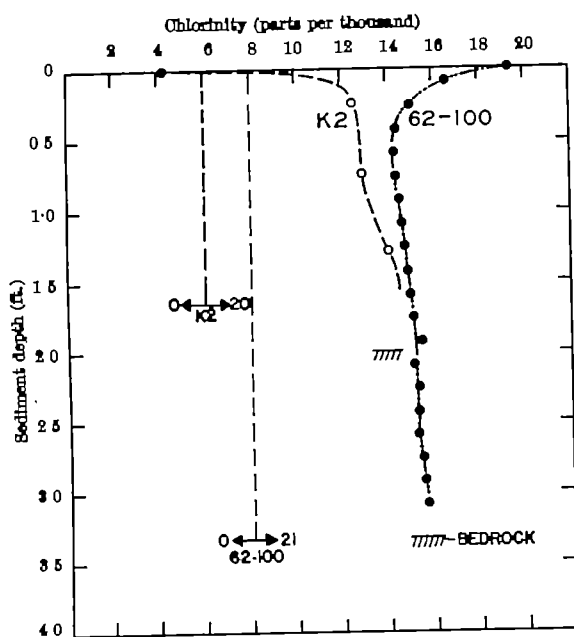
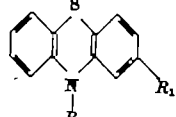


Fig. 1. Vertical distribution of interstitial water chlorinity measured in two sediment cores taken from the coastal mangrove swamps of south-western Florida. The vertical dashed lines indicate the approximate average ( $\pm 1$  part per thousand) bottom-water chlorinity at stations K2 and 62-100. Values inserted on either side of the arrows at the base of the lines show the range in monthly chlorinity averages.



Table 1

	Phenothiazine	Chlorpromazine hydrochloride	Chlorpromazine base	'Torecan'	'Stelazine'
					
$R_1$	H	Cl	Cl	$SC_2H_5$	$OC_2H_5$
$R_2$	H	$(OH)_2N \begin{array}{c} \diagup CH_3 \\ \diagdown CH_3 \end{array} + HCl$	$(OH)_2N \begin{array}{c} \diagup CH_3 \\ \diagdown CH_3 \end{array}$	$(OH)_2N \begin{array}{c} \diagup CH_3 \\ \diagdown CH_3 \end{array}$	$(OH)_2N \begin{array}{c} \diagup CH_3 \\ \diagdown CH_3 \end{array}$
Formula	$C_{15}H_9N_3S$	$C_{17}H_{19}N_3SO_2$	$C_{17}H_{17}N_3SO$	$C_{18}H_{19}N_3S_2$	$C_{18}H_{17}N_3FS$
Molecular weight	199.266	356.325	318.361	399.508	407.492
Density					
Flotation liquids	Water + mercury potassium iodide	Benzene + carbon tetrachloride	Water + mercury potassium iodide	Water + silver nitrate	Water + silver nitrate
g/c.c. meas.	1.865	1.31	1.289	1.21	1.27
g/c.c. calc.	1.842	1.25	1.285	1.19	1.29
No. of mols.	4	8	8	4	8
Crystal system	Orthorhombic	Monoclinic	Orthorhombic	Orthorhombic	Monoclinic
Space group	$Pnma$	$P2_1/c$	$Pbca$	$P2_12_12_1$	$Cc$
$a$ (Å)	$7.94 \pm 0.01$	$11.99 \pm 0.02$	$23.50 \pm 0.04$	$13.07 \pm 0.01$	$18.86 \pm 0.04$
$b$ (Å)	$21.02 \pm 0.01$	$32.83 \pm 0.06$	$15.20 \pm 0.02$	$19.99 \pm 0.02$	$9.27 \pm 0.03$
$c$ (Å)	$5.91 \pm 0.01$	$9.89 \pm 0.02$	$9.23 \pm 0.01$	$9.25 \pm 0.01$	$25.67 \pm 0.07$
$\beta$	—	99°	—	—	$111^\circ 01' \pm 7'$
Volume (Å <sup>3</sup> )	986.4	8,787	3,297	2,232	4,193
Solvent	Carbon tetrachloride or methyl alcohol	Benzene + ethanol under nitrogen	Low b.p. petrol ether under nitrogen	Petrol ether	Low b.p. petrol ether

Note: The limits of error may be slightly larger than those quoted, as allowance was not made for film shrinkage.

**Chlorpromazine base.** Crystals were supplied by the Smith, Kline and French Laboratories (United States), the most suitable of which for single-crystal work were those which had been crystallized from low b.p. petrol ether. As in the case of chlorpromazine hydrochloride, the base was also susceptible to light, and to exposure to X-rays when in air, so it was re-crystallized under nitrogen in the dark from low b.p. petrol ether, and the crystals were mounted in nitrogen-filled fine capillary tubes.

X-ray rotation and Weissenberg photographs taken about the  $y$  and  $z$  axes gave an orthorhombic crystal system, with dimensions as given in Table 1.

The conditions for non-extinction were:  $0kl$ ,  $k = 2n$ ;  $h0l$ ,  $l = 2n$ ;  $hk0$ ,  $h = 2n$ ;  $h00$ ,  $h = 2n$ ;  $0k0$ ,  $k = 2n$ ;  $00l$ ,  $l = 2n$ ; which lead uniquely to the space group  $Pbca$ .

So far as we are aware, no crystal data have yet been published on chlorpromazine hydrochloride or on the base.

**Torecan** (see Table 1) (Santoz, Baale, Switzerland). Completely clear crystals of torecan were re-crystallized easily from petrol ether. From the reflexions observed:  $h00$ ,  $h = 2n$ ;  $0k0$ ,  $k = 2n$ ;  $00l$ ,  $l = 2n$ ; no other conditions; the space group was uniquely determined as  $P2_12_12_1$ . No further work on this compound is intended.

**Stelazine** (see Table 1). Pale yellow crystals were re-crystallized from low boiling point petrol ether. From the observed reflexions:  $h0l$ ,  $h = 2n$ ;  $0k0$ ,  $k = 2n$ ;  $hkl$ ,  $h + k = 2n$ ; two space groups,  $O2/c$  and  $Oc$ , were possible.

The intensity statistics test of Howells *et al.*<sup>2</sup> was made on the  $h0l$  X-ray data and this showed a non-centrosymmetric space group thus ruling out  $O2/c$ . The space group is therefore assumed to be  $Oc$ .

Approximately 1,800 reflexions have been recorded by the multiple-film technique and measured using a microdensitometer. A three-dimensional Patterson function has been calculated and is at present being interpreted.

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## Growth Mechanism of Silicon Carbide

It has been suggested<sup>1,2</sup> that the growth of silicon carbide crystals takes place by the spiral-growth mechanism. Even though this theory, at the time, did explain the formation of the various polytypes, it did not account for the growth of the crystals in the 'a' direction. Recent X-ray work has shown the presence of new polytypes, the formation of which could not be explained by the screw-dislocation mechanism<sup>3,4</sup>.

This communication describes defects observed on some of the crystals which suggest that another mechanism could also play a part in the growth of silicon carbide. Examination of crystals under the optical microscope in some cases showed a series of steps around the edge ('root') which, during the sublimation-growth of the crystals<sup>5,6</sup>, was attached to the wall of the cavity. The steps were decreasing the thickness of the crystal in the direction of the root. In a few cases, such steps covered the whole basal plane.

Growth and evaporation spirals were also observed. The simultaneous presence of evaporation spirals and of steps is shown in Fig. 1a, while Fig. 1b shows part of the surface at a higher magnification. A surface similar in appearance was obtained by Young<sup>7</sup> on thermal etching of silver, when steps caused by evaporation propagated around an evaporation spiral. However, the structure shown in Fig. 1b could not have been formed by the same mechanism: the curvature of the steps suggests that they propagated from right to left; as the root of the crystal is to the left and the crystal is thinner there, the steps must have been formed by growth and not by etching. The interaction between the growth ledges and the spiral must have occurred due to line tension.

On some other crystals, the simultaneous presence of steps and of parallelepiped-shaped occlusions, appearing as light-coloured areas, was observed. The interaction of steps with two such occlusions is shown in Fig. 1c. The steps originating at two edges of the smaller occlusion were of relatively small height. To make them easier to locate, dashed parallel lines were drawn adjacent to them. Their curvature again suggests that they were advancing from right to left and must therefore have been caused by growth.

Steps were also observed to interact with defects that appeared as dark points in the photomicrographs (Fig. 1d). In such cases, the rate of advance of the steps was enhanced by the defects, suggesting the possibility that the defects were small spiral-growth hills.

It is difficult to visualize, even should critical concentrations of impurities be present<sup>8</sup>, how the spiral-growth

up to six months, although there was a greater proportion of polyhedra in the preparation with the most octo-*octearyl* alcohol. Preparations of this type with *cetyl* alcohol tend to develop needle-like crystals quite distinct from those particles shown in Figs. 1 and 2. The formation of polyhedra would not therefore appear to be a crystallization phenomenon.

Martynov<sup>6</sup> has pointed out that a non-spherical surface may have a lower surface energy than a spherical surface enclosing an equal volume. He found that a dodecahedron has a lower energy than spheres of equivalent volume below  $10^{-6}$  cm radius. However, non-spherical particles in our emulsions are at least two orders of magnitude larger than this (Fig. 2) and, while this effect cannot be discounted, we feel that an alternative explanation is required.

Manegold<sup>7</sup> described the formation of honeycomb or "polyhedral drop foams" in unstable close-packed emul-

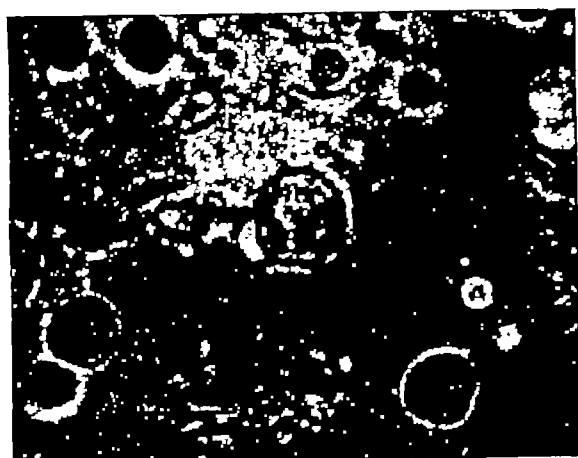


Fig. 1. Photomicrograph of emulsion A, cooled while being subjected to ultrasonic irradiation. Diluted in glycerol containing 0.01 per cent methylene blue. 1 division,  $10\mu$ .



Fig. 2. Electron photomicrograph of emulsion B, allowed to cool undisturbed. Dispersed in bovine plasma albumin Fraction V (Armour) and sprayed on to grids coated with 'Formvar' and carbon films. When dry the grids were placed overnight in a holder over 0.5 per cent osmium tetroxide solution. The grids were then examined in an A.B.I. JEM3 electron microscope (method due to Dr. D. J. Craik, personal communication). 1 division,  $1\mu$ .

sions with disperse phase ratios in excess of 0.74, the theoretical limiting value for close-packing spheres of equal diameter. Our emulsion system has a disperse phase ratio of 0.225 and is apparently stable. Nevertheless, although the system as a whole cannot be close-packed, it appears likely that localized close-packing areas can exist, especially as the system has a wide range of particle sizes. This appears to be confirmed from examination of the undiluted emulsion and from electron photomicrographs when samples have not been adequately dispersed, for example, parts of Fig. 2. Under these conditions some particles or droplets are distorted by being close-packed and in this system these particles retain their original shape acquired during or following the emulsification stage. It is clear that the treatment of the liquid-liquid emulsion during cooling exerts an influence on the number of polyhedra which are seen in the subsequent solid-liquid dispersion. During dilution for microscopic analysis the particles in our system are solid and hence retain their shape. It seems likely that, in others, liquid particles may be able to retain their shape owing to the rigidity of the stabilizing interfacial film<sup>8</sup>. On the other hand, the disperse phase and the stabilizing interfacial film may be sufficiently fluid for the droplet to 'deform', or revert to a spherical droplet, as soon as the restraining conditions of tight packing are removed on dilution.

It may be concluded that some particles in an emulsion system may be irregular in shape. Whatever the cause, this irregularity can exist in systems where the disperse phase ratio is well below 0.74. In any investigation, therefore, of an undiluted emulsion it may not be tacitly assumed that all the disperse phase particles are spherical globules.

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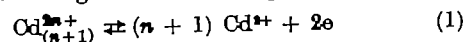
<sup>6</sup> Martynov, V. M., *Kolloid Zh.*, 12, 359 (1950).

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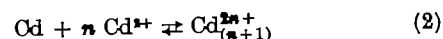
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### Cadmium-Cadmium Chloride Solutions

THE nature of cadmium-cadmium chloride solutions is still the subject of considerable discussion, as also are the other metal-halide mixtures. The redox process between divalent cadmium and the species originating from the dissolution of metallic cadmium implies two electrons<sup>1,2</sup>, according to the following reaction:



This result agrees with a dissolution process the general scheme of which is as follows:



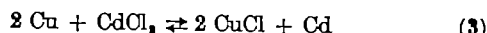
The most widely accepted values for  $n$  in the foregoing scheme are zero and unity; however, lately Topol and Landis<sup>3</sup>, on the basis of cryoscopic measurements by Grijthuis *et al.*<sup>4</sup> and a value of 7.22 kcal/mole for the heat of fusion of cadmium chloride, proposed values of 2 and 3.

In order to contribute to the solution of this problem, we investigated the distribution equilibrium of cadmium between molten cadmium chloride and a molten cadmium-copper alloy.

Weighed quantities of copper, cadmium and cadmium chloride were sealed in evacuated ampoules and main-

tained at 865° K for about 12 h. The ampoules were successively quenched and the two phases separated. The metallic phase was dissolved in nitric acid and the resulting solution analysed polarographically; the salt phase was dissolved in water, thus producing a cadmium chloride solution and a powdered cadmium residue. This residue was analysed in the same manner as the metallic phase and was found to contain some copper.

The copper content of the cadmium powders has been attributed to high-temperature oxidation of copper according to the reaction:



the equilibrium constant of which at 865° K is about  $10^{-4}$  (approximate value calculated from free-energy data<sup>1</sup>).

When the salt phase is dissolved in water, cuprous copper is reduced again to metal by the cadmium powder. This hypothesis is supported by the fact that the quantities of copper found agree well with those calculated on the basis of the foregoing equilibrium constant value. Therefore, the quantity (in moles) of cadmium metal found in the salt phase must be increased by an amount equal to half the quantity of the copper metal found, and the quantity of cadmium chloride must be decreased by the same amount. The mole fractions,  $N$ , of the components of the salt phase, calculated according to the foregoing statements, are given in columns 2–4 of Table 1 as a function of the mole fraction  $x_{\text{Cd}}$  of cadmium in the metal phase (column 1). The presence in the melt of the cuprous chloride is believed not to interfere seriously with the main equilibrium (2).

The ionic fractions,  $X$ , of the species  $\text{Cd}^{2+}$  and  $\text{Cd}_{(n+1)}^{2n+}$  are related to the crude mole fractions,  $N$ , of  $\text{CdCl}_2$ ,  $\text{Cd}$  and  $\text{CuCl}$  in the salt phase by the following equations, which are easily obtained from the stoichiometry of the reaction (2):

$$X_{\text{Cd}_{(n+1)}^{2n+}} = \frac{N_{\text{Cd}}}{N_{\text{CdCl}_2} - (n-1)N_{\text{Cd}} + N_{\text{CuCl}}}$$

$$X_{\text{Cd}^{2+}} = \frac{N_{\text{CdCl}_2} - nN_{\text{Cd}}}{N_{\text{CdCl}_2} - (n-1)N_{\text{Cd}} + N_{\text{CuCl}}}$$

The equilibrium ratio for the reaction (2) is thus:

$$K_s = \frac{X_{\text{Cd}_{(n+1)}^{2n+}}}{(X_{\text{Cd}^{2+}})^n \cdot a_{\text{Cd}}} = \frac{N_{\text{Cd}} \cdot [N_{\text{CdCl}_2} - (n-1)N_{\text{Cd}} + N_{\text{CuCl}}]^{n-1}}{[N_{\text{CdCl}_2} - nN_{\text{Cd}}]^n \cdot a_{\text{Cd}}}$$

where the ionic fractions of  $\text{Cd}^{2+}$  and  $\text{Cd}_{(n+1)}^{2n+}$  have been used in place of the activities on the hypothesis of the approximate constancy of the activity coefficients in the composition range investigated.

The activity values of cadmium in the metal phase,  $a_{\text{Cd}}$ , have been obtained from the literature<sup>2</sup> and are given in column 5, Table 1.

The values of the equilibrium ratios,  $K_s$ , calculated for the various values of  $n$  from the experimental data, are given in columns 6–9 of Table 1.

As can be seen, the values of  $K_2$  and  $K_3$  vary with the composition over a wide range, thus rendering very questionable the presence of the species  $\text{Cd}_2^{4+}$  ( $n=2$ ) and  $\text{Cd}_3^{6+}$  ( $n=3$ ). It is difficult to distinguish between the two species  $\text{Cd}$  ( $n=0$ ) and  $\text{Cd}_1^{2+}$  ( $n=1$ ) since the variations of  $K_0$  and  $K_1$  are of a similar order of magnitude; our data, however, seem to indicate, although not

in a conclusive manner, the presence in the melt of the species  $\text{Cd}_1^{2+}$ .

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### Series Approximations to the Equation of Thermogravimetric Data

THE equation of the plot of thermogravimetric data is useful in kinetic analysis<sup>(1-12)</sup>, but since it contains an exponential integral, many workers prefer approximate expressions. The best balance of accuracy and convenience is achieved by the use of series taken to as few terms as possible, and it is the purpose of this communication to show which of four such abbreviated series approximations most nearly approaches this ideal.

In my notation<sup>1,12</sup>, the equation of the thermogravimetric data plot is:

$$g(h) = AE (BR)^{-1} p(x)$$

where  $h = (W-G) H^{-1}$

$$p(x) = e^{-x} x^{-1} - \int_x^\infty e^{-x} x^{-1} dx$$

and  $x = E (RT)^{-1}$

and where the specific form of  $g(h)$  depends on the type of kinetic process at hand<sup>1</sup>, and where  $A$ ,  $E$ ,  $R$  and  $T$  have their usual meanings as defined for the Arrhenius equation,  $B$  is the constant heating rate used in the analysis,  $W$  is the instantaneous residual sample weight and  $H$  and  $G$  are the total weight lost during the  $i$ th weight-loss step and the weight remaining after the step has been completed.

The series most commonly used in deriving approximations to  $p(x)$  are the asymptotic expansion (refs. 1, 2–7, 10–12) and Schlömilch's expansion (refs. 2, 8, 9). The expressions which result from taking these series to two and three terms are:

$$p(x) \sim x^{-1} e^{-x} \quad (\text{asymptotic, 2 terms}) \quad (\text{refs. 7, 10})$$

$$p(x) \sim (x-2)x^{-2} e^{-x} \quad (\text{asymptotic, 3 terms}) \quad (\text{refs. 3, 11})$$

$$p(x) \sim (x+1)^{-1} x^{-1} e^{-x} \quad (\text{Schlömilch, 2 terms})$$

$$p(x) \sim (x+2)^{-1} x^{-1} e^{-x} \quad (\text{Schlömilch, 3 terms}) \quad (\text{refs. 8, 9})$$

(It should be noted that the equations in refs. 8 and 9 are in error, owing to the use of the equivalent of  $1+2x$  rather than  $x+2$ .)

All these approximate expressions are convenient, save the second, which leads to an equation of the form:

$$x_s^2 (x-2_s)^{-1} \sim n$$

where  $n$  is a number derived from the experimental data, and  $x_s$  is evaluated at a particular value of  $T$ .

As for accuracy, the four approximate expressions can be compared<sup>12</sup> by taking the ratio between true and approximate values of  $p(x)$  for various values of  $x$ , as given in Table 1.

Table 1. RATIOS OF TRUE TO APPROXIMATE VALUES OF  $p(x)$

x	Asymptotic expansion		Schlömilch expansion	
	2 terms	3 terms	2 terms	3 terms
10	0.844	1.055	0.928	1.013
15	0.888	1.025	0.947	1.007
20	0.913	1.014	0.958	1.003
25	0.928	1.009	0.965	1.002
30	0.939	1.006	0.970	1.001
35	0.947	1.004	0.974	1.000
40	0.954	1.004	0.978	1.000
45	0.958	1.003	0.979	1.000
50	0.962	1.002	0.981	1.000

Table 1

1	2	3	4	5	6	7	8	9
$x_{\text{Cd}}$	$N_{\text{Cd}}$	$N_{\text{CuCl}} \cdot 10^4$	$N_{\text{CdCl}_2}$	$a_{\text{Cd}}$	$K_0$	$K_1$	$K_2$	$K_3$
1.000	0.143	0.000	0.857	1.000	0.143	0.200	0.213	0.505
0.950	0.135	0.068	0.803	0.950	0.143	0.197	0.208	0.532
0.881	0.130	0.167	0.803	0.881	0.145	0.196	0.200	0.493
0.821	0.125	0.251	0.872	0.856	0.146	0.196	0.203	0.464
0.768	0.120	0.300	0.877	0.813	0.148	0.195	0.276	0.438
0.596	0.104	0.590	0.890	0.660	0.157	0.200	0.268	0.386
0.404	0.062	1.120	0.897	0.525	0.176	0.218	0.281	0.384

Clearly, the 3-term Schlömilch expression is the best of all.

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### Electron Spin Resonance Absorption of Solid Ion-radical Salt of *N,N,N',N'*-Tetramethyl-*p*-phenylenediamine and 7,7,8,8-Tetra-*p*-quinodimethane

For most charge transfer complexes, the ground-state can be described chiefly by a non-bonding structure  $DA$ , as the contribution of the dative structure  $D^+A^-$  is rather small, where  $D$  is the electron donor and  $A$  is the electron acceptor. However, it has been shown<sup>1-3</sup> for the complexes formed between aromatic diamines and substituted *p*- and *o*-benzoquinones that the dative or ionic state is the lowest one. These complexes may be regarded as salts, and can be prepared from salts,  $D^+X^-$  and  $M^+A^-$ , by a double decomposition reaction, where  $X^-$  and  $M^+$  stand for inorganic ions.

The solid complex of *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) with 7,7,8,8-tetracyano-*p*-quinodimethane (TCNQ) has been taken as ionic by Foster and Thomson<sup>4</sup> on the basis of its visible absorption spectrum. We prepared the 1:1 TMPD<sup>+</sup>=TCNQ<sup>-</sup> salt from Würster's blue perchlorate (TMPD<sup>+</sup>ClO<sub>4</sub><sup>-</sup>) and lithium salt of TCNQ (Li<sup>+</sup>TCNQ<sup>-</sup>) by the double decomposition reaction. In contrast to the well-known TCNQ anion radical salts<sup>4,5</sup> in which the positive ion is diamagnetic, this salt is composed of paramagnetic positive and negative ions.

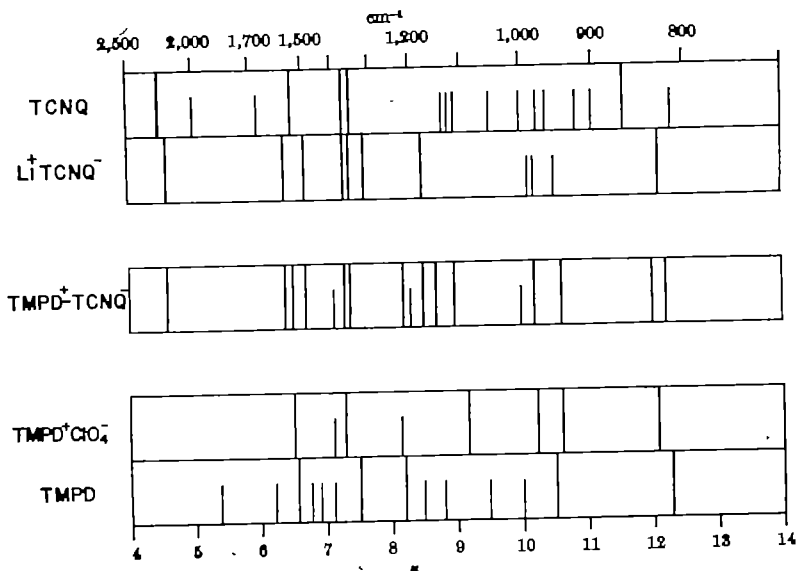


Fig. 1. Schematic illustrations for the infra-red absorption spectra of the salt and its related compounds, TCNQ, Li<sup>+</sup>TCNQ<sup>-</sup>, TMPD<sup>+</sup>ClO<sub>4</sub><sup>-</sup>, and TMPD

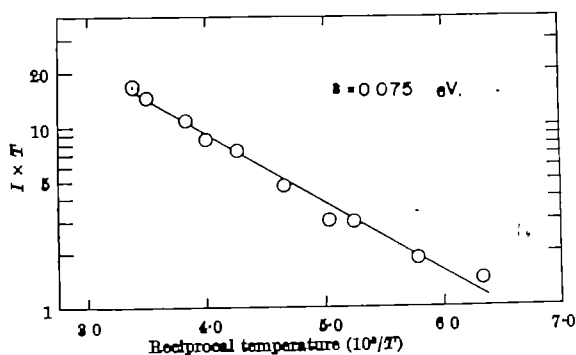


Fig. 2. Variation of the electron spin resonance absorption intensity of the salt with temperature

The infra-red absorptions for the salt and its related compounds were examined by the method of Nujol mulls. The results are shown schematically in Fig. 1. By comparing the spectra with each other, it can be concluded that all the TCNQ and TMPD molecules are present as ion radicals in the salt. This observation is consistent with Foster and Thomson's conclusion for the complex prepared from neutral TMPD and TCNQ.

The electron spin resonance measurements were carried out on the freshly prepared polycrystalline sample of the salt. Temperature was varied by a flow of nitrogen gas at various temperatures between 150° and 300° K through the cavity. The sample showed a very sharp absorption of 6 gauss in over-all width, which was composed of three asymmetrically distributed lines. In the complex of *p*-phenylenediamine with *p*-chloranil, Matsunaga and McDowell<sup>6</sup> found that the electron spin resonance absorption consists of two peaks, which they assigned to each of the ionized component molecules. Recently, however, these two peaks have been shown to be due to the *g*-factor anisotropy<sup>7</sup>. In our case, the three peaks are also accounted for by the same cause.

The ratio of the electron spin resonance intensity of one pair mole of the salt to 2 moles of DPPH was found to be 0.17 at 23° C. If it is assumed that each ion radical in the salt contributes independently to the paramagnetism, this ratio should be one, because both component molecules are completely ionized. The observed ratio is inconsistent with this. Therefore it is likely that the paramagnetic state is an excited triplet state as has been suggested by Bijl, Kainer and Rose-Innes<sup>8</sup>. If the triplet state is thermally accessible, the variation of the electron spin resonance intensity  $I$  with temperature is given by:

$$I = C/T[3 + \exp(\delta/kT)] \approx \frac{C \exp(-\delta/kT)}{T} \quad (1)$$

where  $C$  is a constant and  $\delta$  is the separation between the singlet and triplet levels. As shown in Fig. 2, the observed temperature dependence is described by equation (1) with  $\delta = 0.075$  eV. Similar dependences on temperature for electron spin resonance intensity have been observed in the complexes of *p*-chloranil with diaminodurene<sup>4</sup> and *p*-phenylenediamine<sup>7</sup> with  $\delta$ -values of 0.16 and 0.13 eV, respectively.

The *g*-values for the salt and the component radicals were determined as follows:  $g(\text{salt}) = 2.0029 \pm 0.0001$ ,  $g(\text{TMPD}^+\text{ClO}_4^-) = 2.0032 \pm 0.0001$ , and  $g(\text{Li}^+\text{TCNQ}^-) = 2.0025 \pm 0.0001$ . The *g*-value of the salt agrees well with the mean of the *g*-values for TMPD<sup>+</sup> and TCNQ<sup>-</sup>. This indicates that the exchange interaction, which determines

the  $\delta$ -value, should occur between the unpaired electrons on TMPD<sup>+</sup> and TCNQ<sup>-</sup> radicals.

Although the assignment of the electron spin resonance absorption to the triplet state is a tentative one because of the lack of the detection of the  $\Delta m = \pm 2$  transition or single crystal work, it seems certain that a spin correlation exists between the unpaired electrons on the different ion radicals which enable the salt to be prepared. We also obtained the same results for the complex prepared from neutral components.

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## BIOCHEMISTRY

### Dialysis of Oligosaccharides

IDENTIFICATION of oligosaccharide intermediates liberated during the enzymatic hydrolysis of a polysaccharide provides information on both the structure and mode of hydrolysis of the polymer. Isolation of these intermediates in good yield is facilitated if they can be removed from the enzyme site before further hydrolysis occurs. Perila and Bishop<sup>1</sup> accomplished this removal by hydrolysing a glucomannan in a dialysis sac to give a good yield of disaccharides (28 per cent) and lesser yields of tri- and tetra-saccharides (8 per cent each), and Timell<sup>2</sup> likewise hydrolysed a glucuronoxylan to moderate yields of oligosaccharides (none of these authors specified the grade of dialysis tubing used). Similarly, with a protozoal xylanase, we found that a convincing demonstration of the liberation of xylooligosaccharides of a degree of polymerization (DP) > 3 was only obtained when the xylan was hydrolysed by the enzyme with concurrent dialysis in 20/32 'Visking' tubing (Union Carbide Corp., N.Y.). Oligosaccharides of DP 3-5 are of relatively low molecular weight, but in our experience they dialysed at far slower rates than those reported for comparable peptides (see Table 2, Craig and King<sup>3</sup>). The dialysis of various oligosaccharides was therefore investigated in a simple dialysis chamber consisting of a piece of glass tubing (7 × 3.5 cm diam.) closed at one end with a sheet of opened-out dialysis tubing. The closed end dipped just below the external solution (10 ml.), the oligosaccharide solution (10 mg in 2 ml.; 3.3 cm<sup>3</sup> of membrane per ml.) was placed inside the chamber and both solutions were gently stirred with magnetic stirrers. At intervals, portions (100  $\mu$ l.) of external solution were removed for the measurement of dialysed oligosaccharide so that the time for the diffusion of half the initial oligosaccharide (half-escape time) could be calculated. Three grades of 'Visking' seamless cellulose tubing were used; 20/32, 36/36 and O-75 heavy wall.

The half-escape times obtained at room temperature (20° C) under these conditions for various oligosaccharides are given in Table 1.

With the maltodextrins a plot of these results against DP shows, for each grade of tubing, a linear increase in half-escape time with increasing DP. While the dialysis of these neutral oligosaccharides appears to be

Table 1. DIALYSIS OF OLIGOSACCHARIDES

Oligosaccharide (mol. wt.)	Solvent	Half-escape time (h) at 20° or (37°)		
		20/32	Tubing 36/36	O-75
Maltose (342)	Water	0.45	1.15	1.81
Maltotriose (504)	Water	0.87	1.66	2.31
Maltotetraose (666)	Water	1.25	—	2.76
Maltopentaose (828)	Water	1.60	—	3.00
Maltopentaose	Citrate-phosphate, pH 3.5	(0.80)	—	(1.70)
	Citrate-phosphate, pH 5.0	1.60	—	—
Maltopentaose	Citrate-phosphate, pH 5.0	1.55	—	4.00
Maltohexaose (990)	Water	1.88	3.10	—
Malto-octaose (1314)	Water	2.36	4.00	5.66
Cellopentaose (828)	Water	1.77	—	—
Isomaltopentaose (828)	Water	2.08	—	—
Raffinose (504)	Water	0.85	—	—
Trigalacturonic acid (646)	Citrate-phosphate, pH 7.0	2.30	—	—
Trigalacturonic acid	Citrate-phosphate, pH 3.5	1.40	—	—

independent of pH, the results with trigalacturonic acid suggest that the dialysis rates of acidic oligosaccharides are pH-dependent. It is evident that when good yields of oligosaccharides of DP > 2 are required from such dialysis digests the narrow 20/32 tubing, which would not be the logical choice for large-scale digests, should be used at as high a temperature as the enzyme will tolerate. Alteration of the porosity of dialysis tubing by stretching, as described by Craig and King<sup>3</sup>, might also give faster dialysis of these compounds.

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### A Staining Method for Proteins and Dextran on Cellulose Acetate

AFTER the electrophoresis of human albumin labelled with iodine-131 in 0.06 M veronal buffer pH 8.6 on cellulose acetate, the strips were stained, dried, cleared in 'Dekalin' and scanned. The relationship between counting rate and dye uptake of amidoschwarz 10 B was linear up to, but not above, 30  $\mu$ g/cm, 0.5  $\mu$ l. of 6 per cent albumin applied evenly across 1 cm. Within this limit the method was suitable for the determination of albumin in human serum as percentage total dye uptake times total protein by the biuret method. Thirty-three sera checked using an internal <sup>125</sup>I-albumin standard gave results by this method and by the absolute albumin method of Lubran and Moss<sup>1</sup> which agreed within  $\pm 0.2$  g/100 ml. It was further observed that if the human eye could not see through the cleared band of stained albumin (or myeloma protein), then the strip was overloaded and in the non-linear range. No such simple safeguard existed for Ponocau S, where the relationship became non-linear above 15  $\mu$ g/cm, and indeed previous scans using this dye had underestimated the albumin. Accordingly, the following staining method was adopted.

Immediately after electrophoresis the strips are placed one at a time, applied surface downwards, into 0.25 per cent amidoschwarz 10 B in methanol, to each 100 ml. of which has been added 3 g of trichloroacetic acid to fix proteins immediately. After 5 min they are washed twice, for about 5 min, in methanol 90 parts: glacial acetic acid 10 parts, by which time the background is usually white. They are finally rinsed twice in methanol, and dried flat between blotting paper. Within 1-2 h they are ready for scanning, a considerable advantage over the aqueous

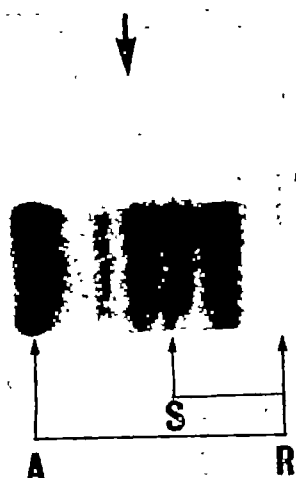


Fig. 1. Ten per cent 'Rheomacrodex' (above) and normal human serum with added 'Rheomacrodex' (below) applied at the heavy arrow and electrophoresed on cellulose acetate.  $SR/AR \times 1.00$  measures the relative mobility of  $\beta_1$ -globulin to albumin.

Ponceau S method, where drying takes many hours. The stain should be prepared afresh each week, or blue backgrounds occur.

During routine use a  $\delta$ -band was observed in a serum strip and was traced to the low molecular weight dextrans ('Rheomacrodex') which had been given to the patient (Fig. 1). Dextrans with no net charge are carried towards the cathode by endosmosis, thus marking the original line of application, and have long been used for the measurement of electrophoretic mobility relative to human albumin, which is taken as 1.00. In previous methods the dextran had to be separately identified<sup>1</sup>, but the foregoing accidental discovery offers a single method for fixing and staining both dextran and proteins. In the figure,  $\beta_1$ -globulin has a relative mobility of  $SR/AR \times 1.00$ . Applications at six different distances from the anode yielded results of  $0.47 \pm 0.01$ , which agree well with Wieme's value of 0.48 in agar.

'Rheomacrodex' is not fixed or stained by aqueous Ponceau S with trichloroacetic acid, but does stain with methanolic amidoschwarz 10 B without trichloroacetic acid, and this also works on chromatography paper, though longer washing is required. This may be applicable in the polysaccharide field.

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### $\alpha$ -Helix in Fibrous Proteins

THE  $\alpha$ -helix was proposed by Pauling *et al.*<sup>1</sup> as a stable configuration of the polypeptide chain, and considerable support for this view was obtained from the X-ray diffraction patterns yielded by naturally occurring and synthetic polypeptides<sup>2,3</sup>. The diameter of the  $\alpha$ -helix was estimated to be about 10 Å and includes the contribution of the side-chains as well as the main-chains of the protein<sup>4</sup>. Direct proof of the existence of  $\alpha$ -helices in the globular protein myoglobin was obtained by Fourier synthesis methods<sup>5</sup>, and there is evidence suggesting that the  $\alpha$ -group of fibrous proteins contains rope- or cable-like assemblies of distorted  $\alpha$ -helices<sup>6,7</sup>.

In a recent investigation of the fibrillation induced in  $\alpha$ -keratin by irradiating iodine-treated wool with ultrasonics, it was found<sup>8</sup> that microfibrils  $\sim 75$  Å across were visible in electron micrographs of negatively-stained samples. In many cases the microfibrils had frayed into

fine protofibrils  $\sim 20$  Å across, an observation which is consistent with the notion of a rope-like assembly of the type proposed by Crick<sup>9</sup>.

Later it has been found that the protofibrils fray still further into either a pair of filaments of unequal diameter as in Fig. 1a or three filaments of equal diameter as in Fig. 1b. In view of the considerable support for Crick's structure from the X-ray data<sup>9</sup> there is good reason to suppose that the finest filaments, which are  $\sim 10$  Å in diameter, represent a direct record of the  $\alpha$ -helix by electron microscopy.

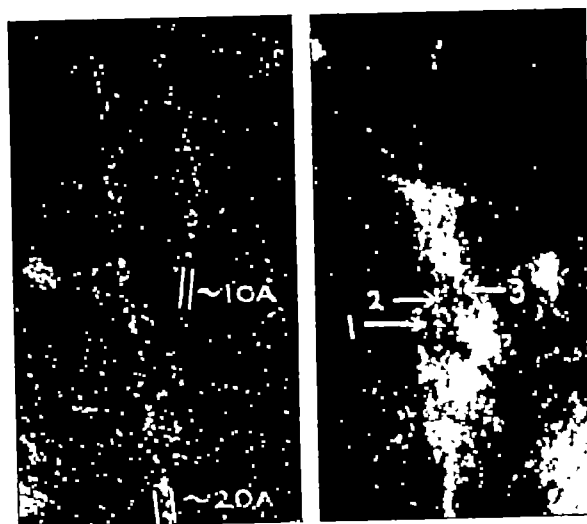


Fig. 1. Single protofibrils fraying into finer filaments ( $\times 600,000$ ).

It is too early for this evidence to be considered conclusive, but the simplest explanation of the situation in Fig. 1a is that one strand of a three-strand rope is detached from the other two, while in Fig. 1b all three strands are separate.

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### Electron Spin Resonance Spectra of Gamma-Irradiated Polycrystalline Glycollic Acid

THE electron spin resonance spectrum of glycollic acid irradiated at 77° K has been reported previously<sup>1</sup>, and consists of two doublets of unequal intensity, each with a separation of about 20 gauss. These were attributed to radicals having the unpaired electrons localized on oxygen atoms and coupling with the different hydroxyl protons.

Glycollic acid irradiated at room temperature gives an electron spin resonance spectrum consisting of a doublet with a separation of 25 gauss<sup>2</sup>. This was shown by deuteration studies, and later confirmed by single crystal experiments<sup>3</sup> to be due to the radical HO-CH-COOH.

We have irradiated polycrystalline glycollic acid at 77° K and observed the changes in spectrum as the crystals are warmed to room temperature. Glycollic acid was purified by repeated sublimation *in vacuo* at 45° C. It was then powdered and thoroughly pumped free of gas in a silica tube, which was finally sealed at a pressure of  $10^{-4}$  torr.

Samples received  $10^4$ – $10^5$  rads from a cobalt-60  $\gamma$ -ray source at a dose rate of  $1.4 \times 10^5$  rads h $^{-1}$ . While maintaining the crystals at 77° K, the electron spin resonance signal was removed from one end of the tube by annealing, and the acid transferred to this end. Electron spin resonance measurements at all temperatures were made using a Varian X-Band spectrometer, 'Model 4200', with accessories, signals being recorded as their first derivative.

Three different signals were observed as the temperature was raised from 77° K to 300° K. These are shown in Fig. 1. Fig. 1a was obtained at 77° K and reveals a doublet of 28 gauss splitting, which agrees with the spectrum published by Gordy *et al.*<sup>1</sup>, with the exception of a further small feature near the centre of the spectrum. This signal is stable for several days at 77° K, but changes gradually at 90° K and rapidly at about 120° K into a more complex spectrum (Fig. 1b), the principal feature of which is a triplet with a total separation of 43 gauss. A weaker signal is also present which appears to be another triplet with a separation of 35 gauss. This spectrum changes slowly at 140° K but rapidly at 170° K into the third spectrum (Fig. 1c). The signal is similar in all respects to that obtained on irradiation at room temperature, which has been shown<sup>2,3</sup> to be due to the carboxyhydroxymethyl radical  $\text{HO}\cdot\text{CH}\cdot\text{COOH}$ .

As the temperature is increased it is observed that the growth of one signal follows the decay of the other, indicating that one radical is reacting to form another. All changes caused by increasing the temperature are irreversible. With increasing dose the signals become stronger, but are otherwise unchanged.

The two triplets of Fig. 1b are probably due to the two radicals  $\cdot\text{CH}_2\cdot\text{OH}$  and  $\cdot\text{CH}_2\cdot\text{COOH}$ . The splitting observed in the weaker of the signals is very similar to that of the radical found on irradiating methanol<sup>4,5</sup>. The  $\cdot\text{CH}_2\cdot\text{COOH}$  radical has not been identified in poly-

crystalline material so that its isotropic electron spin resonance spectrum is unknown. The triplet, believed to be due to the  $\cdot\text{CH}_2\cdot\text{COOH}$  radical, is substantially less stable in this system than is the same radical in irradiated single crystals of malonic acid<sup>6,7</sup>. The change to the doublet which represents the  $\text{HO}\cdot\text{CH}\cdot\text{COOH}$  radical would then involve the abstraction by both these radicals of hydrogen atoms from glycollic acid molecules. The doublet observed at 77° K is possibly an ionic precursor of the type suggested previously, that is,  $\text{HO}\cdot\text{OH}_2^+\cdot\text{COOH}$  or  $\text{HO}\cdot\text{CH}_2^+\cdot\text{COOH}$ .

We have examined the products of the radiolysis at room temperature of crystalline glycollic acid and find some evidence to support the foregoing postulates. Methanol and acetic acid are both formed as major products, as would be expected from the abstraction reactions referred to above. Tartaric acid is also present in considerable amounts, by dimerization of the  $\text{HO}\cdot\text{CH}\cdot\text{COOH}$  radical.

In order to form  $\cdot\text{CH}_2\cdot\text{OH}$  and  $\cdot\text{CH}_2\cdot\text{COOH}$  radicals, it is necessary to eliminate  $\cdot\text{COOH}$  and  $\cdot\text{OH}$  respectively. Carbon dioxide is by far the main gaseous product of radiolysis, but as yet no product has been found to account for  $\cdot\text{OH}$  group removal. However, an adequate method for the analysis of water in this system has not been found and it could well be that the  $\cdot\text{OH}$  group is ultimately converted to water.

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### Coding of Polar and Non-polar Amino-acids

BIOLOGICAL properties of proteins depend on the higher levels of their structure, determined by the primary structure. As has been shown by Fisher<sup>1</sup>, the knowledge of the amino-acid composition permits in many cases the tertiary and sometimes the quaternary structure of the proteins to be predicted. The ratio of the polar and non-polar amino-acids seems to be a very important characteristic of the protein. The change of the relative numbers of the polar and non-polar amino-acids as a result of a mutation must produce big changes in the protein structure. The mutational exchange of a polar group for a non-polar one and vice versa must be more dangerous than the transition from one polar group to another or from a non-polar to another non-polar one. The well-known example is the transition from Glu to Val in the case of sickle-cell haemoglobin.

If these speculations are correct, then we have to look at the corresponding differences in the codons for polar and non-polar amino-acids. Let us take the codons of *m* RNA established by Nirenberg *et al.*<sup>2</sup> (the results published by Speyer, Ochoa *et al.*<sup>3</sup> do not differ much from those in ref. 2). These codons are shown in Table 1 together with parts of the nucleotides in all the codons corresponding to the said amino-acid. The compositions of the codons of the first and of the second group are different. This is shown in Table 2.

We see that the polar amino-acids are coded mainly by triplets containing adenine and cytosine, and the non-polar

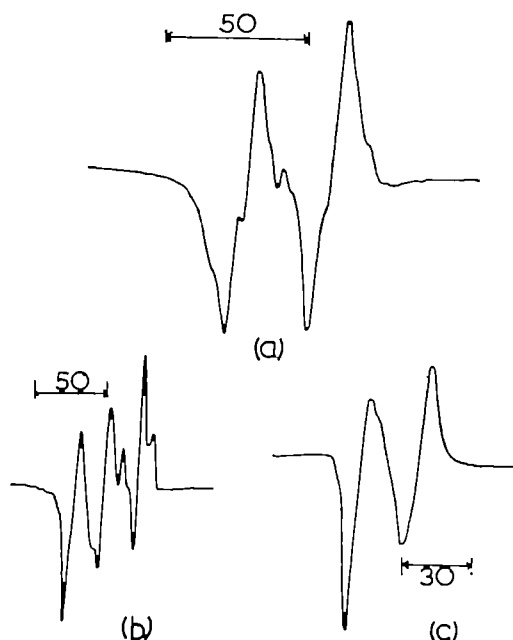


Fig. 1. The first derivatives of the electron spin resonance spectra obtained from glycollic acid irradiated at 77° K and examined at (a) 77° K, (b) 130° K and (c) 300° K. Scales are in gauss.



Table 1. COMPOSITION OF CODONS

Amino-acids		Codons				A	U	G	C
I. Polar									
1. Arg	OGC	AGA	UGO	OGA		0.25	0.08	0.33	0.33
2. Asp	GUA	GUA	GAA			0.45	0.11	0.33	0.11
3. Asp-N	ACA	AUA	ACU			0.50	0.23	0.00	0.22
4. Glu	GAA	GAU	GAO			0.45	0.11	0.33	0.11
5. Glu-N	AAO	AGA	AGU			0.50	0.11	0.23	0.11
6. His	AOO	AOU				0.33	0.17	0.00	0.50
7. Lys	AAA	AAU				0.83	0.17	0.00	0.00
8. Ser	UOU	UOO	UOG	AOG		0.08	0.33	0.17	0.43
9. Thr	CAO	CAA				0.50	0.00	0.00	0.50
10. Tyr	AUU					0.33	0.67	0.00	0.00
		Average over group I				0.43	0.20	0.14	0.23
II. Non-polar									
11. Ala	OGG	UOG	AOG			0.11	0.11	0.33	0.45
12. Cys	UUG					0.00	0.67	0.33	0.00
13. Gly	UGG	AGG	OGG			0.11	0.11	0.67	0.11
14. Ileu	UAA	UAA				0.50	0.50	0.00	0.00
15. Leu	UUG	UUC	UOC	UUA		0.08	0.58	0.08	0.25
16. Met	UGA					0.33	0.33	0.33	0.00
17. Phe	UUU	OUU				0.00	0.83	0.00	0.17
18. Pro	OOO	COU	CCA	OOO		0.08	0.08	0.08	0.75
19. Try	GGU					0.00	0.33	0.67	0.00
20. Val	UGU	UGA				0.17	0.50	0.33	0.00
		Average over group II				0.14	0.40	0.33	0.17
		Total average				0.29	0.30	0.21	0.20

Table 2. THE RATIOS OF THE NUCLEOTIDES IN THE CODONS

Ratio	Polar amino-acids	All amino-acids	Non-polar amino-acids
U/A	0.47	1.04	2.35
G/C	0.61	1.05	1.64
U/C	0.87	1.50	2.35
G/A	0.33	0.73	2.00
U+G/A+O	0.82	1.02	2.20

amino-acids—by the triplets containing guanine and uracil.

This statement is very rough, being based on simple averaging. There are also few exclusions. However, the difference between the codons of the two groups is well pronounced.

It is easy to show that a mutation of the kind called transition means a transition from A to G or from C to U. The transition can therefore exchange a polar amino-acid for the non-polar one and vice versa. It is not always so in the case of transversion. We can think that the transitions are more dangerous than the transversions. Perhaps the probability of transitions is lower. Our knowledge of these problems is, as yet, very limited. But Freese<sup>4</sup> points out that 80 per cent of the spontaneous mutations of the T4-phage are not connected with transitions.

The causes of the differences stated here cannot be explained at present. The structures of A and C have common features—they contain an NH<sub>2</sub>-group at the greatest distance from the N-atom linked with ribose. U and G contain a CO-group at the same positions. Perhaps these differences in structure play some role in the interactions of the s-RNA with the corresponding aminoacyladenylate at the surface of specific enzyme.

The complete deciphering of the compositions of the codons and of the sequences of the nucleotides in them will make possible a more detailed analysis of this problem.

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## Helix-rich Fraction from the Low-sulphur Proteins of Wool

MUCH has been learnt about the architecture of fibrous proteins such as myosin by controlled proteolysis and the application of physico-chemical and analytical techniques to the fragments obtained in this way<sup>1</sup>.

The low-sulphur proteins of wool (S-carboxymethyl-keratin A; SCMKA) resemble myosin in the relatively high proportions that assume the  $\alpha$ -helical conformation.

Harrap<sup>2</sup> found by optical rotatory dispersion measurements that SCMKA contains about 50 per cent  $\alpha$ -helix when dissolved in water and about 60 per cent when dissolved in 2-chloroethanol. It has now been possible, using controlled proteolysis, to prepare fractions containing greater amounts of  $\alpha$ -helix.

Solutions of SCMKA, prepared as described previously<sup>3</sup>, were digested with crystalline trypsin (1 mg to 100 mg SCMKA) or with pronase P (0.1 mg to 100 mg SCMKA) at pH 8.6 in the presence of 0.1 M CaCl<sub>2</sub>. The pH was kept constant and the progress of the reaction was followed by means of a pH-stat. With each enzyme the rate curve was characterized by an initially rapid digestion followed by a slower digestion. The digestion was terminated at the desired point, and the larger molecular species precipitated, by adjusting the pH to 4.2. The precipitate was recovered by centrifugation, dissolved in 0.05 M sodium borate, dialysed against a portion of the same buffer solution overnight and optical rotatory dispersion measurements made on the solution over the wave-length range 334 to 578 m $\mu$  using a Stanley photoelectric polarimeter.

When the optical rotatory dispersion data were plotted in terms of the Moffitt<sup>4</sup> equation, maximum values for  $-b_0$  were obtained when the initial rapid digestion was just complete. Pronase digestion produced fractions with  $-b_0$  values up to 520 corresponding with approximately 84 per cent  $\alpha$ -helix, whereas the product from tryptic digestion gave a  $-b_0$  value of 370, indicating an  $\alpha$ -helix content in water of only about 60 per cent. The maximum yield of the pronase digestion product prepared under these conditions was about 18 per cent by weight. The  $b_0$  values suggest that the wider specificity of pronase<sup>4</sup> enables the enzyme to rupture peptide linkages nearer to the helical portion of the molecule than is possible with trypsin.

Physico-chemical and analytical data relating to the helix-rich material will be published elsewhere.

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## Synthesis of Cellulose by Enzyme Preparations from the Developing Cotton Boll

RECENTLY an enzyme system from mung bean seedlings was described which catalyzes the incorporation of radioactivity into cellulose from guanosine diphosphate D-glucose labelled with <sup>14</sup>C in the D-glucosyl moiety<sup>1</sup>. The extent of this incorporation was considerably stimulated by the addition of GDP-D-mannose to the reaction mixtures. This effect was later shown to result from the formation of some as yet uncharacterized polysaccharide, probably a glucomannan<sup>2</sup>. The synthesis of this compound also appeared to proceed at a rate many times that at which cellulose was formed by the same preparations. Sugars other than D-glucose, chiefly D-xylose or D-mannose, have been found associated with the cellulosic fractions of most plant species, but cotton fibres consist almost entirely of  $\beta$ -1,4 linked D-glucosyl residues<sup>3</sup>. It was therefore of interest to learn whether <sup>14</sup>C-labelled D-glucose from GDP-D-glucose <sup>14</sup>C could be incorporated into the cellulose of cotton fibres *in vitro*

and whether other polysaccharides were formed by the same cell-free enzyme system. The experiments reported here demonstrate that both these processes are brought about by enzymes isolated from the developing cotton boll.

Bolls at various stages of development were obtained from cotton plants (*Gossypium hirsutum*) through the courtesy of Drs. R. E. Johnson and F. T. Addicott, of the Department of Agronomy, University of California, Davis. The ages of the bolls were calculated from the time the flowers opened. The bolls were picked in the late afternoon, refrigerated overnight and sent to this laboratory in crushed ice the following morning. In each case enzymes were isolated from the plant material and incubated with the substrate on the day the material was received. Carpel walls were peeled from the bolls, and the segments, composed of seed hairs and immature seeds, were removed. In some experiments hairs were separated from the seeds by teasing out the seeds from the fibrous mass. The material was ground in a chilled mortar with sea sand and an equal weight of a buffer which contained 0.1 M *tris* HCl, pH 7.5, and 5 per cent polyvinylpyrrolidone (PVP)\*. The homogenate was squeezed through two layers of cheesecloth and centrifuged at 1,000g for 5 min; the precipitate was taken up in a small volume of the same buffer. The supernatant solution was centrifuged at 20,000g for 20 min and that precipitate was also suspended in a small volume of the PVP buffer. Both centrifugal residues were used as sources of the enzyme in most of the experiments.

<sup>14</sup>C-labelled substrates were synthesized and purified, and the incorporation of radioactivity into the product was estimated as described in previous publications<sup>1,2</sup>.

Like the cell-free preparations from other plant species and organs<sup>3</sup>, particles obtained from developing cotton bolls catalysed the incorporation of D-glucose-<sup>14</sup>C into cellulose only from GDP-D-glucose-<sup>14</sup>C. No incorporation of radioactivity could be observed when GDP-D-glucose was replaced in the reaction mixture by any of the following <sup>14</sup>C-labelled compounds: UDP-D-glucose, ADP-D-glucose, CDP-D-glucose, D-glucose 1-phosphate or D-glucose.

The extent of incorporation of radioactivity from GDP-D-glucose-<sup>14</sup>C into alkali-insoluble polysaccharide is shown in Table 1. The most active preparations were from bolls between 4 and 8 days old. The catalytic activities of particles precipitated with quite diverse centrifugal forces were about the same. This suggests that no discrete organelle is involved in this process.

In all cases the incorporation of label from GDP-D-glucose-<sup>14</sup>C into the product was stimulated by the addition of GDP-D-mannose to the reaction mixtures. Furthermore, the data in Table 2 demonstrate that when particles prepared from seed hairs alone were used, GDP-D-mannose still enhanced the incorporation of <sup>14</sup>C from GDP-D-glucose-<sup>14</sup>C into the alkali-insoluble product. By analogy with the results obtained earlier<sup>3</sup> this stimulation indicates the formation of another polysaccharide in addition to cellulose. The properties of the polysaccharide-synthesizing system from the cotton boll thus appear to be about the same as those of similar systems isolated from the roots and hypocotyls of mung bean seedlings<sup>4</sup>.

Cotton fibres are formed from specialized cells in the outer layer of the seed coat and are made up almost entirely of cellulose; thus it is surprising that enzyme preparations from these cells appear to catalyse the extensive synthesis of one or more polysaccharides other than cellulose. This phenomenon may be an artefact of the process *in vitro*, that is, the enzyme responsible for cellulose synthesis is perhaps unable to distinguish GDP-D-mannose from GDP-D-glucose, whereas GDP-D-

\* When PVP was omitted from the homogenizing buffer no synthesis of polysaccharide by the cell-free preparations could be demonstrated. Since cotton bolls contain large amounts of phenolic substances, PVP probably neutralizes the inhibitory effect of these compounds on isolated enzymes. This subject has been discussed at length by Hulme, Jones, and Wooltorton<sup>5</sup>.

Table 1. EFFECT OF AGE OF THE BOLL, PARTICLE SIZE, AND ADDITION OF GDP-D-MANNOSE ON SYNTHESIS OF POLYSACCHARIDE *in vitro*

Age of bolls (days)	Alkali-insoluble polysaccharide (c.p.m.)			
	1,000g fraction		20,000g fraction	
	- GDP-D-mannose	+ GDP-D-mannose	- GDP-D-mannose	+ GDP-D-mannose
4	190	390	200	655
6	170	340	220	610
8	306	560	220	545
12	85	330	180	415
16	120	315	180	530
21	120	200	30	95

Reaction mixtures contained 0.2 ml. of the particle suspension (derived from 2-4 g of immature cotton seeds and hairs) in 0.1 M *tris* HCl/5 per cent PVP buffer, pH 7.5,  $2 \times 10^{-4}$   $\mu$ moles (0.02  $\mu$ g; 6,000 c.p.m.) GDP-D-glucose-<sup>14</sup>C, and, when added,  $1 \times 10^{-3}$   $\mu$ moles GDP-D-mannose in a total volume of 0.25 ml. Mixtures were incubated for 1 h at 23-25° and the reaction stopped by immersing the tubes in boiling water for 2 min. Alkali-insoluble polysaccharide was isolated and its radioactivity estimated as described previously<sup>1</sup>.

Table 2. SYNTHESIS OF POLYSACCHARIDE *in vitro* BY ENZYMES PREPARED SEPARATELY FROM SEEDS AND SEED HAIRS

Source of enzyme	Alkali-insoluble polysaccharide (c.p.m.)	
	- GDP-D-mannose	+ GDP-D-mannose
Seeds	220	500
Seed hairs	180	460

The seeds (with most of the fibre removed) and the seed hairs from 14-day-old cotton bolls were homogenized separately in PVP buffer as described in the text. The reaction mixtures contained 0.2 ml. of particles precipitated at 20,000g and derived from 2 g of seed hairs or 4 g of seeds. The other conditions of the experiment were as given in Table 1.

mannose is not encountered by the enzyme *in vivo*, and hence that compound does not act as a glycosyl donor. If purification of the enzyme or enzymes involved in this synthesis can be achieved and the nature of the D-glucosyl acceptor can be elucidated, undoubtedly a more heuristic hypothesis will offer itself.

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## Nucleoside Incorporation in Animal Cell Cultures

In a recent paper Cleaver<sup>1</sup> has investigated the uptake of nucleosides by tissue culture cells in physiological saline. From the results he states: "This suggests that the metabolism of these cells is markedly disturbed by brief exposures to a physiological saline and in particular the biosynthesis of the acid-insoluble fractions (for example, DNA and RNA) comes to a standstill. It has been assumed that under these conditions tritiated thymidine is incorporated into DNA and tritiated cytidine is incorporated into both DNA and RNA<sup>1,2</sup>".

This reference to my experiments<sup>1,2</sup> is misleading, and may give the impression that I incubated cell cultures in saline. In fact, as I stated clearly, in the procedure described<sup>1</sup> in my original papers<sup>1,2</sup> on nucleoside uptake, the cells were incubated in a mixture of three parts growth medium (containing 20 per cent serum plus amino-acid supplement) with one part Tyrode containing the nucleoside; thus the final concentration of serum in the medium was 15 per cent, which is in fact higher than that used (10 per cent) by Cleaver<sup>1</sup> in his control experiments.

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# Alternative Pathway of Androgen Biosynthesis in the Human Adrenal Gland

THE major pathway of androgen biosynthesis in the human adult adrenal gland is well established: pregnenolone  $\rightarrow$  17 $\alpha$ -hydroxypregnenolone  $\rightarrow$  dehydroepiandrosterone  $\rightarrow$  androstenedione<sup>1</sup>. Previous results indicate that the adult adrenal gland converts a higher percentage of 17 $\alpha$ -hydroxypregnenolone than of progesterone to the product, androstenedione<sup>1</sup>. The data suggest that a pathway is possible which by-passes progesterone to form androgens: pregnenolone  $\rightarrow$  17 $\alpha$ -hydroxypregnenolone  $\rightarrow$  17 $\alpha$ -hydroxyprogesterone  $\rightarrow$  androstenedione. This report describes the results of an incubation to determine the significance of 17 $\alpha$ -hydroxyprogesterone in the biosynthesis of androstenedione. Cortisol synthesis was determined also, for comparison. An adenoma removed at operation from a 35-year-old female with Cushing's syndrome was used for these studies.

Adrenal adenomatous tissue slices were incubated in a Krebs-Ringer bicarbonate buffer (30 ml. buffer per g slice) which contained 7 $\alpha$ -<sup>3</sup>H-17 $\alpha$ -hydroxypregnenolone SA-L <sup>6</sup> $\mu$ o. per  $\mu$ mo and 4-<sup>14</sup>C-17 $\alpha$ -hydroxyprogesterone SA-L <sup>12</sup> $\mu$ o. per  $\mu$ mo separately, and in equimolar amounts (<sup>3</sup>H : <sup>14</sup>C 1:22:1). All flasks contained 0.2 ml. propylene glycol. Beef adrenocorticotrophic hormone (ACTH) (6 units) was added to certain flasks. The incubations were carried out for 3 h in an atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide. <sup>14</sup>C or <sup>3</sup>H-labelled tracers were added to respective flasks for correction of manipulative losses. The methods of extraction, isolation and measurement of cortisol and androstenedione were as reported previously<sup>1,2</sup>. The radiochemical purity of the isolated steroids was determined by the constancy of the <sup>3</sup>H : <sup>14</sup>C ratio of the free compounds and derivatives following paper, or column<sup>3</sup> and thin-layer<sup>4</sup>, chromatography.

The <sup>3</sup>H : <sup>14</sup>C of cortisol determined after separation in the toluene: propylene glycol and Bush C systems<sup>4</sup> was 0.88 and 0.87 in the presence and absence of ACTH respectively. On the other hand, the <sup>3</sup>H : <sup>14</sup>C of androstenedione isolated from the same incubation media was 1.27 and 1.31.

The results of the identification data of cortisol and androstenedione are listed in Tables 1 and 2 respectively. The data indicate that 17 $\alpha$ -hydroxyprogesterone is an important intermediate in the biosynthesis of the adrenal androgen, androstenedione. The starting ratio of <sup>3</sup>H : <sup>14</sup>C of the  $\Delta^5$  :  $\Delta^4$  substrates is maintained in the isolated androstenedione, suggesting that the pathway from the 17 $\alpha$ -hydroxy- $\Delta^5$ -precursor may be via the intermediate 17 $\alpha$ -hydroxyprogesterone. However, the small amount of radioactivity in 17 $\alpha$ -hydroxyprogesterone precluded its identification. The addition of ACTH to this adenomatous slice system did not affect the <sup>3</sup>H : <sup>14</sup>C in androstenedione

Table 1. RADIOCHEMICAL PURITY OF CORTISOL DERIVED FROM 7-<sup>3</sup>H-17 $\alpha$ -HYDROXYPREGNENOLONE AND 4-<sup>14</sup>C-17 $\alpha$ -HYDROXYPROGESTERONE

Cortisol	Chromatography systems	<sup>3</sup> H : <sup>14</sup> C*
	B <sub>2</sub> B (ref. 5)	0.88
	Bush C	0.84
Monoacetate derivative	Toluene: propylene glycol	0.85
	Benzene: cyclohexane	
	Methanol: water	
	50:100:100:25 (ref. 6)	0.86
	Thin-layer	0.84

\* The ratio of the substrates before incubation was 1:22. Measurements were made only after successive paper chromatography in the toluene: propylene glycol and Bush C systems.

Table 2. RADIOCHEMICAL PURITY OF ANDROSTENEDIONE DERIVED FROM 7-<sup>3</sup>H-17 $\alpha$ -HYDROXYPREGNENOLONE AND 4-<sup>14</sup>C-17 $\alpha$ -HYDROXYPROGESTERONE

Androstenedione	Chromatography systems	<sup>3</sup> H : <sup>14</sup> C*
	Heptane: 95 per cent methanol (ref. 7)	1.31
	Toluene: propylene glycol	1.29
	Thin-layer	1.25
Mono-2,4-dinitro-phenyl-hydroxone derivative	5:6 per cent hydrated neutral 1:25, 1:23, 1:20†, 1:20, 1:24	
	Alumina column (ref. 1)	

\* See footnote, Table 1.

† Peak and two adjoining fractions on both sides of the peak.

but increased the efficiency of 17 $\alpha$ -hydroxyprogesterone as an intermediate in the biosynthesis of cortisol. These experiments suggest that the pathway 17 $\alpha$ -hydroxypregnenolone  $\rightarrow$  17 $\alpha$ -hydroxyprogesterone  $\rightarrow$  androstenedione is an important one for androgen biosynthesis in the adrenal adenomatous tissues studied.

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## PHYSIOLOGY

### A Proposal for a Reference Standard Preparation for the Hypothalamic Thyrotrophic Hormone Releasing Factor

ANY programme which proposes to purify and isolate a biologically active material for which there is no specific physico-chemical mode of identification is on solid ground when it has at its disposal a bioassay and some reference standard preparation. The bioassay must be specific, reliable (reproducible), preferably sensitive, and able to demonstrate a linear increment of the measured metameter as a function of the log of the dose of the biological material injected. A reference standard preparation must be an isologous material which will give responses as a function of the doses injected, varying with slopes of that function identical to those observed when comparing the responses obtained with injection of purified materials along the course of the isolation procedure.

With the definition of a unit of biological activity, it is possible to follow quantitatively the various steps of a purification method and to calculate the specific activity (units/mg) of materials of various degrees of purity. A reference standard gives, furthermore, the possibility of comparing preparations obtained in various laboratories by calculating their specific activities in terms of a common unit. To our knowledge a reference standard has never been proposed for any of the hypothalamic hypophyseotropic hormones. Thus, the various programmes on the purification of the hypothalamic releasing factors have so far reported the biological activity of the material prepared by quoting the dry weights for minimal effective doses or by showing the very responses obtained in the various bioassays or tests (adrenal ascorbic acid depletion, plasma or adrenal corticosterone concentrations, ovarian ascorbic acid contents or variations, uterine weights, plasma iodine-131 radioactivity, etc.).

The inherent limitations to such procedures for quantitative studies on a purification programme are all too obvious, and the use of the (corresponding) pituitary hormone standards as reference preparations to express the hypophysiotrophic activities is not a satisfactory answer to the problem. The activity of the hypophysiotrophic factors is transhypophysial, that is to say, their effects are mediated by an adrenohypophysial response, which would not be reflected or reproduced with the use of pituitary hormone standards. This is even more evident where the hypophysiotrophic activities are examined by measuring the plasma concentrations of circulating pituitary hormones, as can now be done for adrenocorticotrophic hormone (ACTH), luteinizing hormone (LH) or thyrotrophic hormone (TSH).

We propose and describe here a reference standard preparation of hypothalamic origin to be utilized in the purification of the hypothalamic factor TRF related to the secretion of thyrotrophin. This material is the lyophilized fraction with TRF-activity obtained by gel-filtration through 'Sephadex G25' of the 2 N acetic acid extracts of ovine hypothalamic tissues, as in the conditions originally described<sup>1</sup>. This material is crude enough to be reasonably stable when kept as a dry powder in a vacuum at +4°C or at room temperature. It is devoid of TSH activity and has no vasopressor activity<sup>1</sup>. It gives a linear response in the bioassay as a function of the log of the dose of material injected<sup>1,2</sup>. The linear functions relating responses to log doses of the standard are parallel to those obtained with purified TRF.

It is proposed that the biological activity (stimulation of the release of TSH) of 1.0 mg of this preparation be attributed the value of 1.0 unit. The assay used to characterize this TSH-hypophysectrophic activity is the bioassay described earlier by Yamazaki, Sakiz and Guillemin<sup>3</sup>, or a recent modification thereof in which the pre-treatment with thyroxine (see ref. 2) is omitted. Approximately six months ago, several grams of a material with TRF activity prepared as above were set aside from our current purification programme and considered as a reference standard. So far we have no evidence of loss of biological activity. In the bioassay used here<sup>4</sup> this TRF preparation gives a statistically valid response with doses  $\leq 1.0$  mg or 1.0 unit, with a linear segment of the response curve up to 10 units. The use of this reference preparation over several months has shown us that the sensitivity of the bioassay is quite variable from one group of animals to another.

Similar preparations as internal standards could be made simply in the various laboratories interested in the purification of hypothalamic hypophysectrophic factors. For reference standard preparations of CRF (corticotrophin releasing factor) and LRF (LH-releasing factor) we have been investigating the same type of material (that is, the 'Sephadex G25' concentrate of CRF and LRF) and an even simpler material, that is, the boiled 0.1 N hydrochloric acid extract of rat stalk median eminence. Two problems have not been satisfactorily resolved as yet: (a) the linearity of the pituitary secretion of LH as a function of the dose of injected LRF (of whatever origin); (b) the stability over several months of the LRF- and CRF-activity in the crude extracts so prepared.

To facilitate meaningful comparisons, between investigators, for sensitivity of biological assays, specific activity of various preparations, etc., our laboratory will consider making available on request aliquots of the TRF reference preparation described here to allow expressing any other local standards in terms of a common unit.

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### Plasma Disappearance of Various <sup>125</sup>I-labelled Growth Hormone Preparations in Man and Rabbit

STUDYING the properties of pepsin-digested bovine and sheep growth hormone<sup>1</sup> it was found that one fraction of the digest possessed electrophoretic and immunological properties similar to human growth hormone. The purpose of the present work was to compare the *in-vivo* behaviour of untreated and digested sheep growth hormones with the human hormone. This was tested by

following the kinetics of the plasma disappearance of the radio-iodinated hormones.

The growth hormone preparations used were: human growth hormone (HGH), sheep growth hormone (SGH, batch S<sub>1</sub>) and chymotrypsinized bovine growth hormone (BGH-CY-75). The sheep growth hormone was used untreated or after pepsin digestion<sup>1</sup>. The labelling procedure with <sup>125</sup>I was performed according to Hunter and Greenwood<sup>2</sup>. By this method we obtained a specific activity of growth hormone in the range of 50–100  $\mu$ Ci/ $\mu$ g. The patient material comprised 12 adolescent boys and 2 girls. The day before, and on the morning of the experiment, they received stable iodide (Lugol solution) in order to block the thyroid gland. The experiments were performed in the fasting state and labelled growth hormone preparations were administered intravenously in a dose range of 20–50  $\mu$ Ci per patient. Blood samples at predetermined intervals were collected in heparinized test-tubes for 3–4 h. Aliquots of the plasma were counted in a well-type NaI ('TI') scintillation detector, and the proteins were precipitated by a 20 per cent solution of cold trichloroacetic acid (TCA). The values of the TCA-precipitable radioactivity were compared with the injected dose and the ratio was plotted on a semilogarithmic paper against time. Each study group comprised four patients. Serum albumin was given only to two.

The plasma disappearance curve showed three phases: an extremely fast-mixing phase which was followed by a second phase (designated 'A') and a slow third phase (designated 'B'). The results of the individual experiments were in good agreement. The plasma disappearance of the various labelled growth hormones studied and of radio-iodinated human serum albumin (RISA) is illustrated in Fig. 1.

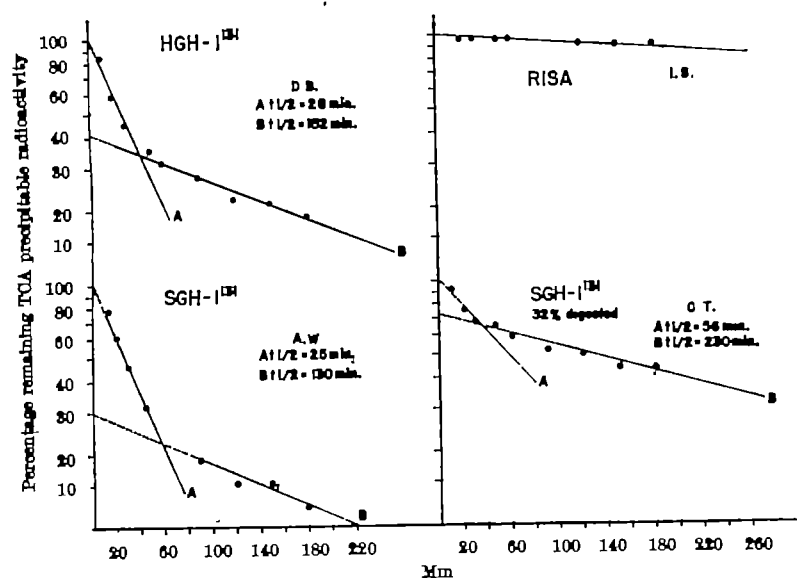
For HGH the  $t_{1/2}$  of phase A ranged between 20 and 30 min. This half-time correlates well with the metabolic degradation rate of endogenous HGH<sup>3,4</sup> and with other studies using HGH-<sup>125</sup>I (refs. 5 and 6). Phase B showed a slower plasma disappearance with a  $t_{1/2}$  ranging between 150 and 180 min. This phase is at present considered to represent the fate of 'damaged' HGH. The plasma disappearance of radio-iodinated SGH was in the similar range with HGH-<sup>125</sup>I: phase A -  $t_{1/2}$  = 20–25 min; phase B -  $t_{1/2}$  = 120–130 min. The similarity of the plasma disappearance of HGH and SGH, in what is considered to represent the metabolic phase of HGH, is of interest as SGH is biologically not active in man. The plasma disappearance of <sup>125</sup>I-labelled SGH after pepsin digestion showed a prolongation of both phases, A:  $t_{1/2}$  = 55–70 min and of phase B:  $t_{1/2}$  = 200–250 min. No significant amounts of TCA-precipitable radioactivity were found in the urine collected for 24 h.

A series of experiments was performed in rabbits. Groups of three rabbits were injected intravenously with labelled untreated SGH, pepsin-digested SGH, chymotrypsinized BGH and RISA. As in the human subjects, three phases of the plasma disappearance of the labelled hormones were found. The results are summarized in Table 1.

The metabolic phase A of HGH in the rabbit as found in the present study and by Salmon *et al.*<sup>7</sup> shows a disappearance range comparable to that in man. Though both HGH and BGH or SGH are metabolically active in the rabbit, the metabolic phase of SGH is slightly longer than that of HGH. The significance of the prolonged second-stage disappearance curve (phase B) remains an open question.

Table 1. PLASMA DISAPPEARANCE IN THE RABBIT OF <sup>125</sup>I-LABELLED GROWTH HORMONE

Hormone preparation	No. of rabbits	Phase A - Range of $t_{1/2}$ (min)	Phase B - Range of $t_{1/2}$ (min)
HGH	3	20–25	120–180
SGH	3	30–40	150–180
SGH pepsin digested	3	40–45	250–280
BGH chymotrypsin digested	1	40	200

Fig. 1. Plasma disappearance in man of  $^{125}\text{I}$ -labelled growth hormones

The sheep growth hormone was kindly donated by the Endocrinological Study Section of the National Institutes of Health by courtesy of Dr. A. E. Wilhelmi. The chymotrypanized bovine growth hormone was supplied by Dr. E. Vairel, Choay Laboratories, Paris.

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### Spinal Cord Convulsions in the Developing Rat at Altitude (12,470 ft, 3,800 m)

PREVIOUS investigations in this laboratory have demonstrated that maximal electro-shock seizure responses are more severe in adult rats brought to high altitude<sup>1</sup> and in developing rats born at high altitude<sup>2</sup> than in corresponding sea-level controls.

Spinal reflex systems play an important part in integrating maximal seizure discharge into the motor pattern<sup>3</sup>. The spinal cord of the rat matures, functionally, more rapidly than do higher centres of the central nervous system<sup>4</sup>. In view of this, the present study was undertaken to determine whether the spinal cord is more active in rats born and maintained at altitude than in their sea-level controls.

This study was done at the University of California, Berkeley, elevation 250 ft. (76 m), 750 mm mercury, and at the Barcroft Laboratory, White Mountain Research Station of the University of California, elevation 12,470 ft. (3,800 m), 480 mm mercury.

Barcroft Laboratory has a Long Evans rat colony. Sea-level rats are continually taken to the station for

breeding. The animal quarters at altitude are similar to those at sea-level as regards food, caging, temperature and lighting.

The experimental animals were divided into 2 different age groups, that is, 11- and 28-day-old rats. The brain of 11-day-old rats is less mature than that of 28-day-old rats<sup>5</sup>. The following numbers of rats were used: in the 11-day-old group, 13 sea-level controls and 12 second-generation rats born at altitude ( $F_2$ ); in the 28-day-old group, 19 sea-level controls and 12  $F_2$  rats.

Spinal cord convulsions were produced by direct electrical stimulation of the cord as described by Esplin and Preston<sup>6</sup> and modified by Vernadakis<sup>7</sup>. All rats were decapitated at an upper cervical level and a stimulating electrode, cathode, was inserted 4–5 mm into the spinal cord. The anode was attached to the exposed tissue of the neck. Square wave stimuli, 1 msec in duration, at an intensity of 30 V and a frequency of 100 pulses/sec were delivered within 10 sec after decapitation. Duration of spinal cord stimulation was 10 sec. A

series of electronic timers were activated by the stimulator and measured in msec the duration of each of the seizure components—tonic flexion and tonic extension.

Significance of differences between sea-level and altitude-exposed animals was calculated by the *t*-test.

Table 1. DURATION (SEC) OF SPINAL CORD CONVULSIONS IN TWO DIFFERENT AGE GROUPS OF YOUNG  $F_2$  RATS AT SEA-LEVEL OR AT ALTITUDE (12,470 ft)

Age	Spinal cord convulsions (duration of phases in sec)	No. of animals	Sea-level controls	No. of animals	$F_2$ rats born at 12,470 ft.
11 days of age	Flexion $\uparrow$	13	1.30 $\pm$ 0.10*	12	2.50 $\pm$ 0.25 <0.001
	Extension $\uparrow$		8.85 $\pm$ 0.30		7.17 $\pm$ 1.15
28 days of age	Flexion $\uparrow$	19	1.96 $\pm$ 0.54	12	1.29 $\pm$ 0.07
	Extension $\uparrow$		4.14 $\pm$ 0.07		9.69 $\pm$ 0.09 <0.001

\* Mean  $\pm$  standard error.

<sup>†</sup> Values based on *t*-test for significance of differences between sea-level and altitude rats.

Durations of flexion and extension in 11- and 28-day-old rats born at altitude and sea-level controls are presented in Table 1. The duration of flexion in 11-day-old altitude-born rats was significantly longer and duration of extension did not change significantly when compared with sea-level controls (Table 1). Duration of extension was significantly increased in the 28-day-old rats born at altitude as compared with sea-level controls; duration of flexion was decreased but not significantly (Table 1).

The shorter the duration of flexion and/or longer the duration of extension the more severe the convulsion and vice versa<sup>8</sup>.

The longer flexion and the shorter extension in 11-day-old altitude rats indicate a decreased activity of the spinal cord. In contrast significantly longer extension of the 28-day-old rats indicates more intense activity of the spinal cord. It is suggested that the longer flexion in 11-day-old altitude-born rats may be due partially to a delay in maturation of the spinal cord. Vernadakis has shown that flexion is longer in less mature rats<sup>7</sup>. The longer extension in 28-day-old altitude rats indicates an increased reflex excitability of the spinal cord. These observations are in agreement with previous studies from this laboratory. It was found that development of the central nervous system as assessed by brain electrical stimulation is delayed at altitude, and once maturation is achieved increased excitability is observed<sup>9</sup>.

It can be concluded that delay in central nervous system maturation in  $F_2$  rats born at an altitude of 12,470 ft. (ref. 2) may be attributed, in part, to changes in the function of the spinal cord.

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# Mechanism of Muscular Paralysis by Insulin with Particular Reference to Familial Periodic Paralysis

OFFERIJNS *et al.*<sup>1</sup> have shown that insulin exerts a paralyzing effect on the isolated diaphragm of rats fed on a potassium-free diet. A close similarity seems to exist between this experimental muscle paralysis in rats and the familial hypokalaemic periodic paralysis in man. In both cases, the contraction in response to indirect as well as to direct stimulation is depressed, insulin and low potassium concentration in the extracellular fluid are important causative factors, and the contraction is restored when the extracellular potassium concentration  $[K]_o$  is increased. These facts led us to investigate further the mechanism of the muscular paralysis induced by insulin.

Albino rats weighing about 90 g were fed for more than three weeks on a potassium-free diet *ad libitum*. The composition of the potassium-free diet was similar to that used by Offerijns *et al.*, and consisted of sugar, casein, soybean oil, salt mixture and vitamins. Phrenic nerve-diaphragm preparations were dissected out and placed in the bath filled with Krebs solution saturated with 95 per cent oxygen and 5 per cent carbon dioxide. The temperature of the bathing solution was kept at 36° C. Isometric tension, produced by direct or indirect stimulation, was recorded with a mechano-electronic transducer (ROA 5734), and the membrane potential of diaphragm muscle fibres was measured with 3 M-KCl microelectrodes.

When  $[K]_o$  was low (0-2.4 mM), twitch tension of the diaphragm produced by direct or indirect stimulation was abolished or greatly reduced by adding insulin (0.1 to 1.1 i.u./ml.) in several min, and the contraction was restored in a few minutes when  $[K]_o$  was increased (4.8 to 6 mM), the findings of Offerijns *et al.* being thus confirmed. Intracellular recording of the membrane potential revealed that at the stage where the contraction was depressed by insulin, the resting potential of muscle fibres was markedly reduced (Table 1 and Fig. 1). At this time, the muscle action potential was greatly decreased

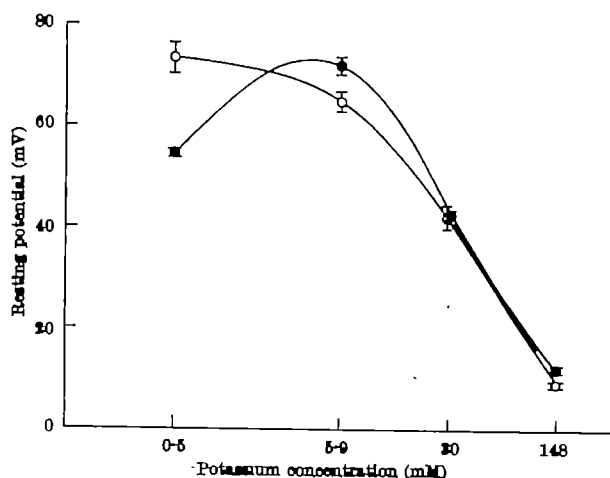


Fig. 1. Relation between resting potential and external potassium concentration in diaphragm muscle fibres of a potassium-deficient rat. Ordinate: O, membrane potential before insulin, and ●, after adding insulin 1.0 i.u./ml. Abscissa: potassium concentration in mM, on logarithmic scale. Potassium concentrations were changed by replacing equivalent amounts of sodium. Each point and vertical line represent a mean and  $\pm$  S.E.

in size, and in most fibres could not be produced by electrical stimulation. When  $[K]_o$  was increased to 5.9 mM, the resting potential was increased to more than 70 mV and the action potential of more than 100-mV amplitude could again be observed.

In the diaphragms from control rats fed on a normal potassium-containing diet, insulin neither influenced the muscle contraction nor produced the depolarization of muscle fibres at low  $[K]_o$ . The resting potential at which mechanical response disappears was determined by increasing  $[K]_o$  of the Krebs solution. The contractions to direct and indirect stimulations were abolished when the mean resting potential fell to 55 mV in 16 mM  $[K]_o$  from the original value of 71 mV in 4 mM  $[K]_o$ .

Fig. 1 shows the relation between the resting potential and  $[K]_o$  in muscle fibres of the diaphragm of a potassium-deficient rat. Insulin evidently exerted a depolarizing effect at low  $[K]_o$ . In 5.9 mM  $[K]_o$ , in contrast, insulin caused hyperpolarization. The latter fact is consistent with the previous findings in rat and frog muscles<sup>2,3</sup>. An explanation of the hyperpolarizing action of insulin might be the acceleration of electrogenic sodium extrusion<sup>4</sup>. However, the hyperpolarizing action was not abolished in the presence of ouabain,  $10^{-5}$  g/ml.

It is clear that the decline in resting potential following the application of insulin at low  $[K]_o$  is the main factor contributing to the failure of contraction. Further attempts were made to elucidate the mechanism of the depolarization by insulin. The depolarization is not dependent on the action of insulin to increase the glucose uptake of the muscle, because the depolarization by insulin, and consequently the decrease in the contractility, occurred in glucose-free Krebs solution. Similarly, the acceleration of active cation transport by insulin<sup>5</sup>, particularly the increased potassium influx<sup>6</sup>, cannot provide an explanation of the depolarization, because the depolarization occurred in the presence of ouabain  $10^{-5}$  g/ml, as well as in potassium-free solution. When sodium in the extracellular fluid was replaced by choline, the depolarizing action of insulin at low  $[K]_o$  was abolished (Table 1). The most plausible explanation of the depolarization by insulin, therefore, would be that insulin increases the permeability of the muscle membrane to sodium ions. Then the effects of  $[K]_o$  can be explained in the following way. A marked depolarizing action of insulin takes place at low  $[K]_o$ , where the permeability to potassium ions is reduced<sup>7</sup>. On the other hand, a rise of  $[K]_o$  restores the resting potential by increasing the potassium permeability<sup>8,9</sup>.

Table 1. DEPOLARIZATION OF DIAPHRAGM MUSCLE FIBRES OF POTASSIUM-DEFICIENT RATS IN VARIOUS EXPERIMENTAL CONDITIONS

Experimental conditions	$[K]_o$ (mM)	Concentration of insulin (i.u./ml.)	Resting potential* (mV)	
			Before insulin	After insulin
Krebs solution	1.2	0.14	70 $\pm$ 4	35 $\pm$ 2
Glucose-free solution	0.5	1.0	73 $\pm$ 2	54 $\pm$ 3
Ouabain $10^{-5}$ g/ml.	0.5	1.1	67 $\pm$ 2	52 $\pm$ 1
K-free solution	0	1.0	81 $\pm$ 4	50 $\pm$ 4
[Na] <sub>o</sub> 148 mM	0.5	0.5	73 $\pm$ 2	57 $\pm$ 3
[Na] <sub>o</sub> 50 mM†	0.5	0.5	73 $\pm$ 2	68 $\pm$ 2
[Na] <sub>o</sub> 0 mM†	0.5	0.5	73 $\pm$ 2	76 $\pm$ 2

\* Each value represents mean  $\pm$  S.E.

† Sodium was replaced by an equivalent amount of choline. ‡ Tubocurarine was added in a concentration of  $2 \times 10^{-4}$  g/ml.

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## PHARMACOLOGY

### Transfer of Habituation by Material extracted from Brain

As an extension of our recent experiments on transfer of morphine tolerance<sup>1</sup>, we tried to explore the possibility of a similar transfer of the tolerance which develops to repeated physical stimuli. Such tolerance, called habituation, is defined as the loss of an innate response to a stimulus repeated without reinforcement<sup>2</sup>. It is considered to be one of the most elementary forms of learning.

The stimulus selected was sound which, when first heard, elicits a 'startle response' consisting of the contraction of various groups of skeletal muscles, accompanied by some autonomic changes<sup>3,4</sup>. The response is easily discernible even by untrained observers.

The sound was produced by a steel hammer hitting a metal plate. The hammer was connected to an electric timer so as to produce a sound every 5 sec. The loudness of the sound was 72 decibels at a distance of 1 m (with the reference pressure of  $2 \times 10^{-4}$   $\mu$ b) and its fundamental pitch was about 400 c/s.

Habituation was induced in rats (Sprague-Dawley strain, all males, weighing between 200 and 300 g) by submitting them to this stimulus for two 1-h periods daily. An additional 100 stimuli were given every day to follow the progress of the habituation. The test was recorded by an observer who pushed the button of an electric signal every time the animal failed to exhibit the startle response. This record was superimposed on the tracing made by the timer activating the hammer. Habituation was considered completed when the animals responded to 10 or less of the 100 stimuli applied on each of three consecutive days. Complete habituation was obtained in rats in  $9.0 \pm 4.5$  (S.D.) days. Mice lost their responses much more slowly; they failed to reach the criterion in 20 days.

The habituated rats were killed by bleeding. The brain was rapidly removed and frozen on dry ice. In the first series of experiments, a homogenate of pooled brains taken from sound-habituated rats was prepared and injected intraperitoneally to albino mice (Swiss strain, all males, weighing 20–30 g). A control group was injected at the same time with a similarly prepared homogenate of brain taken from normal, non-habituated rats. Prior to the injection, all the mice had been tested with 100 stimuli to ascertain that they responded normally to sound. About 16 h after the injection, the mice were tested again before being subjected to the two periods of stimulation. This schedule was followed every day until the end of the experiment. It should be noted that the observers who recorded the responses did not know to which group each mouse belonged.

Owing to the slowness of habituation in mice, the 10 per cent criterion was impracticable in these animals. The results of these experiments are expressed in terms of the mean number of days required to reduce to 50 per

Table 1. EFFECT OF DIFFERENT AMOUNTS OF VARIOUSLY PREPARED BRAIN EXTRACTS ON SOUND HABITUATION IN MICE

Preparation	Dose (mg)	Sound-habituated rat brain			Normal rat brain		
		Days to 50% criterion	$\pm$ S.D.	N	Days to 50% criterion	$\pm$ S.D.	N
Homogenate	300	2.1	2.5	7	12.0	1.5	7
Dialysate	400	1.25	0.5	4	11.0	0.8	4
Dialysed acetone precipitate	500	—	—	—	12.5	1.9	8
" "	240	1.4	0.9	5	—	—	—
" "	280	1.2	0.4	6	—	—	—
" "	140	2.0	1.5	4	—	—	—

N, No. of mice in each group.

cent the responses of a group of mice. Table 1 lists the results obtained with different types of brain extracts and different amounts of brain administered.

It is seen that in the first experiments, using homogenates, there was a striking difference between the group which received the brain from sound-habituated rats and those which were given normal brain. However, homogenates are inconvenient to handle and to administer and they caused a high mortality in both the experimental and control groups. The first experiment lasted only six days.

Since the factor responsible for morphine tolerance was dialysable, we submitted the brain homogenates to dialysis against 20 vol. of distilled water at 4° C for 48 h. The volume of the dialysates was reduced by lyophilization and made up to contain the equivalent of 1 g of brain per ml. Two groups of mice were injected with these preparations: one with the dialysate of sound-habituated rat brain and the controls with normal rat brain. Table 1 shows essentially the same results as those obtained with the homogenates, but there were no deaths and the experiment was continued for 14 days.

Fig. 1 shows the habituation curve constructed with the pooled results of the first two experiments. The number of startle responses was spectacularly reduced in the experimental animals from the first post-injection test onwards. However, after the initial drop in the responses, the rate of habituation slowed down, suggesting that the effect of the treatment is of short duration. In some later

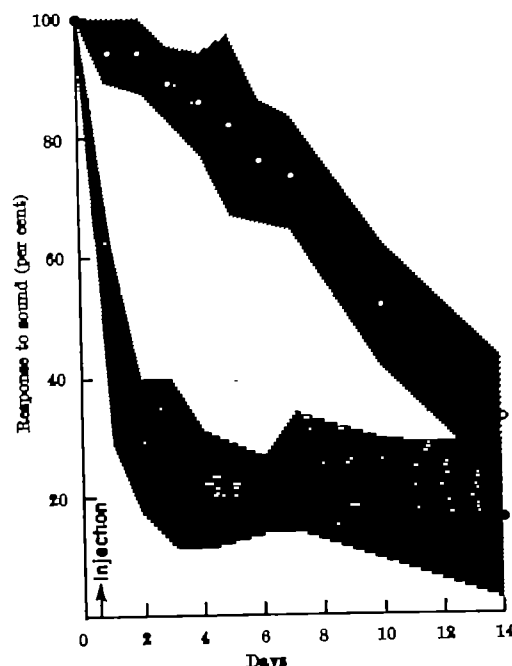


Fig. 1. Habituation curve of mice injected with extracts of normal rat brain (○) and brain from sound-habituated rats (●). Pooled data from the first two experiments shown in Table 1. Abscissa, days, starting from the day of injection; ordinate, mean number of startle responses to sound stimulus. Shading indicates the standard deviation of each mean value of 11 animals for days 1–6 and 4 animals for days 7–14.



experiments, a second injection given on the sixth day increased the habituation rate again.

Further purification of the transfer factor was done by treating the dialysate with 20 vol. of cold acetone. The precipitate was redissolved in water and injected into mice. Table 1 shows that this fraction was active when amounts equivalent to 140–340 mg of brain per mouse were given. The control preparation was inactive at 500 mg.

The transfer factor was found to be soluble in water, insoluble in acetone and 95 per cent ethanol. It is dialysable and, on partition with an equal volume of 88 per cent phenol, it goes into the phenol phase. These properties suggest that it is a peptide or small protein. This has been further confirmed by the disappearance of the activity when the preparation was incubated with crystalline chymotrypsin (1 mg/ml. at pH 8 for 1 h at room temperature). A similar incubation with pancreatic ribonuclease at pH 7 did not affect the activity.

Transfer of habituation has been described in *Planaria*<sup>4</sup>, but, to our knowledge, there is no reference in the literature to the possibility of transfer of any type of learning in higher invertebrates or in vertebrates. The results summarized here suggest that information is recorded and stored in the nervous system in terms of protein structure. Most chemical theories of learning and memory emphasize the role of RNA in information coding<sup>5</sup> and it is probable that persistence of the protein code requires some self-replicating mechanism, through the nucleic acids.

The preliminary experiments reported here show that an elementary form of learning, habituation to sound, can be transferred to untrained animals by injecting them with a peptide-type material extracted from the brain of habituated animals. This factor is absent from the brain of non-habituated animals. Further experiments are in progress in other learning situations, other routes of introduction of the transfer factor and with extracts of circumscribed areas of the brain.

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### Effect of Calcium and Other Ions on Vasopressin Release from Rat Neurohypophyses Stimulated Electrically *in vitro*

THE rat posterior pituitary *in vitro* secretes vasopressin in response to a rise in extracellular potassium concentration. This effect of excess potassium is inhibited by magnesium and sodium ions and is strongly dependent on calcium. Secretion is abolished by omitting calcium from the incubation medium; it increases with increasing calcium concentration over a wide range, and is accompanied by uptake of calcium-45. From such evidence it has been concluded that calcium entry into the neurosecretory terminals on their depolarization by action potentials is the normal physiological event initiating secretion<sup>1-3</sup>. This concept is supported by the demonstration of vasopressin release from rat<sup>3,4</sup> and guinea-pig<sup>5</sup> neurohypophyses *in vitro* in response to electrical stimuli and of action potentials recorded from the supra-optico-hypophyseal tract<sup>6</sup>. It would clearly strengthen the hypothesis if it were shown that the effects of calcium and other

ions on vasopressin release, elicited by electrical stimulation, were similar to their effects on secretion elicited by excess potassium. This communication presents evidence that this is so.

In each experiment, neurohypophyses from five male Sprague-Dawley rats (200–350 g) were dissected out and incubated in bicarbonate Locke's solution at 37°C and stimulated at 40-min intervals for 5 min at a frequency of 20 shocks/sec using an adaptation of the procedure already described<sup>3</sup> that allowed the five glands to be stimulated simultaneously. Vasopressin released into the medium was assayed on the rat blood pressure.

In 53 experiments the mean 'resting' release of vasopressin between the 30th and 40th min after setting up the preparation was  $1.48 \pm 0.35$  (S.E.)  $\mu\mu/5$  glands/10 min. Electrical stimulation for the first 5 min of the succeeding 10-min period raised the mean output in this period to  $18.79 \pm 3.3$   $\mu\mu/5$  glands/10 min. This effect could be abolished reversibly by introducing 0.1 per cent procaine into the medium, and was therefore probably due to the production of electrical activity in the neurosecretory fibres.

The secretory response to electrical stimulation was completely abolished when the calcium concentration in the incubation medium was lowered to about 0.1 mM; it increased with increasing calcium concentration until this reached about 4 mM; and diminished as the calcium concentration was further raised to 8 or 16 mM. The curve relating vasopressin output in response to electrical stimulation to calcium concentration, obtained from experiments on 22 groups of glands, closely resembled that obtained previously using excess potassium to evoke secretion<sup>3</sup>.

An increase in the magnesium concentration of the medium from 1 to 10 mM approximately halved the output of vasopressin in response to electrical stimulation. This effect could be reversed by raising the calcium concentration of the medium ten-fold.

Reduction of the sodium chloride concentration of the medium from 160 to 10 mM (tonicity being maintained with sucrose) enhanced vasopressin output. Thus in five sets of glands stimulated in the low sodium medium vasopressin output was  $44.2 \pm 7.1$   $\mu\mu/5$  glands/10 min while the corresponding control values from the same sets of glands in the high sodium medium was  $22.5 \pm 1.7$ .

The effects of calcium, magnesium and sodium on vasopressin output in response to electrical stimulation thus closely parallel their effects on neurohypophyses stimulated with excess potassium<sup>1,3</sup>. The experiments thus lend further support to the hypothesis that the sequence of events involved in stimulus-secretion coupling in the neurosecretory terminals of the hypothalamo-hypophyseal tract may be: the arrival of impulses, depolarization, and the entry of calcium ions.

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### Acute Toxicity of Extracts of Morning-glory Seeds in Mice

THE presence of alkaloids derived from lysergic acid in the seeds of the morning-glory varieties *Ipomoea violacea* (L.) and *Rivea corymbosa* (L.) Hall f. was discovered by Hofmann<sup>1</sup> and confirmed by several workers<sup>2-4</sup>. Reports of alteration of mental state after ingestion of morning-glory seeds have been made to this Directorate and have

also appeared in the public press. Osmond<sup>6</sup> reported an abnormal mental state after ingestion of the seeds of *Rivea corymbosa*, but Kinross-Wright<sup>7</sup> was not able to induce any such changes in eight subjects. Cohen<sup>8</sup> reported that some individuals appear to have no reaction whatsoever while others may respond as to *d*-lysergic acid diethylamide (LSD). In this same communication, a case of suicide following a recrudescence psychic reaction to ingestion of morning-glory seeds was also reported.

Analysis of the alkaloids of *Ipomoea violacea* by Hofmann<sup>9</sup> showed the largest component to be *d*-lysergic acid amide. There were smaller quantities of *d*-iso-lysergic acid amide, chanoclavine, elymoclavine and ergometrine. Solms<sup>10</sup> and Hofmann<sup>9</sup> reported on psychopharmacological experiments with *d*-lysergic acid amide in man. They observed psychotomimetic activity and a marked sedative effect after doses of 0.5 to 1.0 mg. Yui and Takeo<sup>11</sup> found that elymoclavine caused excitation in mice. Since studies of the total alkaloidal fraction rather than the individual components of morning-glory seeds would appear to be more relevant to the possible toxic effects in man, the following experiment was undertaken.

The alkaloidal fractions were prepared as follows: The defatted, ground (40 mesh) and wetted (10 per cent ammonium hydroxide) seeds were exhaustively extracted with ethyl ether in 30-g lots. The ether was shaken with 0.1 N sulphuric acid, the aqueous phase made ammoniacal, extracted with chloroform, the chloroform evaporated and the residue dried *in vacuo*. In Table 1 are shown the three varieties of morning-glory which were chosen—one variety with high, one with medium and one with very low alkaloid content. The latter served as a control to observe the possible effects of material other than alkaloids.

Homogenous aqueous suspensions of the solid residues of the alkaloidal fractions were injected intraperitoneally into Swiss mice. Table 2 shows the quantities injected and the alkaloidal content, calculated as ergometrine. It also shows the  $LD_{50}$  for two of the varieties. These were calculated by the probit method of Bliss<sup>12</sup>.

About 3 min after injection of the 'Pearly Gates' fraction there occurred loss of motor co-ordination, coarse tremors of the entire body, ataxia, piloerection, ptosis, slight cyanosis and hypersensitivity to touch. At lethal doses there were generalized convulsions and respiratory arrest. Among the surviving animals periodic bouts of fighting behaviour occurred, but these were ineffectual because of the motor impairment. Motor co-ordination improved 3–5 hours after injection, during which time fighting behaviour became more frequent. Finally the mice went into a deep sleep from which they could be easily aroused. There were no delayed deaths. The general impression was of a state of central excitation over-

laid by severe motor dysfunction at sub-lethal doses. At lethal doses severe depression supervened with death due to respiratory arrest. Extracts of the seeds of the 'Wedding Bells' variety produced, the same symptoms, but the excitation component of the reaction was much more intense.

Injection of 125 mg/kg of the 'Scarlet O'Hara' fraction was followed by slight depression and ptosis. The depression became more profound during the following hour, after which recovery began. At 200 mg/kg the above symptoms were noted along with piloerection and forward position of the ears. All the mice appeared normal after 24 h, but there was one death at 200 mg/kg 4 days later.

For comparison, mice were injected intraperitoneally with LSD-25, 40 mg/kg. This produced coarse tremors of the head and neck, piloerection, loss of motor co-ordination and ataxia. These effects were similar to those produced by the morning-glory seed extracts but were much less intense.

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### Central Action of Intravenous Curare

THE use of muscle relaxants as an adjuvant for immobilization in experimental neurology is widely accepted as standard technique. These agents are used because, when given parenterally under the usual experimental conditions, they apparently have little or no effect on central nervous activity<sup>1–7</sup>. Since it is known that neuromuscular blocking agents (especially *d*-tubocurarine and gallamine triethiodide) can markedly alter central nervous system activity when applied directly to the brain (pertinent references reviewed by Winterstein<sup>8</sup>), failure to observe central nervous system effects following intravenous administration of these drugs has been attributed to the existence of a functional barrier interposed between the blood and the brain<sup>9</sup>. Notwithstanding, and in apparent contradiction to this general consensus, several workers have reported a marked and well-defined central action of curare when administered parenterally<sup>11–13</sup>.

Paton<sup>14</sup> has suggested that failure of the barrier mechanism might account for those instances in which significant activity occurs. In line with this suggestion, it has been reported that alteration in the selectiveness of the blood-brain barrier can occur as the result of certain experimental procedures<sup>15,17</sup>. However, Cohen<sup>8</sup> was unable to demonstrate the presence of parenterally administered curare in central nervous tissue following large intravenous doses, hypoxia, hypercarbia, hyperalkalemia, or shock.

As an alternative approach to the problem, a study of the effect of intravenously administered *d*-tubocurarine on

Table 1. ALKALOID CONTENT OF THE SEEDS OF THREE MORNING-GLORY VARIETIES

Species	Variety	Quantity of seeds processed (g)	Weight of residue of alkaloidal fraction (mg)	% Total alkaloids*
<i>Ipomoea violacea</i>	'Pearly Gates'	254	187	0.041
<i>Ipomoea violacea</i>	'Wedding Bells'	400	290	0.023
<i>Ipomoea Nil</i>	'Scarlet O'Hara'	193	50	0.002

\* Determined with Ehrlich's reagent and calculated as ergometrine<sup>9</sup>.

Table 2. QUANTITIES OF RESIDUE INJECTED AND MORTALITY

Variety	Solid residue of alkaloidal fraction injected (mg/kg)	$LD_{50} \pm S.E.M.$ (mg/kg)
'Pearly Gates'	125.0 (73.5)*	164.3 $\pm$ 15.1
	156.0 (90.5)	
	171.5 (99.5)	
	187.0 (108.5)	
'Wedding Bells'	184.0 (74.0)	214.1 $\pm$ 15.8
	195.0 (78.0)	
	207.0 (83.0)	
	230.0 (83.0)	
'Scarlet O'Hara'	125.0 (10.0)	1 delayed death at 200 mg/kg
	200.0 (16.0)	

\* Figures in brackets refer to alkaloidal content calculated as ergometrine in mg/kg.

the central nervous system of an animal in which the blood-brain barrier mechanism was naturally ineffective was felt to be of value. By such an approach, the role of the blood-brain barrier mechanism in the exclusion of curare from the brain might be better understood. The young chicken was selected as the experimental animal because: (1) it does not develop a functionally mature blood-brain barrier until two to three weeks after hatching<sup>11,12</sup>; (2) the electrical activity of the brain shows essentially adult characteristics prior to this time<sup>13-15</sup>.

The present observations initially were obtained from 20 Rhode Island Red chickens, including both sexes, aged 10 days post-hatch (average weight  $72 \pm 8.0$  g). The findings afterwards were confirmed in birds from new hatch to 14 days.

Spontaneous electrical potentials from the optic tectum (TSA) and cerebral hemispheres (hyperstriatal area, HS), the photic evoked electrical activity from the optic tectum (PER) and retina (ERG) were recorded differentially. The reference electrode was in the neck muscles near their occipital insertion. Use of the neck muscles permitted a reliable estimate of the duration of drug action, since the appearance of muscle spikes on the cathode ray-oscilloscope could be used as the end-point.

The electrocardiogram, body temperature, and blood pressure of the cannulated ischiadic artery, were monitored throughout the experiments. A complete description of the techniques used has been published elsewhere<sup>16</sup>.

The *d*-tubocurarine used was the clinical product (Upjohn). The solution, which contained 3.0 mg/ml., was injected intravenously into the ischiadic vein. The initial injection of curare was always administered when the animal first showed signs of recovering from the general anaesthesia (ether). Subsequent injections were given when muscle spikes appeared on the oscilloscope trace, and averaged one every  $45 \pm 10$  min for a period of 8–10 h per experiment. The dose used was 6 mg/kg/injection throughout; this is approximately ten times the amount required to produce total paralysis in the chick for 10 to 20 min<sup>17</sup>.

Buttle and Zaimis<sup>18</sup> observed an immediate flaccid paralysis in the White Leghorn chick following intravenous curare<sup>18</sup>. In contrast, the lightly anaesthetized Rhode Island Red chick showed, at the onset of injection of the initial dose, pronounced body movement. The reaction was characterized by flapping wings and a running motion of the legs. Muscular activity of a fasciculate nature was also evident in the neck and torso. The severity of the reaction was such as to require physical immobilization of the animal to prevent it freeing itself from the stereotaxic apparatus, or dislodging the arterial or venous cannula. The response endured for approximately 2 sec prior to the onset of the usual flaccid paralysis. Subsequent injections of curare failed to re-induce the reaction, even when recovery from the preceding dose had progressed to the stage where muscle spikes generated in the neck muscles appeared on the oscilloscope trace. The possibility that this reaction could be re-induced if the animal were allowed to recover completely from the preceding dose of curare was not tested.

To determine whether the excitation reflected an action of curare within the central nervous system, the sciatic nerve was sectioned prior to the initial injection in ten experiments. In each instance the limb peripheral to the section failed to participate in the body movement. In

four experiments the spinal cord was sectioned at the upper cervical level. This eliminated all body movement.

The effect of curare on the cardiovascular system was inconsistent. There was no effect on the electrocardiogram or on the heart rate; however, changes in blood pressure did occur. The direction of these changes depended on the level of pre-drug pressure. In the lightly anaesthetized chick, the blood pressure was low (80–90 mm mercury). When curare was administered at such time, a rise in pressure of 10–20 mm occurred. But if curare was given when the blood pressure was at a level normal for animals of the age examined (140–120 mm mercury) a fall in pressure resulted. In both instances the change was transient, and pressures returned to pre-drug levels within 1 min. The hypotensive effect usually could be avoided by slow injection of the drug.

Changes in the TSA, HS or ERG were not evident following any of the multiple injections (Fig. 1). However, the photic evoked response (PER) was altered consistently and significantly following the first injection of curare. Changes in the primary negative wave of the potential seldom were evident, but the secondary components of the potential were enhanced markedly both in amplitude and frequency (Fig. 1, PER). A further alteration of the PER was not induced by subsequent injections of curare, even when the frequency of injection was greater than once every 45 min or when an increased dose was given. In no instance did curare alone depress or inhibit the electrical parameters (cp. refs. 12, 14 and 15).

These results strongly indicate that parenterally administered curare is capable of acting upon the central nervous system of the young chicken. Presumably, this is because the incompletely developed blood-brain barrier mechanism fails to prevent the entrance of the drug into the nervous tissue.

That curare does not affect the activity of the central nervous system in the chick in a manner similar to that observed in the mammal<sup>12-15</sup> is of interest. If the action of curare on the mammalian brain is, as suggested by Purpera and Grundfest<sup>19</sup>, suppression of inhibitory mechanisms, followed by inhibition of excitatory mechanisms at higher doses, the present results might indicate that the inhibitory mechanism in the chick brain is not physiologically prominent, or that inhibitory connexions are confined only to those systems giving rise to evoked potentials. In either instance it appears that the excitatory mechanism in the chick is far less sensitive to curare than is that in the mammal.

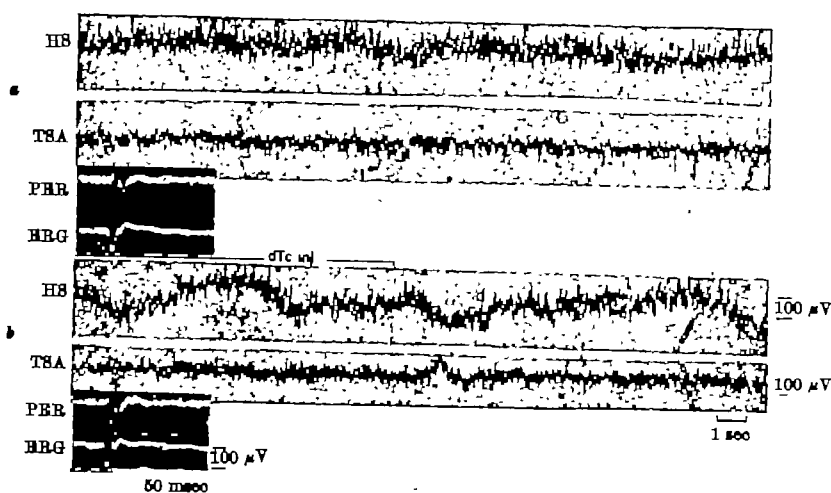


Fig. 1. Effect of parenterally administered curare on the electrical activity recorded from the optic tectum, eye, and cortex of the young chicken

a, control; b, during and immediately following the intravenous infusion of *d*-tubocurarine. HS, hyperstriatum; TSA, tectal spontaneous activity; PER, response evoked in the tectum by photic stimulation of the retina; ERG, electroretinogram. Downward deflections reflect negativity at the critical electrodes

That component parts of the electrical activities recorded from the avian tectum do show different pharmacological sensitivities has been suggested before. For example, Scholes and Roberts<sup>24</sup> have shown that  $\gamma$ -amino butyric acid inhibits the evoked potential while having little or no effect on the ongoing spontaneous electrical potentials, while the opposite occurs with pentobarbital.

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## HAEMATOLOGY

### Interaction between Human Serum Complement and Normal Human Red Cells at Low Ionic Strength

Rapp and Borsoe<sup>1</sup>, in their study of the effects of low ionic strength on immune haemolysis, using sheep cells and guinea-pig complement, found that the erythrocytes were haemolysed in the absence of antibody at extremely low ionic strength. The authors gave details for the preparation of a number of isotonic sucrose buffers of various ionic strengths, and the buffers used in this work were those described by those authors.

We found that when normal human red cells were suspended in veronal sucrose buffer of low ionic strength ( $\mu = 0.009$ ) and mixed with fresh serum, diluted 1:20 in the same buffer, the red cells took up protein, most of which appeared to be C' protein. That these red cells absorbed C' protein was ascertained using the anti-globulin test and anti-C' antiglobulin reagent prepared as previously described<sup>2</sup>, which did not agglutinate normal red cells or red cells sensitized with various blood group antibodies but only after such sensitized cells had absorbed C' components.

In the experiments detailed in Table 1, all the sera or reagents were diluted 1:20 in veronal sucrose buffer, pH 7.4 ( $\mu = 0.009$ ). The red cells, obtained as citrated blood, were washed three times in normal saline and twice in the VB sucrose buffer and suspended in it to a strength of 10 per cent. Two volumes of the diluted reagent (Table 1)

Table 1. MAXIMUM AGGLOUTINATION = + + + + +

The reagents were diluted in veronal sucrose buffer, pH 7.4 (1:20), mixed with washed red cells (see text) suspended at 10 per cent in the buffer. Diluted reagent and red cells were mixed together for 0.5 h at 37° C, before the antiglobulin test.

Reagents	Antiglobulin test Anti-C'
R11S	—
R11S+11S	+ + + +
11S	—
R1	—
R1+11S	—
R2	+ + + + +
R1+R2	—
R4	+ + + + +
R4+R1	+
R3	—
Serum heated 56° C, 5'	—
Serum absorbed AA/pt.	—
Fresh serum + NaHDTA	—
Fresh serum	+ + + + +

and one volume of the red cell suspension were incubated at 37° C for 0.5 h and then washed three times in normal saline before the antiglobulin test. The R reagents (except R11S, below) were made using standard methods and were tested using sensitized sheep cells; they showed no lysis but did show ability to re-combine.

Table 1 shows that R1, R2 and R4 failed to coat the red cells and gave a negative antiglobulin test, but mixtures of R1 and R2 and R1 and R4 did coat the cells and the antiglobulin test was positive. Heated serum, even when heated for only 5 min at 56° C, failed to coat the cells, as did serum absorbed with egg albumen-anti-egg albumen precipitate. Serum treated with EDTA and then diluted in veronal sucrose buffer and mixed with the red cells, as described above, also gave a negative result. These results suggest that the protein absorbed on to the red cell is complement protein.

The R3 reagent gave a positive result, but was only weakly positive. However, if 10 volumes of an appropriately diluted R3 reagent (1:20) were mixed with one volume of red cells, the result was strongly positive; other reagents, when mixed with the red cells, as previously described, continued to give negative results when used in the foregoing proportions, that is, 10 volumes of diluted reagent with 1 volume of cell suspension.

The R11S preparation was made by absorbing serum with aggregated  $\gamma_2$  globulin at pH 6.8, otherwise using the method of Muller-Eberhard and Kunkel<sup>3</sup>, as modified by Hinz and Mollner<sup>4</sup>. This reagent gave a negative result when tested under the conditions previously described, but a strong positive result occurred when the 11S material was added to the preparation; it is of interest to note that R1 + 11S gave negative results.

All these tests were done using group O red cells and mixed normal serum from healthy blood donors, but equally strongly positive results were given if red cells of groups A, B, or AB were mixed with fresh serum from an individual of the same ABO group. Experiments also showed that an individual's own red cells would give a positive result in the antiglobulin test under the conditions described when the individual's own fresh serum was used as a test serum.

When the tests, using fresh serum (as described above), were repeated using as a diluent a veronal sucrose buffer  $\mu = 0.037$  in place of the  $\mu = 0.009$  buffer, the result was either negative or only feebly positive (+).

It is of interest to note that the cells and serum can combine only at low ionic strength but, once the combination of certain C' proteins with red cells has occurred, washing in normal saline before the antiglobulin test, which was routinely carried out, did not affect the positive result; thus it is not necessary to maintain a low ionic strength once the union between C' and the red cells has gone beyond a certain stage.

Experiments have been carried out to try to determine whether there is a sensitizer present in normal serum, which must react with red cells prior to the action of C', but the results so far have been inconclusive and further experiments are in progress.

The above experiments suggest that under conditions of low ionic strength complement proteins are absorbed by normal red cells, but it is not known at the moment what these proteins are—nor is it known that all the proteins so absorbed are C' proteins. These results are of practical interest, as it may be possible to build up cells with a known complement constitution without having to use sensitized cells. Rapp and Borsos<sup>1</sup>, when referring to their work, wrote: "The discovery that erythrocytes can be lysed at low ionic strength by complement in the absence of added haemolysin, may have a bearing on the auto-immune phenomenon". The finding that human red cells can take up proteins from their own serum (which appears to be complement protein) adds interest to this suggestion, and it is hoped that the phenomenon will be of both theoretical and practical value.

*Note added in proof.* P. L. Mollison and M. J. Polley (*Nature*, 203, 535; 1964) have recently made similar observations.

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### A Lymphocytosis-stimulating Substance in Mongoloid Plasma

RECENT investigations have again indicated that the thymus produces a hormone which stimulates the lymphoid tissues of the body<sup>1</sup>. In 1958, Metcalf indicated that the plasma of patients with lymphatic leukaemia contained a lymphocytosis-stimulating factor<sup>2</sup>. Metcalf's investigations on the extract from thymic tissue, as well as the work of other research workers, link the thymus with leukaemia<sup>3,4</sup>.

It is known that Mongoloids combine a high incidence of leukaemia with a chromosomal abnormality<sup>5</sup>. This observation suggested that Mongoloid plasma might contain a lymphocytosis-stimulating substance. This communication reports the results of an experiment to investigate this possibility.

Ten ml. of whole blood was obtained by venepuncture from five Mongoloids ranging from 10 to 20 years in age. The sodium salt of ethylenediamine tetraacetic acid was used as the anticoagulant. Control plasma was obtained from a healthy individual. Plasma was separated by centrifugation, placed in sterile tubes and frozen at -15° C until used. All the plasma samples were cultured on blood agar, brain heart infusion and thyol to check for contamination.

Each plasma sample was injected into 12-18 CFL strain of white Swiss mice which were 24-36 h old. Each animal was given 0.015 ml. intracerebrally on the parasagittal plane midway between the ears using a sterile 27 ga. needle and tuberculin syringe. Each day, starting 24 h after injection, blood from 2 mice from each plasma group was taken for total and differential white blood counts (WBC). Blood was obtained by severing the jugular vein with a sharp razor blade. Blood films were stained with Wright's stain. Counts were not continued beyond 9 days.

All five Mongoloid plasmas caused a lymphocytosis. The lymphocytosis reached its maximum on the fourth or fifth day, after which it decreased to a stable plateau. In the control animals, the lymphocyte count changed very little or dropped slightly. This time-treatment interaction was statistically significant (Fig. 1).

The treatment with Mongoloid plasma resulted in a mean of 49.2 per cent lymphocytes (high, 86 per cent;

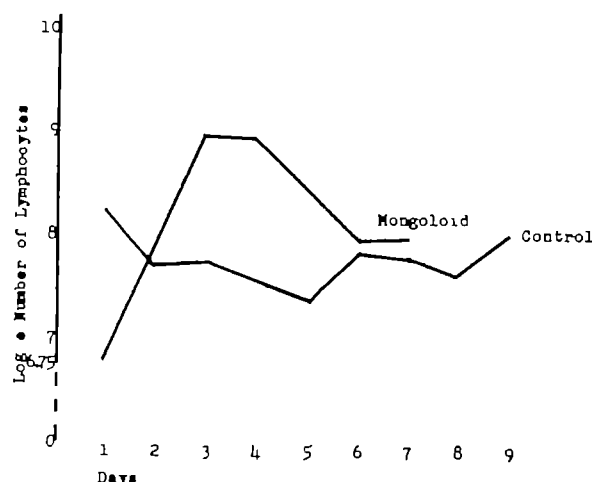


Fig. 1. Comparison of the number of lymphocytes of mice injected with normal plasma, with the number of lymphocytes of mice injected with Mongoloid plasma. The number of lymphocytes is expressed as the log

low, 44 per cent); whereas the treatment with control plasma resulted in a mean of 39.5 per cent lymphocytes (high, 65 per cent; low, 36 per cent) over the 9-day period. This difference is statistically significant. These results are similar to those reported by Metcalf<sup>2</sup> with the exception that in this experiment the lymphocytosis reached its maximum level earlier.

Visual observations, as well as the weights of some animals, revealed that the mice injected with Mongoloid plasmas were smaller and weighed less, on any given day, than comparable controls. Since care and housing were the same for all animals, this difference could only be attributed to the treatment.

The differential smears revealed that the mice injected with plasma from a 12-year-old Mongoloid had an extremely high number of rubricytes. Relatively fewer rubricytes were present on all the other Mongoloid blood smears. On the control smears, rubricytes were seldom found.

We thank Dr. J. H. Gruter for his help with the blood counts, and Dr. W. G. Stover, superintendent, Apple Creek State Hospital, for his co-operation.

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### Effects of Adenosine Diphosphate and Adrenaline on Mean Platelet Shape

If small particles are suspended in a fluid in which there is a velocity gradient, non-spherical particles will become orientated whereas spherical particles will not; this orientation alters the light transmission through a suspension of such particles. Thus the turbulence produced by shaking a test-tube containing a suspension of rod-shaped bacteria can be seen as a 'swirl' whereas a coccal suspension shows no 'swirl'.

Zucker and Zaccardi<sup>1</sup> have claimed that platelets change their shape and become more nearly spherical when exposed to adenosine diphosphate (ADP). This observation can be substantiated and studied in some detail by the following technique. Fresh heparinized or citrated platelet-rich human plasma is placed in a cuvette at 37° C

in an 'Eel' titrometer and stirred at about 500 r.p.m. by a captive magnet. The output, suitably magnified, from the photoelectric cell operates a pen recorder. The platelets are seen to swirl and this is recorded on the tracing as a rapid excursion of moderate amplitude while the mean optical density (*O.D.*) remains constant. Inspection of these platelets under the microscope shows considerable pleomorphism—disks and distorted elongated shapes with some pseudopodia and spicules. If 'Benadryl' or cocaine is added during stirring a drop in the mean *O.D.* occurs as a result of the dilution and then the amplitude of the tracing becomes gradually much smaller (Fig. 1, *BEN*). If the platelets are examined under the microscope at this time they are seen to have become spherical with no spicules. Thus a tracing of large amplitude indicates swirling and non-spherical platelets, and one of small amplitude indicates spherizing. If sufficient ADP is added to a sample of platelet-rich plasma to produce a small degree of aggregation, the mean level of the tracing goes down since the mean optical density decreases with aggregation. The amplitude decreases at exactly the same time as the mean *O.D.* begins to decrease.

Fig. 2 (*ADP*) suggests that the average platelet shape changes towards a more spherical form, and that this occurs simultaneously with the increase in stickiness which is reflected by the beginning of aggregation. As aggregation progresses the platelet masses increase to such a size that they then interfere with the light path and the amplitude of the tracing again increases. Thus a change in amplitude related to a change in shape is only evident before sizable aggregates are formed. If the stirring is continued the platelets disperse and the amplitude of the tracing usually becomes small again but does not revert to the pre-ADP level: this suggests that these dispersed platelets remain more spherical than they were initially. The addition of more ADP does not then alter the amplitude in spite of renewed ADP-induced stickiness and aggregation. Thrombin (Fig. 2, *TH*), 5-hydroxytryptamine, tri-ethyl tin and tendon extract (collagen) (Fig. 1, *COL*) all produce aggregation after a delay of 10–360 sec depending on which compound is used. In each case a decrease in amplitude occurs, not when the compound is added but at precisely the same time as the beginning of aggregation. It is probably relevant that all the foregoing compounds are thought to produce aggregation by causing

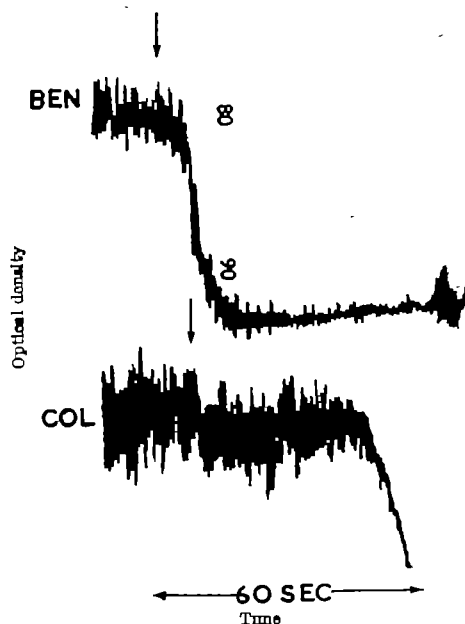


Fig. 1. *BEN*, 2 ml. platelet-rich plasma was stirred. The arrow indicates the addition of 0.2 ml. saline containing 2 mg. of 'Benadryl'. This tracing illustrates spherizing with no aggregation. *COL*, The arrow indicates the addition of 0.1 ml. of a saline suspension of homogenized human tendon.

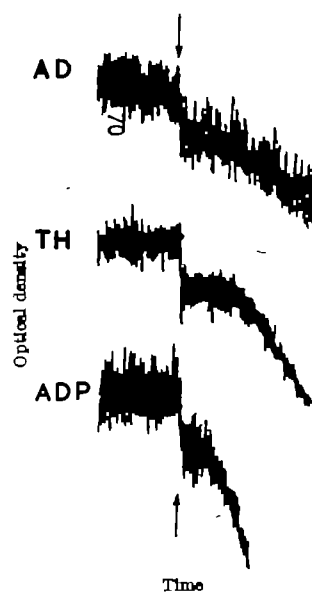


Fig. 2. Experiments and recording similar to Fig. 1. The additions were: *ADP*: 0.1 ml. of saline containing 0.5  $\mu$ g. of adenosine diphosphate. *TH*: 0.1 ml. of saline containing 0.0025 units of human thrombin. This tracing and that of *ADP* and *COL* illustrate the 'rounding up' of platelets at the same time as aggregation begins. *AD*: 0.1 ml. of saline containing 0.11  $\mu$ g. of adrenaline acid tartrate. This tracing illustrates aggregation in the absence of a change in platelet shape.

the liberation of intrinsic platelet ADP. As reported<sup>1</sup>, adenosine added before the ADP prevents both aggregation and change in shape. With the present method no change in amplitude or *O.D.* occurs in such an experiment. Concentrations of ADP too weak to cause aggregation do not cause a decrease in amplitude. Platelets collected into EDTA are not sticky and they regularly give a tracing of small amplitude.

There are many differences between the response of platelets to adrenaline and to ADP<sup>2</sup>. Fig. 2 (*AD*) shows that the addition of adrenaline does not produce a change in the amplitude, and visible swirling in the cuvette persists even though aggregation occurs. Later the amplitude increases as large aggregates form. It seems therefore that no change in shape occurs despite an increased stickiness and aggregation.

Platelets in the early stages of aggregation were fixed in glutaraldehyde and examined under the microscope. The differences observed were not clear-cut, but in general platelets before the addition of an aggregating agent tended to be oval and flattened with a smooth outline. The addition of adrenaline in small concentrations did not materially alter their shape whereas after the addition of ADP the platelets were more rounded, with some spicules. It seems likely that the amplitude of these tracings in the first few seconds and the presence or absence of visible 'swirl' are influenced by the mean platelet shape, although other factors may contribute. When ADP is added the average platelet shape changes simultaneously with the development of stickiness, and the platelets remain more rounded after they have dispersed. This change in shape is probably related to the onset of stickiness even though it persists after dispersion. Conversely, it seems probable that no change in mean platelet shape occurs when platelets become sticky under the influence of adrenaline. These observations may suggest that the adhesive forces in these two situations are different.

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## HISTOLOGY

## Use of Inhibitors in Cholinesterase Histochemistry

PRESENT-DAY histochemical methods for demonstrating cholinesterases require the use of inhibitors in order to distinguish between acetyl- (that is, true, or specific) cholinesterase (AChE) and butyryl- (non-specific) cholinesterase (ChE). The histochemical technique determines AChE and ChE together, so that if either enzyme is inhibited without the other being affected, only the latter can be determined; thus an inhibitor of ChE makes it possible to determine AChE and vice versa.

Holmstedt<sup>1</sup> strongly recommends the use of 'Mipafox' as a selective inhibitor of ChE and of 'BW284c51' as a selective inhibitor of AChE. Pearse<sup>2</sup> recommends 'iso-Ompa' (DPDA) and 'BW62c47', respectively, because of the greater distance between the concentrations that cause 50 per cent inhibition (most readily expressed as pI 50, the negative logarithm of molar concentration of the inhibitor producing 50 per cent inhibition of the enzyme). Later, Holmstedt and Sjöqvist<sup>3</sup> stated that the selectivity depends not only on the distance between the pI-50 values, but also on the slope of the inhibition curves for the two enzymes. Holmstedt<sup>1</sup> published inhibition curves determined under histochemical conditions for the inhibitors 'DFP', 'Mipafox', 'BW284c51', '302IS' and '306IS'. Curves for 'iso-Ompa', 'BW62c47' and 'Lysivane', a useful inhibitor according to Pearse<sup>2</sup>, have not been published.

We determined inhibition curves for 'iso-Ompa', 'BW62c47' and 'Lysivane' titrimetrically at pH 8 with acetylcholine chloride as a substrate. Although our curves were not determined under histochemical conditions as were Holmstedt's, they are nevertheless an indication of the usefulness of the inhibitors (see Figs. 1-3). It must be mentioned, however, that the degree of inhibition is reduced by decreasing the pH (Heilbronn<sup>4</sup>).

The AChE inhibitors 'BW284c51' and 'BW62c47' show but little difference in the slope of their curves and in their pI-50 distances. As the curves of Holmstedt (for 'BW284c51') were determined under histochemical conditions while ours (for 'BW62c47') were not, there is no reason to reject 'BW284c51' as the preferable inhibitor. According to Holmstedt<sup>1</sup> a useful concentration for 'BW284c51' is  $5 \times 10^{-6}$  M.

Of the ChE inhibitors 'iso-Ompa', 'Mipafox' and 'Lysivane', the last is useless as a histochemical agent because of the slope of its inhibition curve for AChE, even though its pI 50-values for AChE and ChE are quite well separated. Both 'iso-Ompa' and 'Mipafox' are well suited, but both show a marked difference in inhibitory power for different animal species<sup>5,6</sup>. Which inhibitor should be used

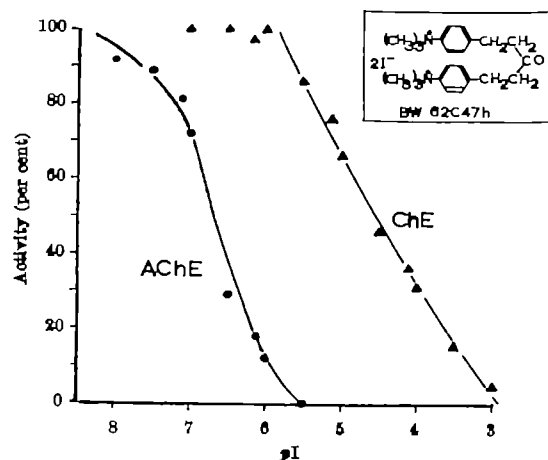


Fig. 1

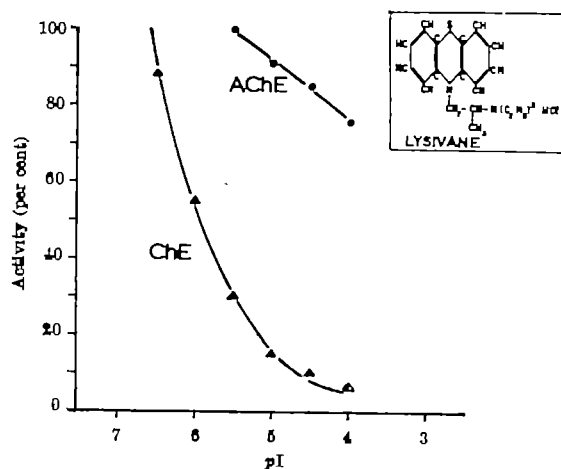


Fig. 2

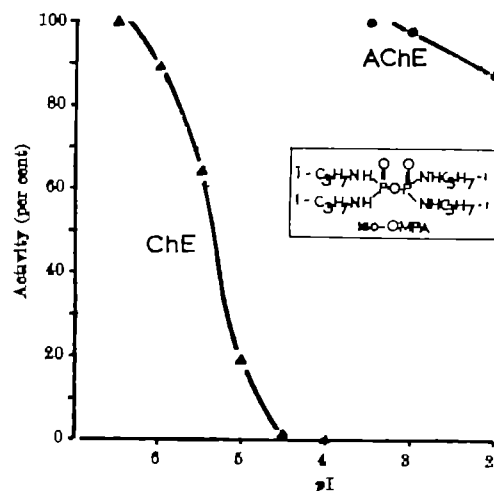


Fig. 3

depends on the species under examination. When no curves of the inhibitor's effect on the enzyme of a certain species are known and where it is difficult to determine them, 'Mipafox' and 'iso-Ompa' may both be used, though in separate experiments. Holmstedt recommends a concentration of  $4 \times 10^{-6}$  M for 'Mipafox'; 'iso-Ompa' can be used at concentrations of from  $3 \times 10^{-5}$  to  $10^{-4}$ .

For AChE we used a purified product from the electric eel (Sigma, St. Louis, U.S.A.), except in our examination of 'iso-Ompa', where fish brain homogenate (three-spined stickleback) was used. For ChE we used a serum fraction from human serum kindly supplied by the Netherlands Blood Transfusion Service, Amsterdam.

'Iso-Ompa' (DPDA, tetra-isopropyl-pyrophosphoramidate) was obtained from Light and Co., England; 'BW-62c47h' (1,5-bis-(trimethylammoniumphenyl)-pentane-3-one-di-iodide) was a gift from the Wellcome Research Laboratories, Beckenham, England; 'Lysivane' (profenamine, diethylamino-2-propyl-10-phenothiazine) was a gift from Specia, Paris.

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# PATHOLOGY

## Growth of Mammary Tumours in $F_1$ Hybrids

TRANSPLANTS from inbred strains to  $F_1$  hybrids derived from one isologous and one foreign parent are generally regarded as compatible. Skin grafts are accepted under these conditions, and most tumours give 100 per cent takes when transplanted by fragments or by inoculation of a heavy suspension. However, Snell<sup>1</sup> reported decreased incidence of takes in  $F_1$  hybrids when small inocula were used, and recently Hellström<sup>2</sup> has described similar findings using induced fibrosarcomas and a spontaneous mammary carcinoma. These reports raise the question whether all tumours exhibit this effect when transplanted in low dosage from their strain of origin to  $F_1$  hybrids.

This prompted the analysis of some previously unpublished work carried out in the Department of Pathology, University of Manitoba. At this time,  $C3H/HeJ$  mice were in very short supply, and  $F_1$  hybrids were produced by mating male  $C3H/HeJ$  and female  $C57L/J$  mice. The experiments were performed originally as part of an investigation of the effect of irradiated cells on transplantation of small numbers of viable cells<sup>3</sup>.

All the mice used for breeding were obtained from the Roscoe B. Jackson Laboratory, Bar Harbor, Maine. The two strains used differ at both  $H_1$  and  $H_2$  histocompatibility loci,  $C3H/HeJ$  having  $a$  and  $b$  alleles and  $C57L/J$  having  $c$  and  $b$  (ref. 4). Only one litter of  $F_1$  hybrids was used from each female. Mice were weaned at 4 weeks and used as tumour hosts at 10 weeks.

Two tumours were used, the  $C3HBA$ , which is a long transplanted and widely used mammary adenocarcinoma, and tumour  $D$ , a mammary carcinoma arising in an aged  $C3H/HeJ$  female, and in its tenth transplant generation in the laboratory at the time of use. Neither tumour had been carried in  $F_1$  hybrids before the experiments. Tumour cell suspensions were prepared by slow stirring of minced tumour in Earle's solution containing a weak concentration of trypsin. Counted numbers of tumour cells (unstained by nigrosin) were injected in 0.1 ml. of volume, intramuscularly into all four legs. All animals were kept under observation until death, or for at least six months. Low doses of inocula were given alone and also mixed with heavily irradiated cells. The latter had received 12,000 r. X-irradiation and never gave rise to tumours by themselves. Results are listed in Table 1 and shown in Figs. 1 and 2 for the  $C3HBA$  tumour, and in Table 2 for tumour  $D$ . Since each animal received four inocula, results show total number of takes and number of animals dying with tumours. Figs. 1 and 2 are survival curves and do not record individual takes.

Table 1. GROWTH OF  $C3HBA$  TUMOUR IN  $C3H/HeJ$  AND  $C3H/HeJ \times C57L/J$  HYBRIDS

Inoculum	Host	No. of takes	No. of animals dying with tumour	Average survival in days*
$10^4$ viable cells	$C3H/HeJ$	55/60	15/15	$33.8 \pm 10.1$
$10^4$ viable cells	$C3H/HeJ \times C57L/J$	59/60	15/15	$33.6 \pm 5.5$
50 viable cells	$C3H/HeJ$	0/40	0/10	—
50 viable cells	$C3H/HeJ \times C57L/J$	0/40	0/10	—
50 viable +	$C3H/HeJ$	23/58†	13/15	$56.2 \pm 14.3$
$10^4$ irradiated cells	$C3H/HeJ \times C57L/J$	35/58†	14/15	$55.0 \pm 11.2$

\* Survival of animals with tumours,  $\pm$  standard deviation.  
† One animal in each group received 2 inocula only.

Table 2. GROWTH OF TUMOUR  $D$  IN  $C3H/HeJ$  AND  $C3H/HeJ \times C57L/J$  HYBRIDS

Inoculum	Host	No. of takes	No. of animals dying with tumour	Average survival in days*
$1.5 \times 10^4$ viable cells	$C3H/HeJ$	24/32	7/8	$88.1 \pm 6.1$
$1.5 \times 10^4$ viable cells	$C3H/HeJ \times C57L/J$	20/40	8/10	$103.6 \pm 17.2$
250 viable cells	$C3H/HeJ$	0/40	0/10	—
250 viable cells	$C3H/HeJ \times C57L/J$	0/40	0/10	—
250 viable +	$C3H/HeJ$	23/40	10/10	$101.1 \pm 14.0$
$1.5 \times 10^4$ irradiated cells	$C3H/HeJ \times C57L/J$	23/40	8/10	$115.2 \pm 20.1$

\* Survival of animals with tumour  $\pm$  standard deviation.

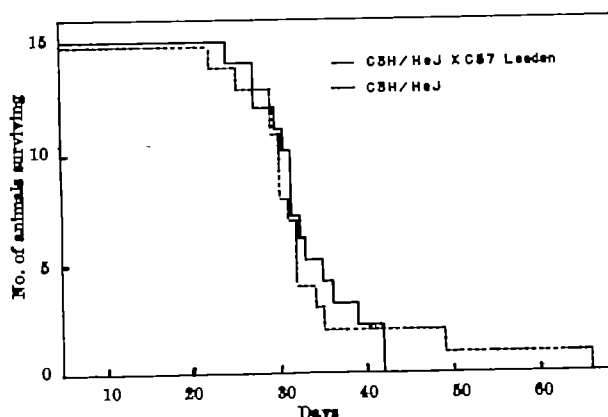


Fig. 1. Survival curve of  $C3H/HeJ$  and  $C3H/HeJ \times C57L/J$  mice following inoculation of  $C3HBA$  tumour,  $10^4$  viable cells into each leg

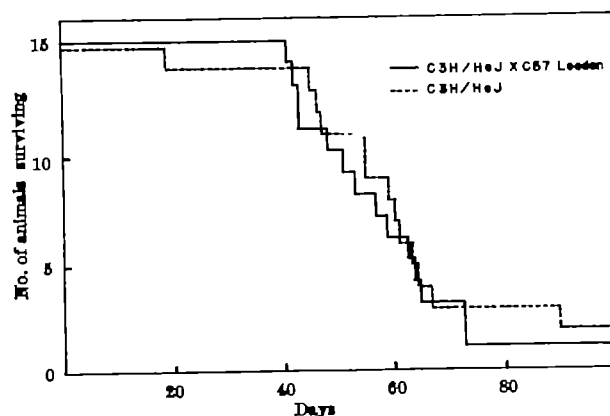


Fig. 2. Survival curves of  $C3H/HeJ$  and  $C3H/HeJ \times C57L/J$  mice following inoculation of  $C3HBA$  tumour, 50 viable +  $10^4$  irradiated cells into each leg

It is seen that the incidence of successful takes is essentially the same with both tumours and at low and high dose ranges. Even the 'high' dose ranges would fall into the category of medium or low doses according to the previously mentioned investigations. None of the groups shows significant differences as to number of takes, number of animals dying with tumour, or average survival time of tumour-bearing animals. Figs. 1 and 2 show the close similarity between the  $C3H$  and  $F_1$  results using the  $C3HBA$  tumour. Tumour  $D$  gave similarly close results in an additional examination using intravenous inocula of  $10^4$  and  $10^5$  cells.

If an immunological factor were involved in the reduced growth in  $F_1$  hybrids found by other workers, one would expect that the presence of large numbers of irradiated cells might reduce the number of takes<sup>4</sup>. However, the results of Snell and of Hellström suggest that the reduced number of takes was not determined by immunological differences. The present results are not necessarily in conflict with those previously obtained, but they do suggest that reduced frequency of takes is not invariable in  $F_1$  hybrids even when very low doses of viable cells are used.

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### Effect of Histidine on the Urinary Excretion of 4-Aminimidazole-5-carboxamide

We have recently shown<sup>1</sup> that patients with folic acid and/or vitamin B<sub>12</sub> (FA-B<sub>12</sub>) deficiency have an increased excretion of 4-aminimidazole-5-carboxamide (AIC) compared with anaemic patients who are not so deficient, or with non-anaemic hospital patients. A FIGLU test, using an oral load of 15 g L-histidine hydrochloride, was performed on many of these patients as a diagnostic procedure; since a haematological response has been observed in such patients following histidine dosage<sup>2,3</sup>, which would imply a partial correction at least of the disordered nucleoprotein metabolism present in these deficiencies, we have taken the opportunity to investigate its effect on the urinary excretion of AIC, itself an intermediary in purine synthesis. For this purpose urine passed between 4 and 8 h after histidine dosage was examined (Period 3); urines collected during one hour in the morning immediately before the test (Period 2) and in some cases for a similar 5-h period the previous afternoon (Period 1) served as controls. For comparison, similar collections were made from 8 normal adult volunteers and 3 patients who had anaemia not associated with FA-B<sub>12</sub> deficiency. No dietary restrictions were imposed during the test. Urines were preserved with 1 ml. 6 N HCl per h of the save and stored at 4° C. AIC was determined by an ion-exchange chromatographic technique<sup>4</sup>. In order to minimize any errors in the timing of the urine collections all results were expressed as AIC µg/mg creatinine.

Table 1 shows the mean AIC excretion in the two groups. In the 11 subjects without FA-B<sub>12</sub> deficiency the excretion was significantly less in Period 2 than in either Periods 1 or 3 ( $P < 0.01$  in each case), but Periods 1 and 3 did not differ significantly. We therefore conclude that histidine does not normally affect AIC excretion and regard the low values in Period 2 as resulting from the diurnal variation reported elsewhere<sup>4</sup>. In the 11 patients with FA-B<sub>12</sub> deficiency where excretion in all three periods was measured, no statistically significant difference in excretion was found between these periods. In these subjects, however, values were slightly lower rather than higher in Period 3 as compared to Period 2. Inclusion of an additional number of subjects from whom urine was collected only over Periods 2 and 3 raised the total to 26, and the fall in AIC excretion during Period 3 was shown to be significant at the 2 per cent level. We therefore provisionally conclude that histidine does produce a fall in AIC excretion in subjects with FA-B<sub>12</sub> deficiency. However, the study of larger and more sharply defined subgroups within this broad classification would be desirable, as would further information on diurnal variations in the excretion of AIC and creatinine.

All the above subjects with FA-B<sub>12</sub> deficiency showed, by virtue of an increased excretion of formiminoglutamic acid and/or urocanic acid following histidine, evidence of a defect in the metabolism of C<sub>1</sub> units. It is difficult to visualize any role for AIC in purine synthesis in man which does not involve C<sub>1</sub> units, and we consider that this metabolic defect must account at least in part for the high excretion of AIC in such subjects, reported previously<sup>1</sup>. The provision of an increased number of potential C<sub>1</sub> units in the form of histidine might well, as a mass action effect, promote a more efficient utilization of AIC, accompanied by a decrease in its excretion. The resulting increase in purine synthesis might provide a basis for

explaining the haematological response noted in megaloblastic anaemia following histidine dosage.

If the high rate of excretion of AIC in anaemia is regarded as due to an impairment in the formylation of AIC ribotide in the absence of adequate quantities of folic acid, as occurs in sulphonamide inhibited micro-organisms, our experimental findings would all appear to be adequately accommodated. If this were the only abnormality, however, an overall depression of purine synthesis would be implied, whereas the exact opposite appears to be the case as judged from recent studies. The finding that patients with vitamin B<sub>12</sub> deficiency, for example, excrete a smaller than normal percentage of orally administered AIC has led to the suggestion that interference with feedback control of purine synthesis may mean that the shunt mechanism of by-passing nucleic acids in purine biosynthesis is functioning at a rate well above normal in these subjects<sup>5</sup>. Interpretation of AIC excretion is further complicated by the possibility that AIC might be a product of purine degradation. If such degradation were inhibited by histidine, as has been shown to occur in micro-organisms<sup>6</sup>, the effects of the latter on the haematological status and on the excretion of AIC could be explained.

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### Lathyrism and 7,12-Dimethylbenzanthracene-Induced Carcinoma in the Rat

This lathyrin agent,  $\beta$ -amino-poprionitrile (BAPN), reduces the tensile strength of mesenchymal tissue and modifies the collagen content<sup>1,2</sup>. Since local tissue factors are believed to be of importance in tumour growth, BAPN was administered to rats in which tumours were induced by a polycyclic hydrocarbon. 7,12-Dimethylbenzanthracene (DMBA) was used because it regularly induces mammary carcinoma in young female Sprague-Dawley rats. The strain of rat, the age, and the hormonal status are important factors in the induction of mammary carcinoma<sup>3</sup>.

50-day-old female Sprague-Dawley rats were given a purified diet with or without addition of 0.5 per cent BAPN. 20 mg 7,12-dimethylbenzanthracene in sesame oil was administered intragastrically at the beginning of the experiment in a single dose<sup>4</sup>. The time of induction, rate of growth and number of tumours were noted for 150 days. Urine samples were collected at 60–70 days after the beginning of the experiment. Oestrogenic substance was extracted, chromatographed on a 3.5 per cent hydrated alumina, and measured by a micro-Kober reaction<sup>5,6</sup>.

The animals were divided into three groups: Group I, intact female rats; Group II, DMBA; Group III, DMBA and BAPN. The lathyrin rats (Group III) developed tumours at an earlier stage than the control rats (Group II). The tumours in the lathyrin group grew faster and the total weight of tumour mass was significantly greater than in the Group II rats. Lathyrin rats did not show any significant increase in the number of tumours. The results are summarized in Table 1.

Table 1. URINARY AIC EXCRETION  
(µg/mg creatinine; mean  $\pm$  S.E.)

	No. subjects tested	Pre-histidine	Period 2	Post-histidine
		Period 1 (afternoon)	(morning)	Period 3 (afternoon)
Normals and non-deficient anaemic patients	11	0.62 $\pm$ 0.029	0.50 $\pm$ 0.029	0.65 $\pm$ 0.029
Folic acid and/or vitamin-B <sub>12</sub> deficient patients	11	2.09 $\pm$ 0.120	1.84 $\pm$ 0.120	1.77 $\pm$ 0.120
	26	—	2.20 $\pm$ 0.164	1.59 $\pm$ 0.164

Table 1. INCIDENCE, INDUCTION TIME, AND WEIGHT OF TUMOURS IN RATS TREATED WITH DMBA AND BAPN

Group	No. rats	No. tumours	Mean tumour induction time (days $\pm$ S.D.)	Mean tumour weight (g $\pm$ S.D.)
I Control	12	0	(days $\pm$ S.D.)	(g $\pm$ S.D.)
II DMBA	12	2.5 $\pm$ 0.4	71.8 $\pm$ 30*	0.80 $\pm$ 0.81
III DMBA + BAPN	12	3.4 $\pm$ 0.4	53.9 $\pm$ 19	3.10 $\pm$ 0.26

\* Standard deviation of the mean.

The tumours were glandular carcinomata. The microscopic appearance did not differ significantly between groups. In all Group III rats there was objective evidence of lathyrium consisting of osteochondral and visceral defects.

It should be noted that these tumours did not metastasize to the internal mammary chain, nor to the lungs or pleura. Although the tensile strength of collagen tissue was reduced, the only change noted was an increase in the local size of the tumour mass. Lymph node hyperplasia in the regional area was a constant finding.

Oestrogen assay at 60 days indicates that DMBA lowers the oestrogen excretion of the rat. BAPN did not have any effect on oestrogen excretion, as noted in Table 2.

Table 2. OESTROGEN EXCRETION 60-70 DAYS AFTER DMBA ADMINISTRATION

Group	I	II	III
Oestrogenic substance ( $\mu$ g/day)	0.176	0.068	0.060

The present results support the contention that BAPN administration accelerates the process of tumour induction and growth<sup>7</sup>. It does not appear to change factors relating to metastatic spread. It also appears that a change in hormonal milieu is an associated factor in tumour induction. Huggins *et al.*<sup>8</sup>, Wong *et al.*<sup>9</sup> and others have reported that adrenal necrosis and lower corticosterone levels follow DMBA administration. Oestrogen excretion may also be lowered.

In summary, lathyrium accelerates the induction and growth of mammary tumours in rats given DMBA. It does not change the microscopic appearance of tumour tissue. DMBA lowers oestrogen secretion.

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### Elastolysis and Giant Cell Reaction against Disintegrated Elastic Fibres

Of the vascular diseases in the surgical field, pulseless disease<sup>1</sup> and giant cell arteritis<sup>2</sup> are histologically characterized by the presence of a granulomatous inflammation as well as elasticophagic giant cells.

Degenerating elastic fibres have been implicated as the cause of granulomatous tissue reaction with formation of giant cells<sup>3</sup>. Since the demonstration by Baló and Banga<sup>4</sup>

that pancreatic extract contains an enzyme (elastase) which dissolves elastic fibres, a considerable number of reports have been published on the elastolytic process by the action of elastase<sup>5</sup> and the production of elasticophagic giant cells *in vitro*<sup>6</sup>, but little is known about the relationship between elastase and elastic tissue *in vivo*, and it remains to be determined whether an intense giant cell reaction is provoked by a primary degeneration of elastic tissue or a secondary elastic injury due to various causes.

The present experiments were carried out to determine whether a cause-and-effect relationship exists between degenerating elastic fibres and granulomatous tissue reaction accompanying giant cell formation *in vivo*. Rabbits weighing 2,000-3,000 g were used.

In order to destroy elastic fibres of the vessel wall, an elastase suspension (0.3, 0.5 and 1 per cent, N.B.C. and Sigma Company) with 0.2 M borate buffer at pH 9.0 was administered to the common carotid arteries of the animals by intravascular infusion under firmly applied ligatures (A) or periadventitial injection (B).

Also an attempt was made to activate the reticulo-endothelial system by typhoid-paratyphoid vaccine which was initially administered intraperitoneally, at a dosage of 3 ml. per kg, and a few days later 0.5 ml. in the neck.

**Experiment A.** After submitting the vessels to the action of elastase for 20 min to 7 days before the ligatures were withdrawn, the segments of the vessels were removed at intervals ranging from 2 to 90 days. Although no noticeable degradation of elastic fibres was seen during the first 20 min attack by elastase, remarkable elastolysis was recognized after 40 min.

One week after the injury there was a concentric or eccentric thickening in the intima, usually depending on whether the whole circumference of the media or only part of it was affected, composed of increased metachromatic material, fibroblasts and newly formed reticular and elastic fibres, and after 2 weeks smooth muscle cells were present.

Multinucleate giant cells phagocytosing the fragments of disintegrated internal elastic lamina were recognized (Figs. 1 and 2). Fibrinoid degeneration was occasionally observed. The media showed disintegrated elastic fibres and a decrease of the metachromatic material. In the adventitia, a proliferation of fibroblasts and mononuclear cells was noticed. There was no elasticophagic giant cell reaction in cases which were suffering a very severe disintegration of elastic fibres. In the cases without injection of the vaccine, there was no giant cell reaction and no noticeable inflammatory cell infiltration.

**Experiment B.** Neither elasticophagic giant cell nor thrombus was present, although an intimal thickening as well as destruction of the media and adventitia were seen.

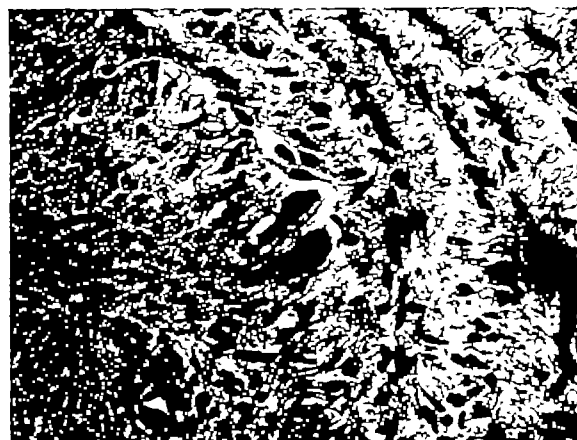


Fig. 1. Section of common carotid artery, fifteen days after submitting the vessel to the action of 0.3 per cent elastase suspension for 3 h. (Haematoxylin and eosin,  $\times$  402)

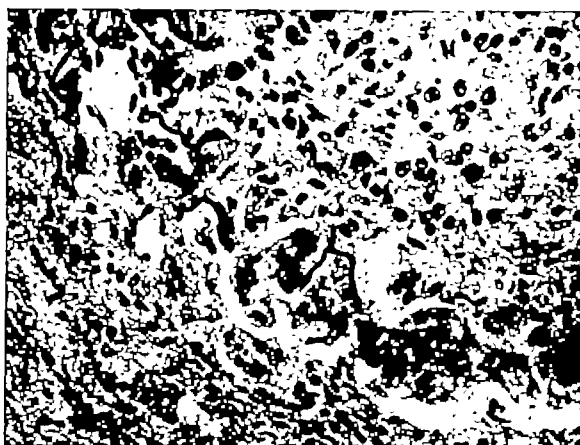


Fig. 2 Section of common carotid artery, twenty-one days after the same experiment as presented in Fig. 1. (Weigert's resorcin fuchsin and Van Gieson,  $\times 402$ )

From these results, it is clear that elastase has an elastolytic activity *in vivo* and the fragments of elastic fibres exist as foreign bodies, and it is reasonable to presume that the following conditions are necessary for the occurrence of elasticophagic giant cells: (1) a specific destruction of elastic fibres to provoke giant cells—for example, hard and slightly soluble fragments of internal elastic lamina; (2) a mesenchymal activation brought about by some factors.

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## IMMUNOLOGY

### Anti-elastin Antibodies in Normal and Pathological Human Sera

SOLUBLE peptides obtained by alkaline-alcoholic degradation of bovine aorta-elastin were shown to be antigenic in rabbits<sup>1</sup>. Though these peptides (the so-called  $\alpha$ -elastin<sup>2</sup>) are of relatively low molecular weight, passive haemagglutination titres up to about 1/1000 could be obtained<sup>3</sup>.

Specific precipitation tests were then performed on normal and immunized rabbit sera, using <sup>125</sup>I-labelled  $\alpha$ -elastin<sup>4</sup>. These experiments showed that even normal rabbit serum precipitated with labelled elastin peptides if incubated for 1 week in the cold. The antibody titres were quite low, and only 1–2 per cent of the labelled peptides were present in the precipitate<sup>4</sup>. These results were similar to those obtained by Grabar for antigelatine antibodies<sup>5</sup>. The experiments were then extended to normal and pathological (atherosclerotic) human sera in order to explore a possible relationship between anti-elastin antibodies and pathological modification of the vessel wall.

Rabbit red cells were coated with  $\alpha$ -elastin prepared as described<sup>1,2</sup>, using tetrazotized benzidine as a coupler, and the passive haemagglutination test<sup>6</sup> performed with serial dilutions of decomplexed sera. Controls for agglutination of uncoated red cells and for self-agglutination were included in every run. We report here the

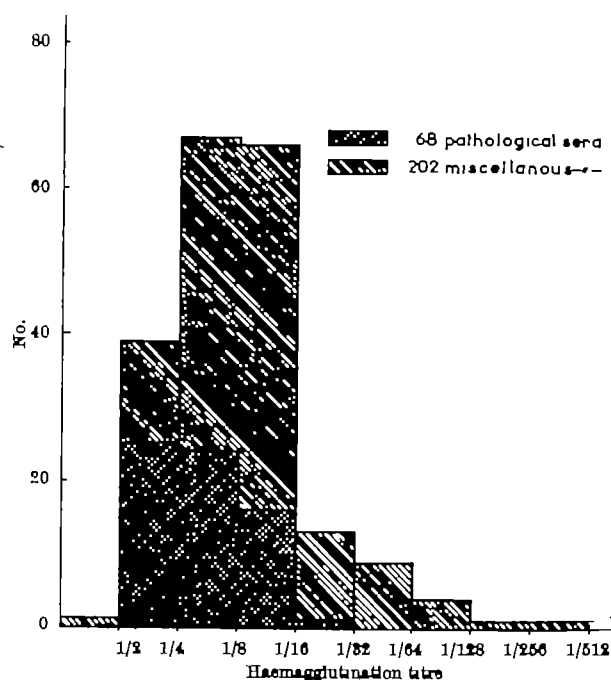


Fig. 1. Frequency distribution of anti-elastin titres in human sera

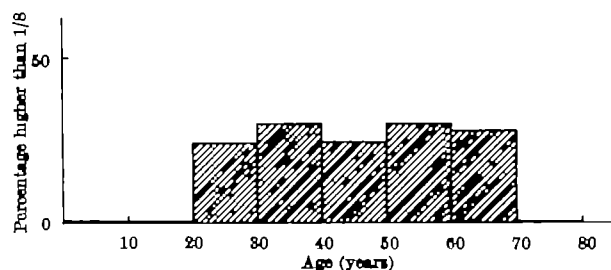


Fig. 2. Anti-elastin titres in 100 human sera as a function of age

results obtained on the first 200 sera examined. Fig. 1 shows the frequency-distribution curves of haemagglutination titres for 68 severe atheromatous patients and 134 miscellaneous sera ('normal' sera, or from patients without recognizable signs of atheromatosis). Though most of the sera studied showed a very low titre (1/2–1/16), about 15–20 per cent of the normal population exhibited significantly higher titres (1/32 to 1/512). In the atherosclerotic sera, the distribution frequency of the low titres was similar to that in the normal population, but titres higher than 1/32 were not encountered. Fig. 2 gives the age distribution of titres higher than 1/8 for the whole population studied. It can be seen that sera with titres higher than this arbitrarily chosen level belong to individuals between 20 and 70 years of age. Younger and older ones have titres between 1/2 and 1/8.

The specificity of anti-elastin antibodies was investigated by absorbing them to fibrous elastin (prepared by the alkaline extraction procedure<sup>1</sup> from bovine aorta), to insoluble collagen (bovine hide powder), or to bovine serum albumin (Armour). Twenty mg of each protein was added to 1 ml. of serum, incubated for 1 h at 37° C and overnight in the cold, then centrifuged. The supernate was tested for anti-elastin antibodies and the adsorption cycle repeated until constant titres were obtained. After 1–2 adsorptions of sera on fibrous elastin, the haemagglutination titre vanished (decreased to 1/2 or even below). Neither collagen nor BSA had any effect on the haemagglutination titres.

These results seem to indicate that anti-elastin antibodies are present in human sera. The low titres observed are probably due to the fact that only a fraction of the

peptides of  $\alpha$ -elastin carries the antigenic determinant. The absorption of these antibodies from the circulating blood to the elastic fibres in the vessel wall or elsewhere has also to be considered. We believe that desmosine and isodesmosine, the particular amino-acids involved in the cross-linking of elastin<sup>1</sup>, may play an important part as antigenic determinants<sup>1,2</sup>. If this is so, wide cross-reaction between elastins of different species (carrying these same amino-acids) could be expected.

It seems probable that the anti-elastin antibodies occurring in human sera can be considered as auto-antibodies. It can be hypothesized that cathepsin-like enzymes slowly degrade elastin<sup>3</sup> and release soluble peptides into the circulation. These peptides would elicit the antibody response. As elastin is an insoluble structural protein, its soluble derivatives might not be recognized as 'self' by the competent cells. These antibodies would have a tendency to react with elastic fibres, mainly at places where the degradative process renders them more accessible. An antigen-antibody complex could be formed by such a mechanism in the vessel wall. Such complexes may very well play a part in the formation or spreading of a local tissue lesion. One example of such a reaction is, for example, the immune nephritis<sup>4,5</sup>.

According to our hypothesis, the degradation of elastic tissue followed by antibody formation against the degradation products may be important in the pathological alteration of the vessel wall and especially in atheromatous plaque formation. This would explain the presence of anti-elastin antibodies in most of the human sera that were examined, as well as the absence of higher titres in the sera of severely atheromatous patients. In the latter, according to the hypothesis, the elimination of antibodies by adsorption to the eroded elastic structures would be more intensive than in normal individuals.

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## Influencing the Survival of Skin Homografts by a Lymphatic Fistula

The lymphatic system plays an important part in a number of processes evoked by homografts. By an extirpation of the regional lymph nodes it is possible to prolong the survival of a skin graft<sup>1-3</sup>. A similar effect can be obtained by injuring the nodes by radioactive radiation or nitrogen mustard<sup>4,5</sup>.

The influence of the extirpation of nodes is of relatively short duration because further nodes are then linked with the lymphatics and take over the task of regional nodes<sup>6,7</sup>. We therefore tried to develop a lymphatic fistula in the regional lymphatic node and to evoke an interruption of the lymph system lasting for a longer period.

Table 1. INFLUENCE OF LYMPHATIC FISTULA ON SURVIVAL OF SKIN HOMO-GRAFTS

Skin graft only (days)	Drainage (days)	Air-pump drainage (days)	Air-pump drainage and irrigation (days)
14	13	17	27
16	16	52	19
12	23	23	34
16	13	23	51
10	12	20	35
12	51	70	18
	16	18	23
	23	23	19
	23	40	
		12	
Mean 13.1	21.0	30.7	28.2

Chinchilla rabbits weighing 2,500–3,000 g were used. Full-thickness skin grafts were transplanted from black donors to the ear of the Chinchilla recipients which were of different colour. Fistulae were developed in such a way that half the regional lymphatic node was extirpated after ligation of the blood supply and with a patent afferent lymphatic vessel; a drain of an inner diameter of 7 mm was sutured to the area which was thus created.

The first group consisted of control animals without drainage of the lymph system. In the second group we left only animals with drainage. In the third group we connected the drainage to an air-pump of a constant pressure of  $-0.2$  kp/cm<sup>2</sup>. In the fourth group we irrigated the injured surface of the node with a physiological solution containing heparin. After seven days the fistula was removed.

During the experiments all rabbits were kept in a special harness and injections of 'Heparin Retard Spofa' in doses of 4,000 units were administered subcutaneously twice daily.

Table 1 shows the results of the experiments. In the control group skin homografts on the ear of the rabbit survived an average of 13.1 days (10–16 days) until complete necrosis. In the second group the survival was 21.0 days on average (13–51 days). In the third and fourth groups the survival was practically the same, averaging 30.7 days (12–70 days) without irrigation and 28.2 days (18–51 days) with irrigation.

The results show that a long-term fistula with the possibility of lymph flow considerably prolongs the survival of grafts as compared with a control group as well as with a group without suction. We can therefore assume that in these two series the integrity of the lymph system is renewed much later as compared with experiments where only extirpation of the lymph nodes was performed.

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## Chromosome Marker Studies in the Graft-versus-Host Reaction in the Chick Embryo

The production of splenomegaly and other lesions by the injection of adult avian blood into the chick embryo is due to an immunological reaction between donor blood cells and host embryo tissues<sup>1,2</sup>. Biggs and Payne<sup>3</sup> have shown that the reaction is accompanied by proliferation of donor cells within the host spleen. They were able to distinguish donor (male) cells from host (female) cells by

identifying the respective sex chromosome complements. Cells of male sex have a ZZ chromosome constitution, whereas female cells have a single Z chromosome<sup>4,5</sup>. The Z chromosome is the only large mediocentric element in avian cells and is therefore readily identified in good chromosome preparations. Thus Biggs and Payne demonstrated proliferation of donor (ZZ) cells in the spleens of female embryos injected with cockerel blood. However, they found that the majority of dividing cells were of host type and they suggested that increased proliferation of host as well as donor elements must be responsible for splenic enlargement. Their investigation was limited to an analysis of 170 cells sampled from 9 embryo spleens removed 4 days after injection. Despite the obvious desirability of obtaining further information regarding the relative roles of donor and host proliferation in producing splenomegaly, no subsequent work has been reported. It would appear that technical difficulties involved in the preparation of clear metaphase figures from avian tissues have prevented further application of the method to the identification of cells.

Recently a technique modelled on those at present applied in mammalian cytogenetics<sup>6</sup> has been used in this laboratory for the routine preparation of excellent metaphase figures of various organs of the chick embryo<sup>7</sup>. It has therefore been possible to make a more comprehensive study of the relative dynamics of host and donor proliferation within the embryo following the injection of blood, using the sex chromosomes as cell markers.

In the initial experiment eggs of hybrid White Leghorn  $\times$  Light Sussex lines of 13 days' incubation were injected intravenously with 0.1 ml. whole blood taken from a 12-week-old White Leghorn cockerel. In a second experiment eggs of known B blood group type were injected at the same incubation time with 0.1 ml. blood taken from a 6-week-old cockerel also of known B blood group. At daily intervals between 16 and 19 days incubation, groups of embryos were injected via the allantois with 0.1 ml. demecolcine solution (containing from 0.05 to 0.1 mg demecolcine according to the stage of incubation). Three hours later, embryos and their spleens were weighed, and spleens of female embryos were prepared for chromosome analysis as follows.

A disaggregated suspension of cells was obtained by gently 'kneading' the spleen in hypotonic solution (0.95 per cent sodium citrate). After standing for 20 min the suspension was centrifuged and the supernatant was replaced by fixative (3 parts ethanol: 1 part glacial acetic acid). After a further 30 min the cells were resuspended in an aqueous solution of 45 per cent acetic acid and a drop of the suspension was placed on a clean slide and 'air-dried' by warming gently over a low flame. The final stage of resuspension in aqueous solution is particularly important for the effective spreading of avian chromosomes by the 'air-drying' technique. Staining was carried out in lactic-acetic-orcein<sup>8</sup> and the preparations were viewed by phase-contrast microscopy.

In Table 1 means of spleen and whole body-weight are presented for the period under study. The marked rise in spleen weight following the injection of whole blood has been noted by a number of workers<sup>4,10</sup> and strongly contrasts with the small increase found in control embryos and in embryos injected with blood of the same B locus type. Table 2 shows the proportions of dividing cells of donor and host origin respectively in a number of female embryo spleens sampled at successive incubation periods. It is apparent, first, that donor cells form a progressively greater proportion as incubation proceeds. For example, after 16 days incubation (3 days after injection) up to 8 per cent of the dividing cells in spleens of female embryos are of male sex, but by 19 days incubation donor cells form up to 93 per cent of dividing cells, that is, the relative proportion of donor to host has been almost reversed. Secondly, no proliferation of donor cells occurs in spleens of embryos injected with blood of similar B group type.

Table 1  
Sex No. Days of incubation Mean spleen-weight (mg) Mean body-weight (g)

(a) White Leghorn Donor and White Leghorn $\times$ Light Sussex Hosts					
$\delta$	6	16	19.7 $\pm$ 1.15	15.9 $\pm$ 0.61	
$\phi$	11	16	19.1 $\pm$ 2.07	16.4 $\pm$ 0.36	
$\delta$	12	17	54.6 $\pm$ 8.23	18.6 $\pm$ 0.61	
$\phi$	6	17	46.6 $\pm$ 6.47	19.1 $\pm$ 0.88	
$\delta$	11	18	94.0 $\pm$ 12.21	21.8 $\pm$ 0.54	
$\phi$	5	18	111.8 $\pm$ 5.91	23.7 $\pm$ 1.08	
$\delta$	16	19	88.28 $\pm$ 10.84	26.6 $\pm$ 0.79	
$\phi$	28	19	110.8 $\pm$ 10.46	25.8 $\pm$ 0.58	
(b) Controls (White Leghorn $\times$ Light Sussex)					
$\delta + \phi$	7	16	9.3 $\pm$ 1.22	14.6 $\pm$ 1.14	
$\delta + \phi + \phi$	9	17	13.9 $\pm$ 0.87	22.4 $\pm$ 0.87	
$\delta + \phi$	8	18	15.5 $\pm$ 1.46	25.3 $\pm$ 0.90	
$\delta + \phi$	8	19	15.3 $\pm$ 1.48	27.7 $\pm$ 0.61	
(c) 21/21 B Group Donor					
21/21 Hosts	$\phi$	6	18.8 $\pm$ 0.91	20.8 $\pm$ 1.12	
19/19 Hosts	$\delta + \phi$	3	60.1 $\pm$ 14.07	23.8 $\pm$ 1.71	

Table 2. PERCENTAGE OF DONOR CELLS IN SPLEENS OF FEMALE EMBRYOS INJECTED AFTER 13 DAYS INCUBATION WITH 0.1 ML. COCKEREL BLOOD

	Days of incubation	Spleen-weight (mg)	Body-weight (g)	No. cells scored	No. of $\delta$ cells	% Donor cells
White Leghorn Donor ( $\delta$ ) to White Leghorn $\times$ Light Sussex Hosts ( $\phi$ )						
	16	11.5	14.9	100	8	8
	16	14.9	16.0	100	6	6
	16	21.9	15.4	100	5	5
	17	29.0	15.7	100	27	27
	17	57.0	18.9	100	32	32
	17	37.7	18.9	100	26	26
	18	77.2	22.3	100	73	73
	18	233.5	24.3	100	68	68
	18	119.8	25.7	100	76	76
	19	128.3	26.1	100	82	82
	19	77.8	31.1	104	97	93.3
	19	155.8	26.7	119	101	84.9
	19	23.0	28.9	100	90	90
	19	101.5	28.0	100	80	80
	19	45.6	28.2	114	106	92.1
21/21 B Blood Group Donor ( $\delta$ ) to 21/21 B Blood Group Hosts ( $\phi$ )						
	18	13.8	22.9	100	0	0
	18	16.6	20.2	100	0	0
	18	84.7	21.7	100	69	69

Thus in a total count of 200 cells not a single donor cell was found 5 days after injection. On the other hand, when donor and host differed at the B locus 69 per cent of spleen cells were found to be donor in type. Thirdly, when a histocompatibility difference between donor and host exists, spleens of embryos of similar incubation contain similar proportions of donor cells, despite considerable variations in overall spleen weight. For example, after 19 days incubation, spleens of 23.0 mg, 45.6 mg and 155.8 mg contain 90, 92.1 and 84.9 per cent donor cells respectively. There was, in fact, a slight negative correlation between spleen size and percentage of donor cells at this period, but it did not reach a level of statistical significance.

It has previously been shown<sup>11</sup> that the amounts of RNA, DNA, and protein per g wet weight of spleen remain constant throughout the period of splenic enlargement, thereby suggesting that the increased spleen size is directly due to cellular proliferation. Although spleen size therefore gives some broad measure of total cell number, the proportions of donor and host cells blocked in mitosis by demecolcine treatment cannot provide an estimate of the relative numbers of cells of these types making up this total. Thus the proportion of cells of a given type arrested in metaphase during the three-hour period following demecolcine injection depends not only on the total number of such cells present, but also on the rapidity with which they are dividing. Obviously if donor cells are dividing more rapidly than host cells, then for a given number of both, donor metaphases will be found at a frequency exceeding that of the host by a factor dependent on the relative mitotic rates. Another consideration, albeit perhaps a minor one in view of the close correlation of DNA content to spleen size, is that selective destruction of host cells, which might occur in the later periods of

incubation and when the graft-versus-host reaction is intense, would accentuate the increase in relative proportion of donor cells.

Nevertheless, most of the increased spleen weight noted 8 days after the injection of blood must be attributed to host proliferation. At this stage the overwhelming proportion of dividing cells are of host type, although already, on average, spleen weight is increased by a factor of 2-3. The nature of the stimulus for host proliferation in the reaction is uncertain; that is, it may be secondary to the proliferative activity of donor cells or it may represent a concomitant host-versus-graft reaction, albeit occurring during embryogenesis when the host is generally regarded as having little or no immunological capacity.

It is equally clear that the rate of donor proliferation, between 3 and 6 days after injection, must considerably exceed that of the host. While donor cells form an increasingly greater proportion of dividing cells, mean spleen-weight continues to rise rapidly and it eventually exceeds that of the controls by a factor of 7. In these circumstances the possibility of host cell destruction merely underlines the intensity of donor cell proliferation.

It has been shown<sup>13</sup> that splenomegaly does not occur when donor and host are of similar B blood group genotype. The B blood group locus appears to be of major histocompatibility importance in the chick<sup>13</sup>, although other blood group loci may also be involved<sup>14</sup>. The results of this experiment show that when donor and host are of similar B blood group, no proliferation of donor cells occurs and in the absence of splenomegaly it must be presumed that no increased host proliferation occurs either.

The constancy in cell proportions found at any one incubation time, irrespective of spleen-weight, strongly suggests that both donor and host proliferation are interdependent. Thus although the overall rate of cell proliferation is related to the degree of histocompatibility difference between donor and host, the comparative proliferative rate of the two components is not related to histocompatibility but is the same for all embryos at any one time.

In summary it may be stated that, although it is clear that there are obvious difficulties in interpreting variations in the proportion of different cell types in terms of variations in actual number of cells, certain conclusions regarding the nature of the splenomegaly reaction in the avian embryo can be made. The initial increase in spleen weight is largely of host origin although when maximal weight increase is occurring, the rate of proliferation of donor cells exceeds that of host cells. The two rates bear a constant relationship to each other at all stages, host and donor cell proliferation being interdependent. Finally, no proliferation of donor cells occurs within the host spleen when donor and host are of the same B blood group type.

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## ANATOMY

### Isolated Rabbit Neurones: Electron Microscopical Observations

We have shown that neurones isolated in Ringer-Locke or in isotonic sucrose solutions lack the image of a surface membrane over the greater part of the soma and dendrites<sup>1</sup>. Since then, we have developed media in which an electron-dense surface image is maintained<sup>2</sup>. These investigations have been carried out on ox neurones, and while we have made efforts to isolate and fix the cells as soon as possible after the death of the animal, the time lapse involved is considerably longer than when smaller laboratory animals are used. Therefore we have made observations on rabbit neurones isolated by hand dissection and fixed at intervals between 12 and 60 min after the death of the animal. In addition, we have examined rabbit neurones which have been incubated for 2 h at 37° C in the succinoxidase assay mixture used in cytophysiological investigations of isolated nerve cells by Hyden *et al.*<sup>3,4</sup>.

Rabbits were given an air embolus and the carotid artery was severed as soon as they became unconscious. The brain was then rapidly removed, the lateral vestibular nuclei were excised and the neurones were dissected out free-hand in 0.25 M sucrose solution as described by Hyden<sup>5</sup>. Cells for immediate fixation were placed into 0.25 M sucrose solution in siliconized glass cavity slides, the sucrose solution was withdrawn and replaced by 1 per cent veronal buffered osmium tetroxide (pH 7.4) in which the cells were fixed for 30-60 min. The neurones were then prepared for electron microscopy as previously described<sup>1</sup>. Samples of neurones were also placed in siliconized glass cavity slides in which the sucrose solution was replaced by the succinoxidase assay mixture. These preparations were then enclosed in chambers by placing small 'Perspex' rings around the sample and sealing a piece of coverslip glass above. After incubating at 37° C for 2 h, the assay mixture was replaced by fixative solution and the cells were prepared for electron microscopy. Neurones were randomly selected for examination from a total of ten experiments.

As with ox neurones isolated in Ringer-Locke or 0.25 M sucrose solution, it was found that rabbit neurones fixed immediately on isolation lacked an electron micrographic image of a surface membrane over the greater part of the soma and dendrites. Fig. 1 shows part of a longitudinal section of a dendrite near its origin from the soma of a neurone hand-dissected from the lateral vestibular nucleus and fixed 12 min after the death of the rabbit. A surface membrane is lacking except for a small patch to be seen at the bottom left of the figure. Neurones incubated in the succinoxidase assay mixture also lacked a surface membrane over the major part of the soma and dendrites. Furthermore, these cells suffered greater disruption of their cytoplasmic structures than is usually observed in isolated neurones. In Fig. 2, which shows part of the soma of a cell incubated for 2 h in the assay mixture, the granular endoplasmic reticulum is very swollen.

It is clear that the rabbit neurones, when isolated from their supporting glia and stripped of many of their boutons, suffer a loss of the usual surface membrane image only minutes after removal from the brain and that this effect is strikingly similar to that seen in isolated ox neurones. It is clear from our investigations that the essential features of the physical boundary of isolated neurones is disrupted, thus the rates of transport of metabolites would be considerably altered from those occurring *in vivo*. Since some structural integrity would obviously be maintained, isolated neurones may reasonably be considered to be a 'partial homogenate'. It appears that metabolic examination of isolated neurones and glia should be monitored in order to establish the





Fig. 1. Longitudinal section of a dendrite of a neurone hand-dissected from the lateral vestibular nucleus of rabbit brain. Osmium tetroxide fixed 12 min after death of the animal

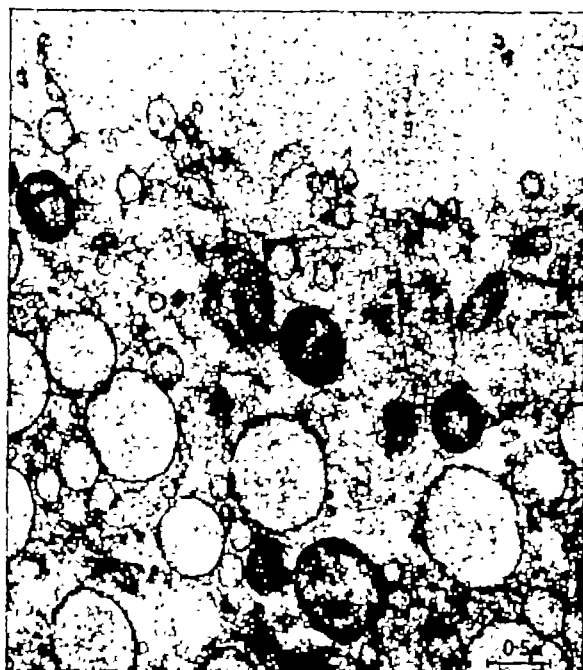


Fig. 2. Part of the soma of a cell similar to that shown in Fig. 1, but fixed after 2 h incubation in succinoxidase assay mixture

extent to which these systems reflect other essential features of the situation *in vivo*.

We thank Mr. David Gunn for assistance with photography.

**Note added in proof.** Since this communication was submitted for publication it has been reported that membrane potentials have been recorded from neurones hand-dissected from Deiter's nuclei of rabbits<sup>6</sup>. This observation is surprising in view of the findings described here. Further investigations are necessary both of the changes

in the surface following dissection and of the mechanism of the development of the recorded potentials.

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## BIOLOGY

### Oscillation in the Simple Logistic Growth Model

THE logistic curve is often used in teaching ecology as a first description of growth of an animal population. For many reasons, frequency related to age structure and time-lag effects, it does not usually fit in practice; and a population may undergo oscillations of one type or another. The causes of oscillations have been discussed in detail by numerous authors (see refs. 1-5), some of whom propose more or less complex conditions which would generate them. Nevertheless, the logistic growth curve is a useful starting point in the study of population ecology, so that the following simple relation between the rate of increase, generation time and the type of approach to the 'saturation' level may be of interest.

If we assume that adult numbers in any generation are determined by the number of adults in the previous generation the type of approach can be predicted immediately from the intrinsic rate of increase ( $r$ ). The logistic equation can then be re-written:

$$N_{s+1} = N_s \cdot e^{r(1-N_s/K)}$$

where  $r$  is expressed per generation,  $K$  is the saturation density and  $N_{s+1}$  is the population size after one generation. Then, if  $\Delta N = N_{s+1} - N_s$ , we find  $d\Delta N/dN = (1 - Nr/K) \cdot e^{r(1-N/K)} - 1$ . From this equation it is seen that when  $N$  is equal to  $K$ ,  $d\Delta N/dN = -r$ .

The relation between  $\Delta N$  and  $N$  allows us to predict the type of approach to equilibrium that will occur<sup>6</sup>. In the present instance the population will (a) approach  $K$  gradually, provided  $r$  lies between 0 and 1, (b) undergo damped oscillations when  $r$  has values between 1 and 2, and (c) show oscillations increasing to the point of extinction when  $r > 2$ .

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### Deposition of Human Polymorphonuclear Leucocytes on Glass

THE phagocytic activity of human polymorphs has been examined by depositing the cells on glass coverslips and observing their behaviour under phase contrast<sup>1</sup>. The coverslips were first prepared for use by boiling in distilled water followed by soaking in glass-distilled water for 3 h and then drying after washing in two changes of absolute alcohol, but it was found that cells collected from the buffy layer of human blood made up within 1 h of venepuncture adhered poorly to the surface of such cover-

slips. In contrast, the cells adhered well to the surface of glass coverslips which were used without any treatment other than rubbing with a paper tissue, though the numbers adhering were somewhat variable. As the principal difference between untreated coverslips and those which had been boiled in water and washed with alcohol was likely to be the existence of a layer of an oily substance at the glass surface, a defined layer of a fatty acid was deposited on the coverslips by a modification of the method of Blodgett<sup>1</sup>.

A glass dish, 20 cm in diameter and 5 cm deep, was filled with distilled water and 0.02 ml. of B.D.H. redistilled oleic acid was dropped on to the surface of the water. The bulk of the oil remained as discrete lenses and the rest spread as a monolayer due to the dipolar nature of the acid<sup>2</sup>. A coverslip, previously cleaned by boiling in water and drying in absolute alcohol, was held in a vertical plane and dipped in the water at a part remote from the oil lenses and the dipping was repeated five times so that a multi-molecular layer of oleic acid was deposited on the glass surface. The excess of water was allowed to drain and the coverslip was dried by standing vertically at room temperature.

A suspension of white blood cells was prepared by dispersing the buffy layer of blood in Medium 199<sup>4</sup>, and the polymorphs were deposited on coverslips within 1 cm × 1 cm areas, marked out by 'Speedry' marking ink (Speedry Products Ltd., Beckenham, Kent) by allowing 0.05 ml. of the suspension to sediment for 5 min in a moist chamber containing an atmosphere of 5 per cent carbon dioxide<sup>1</sup>. Microscopic counts with a calibrated eyepiece graticule showed that the number of polymorphs adhering to a series of multilayered coverslips was more than twice that adhering to a series of alcohol-treated coverslips, when the same cell suspension was deposited.

The oleic acid layer did not appear to affect the behaviour of the polymorphs. Using rice starch as a test particle, the average percentage phagocytosis (87) observed in a series of polymorph populations deposited on multilayered coverslips was no different from the average percentage phagocytosis (89) in a similar series of cells on alcohol-treated coverslips. Similarly, no significant difference was found between serum concentrations required to promote phagocytosis of hydrocarbon particles by 50 per cent of populations of polymorphs<sup>1</sup> deposited on either multilayered or alcohol-treated coverslips. The major difference between preparations deposited on coverslips multilayered with oleic acid and those deposited on alcohol-treated glass thus appeared to be the increased ability of the polymorphs to adhere to glass surfaces on which there had been deposited a layer of the fatty acid. This greater ability to stick made the scoring of quantal phagocytic responses much easier.

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### Observation of Root Feeding by the Nematode *Trichodorus viruliferus* Hooper

OBSERVATION through the glass panels of an underground root observation laboratory<sup>1</sup> and subsequent sampling for detailed study have shown a high proportion of the white growing tips of extending apple tree roots to be attacked by the ectoparasitic nematode *Trichodorus viruliferus* Hooper. Colonies of 10 to more than 100 individuals congregated 1–3 mm behind the apical meristem of suitable roots (about 1 mm in diameter), rasping the epidermis and

hypodermis with their stylets and causing a superficial but characteristic browning. Severely damaged portions of the root often swelled and occasionally their surface split. Root extension usually ceased after 5–15 days of feeding, although the meristem itself was not the focus of attack. Once growth had ceased most of the nematodes dispersed within 1 or 2 days. The damage to the root tip was essentially the same as that associated with the 'stubby root' symptom in many herbaceous hosts, which has been ascribed to other species of this genus in the United States<sup>2</sup>. In extending apple roots, however, it was not accompanied by the characteristic branching and stopping of secondary and tertiary roots.

*T. viruliferus* comprised 95 per cent of the plant-parasitic nematodes recovered from the surface of roots withdrawn through removable panels, and rhizosphere soil from the same roots yielded 93 per cent, the remainder in each case consisting of *Paratylenchus* spp. Extending roots of the same trees, recovered by digging 2–4 ft. away from the trunk, yielded 85 per cent *T. viruliferus*, the remainder being *Paratylenchus* and *Tylenchorhynchus* spp. Soil sampled by auger from the same root zone and elutriated by conventional techniques yielded 7 per cent *Trichodorus* spp. and 81 per cent tylenchid genera (*Paratylenchus*, *Tylenchorhynchus* and *Pratylenchus* spp.). It appears, therefore, that *T. viruliferus* is concentrated on the surface (rhizoplane) and in the rhizosphere of roots to a far greater extent than the tylenchids, which form the majority of the soil population of root parasites. The detection of associations such as this would be difficult by the commonly used soil sampling and processing techniques, illustrating the potential of a root observation laboratory, with its facilities for direct observation and sampling and indirect study by means of time-lapse photography.

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### Parasitism by Larvae of *Unionicola Intermedia* Koenike, and another *Unionicola* sp. (Acarina, Pionae), on Chironomids

THE majority of water-mites for which the life-histories are known have a parasitic larva and free-living nymphs and adults. The family Unionicolidae, however, has always been separated from the remaining families due to the fact that most species have adults and nymphs which are parasitic in the mantle cavities of fresh-water mussels. It has generally been assumed that these mussel parasites have been free-living as larvae and that the larval stage has been of brief duration and has served merely as a means of dispersal. There have, moreover, apparently been no previous records of these larvae being found outside their hosts.

During August and September of the past two years I have collected considerable numbers of the midge *Chironomus plumosus*, L., and on 64 out of 82 examined from three such collections I found 510 mite larvae referable to the genus *Unionicola*. These larvae have been identified as *U. intermedia*, Koenike, and probably *U. aculeata*, Koenike.

The former has been known since the time of Bonz (1783) as a parasite in its nymphal and adult stages of the mussel *Anodonta anatina*, L., while the latter is stated by Mitchell<sup>1</sup> to use mussels as sheltering places for its pupal stages but otherwise to be free-living.

It seems, therefore, that the larvae of these two members of the genus behave in a manner precisely similar to the

remainder of the super-family Pionae and that the parasitism of the adults can be looked on as merely an extension of the relationship mentioned by Mitchell for *U. aculeata*, Koen. This discovery, in fact, makes the whole position of the Unionicolidae much more readily acceptable, since it is no longer necessary to assume that they evolved differently from the remainder of the Pionae.

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### Survival of *Fomes annosus* in Infected Roots in Soil

VARIOUS species of *Fomes* are known to cause considerable root damage to a variety of perennial crops including rubber and several conifer species. In most cases it has been shown that infected root tissue forms a significant source of inoculum for the infection of newly planted crops. Rishbeth<sup>1</sup> has given detailed descriptions of the field symptoms caused by *Fomes annosus* in the Breckland conifer plantations of East Anglia, pointing out that the disease usually appears in patches. By exposing root systems at different stages in the development of the disease, Rishbeth found that lateral spread within such a patch is primarily due to root contact, and that *Fomes annosus* has only a limited competitive saprophytic ability. The disease does, however, sometimes appear in areas not previously planted with conifers, and Rishbeth<sup>2</sup> later established that stump infections are of the greatest significance to the initiation of such new centres of infection.

From these observations it seems obviously desirable to have some measure of the survival capacity of *Fomes annosus* in soil, and to have some knowledge of the factors which might encourage a rapid disappearance of the fungus.

Initial experiments along these lines were based on artificially infected wood pieces, produced by culturing together the fungus and 1-in. lengths of pine stem of approximately  $\frac{1}{2}$  in. in diameter in a cornmeal sand mixture. After having been grown at laboratory room temperature for a period of six weeks, the wood pieces were removed from culture, washed briefly in tap water and buried in containers of Kettering loam soil<sup>3</sup>. Samples were removed from the soil at various intervals and planted on moist sterile sand in Petri dishes. After five days, observations under a binocular microscope showed the presence of the characteristic conidia of *Fomes annosus*. By assessing the degree of sporulation on an arbitrary scale of 0-4, an attempt was made to measure the period of survival of *Fomes* under these specific conditions.

The first experiment along these lines suggested that *Fomes annosus* disappeared from the buried wood pieces within four weeks of burial. However, the experiment was repeated with a three-months-old culture of inoculated wood pieces and, surprisingly, the fungus was replaced by a variety of soil saprophytes within one week of burial. This apparent contradiction did appear to be related to one obvious difference between the first and second experiments, namely, the appearance of the wood pieces before they were buried in soil.

The six-weeks-old inoculated wood pieces remained reasonably firm, with the bark securely in position, whereas after three months in laboratory culture conditions the bark had completely disintegrated on the inoculum used in the repeat experiment. By sectioning some of the original inoculum pieces it was later observed that the fungal invasion was limited to the outer layer of wood, and only penetrated more deeply when such wood pieces showed obvious fracture incurred during the cutting of the wood pieces.

With the knowledge that *Fomes* is known to survive for many years under field conditions, and the possibility that the presence of bark modified the results of the two previous experiments, the following experiment was set up with naturally infected wood pieces collected from newly killed specimens of *Pinus sylvestris* in the Breckland.

A bulk supply of naturally infected root tissue was collected from the Breckland. The trees selected for sampling were those in which the leaves were brown in colour but had remained attached, care being taken not to select those which *Polystichus abietinus* had invaded or those with very resinous roots. The roots were then sawn into 2-in. lengths (approx.) and classified as large, medium or small according to their diameter. Large ones were approximately  $1\frac{1}{2}$  in. diameter, medium ones 1-in. diameter and small ones  $\frac{1}{2}$ - $\frac{3}{4}$  in. diameter.

Twelve samples of wood from each category were then treated in each of the following ways: (a) the bark was removed and the cut end left uncovered; (b) the bark was maintained intact and the cut ends not covered; (c) the bark was left intact and the cut ends sealed with molten sealing-wax. Large boxes of Kettering loam soil were then prepared and all the wood pieces were buried to a depth of 4 in. These boxes were maintained in a heated greenhouse for a period of 18 months. During this time they were watered regularly as a routine operation. At the end of this period the soil surface was well colonized by a species of *Juncus* together with a series of annual weeds. The dominance of rushes was probably a reflection of the mildly acid soil conditions prevailing under these conditions<sup>4</sup>.

At the end of this burial period all wood samples were removed and thoroughly washed, and the degree of survival of *Fomes* was assessed by three different methods. The first consisted simply of splitting each root sample with a sterile scalpel and sub-culturing four wood chips from each sample in an acid 'Dox' medium. Within four days the presence or absence of *Fomes* was recorded for each isolation and the total number of positive records was calculated as an index of percentage survival. The second method consisted of wrapping the two wood segments, previously sub-cultured, in moist newspaper and incubating in a moist chamber for a period of seven days. At the end of this period examination of the wood pieces revealed the characteristic conidia of *Fomes* if the fungus was still viable. By recording the presence or absence of the fungus it was again possible to calculate percentage survival. The third method was simply to count the number of miniature brackets which were present on all wood pieces giving a positive record in either of the previous methods of assessment.

All the data collected in this manner are summarized in Table 1.

A simple examination of these data shows quite clearly that the presence of bark is a marked deterrent to the saprophytic colonization of the infected wood tissue and that the three methods of assessment give an extremely consistent pattern. It should be further noted that, whereas the plating index and the wrapping index give actual percentage survival in terms of the number of wood pieces showing the presence of the fungus, the fruiting index is probably a better index of the surviving inoculum potential.

Table 2 shows the effect of root diameter on the survival of *Fomes*, from which it is obvious that the larger samples survive more effectively than smaller samples, irrespective of the method of treatment. Garrett<sup>4</sup> has shown that the size of the nutritional substrate determines the inoculum

Table 1. SURVIVAL OF *Fomes annosus* IN SOIL AFTER BURIAL FOR 18 MONTHS

Treatment	Survival recorded by various assessment techniques		
	Plating index	Wrapping index	Fruiting index
Without bark	0	0	0
With bark	30	25	38
With bark and seal	85	58	91

Table 2. EFFECT OF SAMPLE SIZE ON SURVIVAL OF *Fomes annosus* AS RECORDED BY THE FRUITING INDEX

Sample size	Without bark	With bark	With bark and seal
Large 1½-in. diam.	0	31	70
Medium 1-in. diam.	0	6	21
Small ¾-in.-1-in. diam.	0	1	0

potential of *Armillaria mellea*. This concept was partially confirmed for *Fomes annosus* in the present series of experiments where the fruiting index for the larger samples far exceeds that for the smaller units.

The experiments reported here have shown quite clearly that the size of root sample, as well as the presence or absence of bark, plays a significant part in the survival of *Fomes annosus* in soil. Since the removal of infected root tissue is one of the methods used to control the spread of this parasite, this information is of considerable practical significance in that any method used which could lead to excessive damage to bark would be more conducive to the rapid disappearance of the parasite.

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## Vitamin B<sub>12</sub> Analogues In Non-legume Root Nodules

It has been shown<sup>1-4</sup> that cobalt must be available for legume nodules to supply fixed nitrogen efficiently to the plant bearing them. It has also been shown that in such nodules cobalt is elaborated into the form of vitamin B<sub>12</sub> analogues. Thus Levin *et al.*<sup>5</sup>, using *Ochromonas* and *Eruglena* assays, reported that appreciable (though not actually stated) amounts of the vitamin were present in nodules of lucerne, clover and pea, very much smaller quantities being detected in the roots of the same plants. Shaikat-Ahmed and Evans<sup>6</sup> reported that *Ochromonas* assay of nodules from soya bean plants supplied intermittently with 0.05 p.p.m. of cobalt indicated the presence of 619 mg vitamin B<sub>12</sub> per g of fresh nodules. By enzymatic assay, Klierer and Evans<sup>7</sup> found appreciable amounts of vitamin B<sub>12</sub> co-enzyme(s) in the nodules of various legumes grown in the presence of the above level of cobalt.

Klierer and Evans also reported the presence of vitamin B<sub>12</sub> co-enzyme(s) in the root nodules of the non-legume *Alnus oregona*, while Bond and Hewitt<sup>8</sup> provided evidence of the necessity of cobalt for the proper functioning of the nodules of *Alnus glutinosa*, and in unpublished work have extended this finding to species of other non-legume genera with nitrogen-fixing root nodules. The question thus arose whether in these further species cobalt is elaborated in the nodules into the form of vitamin B<sub>12</sub> analogues. Data on this aspect are now presented.

Samples of nodules and also of roots of five non-legume species were taken, during the period July to September, 1963, from plants growing in the greenhouse in non-aseptic culture. The plants of *Coriaria* were grown in vermiculite, those of the other species in water culture, the culture solution employed being that of Crone (nitrogen-free formula). Cobalt was supplied intermittently at 0.015 p.p.m., while in addition there may have been a further quantity present as impurity in the salts used in preparing the culture solution since these were not specially purified. Root samples were included in order to permit comparison between the nodules and a normal part of the plant. The roots selected were from the same plants

as the nodules, though roots which had actually borne nodules were avoided. Nodule samples from two legumes were also included for comparative purposes, and were taken from garden plants. After being washed in water, the nodule samples (usually 1 g) were homogenized as completely as possible with 100 ml. distilled water in a Waring blender. With roots a larger sample (usually 10 g) was similarly treated. The homogenates were placed in a deep freeze until required for assay.

The microbiological assay procedure was that described by Hutzner, Bach, and Ross<sup>9</sup> using commercially available medium (Difco) and *Eruglena gracilis* 'Z' strain as the test organism. The samples were assayed against aqueous standards of pure cyanocobalamin. Various dilutions were assayed until a suitable dilution was found, and the results given below are the mean values of two or more assays of such a dilution. Different extraction procedures were tried: (1) the homogenate from the Waring blender was used direct, (2) it was subject to further ultrasonic homogenization, (3) its pH was adjusted to 4.0 and potassium cyanide was added to give a concentration of M/100, after which the mixture was boiled for 15 min, (4) the original homogenate was digested with pancreatin, papain, or ficin. The results obtained by these various procedures with a given sample varied considerably, but the only consistent difference found was that the yield of active substances from root samples was much increased when cyanide was used.

The results obtained by two of the extraction procedures are presented in Table 1. Although the data are shown in terms of vitamin B<sub>12</sub>, it is appreciated that *Eruglena* also responds to pseudo-vitamin B<sub>12</sub> (ref. 10) and (in our experience) to hydroxocobalamin and to at least one of the cobalamin coenzymes in addition, so that it is uncertain to which of these substances any activity shown by the extracts was due.

Table 1. RESULTS OF *Eruglena* ASSAYS  
mg vitamin B<sub>12</sub>/g fresh tissue

Species	Stage of plant development	Nodules		Roots	
		(1)*	(2)*	(1)*	(2)*
<i>Casuarina cunninghamiana</i>	2nd yr.	211	367	0.36	5.5
	1st yr.	307	219	<0.25	<2.5
<i>Alnus glutinosa</i>	2nd yr.	63	185	<0.25	3.4
	1st yr.	181	236	<1.0	<10.0
<i>Myrica gale</i>	2nd yr.	55	85	<0.25	<2.5
	1st yr.	207	185	11.6	23.8
<i>Hippophae rhamnoides</i>	2nd yr.	28	53	4.5	23.4
<i>Coriaria myrsinites</i>	Flowering	238	134	—	—
<i>Pursh sativum</i>	Young pods present	233	254	—	—

\* Numbers refer to extraction procedures (see text).

The data indicate that considerable amounts of one or more of these vitamin B<sub>12</sub> analogues are present in the non-legume nodules, the levels in *Casuarina* and *Hippophae* nodules and in those from first-year plants of *Alnus* and *Myrica* being of the same order as in the legume nodules. The occurrence of active substance is not entirely restricted to the nodules, since some was detected in root samples, especially those of *Hippophae* and *Coriaria*. In a preliminary survey in summer, 1962, a number of samples were assayed using extraction method (1) only. The results were in general similar to those reported here.

The much greater abundance of active substance in the nodules as compared with the roots (except in *Coriaria*) can undoubtedly be attributed to the extensive development within the nodules of the endophyte, which is almost certainly of actinomycoete affinities. It is well known that organisms of this group are active producers of vitamin B<sub>12</sub> analogues. It remains to be seen whether the necessity of cobalt for the proper functioning of these non-legume nodules is due to some essential part played by vitamin B<sub>12</sub> substances.

The activity found in the roots has three possible origins: (a) by leakage from the nodules into the roots; (b) production by contaminant micro-organisms growing on the surface of the roots; (c) synthesis by the root cells

themselves. Whether the cells of higher plants have such a capacity is a moot point<sup>11-13</sup>.

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### Heritability of Heartwood Formation in *Pinus radiata* D. Don

AN important change which occurs in the wood of most trees as they grow older is the transformation of sapwood into heartwood. This change is associated with the death of the ray and vertical parenchyma cells, and definite modifications to the anatomy of both softwoods and hardwoods<sup>1</sup>. As a result, heartwood is more difficult to season, is less readily penetrated by preservatives and pulping liquors, and resinous extractives are troublesome.

Schreiner<sup>2</sup> could not find conclusive evidence of the inheritance of heartwood formation, while Harris<sup>3</sup> thought that the genetical constitution, age and ecology of a tree together contribute to the formation of heartwood in the stem. The latter suggested that a 'heartwood forming' tendency is genetically controlled, but that environment also plays an important part.

The most useful investigation of the inheritance of a characteristic should yield a quantitative measure of the intensity of genetic control. Such a measure is provided by a ratio called 'heritability'<sup>4</sup> which considers genetic variability in relation to phenotypic variability. This term is used in either a broad or narrow sense depending on whether the total or only the additive portion of the genetic variability is taken into account.

In an investigation of this kind it is not practical to investigate the variation in the age at which heartwood begins to form in the tree. Therefore the amount of heartwood was used as the basis for calculating estimates of gross heritability from results derived from trees of a clonal plantation.

Thirteen clones from a plantation of *P. radiata*, established in 1930, in the Australian Capital Territory were sampled by selecting the first, middle and last trees of the row comprising the clone. Only vertical trees were chosen. Increment cores were taken from each tree in the north and the south directions at heights of 1.2 m and 4.6 m.

In a mature cross-sectional disk of *P. radiata*, the coloured heartwood adjacent to the pith and the narrow transition zone, which also contains heartwood substances<sup>5</sup>, stain a deep orange-brown on the application of a diazotized benzidine stain<sup>6</sup>, in contrast to a yellowish stain for the sapwood. This staining technique was used to differentiate the heartwood and transition zone from the sapwood, because the natural colour changes could not be seen clearly in the small samples used.

Because of differences in the pattern of early height growth, sampling at fixed heights in trees produces speci-

mens with differing ring counts. Consequently the growth ring showing the furthest extent of heartwood from the pith could not be used as a comparative measure of heartwood content, and it was decided instead to use the area proportion of heartwood. This was possible because of the poor correlation between specimen ring count and proportion of heartwood in these results. Therefore, the radial extent of the heartwood and the tree radius were recorded for each specimen, so that values for the opposite radii at each height could be averaged and used to express the heartwood area as a proportion of the total cross-sectional area for each sampling height in each tree.

The variation in heartwood content between trees was separated by analysis of variance into within-clones and between-clones components and gross heritability estimated as :

$$\frac{\text{between-clone variance}}{\text{between-clone variance} + \text{within-clone variance}}$$

Estimates of gross heritability for the proportion of heartwood were as follows :

Height (m)	Gross heritability estimate significant at 5% level	S.E.
1.2	0.37	0.18
4.6	0.36	0.18

The value of 0.37 is not very large compared with the estimate of 0.73 obtained for average fibre length on clonal material from the same locality by Dadswell *et al.*<sup>7</sup>. Little gain could therefore be expected to result from the use of seed from parents selected for low heartwood content, and even if vegetative propagation methods were used, the gains achieved would be worthwhile only if the absence of heartwood was very critical to the final product.

It should be stressed that the work recorded here is based on results from 13 clones from a single area. However, the lack of heritability estimates for many wood features is keenly felt and there is some justification for publishing preliminary values in the hope that they may be augmented by others to provide better estimates based on pooled results.

I thank the Director-General, Forestry and Timber Bureau, Canberra, for making available the wood specimens, and particularly Messrs. A. G. Brown and J. M. Fielding for arranging for their collection. I also thank Misses J. Down and J. Sibthorpe for carrying out the detailed measurements on which the results of this investigation have been based, and Miss N. Ditchburne, C.S.I.R.O. Division of Mathematical Statistics, for help in the statistical analyses.

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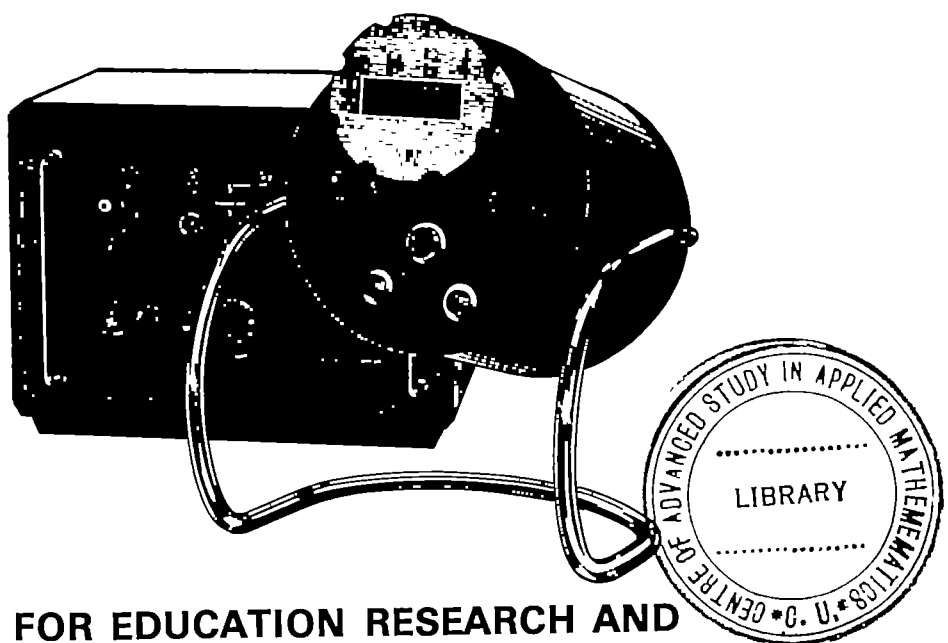
## ENTOMOLOGY

### Purification of the Fire Ant Trail Substance

THE fire ant, *Solenopsis saevissima* (Fr. Smith), utilizes a trail substance as an aid in effective foraging<sup>1</sup>. Workers secrete a substance from the Dufour's gland which is deposited from the sting in the form of minute streaks. These mark a trail from a suitable food source to the nest. Workers are strongly attracted to this substance and follow the trail outward to the food source. The newly recruited workers in turn lay trails to the nest, reinforcing the original trail. The appropriate properties for effective trail substances have been discussed by Bossert and Wilson<sup>2</sup>.

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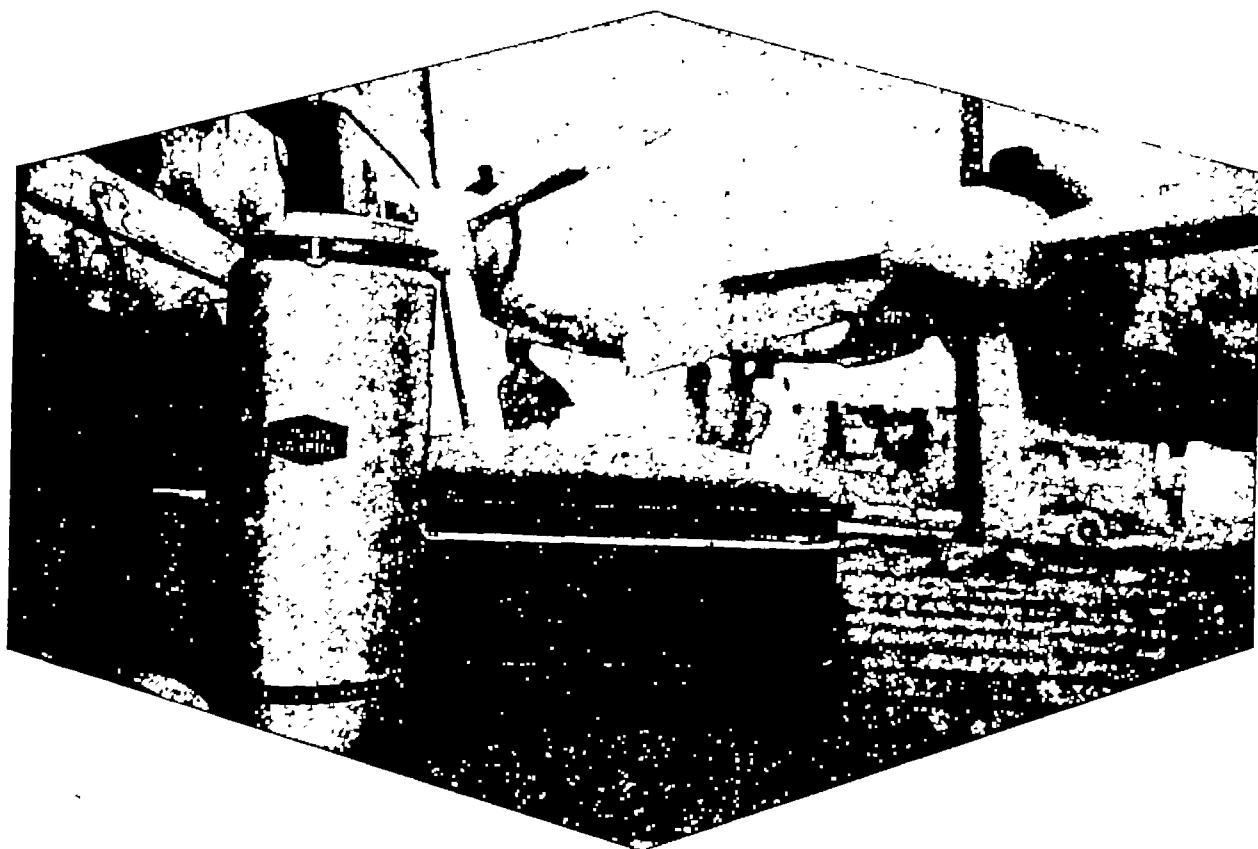
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The trail substance of *S. saevissima* has been purified in the hope of learning something of its chemical properties. For a qualitative assay during purification, wooden splints were dipped into solutions of the fractions to be tested and the splints drawn across a smooth surface near a laboratory colony of *S. saevissima*. Activity was estimated by rough counts of the number of workers which were attracted to and followed the artificial trail.

Workers of *S. saevissima* were collected near Jacksonville, Florida, in the following manner. The nests were quickly spaded into buckets and the sandy contents were poured into still water. Most of the sand and debris sank, while a considerable number of ants rose to the surface, where they aggregated and floated in clumps. The ants were gathered with a tea strainer and transferred to bottles which contained dichloromethane.

Ants (approximately 200,000 workers), along with water, dichloromethane and remaining debris, were poured into a large flask and the whole mass was steam distilled into an ice-cooled receiver until attractive material ceased to distil. For a volume of 1,500 ml. in the distillation flask, about 3 l. of steam distillate (water and dichloromethane) was obtained. This was repeated six times to process all the collected material. The active material could be extracted from the aqueous phase of the steam distillate with dichloromethane. Three extractions of 3-l. portions of the distillate with 200-ml. portions of dichloromethane removed all the attractive substance. The dichloromethane phases were dried over anhydrous sodium sulphate. After filtration the dichloromethane solution could be concentrated cautiously in a distillation flask at atmospheric pressure without extensive loss of active material. In this way 4 l. of solution were reduced to a volume of 2 ml.

Attempts to purify the trail substance by adsorption chromatography on alumina or silicic acid were ineffective, and either resulted in complete loss of the active material or in no adsorption of the active material on the column. Exploratory experiments with gas-liquid chromatography indicated that the active compound could be recovered from columns when polydiethyleneglycol succinate was used as a liquid phase. When the temperature of the column was increased from 50° to 200° at the rate of 5°/min, the active material was collected over the range of 125–140°.

For purification of the trail substance, a column 8 ft. by 0.25 in. packed with 5 per cent polydiethyleneglycol succinate on 'Chromosorb W' was operated at 135° in a Research Specialties model '600' apparatus, equipped with an argon detector. Aliquots (50 µl.) were injected and the effluent compounds were collected in cooled U tubes. The process was repeated until the whole 2 ml. sample had been chromatographed. Fig. 1A shows the chromatogram obtained in this fashion. The collected material was chromatographed a second time (Fig. 1B) to yield homogeneous material. In Fig. 1C, the pure sample is compared with geraniol, which was used as a standard for the calculation of a relative retention time and for estimation of mass. The apparently homogeneous trail substance has a retention time relative to geraniol of 0.8, and the total sample contained about 250 µg of material.

While it is clear that the active material is contained within the peak observed by gas chromatography, the sample may still be contaminated with a large amount of an inactive compound with similar chromatographic properties. Williams and Law<sup>3</sup> have recently shown that a highly purified insect hormone, which gives essentially one peak on gas chromatography, still represents a small amount of active material in the presence of a large amount of an inactive compound.

Considerable losses of active material were encountered in the purification procedure. Furthermore, it was found that either crude or purified material rapidly lost activity, even when stored at –20° in dichloromethane. A sample of the apparently homogeneous material lost about 80 per

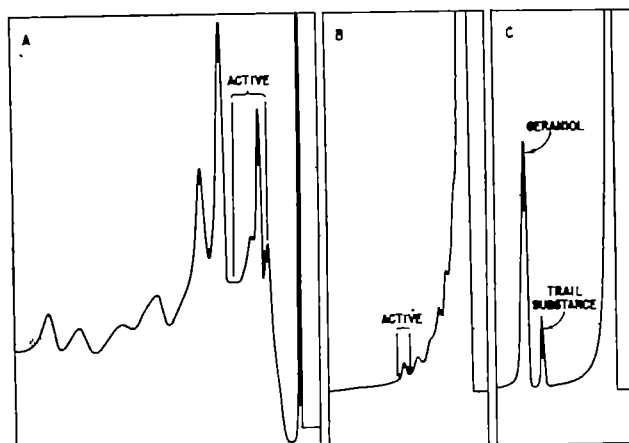


Fig. 1. Chromatographic purification of the fire ant trail substance. The chromatographic column was operated at approximately 135°. Slight variations in temperature and pressure caused the differences in retention time shown in the three curves. Curve A, crude steam distillate. Curve B, active fraction collected from the first chromatographic step. Curve C, pure trail substance collected from two passes on the chromatographic column, compared with geraniol. 10 µg of geraniol was mixed with 1/100 of the total trail substance sample. Comparison of the peak areas indicates that about 8–5 µg of material is present in the trail substance peak.

cent of its activity when stored for three months at –20° C in dichloromethane. The peak observed by gas-liquid chromatography also decreased in area by a similar proportion. A second large batch of crude material representing about 300,000 ants lost almost all activity when stored for two months in dichloromethane at –20°.

While this undesirable behaviour causes some frustration in attempts to identify the active trail substance, it also indicates the possibility that chemical instability may play a part in obliterating the trail substance after it has served its function. Wilson<sup>4</sup> has previously suggested that this is accomplished by using a very volatile material for a trail substance. However, it cannot be ruled out that the substance may also be inactivated by chemical reaction with some substance in the environment, such as oxygen or water.

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## Gland-like Abdominal Structures of Possible Epigamic Function in the Diptera

RECENTLY one of us<sup>1</sup> erected a new genus, *Austrodrapetis*, to receive an undescribed empidid fly from the Cook Islands. Striking features of this species were the presence of abdominal structures of unknown function in the male coupled with certain secondary sexual characters in the coxae of both sexes and the tibiae of the male.

The abdominal organs (Figs. 1a and b) are in two pairs beneath the third and fourth terga. There is no apparent opening to the surface, but a small one may be present in view of the duct-like anterior region of each organ. The organs seem too strongly sclerotized to be eversible especially as there is no obvious opening to the surface. There is no apparent connexion between the organs and any of the few large tracheal trunks in their vicinity. The

## CYTOLOGY

## Ribosome Numbers in a Fission Yeast

THERE is a need for a more confined and precise use of the word ribosome. It would clarify molecular biology if the word were reserved for particles in the cell which measure 100 Å–300 Å in diameter, are composed of RNA and protein, and have been shown to be active as sites for the synthesis of proteins. However, such criteria are not easily available to, say, an electron microscopist. This communication describes an attempt to compare the number of ribosomes in a cell, as calculated from biochemical data, with the number of particles in the same cell type, visible in the electron microscope after osmium tetroxide fixation, and, in terms of size and distribution, categorized as ribosomes in contemporary electron microscopy.

The cell used in this work was a fission yeast, *Schizosaccharomyces pombe*. An electron microscope investigation of this cell has already been published<sup>1</sup>, and the RNA content in a variety of growth conditions is known<sup>2</sup>. *S. pombe* (National Collection of Yeast Cultures, 132) is a small yeast, cylindrical in shape, with the ends of the cell hemispherical, varying between 5 and 15 μ in length, and maintaining a fairly constant diameter of 3.5 μ. All cells used were growing vegetatively, cultured in 2 per cent w/v Oxoid 'Wort Broth' (malt extract broth) in distilled water, and in the exponential phase of growth from cultures containing between 3 and 6 × 10<sup>6</sup> cells per ml. Cultures were grown at 33° C (the generation time at this temperature is slightly more than 2 h) in 400 ml. of medium in 1-l. conical flasks, with no agitation or aeration.

Since *S. pombe* grows, in general, only in length, an estimate of the stage of a single cell in the cell cycle can be assessed from its length. But if lengthwise measurements are to be made on sections viewed in the electron microscope, it is essential that the sections be truly longitudinal. This implies that the cells must be aligned in the embedding procedure so that they are flattened in a monolayer and present their total length to the microtome knife. The following method of preparation was used.

Cells were gathered by centrifugation, washed twice in 0.25 M sucrose buffered with veronal acetate at pH 7.2, and spun down in the sucrose to a concentration of 2 × 10<sup>6</sup> cells per ml. Drops of this suspension were now placed on square cover slips, size 1, and the solution evaporated almost, but not quite, to dryness. With the cover slips in a glass Petri dish, cells uppermost, and some 8 cover slips per dish, drops of 2 per cent osmium tetroxide in the buffered sucrose were applied in sufficient quantity to flood each cover slip, and the Petri dish cover put in place. After 30 min at room temperature, the Petri dish cover was removed and the dish flooded with distilled water. Numerous washings with water were carried out, followed by washings in increasing concentrations of alcohol, and ending with three washings in absolute alcohol. The cover slips were not moved during this procedure. Following this dehydration, the Petri dish was flooded once with liquid methacrylate, which, on removal, was replaced by a final pool of liquid methacrylate which consisted of 91 per cent butyl methacrylate, 8 per cent methyl methacrylate and 1 per cent benzoyl peroxide. Polymerization was carried out at 58° C with the Petri dish cover in place, and was normally complete in 24 h.

After polymerization, the bottom section of the Petri dish was splintered with a hammer and removed, leaving a flat block of plastic with the cover slips applied to the flattened surface. The cover slips were removed by contact with solid carbon dioxide, causing them to break away from the plastic. With the cover slips removed, the monolayer of cells which had been applied to them is found embedded in the plastic surface. Groups of cells are located on the surface by a low-power microscope and

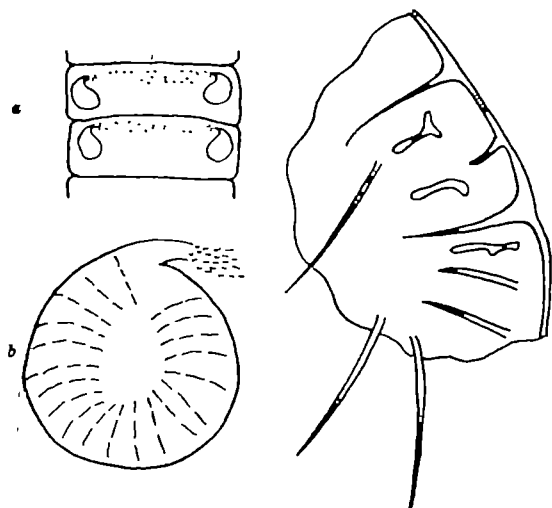


Fig. 1

organs are hollow and the inner surface of the outsole is produced into hair-like structures interspersed with some shorter blunter processes (Fig. 1c).

It is possible that these are scent-producing glands, perhaps defensive, but more probably epigamic. The presence of numerous hairs within the cavity and the setose area on each segment between the two 'openings' would be an efficient method of conducting any liquid secretion to the exterior and providing it with a large surface area for evaporation. The position of the organs, fairly close behind the wings, would also ensure an efficient dispersal of the scent in the alipstream.

Feuerborn<sup>3</sup> has described glandular organs of probable epigamic function on the thorax of some Psychodidae (again behind the wings). Lamb<sup>4</sup> has described some curious structures in the basal abdominal segment of males of the dolichopodid fly *Oraterophorus mirus* Lamb and it may be significant that the structure of the squamae and the shape of the hind-margin of the wing are modified, perhaps affecting the alipstream. Parent<sup>5</sup> has also described abdominal organs of unknown function in *Syntormon* (Dolichopodidae) from New Zealand. Edwards<sup>6</sup> has described eversible glandular structures in the abdomen of females of the midge *Palpomyia brachialis* Hal. The females of this midge dance in swarms with the abdominal glands everted and males fly into the swarms, the paired insects then dropping out to mate. Later Edwards<sup>7</sup> reported these glands as occurring throughout the genera *Palpomyia* and *Bezzia*. Scent-distributing organs occur widely in the Lepidoptera (*vide* Varley<sup>8</sup>, Philpott<sup>9</sup>), but are usually of more elaborate structure. A comparative study of sense organs in the antennae and palpi of Diptera has been published<sup>10</sup>, and we suggest that a comparable study of abdominal structures would be a suitable topic for further investigation.

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0.25-in. cubes of the plastic are cut from these areas, and trimmed under the microscope to leave a flat face of about 1 mm square to be presented to the microtome blade. Great care was necessary in cutting these blocks, since, if the microtome was not properly set, all the cells could be lost in a few primary sections which were too thick to be of use. The plastic blocks were held in a small chuck for cutting in a Huxley microtome and sections were floated on to a water reservoir, expanded with xylene vapour, and picked up on carbon-coated grids in the usual way. Sections showing silver interference colour were removed for examination in the microscope.

Numbers of ribosomes per cell were calculated from sections in the following way. Since a certain degree of detail and contrast was invariably lost in printing and enlarging the photographic plates, the ribosome counts were made by direct examination of the plates themselves. Using a high-power hand lens for examination, plates taken at instrumental magnifications of 20,000 were illuminated from below by means of a cold light illuminator, and particle numbers and distribution determined by placing a transparent plate, marked off in 1 mm squares, over the photographic plate. By this means a cell section at a magnification of 20,000 was subdivided into some 1,400 squares, permitting assessment of the comparative area occupied by cytoplasm, nucleus and vesicles, and the distribution and absolute numbers of the ribosomes themselves. Actual counts of ribosome distribution and numbers were made by numbering the squares on the grid and counting the ribosomes in 40 squares per section, the squares for examination being selected at random by the use of random number tables. Squares falling entirely or partly on cell organelles (nuclei, vacuoles and vesicles) were omitted from counts, and particles on the line were scored on only two sides of the square. The standard deviation of ribosomes per square was  $\pm 0.1$  to  $\pm 0.5$  for 40 counts, and the mean of 40 squares multiplied by the total number of squares covering cell, minus cell wall, gives the figure for total numbers of ribosomes per section. It was assumed that all cell sections examined represented 800 Å of the cell when cut<sup>2</sup> and thus the number of particles per section was multiplied by 37 to give the number per cell, an average cell thickness, minus cell wall, being taken as 3µ. Shrinkage of the cells during preparation and examination appeared to be slight and was ignored. Using the grid plate examination technique described, the ratio of mean area of cytoplasm to area of nucleus and cell inclusions was found to be 9-1, and the total ribosome number per cell was assessed for 100 per cent cytoplasm and rectified in accordance with this ratio.

The ribosomes of *S. pombe* appear to be freely dispersed in the cytoplasm<sup>1</sup>, and all darkly stained particles in the cytoplasm of about 150 Å diameter were scored as ribosomes. Of course, the number of assumptions inherent in this counting scheme is considerable, as are the possible sources of error, for example, deviation from the estimated ratio of volume of cytoplasm to volume of cell inclusions, varying cell thickness, thickness of section, and visibility of ribosomes, and the possible existence of ribosome dimers scored as single ribosomes.

Fig. 1 shows the number of ribosomes calculated from the electron micrographs as being totals per cell, plotted against cell length of the section. From this figure it would seem that an average cell of *S. pombe* in logarithmic growth possesses about 500,000 ribosomes. Although it would be of considerable interest to know the variation in ribosome numbers during the cell cycle, the inadequacy of the results presented in Fig. 1 does not permit drawing any conclusions on this question.

Since the sum of the molecular weights of the RNA molecules in a single ribosome is believed to be about  $1.7 \times 10^6$  for a 70 or 80 S ribosome<sup>4</sup>, it is possible to calculate that such a ribosome must contain  $2.7 \times 10^{-18}$  g of RNA (using Avogadro's number). With 500,000

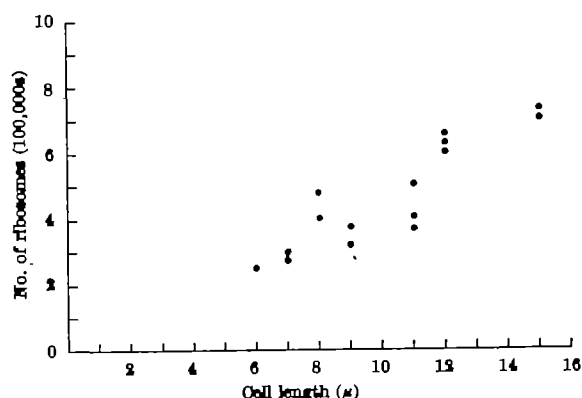


Fig. 1. Total numbers of ribosomes in *S. pombe* cells of different sizes

ribosomes in a cell, a cell of *S. pombe* should possess  $1.35 \times 10^{-13}$  g of ribosomal RNA, and assuming 80 per cent of the total cell RNA to be ribosomal,  $1.7 \times 10^{-13}$  g of total RNA. Mitchison and Lark<sup>3</sup>, using biochemical methods, have determined that *S. pombe* cells in logarithmic growth at a cell density of  $5.7 \times 10^6$  cells per ml. contain  $2.13 \times 10^{-13}$  g RNA. These results show that, at least on numerical grounds, there is some basis for believing that the ribosomes of the electron microscopist and the biochemist are the same particles.

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## VIROLOGY

### Morphology of Components of Sendai Virus

It has been shown by using a negative contrasting method that Sendai virus particles have an envelope which is formed of spikes of haemagglutinin and covers a tubular nucleocapsid with a diameter of helix of about 170 Å<sup>1</sup>. Sub-units of this virus (*S* and *V* antigens) resemble those of other myxoviruses, particularly of Newcastle disease virus<sup>2,3</sup>.

We have examined some details of the structure of Sendai virus in native and purified preparations treated by several methods. 11-day-old chick embryos were infected by the allantoic route with 100-1,000 *ID*<sub>50</sub> of the LM-1 strain of Sendai virus; the latter was collected after incubation at 35°C within 72 h. Allantoic fluid virus, native and purified by differential centrifugation and subsequent 'Sephadex'-gel filtration<sup>4</sup>, was examined in an electron microscope.

To reveal the structure of virus particles the preparations were treated with ethyl ether and trypsin. Ethyl ether was refined and freed from hydrogen peroxide, added gradually to an equal volume of virus preparations at 17°C in a glass with magneto-mixer. A layer of ether was removed after centrifugation at 1,600 r.p.m. for 15-20 min, and water suspension was freed from ether by passing gaseous nitrogen. In experiments with trypsin a crystalline enzyme was added to virus suspensions in 0.007 phosphate balanced solution, pH = 7.8, so that concentration of enzyme and substrate related as 1:50. Specimens were taken at intervals of 30 min for haemagglutination tests and electron microscopic examination.

Preparations for electron microscopy were prepared by means of thermal fixation<sup>4</sup>. Our modification<sup>5</sup> of the Brenner and Horne<sup>6</sup> negative contrasting method was used.

Besides the usual tubular structures of the nucleocapsid which are characteristic for this virus, we have found rod-like structures about 380 Å in diameter and 2500–3000 Å in length (Fig. 1). They are not sensitive to treat-



Fig. 1. A particle of Sendai virus. Usual helical rod-like structures and a wider structure (arrow). Negative contrasting with phospho-tungstic acid,  $\times c. 200,000$



Fig. 2. A preparation of Sendai virus after treatment with trypsin. Helical rod-like structures and a wider structure partly released from a capsule. Negative contrasting with phospho-tungstic acid,  $\times c. 187,000$



Fig. 3. A preparation of Sendai virus after treatment with ethyl ether. Secondary arrangement of haemagglutinin. Negative contrasting with phospho-tungstic acid,  $\times c. 100,000$

ment with trypsin which destroys the external envelope of the virus. In preparations treated with trypsin one can see clumps of viral envelopes, granular substrate, rod-like helical structures of nucleocapsid and the structures mentioned above which have also a helical structure with the angle of pitch about  $45^\circ$  with a partly destroyed capsule (Fig. 2). In preparations treated with ether these structures lose the capsule, which points to the latter being of lipid nature.

After treatment with ether, viral particles are decomposed into their components, and secondary aggregates of haemagglutinin are formed around dense masses of viral material which resemble a typical spike-like arrangement of haemagglutinin at the surface of intact virions (Fig. 3).

These results lead to the conclusion that structurally the nucleocapsid of Sendai virus, besides the usual rod-like dense helical forms, occurs also as more crumbly (pasty) packing which possesses lipotropic properties. The secondary aggregates of haemagglutinin as a typical palisade of spikes demonstrate a self-assembly process which probably occurs during the formation of virions.

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### Determination of the Type of Nucleic Acid in Rubella Virus using 5-Bromo-2'-deoxyuridine

SALZMAN<sup>1</sup> showed that 5-fluoro-2'-deoxyuridine (5-FUdR), when added to virus-tissue culture systems, specifically inhibited the synthesis of DNA viruses but did not affect RNA virus or protein synthesis. The inhibition could be reversed by the addition of excess thymidine. The use of 5-FUdR, or one of its analogues, thus provides an indirect test of whether a virus contains DNA or RNA. An advantage of such a test is its rapidity when compared with conventional techniques of nucleic acid isolation and purification; but in order to avoid misleading results the final concentration of the analogue<sup>2</sup> and the serum content of the tissue culture maintenance medium must be carefully controlled<sup>3</sup>. Media prepared with high concentrations of serum or embryo extract may contain enough thymidine to cause partial reversal of the inhibitory action of the analogue.

The object of the work recorded here was to use 5-bromo-2'-deoxyuridine (5-BUdR) in an attempt to determine the nucleic acid type of rubella virus. The experiments were controlled by testing the effect of 5-BUdR on the growth of two known DNA viruses (adenovirus type 7 and herpes simplex) and an RNA virus (coxsackie A21). The specificity of the effects of 5-BUdR was controlled by seeking reversal of inhibition on addition of thymidine.

Recently, several reports have appeared of isolations of strains of virus from patients with rubella<sup>4-6</sup>. Investigations of the biological characteristics of these strains are, as yet, incomplete, but the information available indicates that they form a serologically homogeneous group. The rubella strain<sup>7</sup> selected for use in the work described here appears to have biological features typical of the group.

Table 1

Virus	Tissue culture system	Tissue culture maintenance medium	Titre of virus inoculum TOID <sub>50</sub> /ml.	Time of virus adsorption (h at 37° C)
Herpes simplex†	RK <sub>13</sub> (ref. 10)	Medium 199+2% calf serum	10 <sup>4.5</sup>	4
Adenovirus type 7†	HeLa	Hanks's saline+2% rabbit serum+0.25% lactalbumin hydrolysate	10 <sup>1.5</sup> *	6
Coxsackie A21	HeLa	Hanks's saline+2% rabbit serum+0.25% lactalbumin hydrolysate	10 <sup>4.5</sup>	4
Rubella‡	RK <sub>13</sub> (ref. 6)	Medium 199+2% calf serum	10 <sup>4.5</sup>	4

\* Five-day end-point reading.  
† and ‡ strains obtained from Virus Reference Centre, Colindale.  
‡ Obtained from Dr. J. S. Porterfield.

The tissue culture system and composition of the maintenance medium used for each virus are shown in Table 1. Preparation of tissue cultures was by conventional methods<sup>6</sup>. For each virus, three parallel growth curve experiments were carried out under the following conditions: (1) in cells with maintenance medium only; (2) maintenance medium + 5-BUDR ( $6.5 \times 10^{-4}$  M); (3) maintenance medium + 5-BUDR ( $6.5 \times 10^{-4}$  M) +

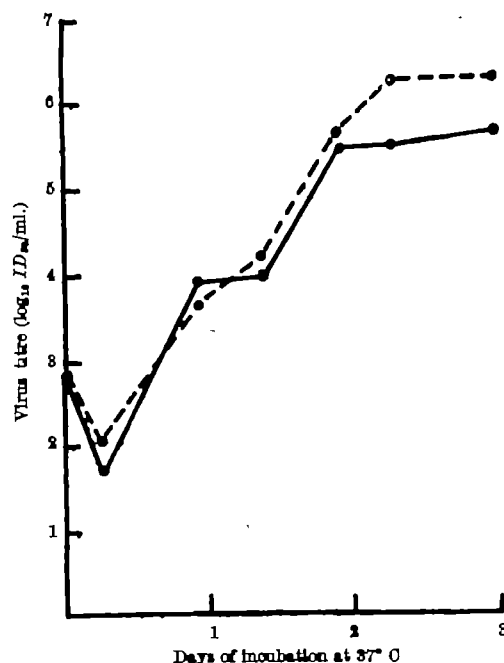


Fig. 2. Multiplication of coxsackie A21.  
Key, as Fig. 1

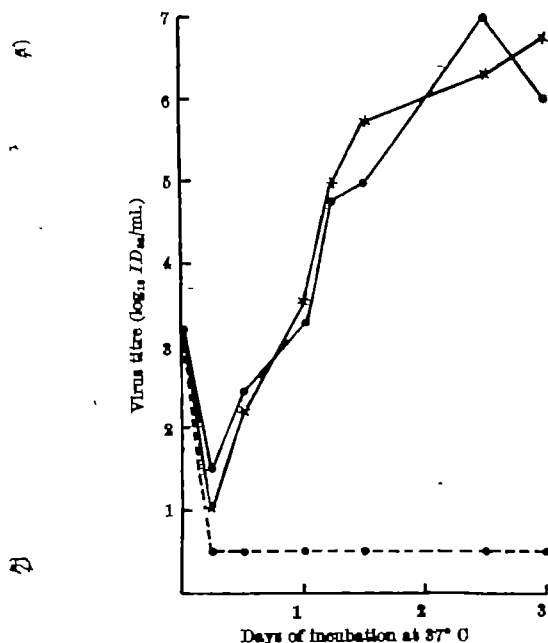


Fig. 1. Multiplication of herpes simplex virus. —●—, Normal maintenance medium; —●—, maintenance medium + 5-BUDR ( $6.5 \times 10^{-4}$  M); —×—, maintenance medium + 5-BUDR ( $6.5 \times 10^{-4}$  M) + thymidine ( $4.1 \times 10^{-4}$  M)

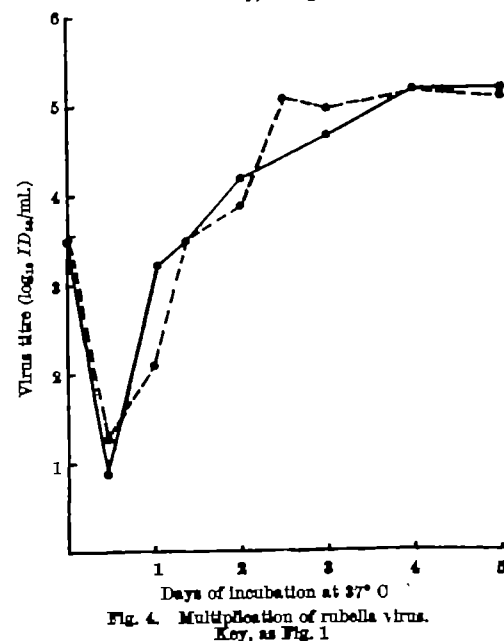


Fig. 4. Multiplication of rubella virus.  
Key, as Fig. 1

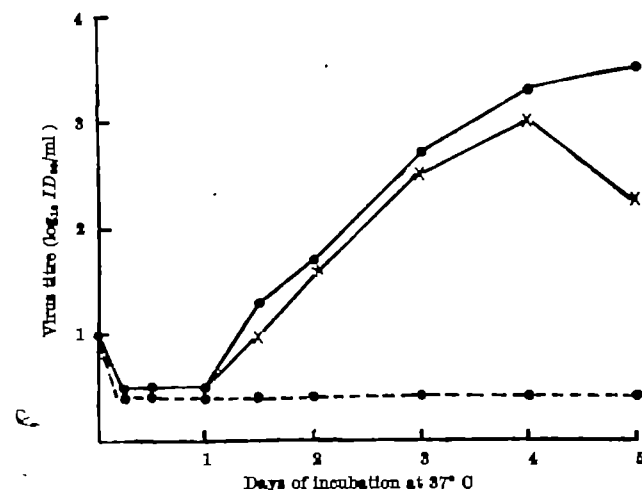


Fig. 3. Multiplication of adenovirus type 7.  
Key, as Fig. 1

thymidine ( $4.1 \times 10^{-4}$  M). In each case the tissue cultures were treated for 24 h with the appropriate maintenance medium prior to inoculation with virus. Replicate test-tube tissue cultures were inoculated with a measured dose of virus and, after a period of virus adsorption (Table 1), residual virus was removed by three washings with Hanks's saline. At selected time intervals, sample groups of tissue cultures were collected by subjecting them to three cycles of freezing and thawing to release cell-bound virus, and the fluids centrifuged (2,000 r.p.m./6 min) and afterwards pooled. Infective virus in the pools was assayed using end-point infectivity titrations<sup>6</sup>.

The growth curves are shown in Figs. 1-4. The multiplication of DNA-containing herpes simplex and adenovirus type 7 was completely suppressed in the presence of  $6.5 \times 10^{-4}$  M 5-BUDR. This inhibitory effect of 5-BUDR was reversed by the addition of thymidine. Thus, herpes simplex virus (Fig. 1) multiplied rapidly 24-60 h after

adsorption both in cells treated with thymidine + 5-BUDR and in control cells maintained with normal medium. No infectious virus was detected over the experimental period in cells treated with 5-BUDR alone. The growth curves for adenovirus type 7 (Fig. 2) are similar to those for herpes simplex and confirm these results for a more slowly multiplying DNA virus in an alternative host cell system.

In contrast, 5-BUDR at the same concentration had no detectable effect on the growth rate or final yield of coxsackie A21 virus (Fig. 3). The multiplication of rubella virus (Fig. 4), similarly to coxsackie A21, was not inhibited to any measurable extent in the presence of 5-BUDR. The most active period of rubella virus multiplication, 24–72 h after adsorption, and the final virus yield, were the same in 5-BUDR-treated cells and in cells maintained in normal medium. The strain of rubella virus tested thus clearly behaved as an RNA-containing virus.

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*Note added in proof.* Since this communication was submitted for publication, H. F. Maassab and K. W. Cochran have reported similar results using the fluorochrome analogue and an indirect viral interference method of estimating the growth characteristics of rubella virus (*Proc. Soc. Exp. Biol. and Med.*, 117, 410; 1964).

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### Reproduction of the Viruses Isolated from Cases of Infectious Hepatitis on Detroit-6(VA) Cell Line

In this report we propose to study certain aspects of the reproduction of viruses isolated from cases of infectious hepatitis<sup>1–4</sup> and at the same time to demonstrate their aetiological role in the development of this disease.

Between 1957 and 1964, we have studied a total of 21 different strains of infectious hepatitis viruses. These viruses were isolated on Detroit-6 cells, while 4 strains (R, V9, V6, 163 S) were adapted to a variant of the Detroit-6 cell line, obtained in our laboratory and given the name of Detroit-6(VA). In the course of our investigations we used a culture medium consisting of two parts of Earle's medium, one part of synthetic medium M 199, and calf serum, at a pH of 7.6. The infected cell cultures were maintained for ten days at a temperature of 37° C on a roller-tube apparatus, and for an additional four days on a stationary stand. With each strain of virus we made 40 successive passages. In order to study the modifications of the cells, they were included in a methacrylate or 'Vestopal' base, sectioned with the aid of a Reichert-type ultramicrotome and examined on a TESLA 'BS 242 A' electron microscope.

Complement fixation reaction and neutralization tests were applied for the serological identification of the isolated virus strains.

Four days after inoculation of the Detroit-6(VA) cells with the virus of infectious hepatitis, we observed swelling, rounding-off of the marginal cells, and the formation of aggregates—a phenomenon which appears after ten days in the form of foci, on the entire surface of the culture. Later on, the affected cells degenerate and detach from the walls of the test-tube; meanwhile the other cells, which have not previously shown any sign of degeneration, are also damaged. The most notable changes are observed 14 days after inoculation, the controls remaining unaffected.

Four days after inoculation, electron micrographs showed the aggregation of dense particles within the nuclei and the appearance of very small granules along the whole length of the nuclear substances. The nucleolus remained unaffected. In the cytoplasm there was an accumulation of dense granular material, similar to, and presumably identical with, the viroplasm, from which the virus particles form.

Ten days after inoculation the granular substance of the nucleus became rarefied, with the appearance of individualized dense granules of approximately 200–300 Å, dispersed irregularly in the nucleoplasm. During this period the cytoplasm shows a marked vacuolation, with diminished mitochondria (compared with the control cells), and loss of internal structure. The endoplasmic reticulum is imperceptible. During this phase we should like to emphasize the appearance of dense corpuscles about 150 Å diameter, occurring in compact masses, most frequently in formations of 4–6 particles, with the form of a rosette with a hollow centre, which fill parts of the cytoplasm, without a regular distribution. On the 14th day the rosette-like formations become prominent within the cytoplasm and acquire a symmetrical arrangement in parallel lines (Fig. 1).

Similar particles were also detected in the liver of patients suffering from infectious hepatitis. The supernatant fluid of the Detroit-6(VA) cells, infected with R and 163 S viruses after purification, contains certain formations similarly formed by 4–6 small particles. These formations appear to attain a similar size, about 150 Å, in all the strains of viruses studied. This results in the accumulation of RNA in the cytoplasm (studied with an acridine-orange staining), the particles resisting treatment with ether, as well as a temperature of 60° C.

The complement-fixation reaction made with the viruses isolated by ourselves, and the sera of 260 patients suffering from infectious hepatitis, proved to be positive in 91 per

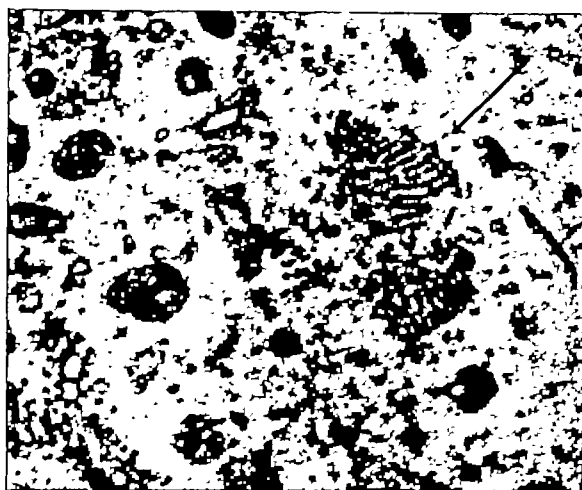


Fig. 1. Detroit-6(VA) cell, infected with the 163 S strain of hepatitis virus. The arrow indicates the symmetrical arrangement of the viruses ( $\times 17,500$ ).

cent of cases. The seroneutralization of the virus with 32 sera of convalescent patients indicated that there is a certain serological relationship between all our strains of viruses, although frequently the titres were quite low. Even so, in two cases titres of 1 : 640 were obtained.

From these findings, and from the fact that two accidental infections occurred in the laboratory due to two strains (R, V9), and having regard to the typical clinical symptoms of infectious hepatitis and the fact that it was possible to re-isolate these viruses, as well as to neutralize them, by the patients' sera, we consider these agents to be the viruses of infectious hepatitis.

Rightel *et al.*, Taylor *et al.* and Bearcroft have described similar viroic particles in the cell culture<sup>4,5</sup> and in the liver of patients suffering from infectious hepatitis<sup>4-7</sup>. These particles are very similar to those noted in the Detroit-6 (V4) cells inoculated with the strains of viruses isolated in our laboratory.

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## Effect of 5-Fluorodeoxyuridine on the Replication of Tobacco Mosaic Virus in Meristematic Tissue

THE phenomenon by which plant viruses are excluded from proliferating cells may prove to be a valuable means for investigating the mechanism of plant virus replication itself.

It has been shown that incubation of young tomato plants in ethylene diaminetetraacetic acid (EDTA) supplemented medium enables tobacco mosaic virus (TMV) to replicate in root apical meristem, from which it is normally excluded, and it has been suggested that this compound acts by degrading microsomal particles, thus furnishing building blocks for virus formation<sup>1</sup>. On the other hand, TMV replication in leaf disks of *Nicotiana glauca* was depressed by kinetin, a compound known to promote cell proliferation<sup>2</sup>, suggesting that conditions favouring proliferation may prevent virus formation. One of the processes which is obviously correlated to cell proliferation is DNA synthesis itself, and accordingly, the effect of inhibition of this process on the capacity of meristematic cells to sustain TMV replication was investigated.

In this communication, results are presented demonstrating replication of TMV in root tips of young tomato plants incubated with 5-fluorodeoxyuridine. This compound is known to inhibit synthesis of DNA in other systems<sup>3,4</sup> but to have little effect on the formation of TMV in excised leaf disks of Turkish tobacco<sup>5</sup>. Three to five 3-week-old tomato plants grown in soil were transferred to each of a series of 100-ml. jars containing 25 ml. Knopp solution. Twenty-four hours later, substances, the effect of which was to be examined, were added to the jars in the following final concentrations: EDTA,  $6.4 \times 10^{-4}$  M; and FUDR,  $7.6 \times 10^{-4}$  M. After 18 more hours, all plants were infected with TMV (isolate 'Mostar') by rub-

bing leaves with a purified suspension of virus. One week later, root tips, 0.5–0.7 mm long, were collected from the plants. From each category, 25–30 mg of root material was taken and homogenized in 0.7 ml. of a 0.1 M phosphate buffer, pH 7.0, and shaken in chloroform for 30 min. To estimate the concentration of virus particles, 0.6 ml. of each homogenate was used to infect a total of 4 leaves of the test plant *Nicotiana glauca*. After five days, the number of local lesions was counted. Table 1 gives the results, and shows the effect of EDTA and FUDR on the ability of infected plants to promote virus replication in root tips.

Table 1. TOTAL NUMBER OF LESIONS PER 4 LEAVES OF *N. glauca*

Knopp solution supplemented with :		
No addition	EDTA ( $6.4 \times 10^{-4}$ M)	FUDR ( $7.6 \times 10^{-4}$ M)
0	35	41

These results clearly indicate that FUDR, like EDTA, enabled TMV to replicate in tomato root tips, contrary to what happened in control plants. It remains to be established what relations exist between the phenomenon described by Crowley and Hanson<sup>1</sup>, who used EDTA, and that described here. While the action of EDTA on fostering TMV replication in meristematic tissue may be explained on a microsomal level, as microsomal RNA is used for TMV-RNA synthesis<sup>7</sup>, it is equally plausible to speculate that this compound acts by disorganizing chromosomal structure and function; indeed, EDTA has been shown to cause chromosomal aberrations<sup>8</sup> and to increase the frequency of crossing-over<sup>9</sup>. On the other hand, the action of FUDR could be clearly visualized on the chromosomal level, as its action is rather specific and as it is able to induce chromosomal lesions in plants<sup>10</sup>. Thus the possibility exists that a general weakening of the controlling system in the cellular machinery permits virus biosynthesis in cells normally resistant to virus invasion.

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## GENETICS

### Localization of Haptoglobin and ABO

RECENTLY, P. S. Gerald *et al.*<sup>1</sup> published their observations on haptoglobin (Hp) in a family with a child in which one of the D group chromosomes (13, 14 or 15) was deformed into a ring structure, which implies that part of the chromosomal material of the chromosome is deleted. It appeared that the carrier of this chromosome lacked the Hp genetical contribution from her father (father, Hp 2-2; mother, Hp 2-1; carrier child, Hp 1-1). Therefore the authors tentatively proposed that the haptoglobin locus is on the end of the long arm of a D chromosome. We had the opportunity to study a number of families with translocations, and our preliminary results are presented as additional information on the localization of Hp.

Family K. with D-D translocation, studied in the University Laboratory of Genetics in Nijmegen, provided the following information: both parents are heterozygous,



*Hp* 2-1; two out of their seven children are chromosomally normal, *Hp* 2-2; two are carriers of the translocation, *Hp* 1-1; three are heterozygous for *Hp*.

The pedigree of family R-v. d. P. with *D*-21 translocation, also analysed in Nijmegen, contains three brothers: one with the translocation, *Hp* 2-1; one carrier of the translocation, *Hp* 2-2; one normal, *Hp* 2-1. The first of these brothers has seven living children (mother, *Hp* 2-2): four carriers of the translocation, *Hp* 2-2; one carrier, *Hp* 2-1; one non-carrier, *Hp* 2-1; one non-carrier, *Hp* 2-2. This makes a total of 5 non-cross-overs and 2 cross-overs.

Family V. with *D*-21 translocation, studied in the Institute of Human Biology in Amsterdam, consists of the normal father, *Hp* 2-2; the carrier mother, *Hp* 2-1; one normal carrier child and two translocation mongrels, all three *Hp* 2-1.

Combination of the foregoing data amounts to 10 non-cross-overs: 2 cross-overs; this ratio has a chance probability of  $79/4096 = 0.019$ . The observed crossing-over rate is  $2/14 = 0.14$ .

Some further information can be found in publications by Hamerton *et al.*<sup>1</sup> (*D*-*D* translocation; 4 non-cross-overs: 1 cross-over), and by Macintyre *et al.*<sup>2</sup> (*D*-21 translocations; 2 non-cross-overs).

Combination of these results with ours does not change the crossing-over frequency (in total  $12 : 2 + 6 : 1 = 18$  non-cross-overs : 3 cross-overs = 14 per cent crossing-over).

Since a crossing-over frequency of only 14 per cent is very unlikely to occur if the *Hp* locus is situated on the end of the long arm of the *D* chromosome, it seems reasonable to think of a localization on the short arm.

The R-v. d. P. family gives also additional information on the still undecided problem of localization of ABO on chromosome 21. A genotype *A*<sub>1</sub>*O* carrier of the translocation married to a normal group *O* woman has five carrier group *O* children, one non-carrier group *A*<sub>1</sub> and one non-carrier group *O*. Other families in the pedigree indicate the translocation to be associated with the *O* gene of the ABO system. The resulting linkage information is therefore 6 non-cross-overs : 1 cross-over. The chance probability of this ratio is  $8/128 = 0.0625$ .

We thank Dr. H. K. Prins for carrying out the *Hp* typing, and Dr. J. v. d. Bosch for his advice. This work was supported in part by a grant to one of us (L. E. N.) from the U.S. National Institutes of Health (NB 04321-03).

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## Chromosomes of the Sperm Whale

THIS report describes the results of a chromosomal analysis of the sperm whale by the method of leucocyte tissue culture. During July and August, 1964, fresh sterile blood was obtained at sea for various research purposes from sperm, finback, and sei whales (*Physeter catodon*, *Balaenoptera physalus* and *B. borealis*, respectively) during the course of commercial whaling operations. The method of blood collection, developed by one of us (R. P. A.), will be described elsewhere.

Using this system, blood was collected, aseptically, in heparin, within 10 min of cessation of respiration, from a 56-ft. male sperm whale captured at 2.45 p.m. on August 20, 1964, in lat. 65° 20' N., long. 30° 10' W.

Peripheral blood leucocytes were grown in tissue culture by a modification of the method of Moorehead *et al.*<sup>1</sup>, using a medium composed of 80 per cent Eagle's minimum essential medium, 20 per cent autologous serum, phytohaemagglutinin and antibiotics.

The distribution of chromosome numbers in twelve suitable metaphase spreads showed a mode of 42 chromosomes:

Number of chromosomes	38	39	40	41	42
Number of cells counted	1	1	2	0	8

A karyotype constructed from one well-spread cell containing the modal number and an intact cytoplasmic halo (Fig. 1) showed fourteen metacentric or submetacentric chromosome pairs, six subacrocentric pairs, and two chromosomes, one a large metacentric, the other a small submetacentric or subacrocentric with uneven short arms, which could not definitely be paired. In the absence of a female karyotype it is not possible to decide which are autosomes and which are sex chromosomes.

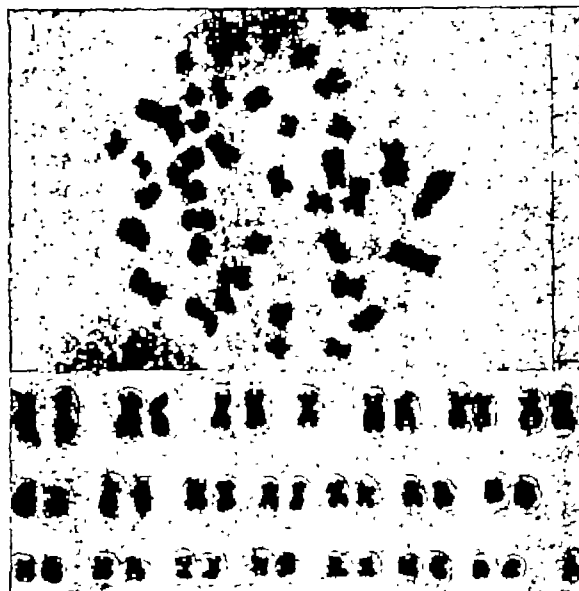


Fig. 1. Metaphase spread and karyotype from peripheral blood leucocytes of male sperm whale (*Physeter catodon*).

The relatively low diploid number and the predominance of metacentric with absence of acrocentric chromosomes is interesting in view of suggestions, made by various authors<sup>2-4</sup>, that evolutionary specialization is associated with reciprocal translocation between acrocentric pairs and a balanced reduction in diploid number (Robertsonian-type variation<sup>5</sup>). Such a process would be likely to occur in a group of mammals as highly specialized as the Cetacea.

Since this communication was prepared for publication Cortman and Richart<sup>6</sup> have described the chromosomes of a male ring seal, *Phoca hispida krascheninikovi*, in which a similar reduction in diploid number and tendency to metacentricity was found.

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## Brilliant Cresyl Blue as a Stain for Plant Chromosomes

THE usefulness of cresyl violet as a stain for rapid preparations of root tip squashes has been recently reported<sup>1</sup>.

For the past two years I have used the related stain brilliant cresyl blue in aqueous solution, in preference to conventional staining procedures which have generally been more time-consuming. More than forty species of flowering plants have been tested with aqueous brilliant cresyl blue without any difficulties being encountered.

In Amirkhanian's method<sup>1</sup>, cresyl violet was dissolved in 50 per cent acetic acid. This solvent proved unsuitable with brilliant cresyl blue which was finally prepared as a 10<sup>-4</sup> M solution in distilled water.

Consequently the same solution of brilliant cresyl blue may be used with vital staining of dividing nuclei as well as with normal fixation procedures. It is thus possible to compare fixed and living chromosomes (or other cellular components) which may be an important consideration in some cytological studies.

Brilliant cresyl blue is classified as a quinone-imine dye belonging to the sub-group of oxazines. According to Conn<sup>2</sup> "they are not stains having very general application". The dye marketed under the name of cresyl violet is of uncertain chemical structure, but is probably closely related to cresyl violet acetate, the formula of which has been characterized from the chemical synthesis of the material. Since the chemical formula of brilliant cresyl blue is definitely known, it would be preferable to use this dye in place of cresyl violet in cytological studies where a knowledge of the underlying histochemical reactions is desirable.

In squash preparations involving refractory material (for example, shoot and root meristems of grasses) brilliant cresyl blue gives good results after pectinase treatment<sup>3</sup>. It is also compatible with colchicine and other chemicals used in pre-treatment<sup>4</sup>.

The procedure in squashing is as follows: After pre-treatment (if any) root tips or other meristematic tissues are fixed in aceto-alcohol (1 part glacial acetic acid : 3 parts ethanol). Other appropriate fixatives may be used<sup>4</sup>.

After fixation, the specimen is hydrolysed in 1 N HCl for 5 min or longer at 60° C and transferred to a drop of brilliant cresyl blue on a slide (a 10<sup>-4</sup> M solution in distilled water, or stronger solutions up to 10<sup>-3</sup> M). The specimen is teased out, tapped and squashed under the coverslip in the usual way<sup>4</sup>. By warming the slide gently over a spirit lamp the cytoplasm may be cleared to the degree desired. Permanent preparations may be made by inverting the slide in 80 per cent ethanol in a smearing dish, dehydrating rapidly in 100 per cent ethanol, and mounting in 'Euparal' or Gurr's neutral mounting medium. Other methods of making the preparation permanent may equally well be applied<sup>4</sup>.

Brilliant cresyl blue in dilute aqueous solution provides the means for rapid squash preparations and is applicable to a wide range of different species of flowering plants and, if required, the concomitant metachromatic staining of living cells<sup>5</sup>.

An aspect of added interest is the report of retardation of the growth of tumours by brilliant cresyl blue<sup>6</sup>; further work with this dye might throw valuable light on the course of cell division, not only in cancerous growths but also in normal tissues.

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## VETERINARY SCIENCE

### Restricted Protein Intake and Avian Atherosclerosis

ALTHOUGH low-protein diets in the presence or absence of supplementary dietary cholesterol give rise to a marked hypercholesterolaemia in Leghorn cocks, nevertheless, we observed<sup>1,2</sup> a reduction in the severity of abdominal atherosclerosis after 10 or 20 weeks on the experimental low-protein diets. We have examined the effects of prolonged restrictions (20 months) of protein intake in relation to plasma cholesterol, body composition and abdominal aortic and coronary atherosclerosis.

Day-old Leghorn cocks from the same random-bred strain used in previous investigations of avian atherosclerosis<sup>3,4</sup> were divided into two groups of sixty, one being given an adequate and the other an inadequate protein-level. The basic diet was composed of soy-bean meal, glucose, maize oil, vitamins and minerals. Its protein content was continuously diminished with increasing age and the average daily intake per bird throughout the experiment is listed in Table 1.

Mortality was 31 and 25 birds from the adequate and inadequate protein-intake groups, respectively. During the course of the examination another 14 and 20 cocks from each group were removed for nitrogen metabolism studies. Blood was taken from the 15 survivors in each group for plasma-cholesterol analysis prior to the termination of the experiment. The birds were killed with chloroform, and the liver and aorta removed for further analysis. Liver cholesterol was determined<sup>5</sup> on a portion of the lipid extracted with chloroform-methanol (2:1). The aorta was macroscopically graded<sup>4</sup> on a scale of 1-3 and then examined and graded histologically<sup>4</sup>, as were also the

Table 1. COMPARISON BETWEEN SEVERITY OF ACETIC AND CORONARY ATHEROSCLEROTIC LESIONS IN LEGHORN COCKS ON NORMAL OR RESTRICTED LEVELS OF DIETARY PROTEIN OVER A PERIOD OF 20 MONTHS

Measurements	Protein Intake Normal	Restricted
Number of birds: at start	60	60
removed*	14	20
died	31	25
Protein intake (g/bird/day).		
1 day-1 month	4.1	2.5
1 - 2 months	7.7	4.6
2 - 4.5 months	11.2	7.1
4.5 - 7 months	13.1	7.8
7 - 14 months	11.2	6.2
14 - 20 months	7.4	4.3
Number of survivors	15	15
Body-weight, final (g)	2,504 ± 28†	2,234 ± 40
Oxazone N (g/100 g dry weight)	9.6 ± 0.3	9.7 ± 0.3
Oxazone extractable lipid (g/100 g dry weight)	20.9 ± 2.2	19.8 ± 1.5
Plasma cholesterol (mg/100 ml.)	110 ± 5	135 ± 7
Liver cholesterol (g/100 g wet weight)	0.52 ± 0.02	0.51 ± 0.03
Abdominal aorta:		
Macroscopic score	2.07 ± 0.15	2.07 ± 0.15
Weight-area ratio	68.1 ± 2.3	65.6 ± 2.4
Histological score	1.53	1.52
Coronary arteries:		
Histological score	1.45 ± 0.12	0.78 ± 0.10
Number of birds with severe scores	8 (53%)	1 (7%)

\* For nitrogen metabolism investigations.

† Mean value with its standard error.

coronary arteries. The remainder of the carcass, devoid of aorta and liver, was dried, and nitrogen and extractable lipid determined<sup>8</sup>.

All the results are given in Table 1. The protein intake of the restricted group was approximately 60 per cent of that of the normal group. This was reflected in a significantly lower body-weight at the end of the 20-month experimental period. There was no difference between the two groups, however, in the percentages of body nitrogen and extractable lipid.

As in our previous studies<sup>1,2</sup>, there was a significantly higher plasma cholesterol concentration in the group of cocks on the restricted protein intake. This difference was not reflected in differences in liver cholesterol values. There was no difference in aortic atherosclerosis as judged by either macroscopic or histological scoring. The coronary arteries, however, showed markedly greater severity of atherosclerosis for the cocks on the normal protein intake in comparison with those on the restricted intake. These observations lend further emphasis<sup>4,5</sup> to the poor correlation between cholesterol values and the incidence and severity of atherosclerosis.

In contrast to the present observations, Stamler *et al.*<sup>6</sup> observed an increased incidence of lesions with low-protein diets; their data indicate, however, that the protein-level considered 'low' was adequate for normal growth as judged by a comparison of the final weights of the 'low' and high-protein groups.

In the fowl, spontaneous coronary atherosclerotic lesions do not develop in parallel with, and are generally less severe than, those of the abdominal aorta<sup>7</sup>. It may be that in the fowl, as in man, where the coronary arteries and the aorta are often affected to different degrees by atherosclerosis, these two sites react differently to atherogenic stimuli. This may be why less-severe coronary lesions were observed in birds after a restricted protein intake while no difference was seen in the aortic lesions.

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### Premature Delivery and Survival in Lambs

BETWEEN 105–110 days gestational age (term is about 147 days) it is impossible to obtain independent survival of lambs delivered by Caesarean section, even on ventilation with 100 per cent oxygen<sup>1</sup>. This estimate of the ultimate limit of viability in sheep agrees well with the histological changes which begin in the lungs at about 90 days gestation<sup>2</sup>. The lamb is very mature at birth, so there was every reason to suppose that most lambs delivered prematurely would survive. However, 23 Clun-Hampshire lambs survived after being delivered (by Caesarean section under local or epidural anaesthesia) at 142 days gestation or more, whereas 4 of 23 that were delivered at 138–141 days gestation did not survive. The weights of these 4 lambs were 3.3–4.2 kg and they all appeared well developed. But they failed to maintain spontaneous breathing for more than a few minutes after the umbilical

cord was tied, in spite of prolonged efforts at resuscitation by positive pressure ventilation with oxygen.

This large mortality rate was surprising, because 138 days represents 94 per cent of normal term in sheep, and one expects that all infants should establish normal respiration on premature delivery after only 90 per cent of term in man or rhesus monkeys, when instances of gross anomalies and disease are excluded. However, the numbers involved were small and therefore enquiries have been made into the losses of newborn lambs in flocks where accurate records of tupping dates were available.

The causes of perinatal wastage in lambs under field conditions are various. Hence, in examining that portion of the perinatal mortality rate which may fairly be attributed to temporal prematurity, certain criteria must be met. The data presented below refer only to sheep in supervised recorded flocks with individual mating dates and a high standard of antenatal care under competent daily inspection; the flocks have been free for at least 5 years from any clinical evidence of contagious illness known to affect pregnancy; the pregnancies were uncomplicated and lasted more than 90 per cent of the normal gestation length (132–135 days), and with normal parturition yielding lambs free of physical defects and of minimal viable birth weights (2.75 kg for males and 2.25 kg for females of the breeds concerned); they include cases only of recent stillbirth, death during parturition, and death in the first 24 h after birth.

Data meeting these criteria have been assembled for 1,077 pregnancies during 1960–64 in four flocks of two pure breeds, breed A being similar to the Clun-Hampshire and breed B being somewhat smaller. The mean gestation length of breed A is said to be 147 days and our figures endorse this estimate; that for breed B is thought to be longer and may be 149–150 days, with which our limited data concur.

For preliminary study over some years we have accepted a variation of 5 per cent about the mean gestation length as the normal range, that is, 144–150 days in breed A and 146–154 days in breed B. A lamb born after a gestation shorter than this is considered premature, a 2.5 per cent shortening being considered slight temporal prematurity (141–143 days for breed A and 144–146 days for breed B) while a gestation only 95–90 per cent of the mean was considered as marked temporal prematurity in the lambs.

Table 1. EFFECTS OF PREMATURE DELIVERY ON SURVIVAL IN SHEEP

	Breed and flock				
	A8	A20	A53	Total A	B2
Delivered at term $\pm 2.5\%$ :					
Mean gestation age (days)	147.2	147.0	147.6	147.3	149.8
Average litter size	1.81	1.87	1.87	1.86	1.51
No. lambs	261	1,181	389	1,731	226
Deaths	27	107	53	167	26
% dead	10.3	9.5	9.7	9.7	11.5
Delivered at term less 2.5–5%:					
Average litter size	1.67	1.87	1.73	1.80	1.61
No. lambs	10	28	14	52	21
Deaths	2	6	5	13	12
% dead	20	21	36	25	57
Delivered at term less 5–10%:					
Average litter size	—	1.8	2	1.8	1.5
No. lambs	—	9	2	11	3
Deaths	—	8	2	10	2

<sup>2</sup> Tests on the total data from breed A show that there is a significant association between survival at birth and maturity ( $P < 0.001$ ).

Table 1 shows that for similar average litter sizes, the perinatal mortality rises from about 10 per cent for full-term lambs to 25 per cent or more for slightly premature lambs, and even higher for markedly premature lambs. Hence lambs of gestational age less than 95 per cent of the normal, that is, of 5 per cent prematurity, are not of normal viability. In this respect the lamb appears to differ from man, rhesus monkey and cattle. The mortality rate on delivery at term also compares very unfavourably with that in man.

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## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

**SENIOR RESEARCH ASSOCIATE** (with an honours degree in soil science or equivalent, preferably with some postgraduate experience) in the DEPARTMENT OF SOIL SCIENCE for work in connection with a landscape reclamation research project—The Registrar, University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne, 2 (July 24).

**RESEARCH WORKER IN INDUSTRIAL SOCIOLOGY** in the DEPARTMENT OF SOCIAL SCIENCE—The Registrar, The University, Liverpool, quoting Ref. OV/186 (July 28).

**STAFF TUTOR IN PSYCHOLOGY** in the DEPARTMENT OF ADULT EDUCATION AND EXTRA-MURAL STUDIES, to conduct courses principally for voluntary and professional workers, and other professionally engaged students—The Registrar, The University, Liverpool, quoting Ref. OV/184 (July 28).

**TUTORIAL ASSISTANTS (2)** (recent graduates with qualifications in sociology, social anthropology or in a related discipline) in the DEPARTMENT OF SOCIOLOGY AND SOCIAL ANTHROPOLOGY, to help in the teaching of sociology—The Registrar, The University, Hull (July 28).

**DEMONSTRATOR (Hydrology)** in the DEPARTMENT OF FORESTRY AND NATURAL RESOURCES, to assist in the teaching of the hydrological aspects of land-use ecology, and to pursue research studies in a field of hydrology related to rural land use—The Secretary, The University, Edinburgh (July 28).

**DEMONSTRATOR** (with, or taking, an honours degree in botany and, either have studied, or have an interest in, bacteriology or microbiology) in the DEPARTMENT OF BOTANY—The Registrar, The University, Nottingham (July 30).

**GALLAHUE FELLOW** in the DEPARTMENT OF MYCOLOGY AND PLANT PATHOLOGY to carry out research on the microbiology of tobacco, and will have opportunities for discussing research problems and co-operation with the personnel of the Gallahue factories in Northern Ireland—The Deputy Secretary, Academic Council, The Queen's University of Belfast, Belfast, 7, Northern Ireland (July 31).

**LECTURER** (with a good honours degree and/or a higher degree in psychology, and preferably experience in remedial education) in the DEPARTMENT OF PSYCHOLOGY—The Registrar, University College of North Wales, Bangor, North Wales (July 31).

**LECTURER OR ASSISTANT LECTURER** in the DEPARTMENT OF PURE MATHEMATICS—The Registrar, University College of Wales, Aberystwyth (July 31).

**RESEARCH ASSISTANT** in the DEPARTMENT OF PHYSICS for work on plasma heating by lasers—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (July 31).

**RESEARCH FELLOW OR RESEARCH ASSISTANT** (with an honours degree in psychology) in the DEPARTMENT OF PSYCHOLOGY, to work in a small team sponsored by the M.R.C. to study various aspects of attention in normal and abnormal subjects—A. B. Burnell, Department of Psychology, University of Aberdeen, Taylor Building, Old Aberdeen, Scotland (July 31).

**RESEARCH OFFICER** (with a pass degree, H.N.C., H.N.D. or equivalent, and experience of crystal growth or metallurgy) in PHYSICS—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (July 31).

**SENIOR LECTURER** in the DEPARTMENT OF PSYCHIATRY—The Secretary, The University, Edinburgh (July 31).

**ASSISTANT LECTURER** in BOTANY—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (August 2).

**RESEARCH ASSISTANTS (2)** (honours graduates in sociology, physiology or anatomy) with the NEUROLOGICAL RESEARCH GROUP in the DEPARTMENT OF SOCIOLOGY, to work with Prof. D. Barker on problems connected with the innervation of vertebrate skeletal muscle, for example, investigations into the process of motor end-plate replacement—The Secretary, University of Durham, Old Elvet Hall, Durham (August 2).

**L.B.M. RESEARCH FELLOW** in the DEPARTMENT OF PSYCHOLOGY, to carry out research into the stimulation of the retrieval of linguistic information by the brain, and will be responsible for the programming of the stimulation studies—The Registrar, The University, Sheffield (August 9).

**PROFESSOR OF SOCIOLOGY** in the SCHOOL OF HUMANITIES AND SOCIAL SCIENCES—The Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol, 7 quoting Ref. CST 65/62 (August 9).

**CHAIR OF CONTROL ENGINEERING** (candidates may have interests in any branch of control engineering—The Deputy Secretary, The University, Southampton (August 14).

**LECTURER IN BIOLOGICAL CHEMISTRY**—The Registrar, The University, Manchester, 13, quoting Ref. 140/65 (August 14).

**LECTURER** (with medical qualifications registrable in the United Kingdom) in MEDICINE—The Registrar, The University, Manchester, 13, quoting Ref. 146/65 (August 14).

**POST-DOCTORAL ASSISTANT (Chemist)** in the DEPARTMENTS OF CHEMISTRY AND BIOCHEMISTRY for work on the effects of radiations on polymers of biological importance, under the immediate direction of Dr. G. O. Phillips—Prof. K. S. Dodgson, Department of Biochemistry, University College of South Wales and Monmouthshire, St. Andrew's Place, Cardiff (August 14).

**RESEARCH ASSISTANT** in the DEPARTMENT OF BIOCHEMISTRY—Prof. K. S. Dodgson, Department of Biochemistry, University College of South Wales and Monmouthshire, St. Andrew's Place, Cardiff (August 14).

**RESEARCH ASSISTANT** (with a good knowledge of mathematics, preferably at the university level, and typing and general secretarial experience) for the *Journal of Applied Probability*—The Registrar, The University, Sheffield, 10 (August 14).

**UNIVERSITY LECTURER IN THEORETICAL CHEMISTRY**—Prof. R. H. Richards, F.R.S., Physical Chemistry Laboratory, The University, South Parks Road, Oxford (August 14).

**LECTURER** (with experience of electrical machine design, operation or control) in ELECTRICAL ENGINEERING—The Registrar, University College of Swansea, Singleton Park, Swansea (August 15).

**SENIOR LECTURER** in PHARMACOLOGY in the DEPARTMENT OF MATERIA MEDICA AND THERAPEUTICS—The Secretary, The University, Aberdeen (August 21).

**POST-DOCTORAL RESEARCH FELLOW** in ORGANIC CHEMISTRY—The Registrar, The University, Hull (August 28).

**RESEARCH ASSISTANT** (preferably with experience in geology and/or microprobe analysis) in the DEPARTMENT OF GEOLOGY AND MINERALOGY to be concerned with electron probe microanalysis—The Reader in Mineralogy, Department of Geology and Mineralogy, University of Oxford, Parks Road, Oxford (August 28).

**LECTURER** (with the ability to lecture in physics) in the SUB-DEPARTMENT OF GEOPHYSICS—The Registrar, The University, Liverpool, quoting Ref. OV/182 (August 30).

**JUNIOR LECTURER/LECTURER** in PHILOSOPHY at Rhodes University, Grahamstown, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, August 31).

**LECTURER** (preferably with some experience of hospital laboratory work, particularly in a biochemical department) in the DEPARTMENT OF CLINICAL CHEMISTRY—The Secretary, The University, Edinburgh (August 31).

**LIBRARIAN** at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 31).

**SENIOR LECTURER/LECTURER** (preferably with a knowledge of, and special interest in, bacteriology) in MICROBIOLOGY at Rhodes University, Grahamstown, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, August 31).

**EXECUTIVE DEAN** (with medical qualifications, and also academic and administrative experience) of the FACULTY OF MEDICINE—The Secretary, The University, Edinburgh (September 7).

**CHAIR OF PHILOSOPHY** at the University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 18).

**ADMINISTRATIVE ASSISTANT** (with a university degree or equivalent qualification, and preferably not more than 30 years of age) in the DEPARTMENT OF AERONAUTICAL AND AUTOMOBILE ENGINEERING—The Registrar, Loughborough College of Technology, Loughborough, Leicestershire.

**ANIMAL NUTRITIONIST** (national of the United Kingdom or the Republic of Ireland, with a degree in agriculture and postgraduate training or experience in biochemistry of agricultural biochemistry) with the East African Agriculture and Forestry Research Organization, to investigate metabolism of tropical and temperate type cattle with a tropical environment—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. R.O. 213/214/016.

**ASSISTANT CONSERVATORS OF FORESTS** (nationals of the United Kingdom or the Republic of Ireland, between 30 and 40 years, with a degree in forestry or a degree in natural science plus a diploma in forestry) in Tanzania (Tanganyika and Zanzibar), for the administration of normal Districts, including reservations, surveys, enumerations, supervising and directing of harvesting and advice to local authorities on forestry matters—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting Ref. R.O. 324/173/01.

**BIOCHEMIST**—The Medical Superintendent, Maryfield Hospital, Dundee, Scotland.

**CHAIR OF CHEMISTRY** (preferably candidate who has specialised interests in organic chemistry or in biological chemistry)—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 29/6.

**HONOURS GRADUATES (first- or second-class) in the MECHANICAL ENGINEERING DEPARTMENT**, to undertake directed research in the field of turbine blade vibration—The Head of the Department of Mechanical Engineering, Battersea College of Technology, London, S.W.11, quoting Ref. D 18.

**LECTURER** (with teaching interests in stereo-chemistry and physical organic chemistry) in ORGANIC CHEMISTRY to assume responsibility for the organisation and development of teaching of the subject in the Department—The Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol, 7, quoting Ref. CST 65/61.

**LECTURER OR ASSISTANT LECTURER** (preferably with interests in (i) numerical analysis or computer science; (ii) fluid dynamics or any other branch of continuum mechanics) in APPLIED MATHEMATICS—The Registrar (Room 22, O.B.B.), The University, Reading.

**MASTER** to teach Mathematics to scholarship level—The Headmaster, Monkton Combe School, near Bath.

**PHYSICIST, MECHANICAL OR MECHANICAL ENGINEER, or METEOROLOGIST** (recently graduated) in the BUILDING CLIMATOLOGY RESEARCH UNIT, DEPARTMENT OF BUILDING SCIENCE, to assist in investigations of the characteristics of turbulence in the natural wind—The Registrar, The University, Liverpool, quoting Ref. 176/N.

**POST-DOCTORAL ASSISTANTS; TUTORIAL ASSISTANTS; and RESEARCH SCHOLARS** (graduates or holders of an equivalent qualification) in CHEMISTRY—The Secretary, Department of Chemistry, St. Salvator's College (University of St. Andrews), St. Andrews, Fife, Scotland.

**RESEARCH ASSISTANT** (with a first degree in physics, chemistry, ceramics or metallurgy) in the COLLEGE OF CERAMICS, for work on an interesting sponsored research project relating to the sintering of alumina—The Clerk to the Governors, North Staffordshire College of Technology, Stoke-on-Trent, Staffordshire.

**RESEARCH FELLOW, RESEARCH ASSISTANT and a RESEARCH STUDENT** at the Tidal Institute and Observatory, to participate in a three year research project to study the generation, propagation and methods of forecasting storm surges and related tidal phenomena in the North Sea and adjacent areas—The Registrar, The University, Liverpool, quoting Ref. OV/184.

**RESEARCH STUDENTS and POST-DOCTORAL WORKERS** in the DEPARTMENT OF MICROBIOLOGY for studies of microbial chemistry in relation to pathogenicity and immunity—Prof. H. Smith, The University, Edgbaston, Birmingham, 15.

**SENIOR TECHNICIAN** (experienced in the major biochemical techniques including enzymology, radioisotopes, biochemical fractionation and animal care, and preferably experience both in supervising a teaching laboratory and in research) to take charge of an advanced biochemistry laboratory course—Department of Biochemistry, University of British Columbia, Vancouver 8, Canada.

**SENIOR TECHNICIAN** (with a good knowledge of laboratory procedure and holding I.S.T. or I.M.L.T. qualifications) to assist with general laboratory duties and research in a University Department—The Regius Professor of Medicine, Radcliffe Infirmary, Oxford.

**TECHNICAL ASSISTANT** (preferably with previous experience) in the RADIOLOGICAL MICROSCOPE UNIT—Prof. H. J. J. Blackwood, Royal Dental Hospital, 32 Leicester Square, London, W.C.2.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

## Great Britain and Ireland

University of Leeds. Annual Report of the Librarian, Session 1963-64. Pp. 12. (Leeds: Brotherton Library, The University, 1964.) 16

The British Medical Profession's Service to the People. By K. W. Moncreaf. Pp. 15. (Liverpool: The Sir Robert Jones Memorial Workshops, 74 Upper Parliament Street, 1965.) 16

Ministry of Technology: Hydraulics Research Station. Hydraulics Research Paper No. 6: Similarity in Sediment Transport by Currents. By Dr. M. Sedim. Pp. iv+24. (London: H.M. Stationery Office, 1965.) 3s. net. 16

Ministry of Technology. Report of the National Physical Laboratory for the year 1964. Pp. viii+277+14 plates. (London: H.M. Stationery Office, 1965.) 20s. net. 16

- The Association of the British Pharmaceutical Industry. *Pharmaceutical Industry Press Directory, 1965/1966*. Pp. 28. You and the Pharmaceutical Industry: Practical Hints on How to Start on a Successful Career. Pp. 3. (London: The Association of the British Pharmaceutical Industry, 1965.) [16]
- Obba (A.B.I.), Ltd. *Technical Notes, April 1965: Obba Glues in System-Built Houses*. Pp. 8. (Duxford: Obba (A.B.I.), 1965.) [16]
- Annual Report of the British Aerosol Manufacturers' Association for the year ended 31st December, 1964. Pp. 10. (London: British Aerosol Manufacturers' Association, 1965.) [16]
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- The Empire Cotton Growing Corporation. *Progress Reports from Experiment Stations, Season 1963-64*. Northern Nigeria. Pp. 18. 2s. 6d. Western Nigeria. Pp. 19. 2s. 6d. South Arabia. Pp. 23. 2s. 6d. (London: The Empire Cotton Growing Corporation, 1965.) [16]
- The Natural Rubber Producers' Research Association. *List of Publications, 1938-1964*. Pp. 53. (London: The Natural Rubber Producers' Research Association, 1965.) [16]
- Magnesium in Theory and Practice. By Prof. B. P. Wohlfarth. (Inaugural Lecture, 24 November 1964.) Pp. 39-63. (London: Imperial College of Science and Technology, 1965.) 4s. [16]
- The Edinburgh School of Agriculture. *Experimental Work 1964*. Pp. vii+79. (Edinburgh: The Edinburgh School of Agriculture, 1965) 7s. 6d. [16]
- Register of Veterinary Surgeons and Supplementary Veterinary Register 1965. Revised to January 1st, 1965. Pp. xx+491. (London: Royal College of Veterinary Surgeons, 1965.) [16]
- The Institution of Gas Engineers. Publication No. 677: Why Look for Oil and Gas in the North Sea? By Dr. M. J. Sander and Dr. William R. Humphrey. Pp. 17. Publication No. 678: Natural Gas in Canada. By James W. Kerr. Pp. 11. Publication No. 679: The Application of Work Study and Associated Techniques to Plant Maintenance. By H. R. Hart. Pp. 16. Publication No. 680. Some Special Features of the Recent Developments in the East Midlands Gas Board. By O. R. Mills. Pp. 21. Publication No. 682: Keeping up the Pressure. By Fred Bell, B. O. Emmony and P. B. Gallaher. Pp. 17. Publication No. 683: The Production of Gas from Hydrocarbons, Using the O.N.I.A. Continuous Autocatalytic Process. By P. J. Savage. Pp. 17. Publication No. 684: Part and Parcel (An Appliance Spare-Part Service). By W. V. Olson and J. K. Mitchell. Pp. 16. Publication No. 685: Progress in Management Techniques. By R. J. Maher. Pp. 15. (London: The Institution of Gas Engineers, 1965.) [16]
- The Obba Foundation for the Promotion of International Co-operation in Medical and Chemical Research. *Report for 1964*. Pp. 69. (London: The Obba Foundation, 1965.) [16]
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- Arthur D. Little in the United Kingdom—1964 Report. (Inveresk Gate, Musselburgh and London: Arthur D. Little, 1965.) [16]
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- Building Research Station Digest (Second Series), No. 58: Mortars for Joining. Pp. 6. (London: H.M. Stationery Office, 1965.) 4d. [16]
- Planning. Vol. 31 (May 1965): East-West Trade. Pp. 111-188. (London: Political and Economic Planning, 1965.) 10s. [16]
- Bulletin of the British Museum (Natural History). Botany. Vol. 3, No. 5: Marine Algae of Gough Island. By Yvonne M. Chamberlain. Pp. 173-232+plates 16-19. (London: British Museum (Natural History), 1965.) 32s. [16]
- Ministry of Technology. *Water Pollution Research 1964*. The Report of the Water Pollution Research Board with the Report of the Director of the Water Pollution Research Laboratory, and Cumulative Index for the Years 1962-1964. Pp. viii+173+4 plates. (London: H.M. Stationery Office, 1965.) 13s. net. [16]
- National Lending Library for Science and Technology. *Index of Conference Proceedings Received by the N.L.L., No. 1, 1964*. Pp. iv+60. (Wilton, Boston Spa: National Lending Library for Science and Technology, 1965.) [16]
- Department of Agriculture and Fisheries for Scotland. *Freshwater and Salmon Fisheries Research Series, No. 35: The Distribution and Food of the Cormorant in Scottish Inland Waters*. By Dr. D. H. Mills. Pp. 16. (Edinburgh and London: H.M. Stationery Office, 1965.) 5s. 6d. net. [16]
- Rubber and Plastics Research Association of Great Britain. *Forty-fifth Annual Report and Accounts, 1964*. Pp. 45. (Rushbury, Shrewsbury: Rubber and Plastics Research Association of Great Britain, 1965.) [16]
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- The Mechanism of Gravitation. By John Atkinson. Pp. 25. (Ambleside, Stourbridge: John Atkinson, 58 Platts Crescent, 1965.) 10s. [16]
- Government of Northern Ireland. *Ministry of Agriculture, Local No. 108: Bee-Keeping*. Pp. 14. (Belfast: Ministry of Agriculture, 1965.) [16]
- The Lace Research Association. *16th Annual Report, 1964*. Pp. 24. (Bilborough, Nottingham: The Lace Research Association, 1965.) [16]
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- United States Department of Commerce: Coast and Geodetic Survey. *Technical Bulletin No. 24: Satellite Triangulation in the Coast and Geodetic Survey*. Pp. 21. *Technical Bulletin No. 23: Aerotriangulation Strip Adjustment*. By M. Keller and G. O. Towinski. Pp. 24. 80 cents. (Washington, D.C.: Government Printing Office, 1964 and 1965.) [26]
- Chicago Natural History Museum. *Fieldiana: Anthropology*. Vol. 55: *Chapters in the Prehistory of Eastern Arizona*. By Paul S. Martin, John B. Rinaldo, William A. Longacre, Leslie G. Freeman, Jr., James A. Brown, Richard H. Healy and M. B. Cooley. Appendices by Hugh C. Outler and S. F. F. Seaberg. Pp. 261. 6 dollars. *Fieldiana: Botany*. Vol. 51, No. 3: *Agriculture, Tehuacan Valley*. By C. Harle Smith, Jr. Pp. 49-100. 1.50 dollars. Vol. 51, No. 4: *Flora, Tehuacan Valley*. By C. Harle Smith, Jr. Pp. 101-143. 1.50 dollars. *Fieldiana: Zoology*. Vol. 44, No. 21: *Relationships and Zoogeography of the Viperine Snakes (Family Viperidae)*. By Hymen Marx and George B. Rabb. Pp. 161-206. 1.75 dollars. (Chicago: Chicago Natural History Museum, 1965.) [26]
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- Commonwealth of Australia: Department of External Affairs. *ANARE Reports, Series A, Vol. 1: (Narrative)—The ANARE 1963 Expedition to Heard Island*. By G. M. Budd. Pp. 53. (Melbourne: Antarctic Division, Department of External Affairs, 1964.) [26]
- Smithsonian Miscellaneous Collections. Vol. 148, No. 2: *The Brachyopod Superfamily Stromatostomatidae*. By Richard R. Grand. Pp. v+102+24 plates. (Publication 4569.) (Washington, D.C.: Smithsonian Institution, 1965.) [26]
- United States Department of Commerce: National Bureau of Standards. *NBS-2: Thermal Properties of Aqueous Uni-univalent Electrolytes*. By Vivian Barfield Parker. Pp. v+66. (Washington, D.C.: Government Printing Office, 1965.) 45 cents. [26]
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- Records of the Australian Museum, Vol. 20, No. 11 (30th November, 1964): *Sex Determination of Aboriginal Gracils from Coastal New South Wales, Australia*. By S. L. Larnach and L. Freedman. Pp. 236-268+plates 23 and 24. (Sydney: The Australian Museum, 1965.) 4s. [26]
- Forest Research in India, 1960-61. Part 1: *The Forest Research Institute, Dehra Dun*. Pp. ii+118. (Delhi: Manager of Publications, 1964.) Rs. 2.80; 6s. 3d. [26]
- National Academy of Sciences—National Research Council. *Publication 1279: The Growth of U.S. Population—Analysis of the Problems and Recommendations for Research, Training, and Service*. Pp. vii+25. (Washington, D.C.: National Academy of Sciences—National Research Council, 1965.) 1.25 dollars. [26]
- Australia. *Commonwealth Scientific and Industrial Research Organization. Annual Report of the Animal Research Laboratories, 1963-64*. (Comprising Reports of: Division of Animal Genetics; Division of Animal Health; and the Division of Animal Physiology.) Pp. 145. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1964.) [26]
- U.S. Department of Health, Education, and Welfare: Public Health Service. *Publication No. 999-WP-18: Influence on Impoundments on Water Quality—a Review of Literature and Statement of Research Needs*. By James M. Symons, Samuel B. Weibel and Gordon G. Robcock. (Environmental Health Series—Water Supply and Pollution Control.) Pp. vii+78. (Cincinnati, Ohio: U.S. Department of Health, Education, and Welfare, Division of Water Supply and Pollution Control, 1964.) [26]
- Durban Museum Novitates, Vol. 7, Part 9 (31st March, 1965): *A Catalogue of Birds of the South African Sub-Region. Part 1: Families Sphenocidae—Burhinidae*. By P. A. Clancy. Pp. 201-304. (Durban: Durban Museum, 1965.) R. 1.50. [26]
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- World Health Organization. *Technical Report Series. No. 302: Nutrition in Pregnancy and Lactation—Report of a WHO Expert Committee*. Pp. 64. 2 \$w. francs; 5s.; 1 dollar. No. 303: *Mechanism of Action of Sex Hormones and Analogous Substances—Report of a WHO Scientific Group*. Pp. 34. 2 \$w. francs; 5s. 6d.; 0.60 dollars. No. 304: *Neuroendocrinology and Reproduction in the Human—Report of a WHO Scientific Group*. Pp. 19. 2 \$w. francs; 5s. 6d.; 0.60 dollars. No. 306: *Public Health and the Medical Use of Ionizing Radiation—Fifth Report of the WHO Expert Committee on Radiation*. Pp. 41. 2 \$w. francs; 5s.; 1 dollar. (Geneva: World Health Organization; London: H.M. Stationery Office, 1965.) [26]

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## Other Countries

- Australia. *Geological Survey of New South Wales. Report No. 19: Short Notes on Various Building Stone Deposits in New South Wales*. By O. L. Adamson, G. R. Walls and G. A. Frende. Pp. 30. Report No. 21: *Coastal Water Supplies—Nambucca*. By B. J. Griffin. *Underground Water—Wuyong Shire*. By B. J. Griffin. *Underground Water in the Omelette—Merriwa Area*. By J. Ringa. Pp. 16. (Sydney: Department of Mines, 1964.) [26]
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## ORGANIZATION OF BUILDING RESEARCH

THE Trend Committee in its report on the organization of civil science had some reservations about the future position of the Building Research Station and suggested that some consideration should be given to its transfer to the Ministry of Public Building and Works. Such a proposal has been strongly questioned, however, and in the meantime, with the disappearance of the Department of Scientific and Industrial Research, under the Science and Technology Act, the Building Research Station has been transferred to the Ministry of Technology. Discussion on this point alone would give special interest to Dr. M. S. Kaplan's Unwin Memorial Lecture to the Institution of Civil Engineers, on the pattern of building and civil engineering research in Britain last April. Dr. Kaplan, however, surveyed a much wider field and began by pointing out that present-day expenditure on building and civil engineering in Britain has been estimated at more than £3,000 million. Of this about £2,000 million is being spent on new works, and £1,000 million on maintenance. The industry employs some 1.5 million people, or about 6 per cent of the working population.

In a review of Government institutions concerned directly or indirectly with research, two points stand out. The Chief Scientific Adviser's Division of the Ministry of Works, set up in 1944, was transferred to the Department of Scientific and Industrial Research in April 1950, and the Ministry's own Advisory Council on Building Research and Development, which was established in 1947, was disbanded in 1959 and its responsibility passed to the Building Research Board. Only in 1962 was there established a Directorate-General of Research and Development at the Ministry of Public Building and Works with four directorates: (1) of development, to promote new and more efficient ways of building; (2) of building management, to improve management techniques and more effective co-ordination throughout the building process; (3) of research and information, to formulate research needs in conjunction with the industry and the professions, to improve the technical information services and to examine the pattern of education in the construction industries and promoting movements; (4) of economic intelligence, to collect, interpret and disseminate statistical and economic information. Since then, in March 1964, the Government has established the National Building Agency to help the industry to deal with the great upsurge of demand now facing it and to encourage methods of increasing productivity, both in traditional and industrial building. The Agency is financed by the Government but is independent of direct Government control.

The Agency is aware of the dangers of duplication and overlapping, and works in close liaison with various Government departments and inter-departmental working parties to avoid these difficulties. Dr. Kaplan points out that it is not at all clear how the activities of the Association and of the Director-General of Research and Development and of the Building Research Station will develop in relation to each other. He believes that there is a danger of overlapping functions which could be serious because

of the shortage of qualified people. More serious, however, is the need to make a much greater use of the universities. The need for more university research in this field is two-fold. First, a greatly expanded effort in building and civil engineering research will call for many more architects, engineers and others with training in research: at present the number of research students in this field is very small. Secondly, he emphasizes that building and civil engineering should make much more use of the expertise and knowledge of university teachers, and doubts whether full advantage can be taken of the research facilities and skills available in British universities on the 'stop and go' basis which now exists. The establishment of more research units like the Pilkington Research Unit at the University of Liverpool, which is studying environmental requirements of buildings, and the Hospital Engineering Research Unit at the University of Glasgow, may well be the answer.

While there were several Research Associations carrying out research of interest to the construction industry, particularly as regards constructional materials, and others dealing with heating and ventilating, welding, water and hydro-mechanics, the Civil Engineering Research Council was established in November 1960, and Dr. Kaplan was invited to become its first director of research. Following an application to the Department of Scientific and Industrial Research the Civil Engineering Research Council had been offered a grant for an initial period of three years from January 1, 1963. This was conditional on the Council raising an annual income of not less than £40,000, when the Department would make a pound-per-pound grant up to £80,000 per annum. It was agreed to adopt a constitution based on the model for the Research Associations, and the Civil Engineering Research Association came into being on February 3, 1964, taking over the activities of the Civil Engineering Research Council. At present the Research Association is without laboratories of its own, and is using facilities provided by Government research establishments, other research associations, the universities and industry. Besides identifying research projects the Association receives and considers proposals for research from professional institutions, universities, consulting engineers, contractors, suppliers of materials, Government departments and other organizations connected with the industry. Technical advisory panels have been formed, covering particular aspects of civil engineering. The function of these panels is to examine proposals for research and to consider reports on research in progress. Not the least valuable result of the panels' work is that they help research workers and others to become more aware of the research needs of the industry. The income of £158,000 for 1964 exceeded the target figure for that year by more than £38,000, and some 50 approved research projects are being carried out at the Building Research Station, the Road Research Laboratory, and the Hydraulics Research Station, by the Cement and Concrete Association, and in the universities and laboratories of other Research Associations. Dr. Kaplan, however, emphasizes the importance



of disseminating as rapidly and effectively as possible the results of research; shortcomings in this respect could be as serious as shortcomings in the prosecution of research itself. He also recognizes, however, that the construction industry and associated professions must be prepared to study and make use of the results of research.

Dr. Kaplan does not regard the present scale of building and civil engineering research as satisfactory. No reliable and comprehensive information exists at present, but he states that the survey now being carried out by the Ministry of Public Building and Works covers more than two thousand organizations and reveals the existence of more than eight hundred research and development bodies. In May 1962 the Minister of Public Building and Works, Mr. G. Rippon, suggested to the building industry the establishment of a Building Research and Information Association, to be managed and financed jointly by industry and Government. While the Government was willing to play its part in increasing the total effort in this field, Mr. Rippon believed that successful results could not be obtained unless the industry itself played a greater part in both fields of activity. Accordingly, his proposal was that the Government should contribute up to £150,000 a year for three years, if the industry matched this contribution, and conditional on a minimum contribution from the industry of £50,000 per year. It was hoped that the Association's budget would build up to at least £500,000 in five years. In September 1963 the Minister met a deputation of organizations and professions in the building and associated industries to discuss the proposal, which was accepted in principle. It was agreed to set up a working party with Mr. D. Woodbine-Parish as chairman to examine research and information services, the scope of any association to achieve improvements, its constitution and the arrangements for financing it. The working party reported to the Minister in December 1963, but found little support from the industry for the suggestion that a Development Council should be established with the right to impose a statutory levy for research and information.

This working party attempted to assess the scale of research and development and concluded that, excluding the production of materials and components, the annual expenditure on research and development advisory services was about £3 million a year, or 0.2 per cent of the net output of the industry. It was difficult to find a comparable figure for research and development on materials and components used in construction, the annual output of which is estimated at about a further £150 million a year. Annual expenditure on research in the glass, cement and ceramics (including refractories) industries is about £2 million, and in the absence of any sufficient analysis of expenditure in the metals, plastics, paint, and other materials which contribute to construction, the working party suggested that the relevant figure was of the order of £5 million, which would put the total expenditure on research and development for the construction industry and its suppliers at about £10 million a year, or 0.3 per cent of the estimated annual output of about £3,000 million. This supported the opinion of the Advisory Council of Scientific Policy in its report for 1959-60 that expenditure on research in this industry compares unfavourably with that in other industries. At a meeting in July 1964, Mr. Rippon proposed the establishment of a Construction Industry Research and Information Commission, broadly on the lines which had been suggested in the Woodbine-Parish Report. This Commission

would be appointed by the Minister after consultation with the industry, with the main functions of surveying the industry's research and information needs and capacity, advising the Minister on the income to be raised by levy and apportioning the financial resources between the proposed Building Research and Information Association, the Civil Engineering Research Council, and other relevant research organizations. A first task of the Commission would be to advise on a statutory levy on the industry. At this meeting Sir Herbert Manzoni, chairman of the Civil Engineering Research Association, submitted proposals on behalf of the Federation of Civil Engineering Contractors, the Association of Consulting Engineers and the Institution of Civil Engineers. These proposals indicated that further developments should be primarily based on the industry, and informal discussions had revealed the possibility of establishing one research association to cover both building and civil engineering. The Minister agreed to consider these proposals and a further memorandum was submitted by Sir Herbert Manzoni in September 1964, suggesting the establishment of a Building and Civil Engineering Research Association, financed jointly by Government and industry.

In view of the General Election and change in Government, discussions with the new Minister, Mr. C. Pannell, were delayed until December 1964, and as a result of further discussions, proposals were submitted to the Minister on March 10, 1965, suggesting that the industry should raise £170,000 in the first year, rising to £250,000 in the third year, to finance the proposed Research Association. A study of detailed arrangements to obtain these funds and assure continuity of support was being made, and it was anticipated that the Government would make a pound-for-pound grant so that the initial annual income of the Association would be about £350,000, rising to some £500,000 in the third year. This Research Association would develop an information and advisory service with a central organization, together with a regional organization spread over England, Scotland and Wales. Dr. Kaplan emphasizes that final decisions on these proposals, which have now been before successive Governments for almost two years, should be reached in the near future. Almost half the total output of the construction industry is paid for by central and local government and other public bodies, for houses, schools, hospitals, roads, power stations, harbours and so on, and the Government responsibility is very clear. He does not agree, however, that research in this field should be entirely financed and controlled by the Government, although at present about 90 per cent of the cost of the Building Research Station is paid for by the Government. He rightly emphasizes that, as the Building Research Board has pointed out, the prime criterion for the selection of research projects should be the need for the knowledge that they are designed to provide rather than the prospects of external financial support. The Building Research Board is obviously concerned that too great an emphasis on obtaining finance from industry might lead to activities being orientated excessively towards immediate needs. Dr. Kaplan seems to doubt whether larger financial contributions towards research at the Building Research Station itself are likely to be forthcoming from the construction industry. He insists that the Building and Civil Engineering Research Association, if established, will require its own experimental facilities, although the building of a further laboratory might not be justified. He suggests that the Minister of Technology should



consider the possibility of the Building Research Station's facilities being jointly financed and controlled by industry and Government under the aegis of the proposed Building and Civil Engineering Research Association. If such arrangements could be worked out they could well be in the best interests of both Government and industry. Examination of the research projects at present being sponsored by the Association shows that most of them are in the national as well as in the industry's interest and that many are of a long-term, basic character. This experience is confirmed by that of the Fire Research Organization, which is operated and financed jointly by the Government and the fire insurance companies. Finally, he points out that the Royal Institute of British Architects supported the establishment of the Building and Civil Engineering Research Association on the understanding that it would not prejudice the setting up later of a Research Council to study the problems of the built environment and covering the whole field of physical planning, architecture and construction. In his Tavistock Lecture for 1964, Lord Holford, in urging the formation of such a Research Council, suggests that it would draw its membership, to begin with, from the existing research organizations in the construction industry and from the associated professions, including those concerned with urban studies and with the use and development of land. It would be related to this whole field of activities just as other Research Councils are to agriculture and medicine and now also natural resources. It should not be divorced from industry and should be connected with all the sciences in which advances are being made affecting our knowledge of what to build and how to build it. He visualized such a Research Council as creating a demand for new thinking at the same time as it encourages and co-ordinates an increasing supply.

Lord Holford recognized that the relations of any such council would need careful definition if it were decided, as the Government has now done, on the recommendation of the Heyworth Committee, to set up a Research Council for the social sciences as a whole. Clearly there could be some overlap, for among the fields in which the Heyworth Committee sees a need for more research are included problems not only of regional development but also of land use, the social effects of different forms of environmental planning and design, and of urban redevelopment to form satisfactory social settings. Nevertheless, Dr. Kaplan questions whether the idea of such a Council, as Lord Holford proposes, is too broad to be practicable. It would certainly seem that its functions would inevitably overlap with those of the Social Science Research Council, now to be established. It might well be that the more limited project of a Building and Civil Engineering Research Association would best meet the present need to stimulate building research and to handle the special difficulties presented by the position of the Building Research Station, provided effective co-ordination is ensured between the various Research Councils and other organizations.

The Heyworth Committee in its report rejected the idea of a separate Research Council for the built environment, but the Government in turn did not consider that the proposal of the Heyworth Committee for a joint board from the Research Councils concerned with urban planning matters would be satisfactory. The question of how best to meet the needs of research in the field of urban planning in the broadest sense is thus still under consideration.

## KEPLER'S WORKS

Johannes Kepler

Gesammelte Werke. Band 8: *Mysterium Cosmographicum*; Editio altera cum notis. De Cometis. *Hyperaspistes*. Pp. 516. Geheftet 56 D.M.; In Halbpergament 65 D.M. Band 9: *Mathematische Schriften*. Pp. 560. 48 D.M. Herausgegeben und bearbeitet von Franz Hammer. (München: C. H. Beck'sche Verlagsbuchhandlung, 1963 and 1960, respectively.)

APART from the importance of his works as a monument to a great man of science who was also deeply involved in the religious and political struggles of his time, Kepler's search for a synthesis of mechanical and aesthetic explanations of Nature, carried out with a rigorous regard for observational and mathematical facts, may arouse a sympathetic response from modern scientific readers accustomed to Eddington and *SU*, as well as from professional historians. While several of his main works have been translated into German, only some rather brief extracts are available in English, and the complete eight-volume edition in the original brought out by Ch. Frisch between 1858 and 1871 is virtually unobtainable outside long-established libraries. The sumptuous and generously annotated modern edition of the *Collected Works* in 22 volumes, begun in the 1930's by Walther von Dyck and Max Casper and continued after their deaths by Franz Hammer, is therefore very welcome. The present volumes are the fourteenth and fifteenth in the series to have appeared; Volumes 13-18, containing the *Letters*, came out between 1945 and 1959.

Volume 8 contains three works from Kepler's late period. The second (1621) edition of the *Mysterium Cosmographicum*, the youthful work in which he had projected his plan of the five regular solids a quarter-century previously, faced Kepler with the problem of presenting a largely obsolete work which nevertheless embodied the inspiration that had led him to his great discoveries and could, in his mind, still have some validity as a rough framework. He published it unchanged, but with the addition of extensive commentaries after each chapter. The *De Cometis Libelli Tres* (1619) includes earlier work inspired by the apparition of Halley's comet in 1607, which could not be published until public interest had been re-awakened by the famous three comets of 1618. The book describes all four comets in detail, giving Kepler's ideas on their orbits, physical nature and augural significance, notably his suggestion that comets travelled at a non-uniform rate along linear trajectories distorted into apparent curves by the orbital motion of the Earth, and his theory of the expulsion of the material of cometary tails by solar radiation pressure. The third work, *Tychonis Brahe Dani Hyperaspistes*, or "Shield-bearer of Tycho Brahe the Dane" (1625), is an undignified polemic against Scipio Chiaramonti, who had written a treatise *Anti-Tycho* attempting to defend Aristotelian cosmology against Tycho Brahe's criticisms, notably his conclusion from parallax measurements that comets are further off than the Moon and that there could be no solid crystal spheres. An appendix, available in English in *The Controversy on the Comets of 1618* (translated by Stillman Drake and C. D. O'Malley, Philadelphia 1960), sets the record straight with regard to the references made to Tycho Brahe and to Kepler himself in this controversy by Grassi and Galileo (who was no admirer of Tycho Brahe and had quoted Chiaramonti with approval in the *Saggiatore*).

The mathematical works in Volume 9 are *Stereometria Doliorum* (1615), *Messeturus Archimedis* (1616), *Chilias Logarithmorum* (1624) and *Supplementum Chiliadis Logarithmorum* (1625). The *Stereometria*, devoted to the Archimedean problem of determining the volumes of solids of revolution and applications to wine-barrels, is a famous landmark in the history of integration; the results are completed, as well as popularized, in the *Messeturus*,

which has the alternative title of *Oesterreichisches Wein-Visioner Buchlein*.

Kepler's appreciation of the significance of logarithms was delayed for several years by the lack of an opportunity to study Napier's *Descriptio* (published in 1614) at leisure. Afterwards he became an enthusiastic advocate of the new method and wrote his two works on logarithms (which should have been a single book but became accidentally separated) for the two-fold purpose of convincing his colleagues by an independent derivation and of computing a new table with 1,000 values of a uniformly changing numerical argument (as opposed to Napier's table of log sine, etc., for a uniformly changing angle) so as to facilitate their use for non-trigonometric calculations. Kepler's logarithms differ slightly from Napier's and are more accurate; this is due to a small computational error by Napier and not to a mathematical difference between the two derivations as is here claimed by the editor, apparently in accordance with Kepler's own opinion. Like Napier's, they have the very inconvenient property that antilog 0 is  $10^7$  instead of 1, although this disadvantage had in the meantime been removed (at Napier's own suggestion) in the development of dekadische logarithms by Henry Briggs and also in the independent derivation of natural logarithms by Jost Bürgi. Kepler's use of his quasi-Napierian logarithms for the Rudolphine tables unnaturally postponed their obsolescence for a century.

The editor's extended commentaries and notes at the end of each volume provide an excellent account of the history and significance of the preceding works and really make them accessible to the modern reader, especially in Volume 9, in which Kepler's mathematical results are expressed in modern notation. The result is a fine piece of thorough, painstaking scholarship and a notable contribution to the history of science.

B. PAGEL

## DIFFERENTIAL AND INTEGRAL EQUATIONS

### Asymptotic Solutions of Differential Equations and Their Applications

Edited by Calvin H. Wilcox. (Proceedings of a Symposium conducted by the Mathematics Research Center, United States Army, at the University of Wisconsin, Madison, May 4-6, 1964.) Pp. x+249. (New York and London: John Wiley and Sons, Inc., 1964.) 38s.

### Nonlinear Integral Equations

Edited by P. M. Anselone. (Proceedings of an Advanced Seminar conducted by the Mathematics Research Center, United States Army, at the University of Wisconsin, Madison, April 22-24, 1963.) Pp. xii+378. (Madison: University of Wisconsin Press, 1964.) 6.50 dollars.

### Periodic Differential Equations

An Introduction to Mathieu Lamé, and Allied Functions. By F. M. Arscott. (International Series of Monographs in Pure and Applied Mathematics, Vol. 66.) Pp. 284. (Oxford, London and New York: Pergamon Press, 1964.) 60s.

### Operational Calculus based on the Two-sided Laplace Integral

By Balth. van der Pol and H. Brömmer. Pp. xiii+415. (Cambridge: At the University Press, 1964.) 25s.

THAT most differential equations do not possess exact analytical solutions is by no means an unmitigated disaster, since physics often begins where mathematics ends, as demonstrated, for example, by asymptotic approximations in which the dominant term represents a wave or boundary-layer effect which would otherwise be completely obscured. A veritable feast of asymptotic mathematics, dedicated to Prof. R. E. Langer on the occasion of his retirement as first director of the Mathe-

matics Research Center at Wisconsin, has now been prepared as a book by Langer's younger colleague, Prof. Calvin H. Wilcox, utilizing the services of ten leading experts who have contributed as follows: asymptotic expansions for ordinary differential equations, by W. Wasow; solvable related equations pertaining to turning point problems, by H. L. Turrittin; asymptotic methods for the solution of dispersive hyperbolic equations, by R. M. Lewis; asymptotic solutions and indefinite boundary value problems, by R. W. McKelvey; some examples of asymptotic problems in mathematical physics, by C. C. Lin; on the problem of turning points for systems of linear ordinary differential equations of higher orders, by Y. Sibuya; error bounds for asymptotic expansions, by F. W. J. Olver; asymptotic solutions of elastic shell problems, by R. A. Clark; the integral equations of asymptotic theory, by A. Erdélyi; application of Langer's theory of turning points to diffraction problems, by N. D. Kazarinoff. As might be expected, the collection is very heterogeneous, embracing highly specialized reports on present-day research, expository articles of more general interest, and papers concerned with physical applications. The editor would have done well to commission a fairly expansive introduction by some imaginative mathematician capable of drawing all the threads together and stimulating the imagination, for example, on the lines of the American Mathematical Society 1954 lecture by K. O. Friedrichs. Even so, nobody even mildly interested in asymptotics could find this book dispensable. An excellent photograph of Prof. Langer is included.

A somewhat similar collection of articles on *Nonlinear Integral Equations*, originating from an advanced seminar at the same Center, has been prepared by Dr. P. M. Anselone. The contributions are as follows: direct iteration, existence and uniqueness, by A. Wouk; applications of the fixed-point theorem from Russian mathematicians, by A. P. Thielman; equations in partially ordered spaces, by H. F. Bueckner; Newton's method and variations, by R. H. Moore; on nonlinear integral equations of the Hammerstein type, by O. L. Dolph and G. J. Minty; variational methods for nonlinear integral equations, by L. B. Rall; problems in qualitative behaviour of solutions of nonlinear Volterra equations, by J. A. Nohel; the numerical solution of nonlinear integral equations and related topics, by B. Noble; some nonlinear integral equations of hydrodynamics, by D. H. Hyers; nonlinear integral equations of radiative transfer, by T. W. Mulliken. Most of the treatments plunge straight into function space in a difficult attempt to establish existence and uniqueness theorems appertaining to nonlinear analysis. However, even where positive conclusions have been achieved, there still remains a considerable gap between the theory and its applications to the concrete equations of applied mathematics. A different note is struck in the long and well-balanced article by Dr. B. Noble, who provides a thorough account of his topic amply illustrated at each stage by examples taken from life. The two final papers deal in a recondite manner with areas of physics where nonlinear integral equations play an indispensable part. Whether this book succeeds in its professed aim of inaugurating the title subject as a viable autonomous discipline is open to question, but it certainly provides a very fair picture of present-day activities in a central field of analysis.

Mathieu functions were first introduced to the British mathematical public by Whittaker and Watson. Then came the highly readable 1947 monograph by McLachlan, who presented a wealth of new results and an effective symbolism. Now at last, by adequately exploiting the fundamental theorems of Floquet and Ince before actually constructing solutions, Prof. F. M. Arscott has provided a coherent modern account of Mathieu's equation, concentrating largely on essentials but always clearly indicating where further details can be pursued. This work occupies the first half of his book, and paves the way for

an examination of Hill's and Lamé's equation, and allied equations, in the second half. It is difficult to think of any alternative source in English which renders this somewhat recalcitrant material so readily accessible. The author in his preface suggests that Lamé's and allied functions might afford a superior means of solving the boundary- and initial-value problems of mathematical physics than computer supplied numerical solutions. Certainly no mathematician would attempt a numerical solution if moderately tractable analytical machinery were available, but Arscoott's own model example on pp. 228 *et seq.* shows how far this is from being the case for even the simplest problems relating to ellipsoidal domains. If a practical motivation for studying the higher functions must be cited, it ought to be that their Frobenius or asymptotic expansions reveal the approximate structure of potentials and waves at important special neighbourhoods beyond numerical scope.

The book by van der Pol (now deceased) and Brammer is the second impression of the 1959 edition of their well-known text. Its success stems not merely from their treatment of the operational calculus, but also from their refreshing approach to various important modern infinitesimal processes. For example, their remarks on Stieltjes integration make clear just why such an integral should be introduced and what kind of part it could usefully play. An appreciation of this factor is, for most students, the critical step on learning any new technique. Although primarily addressed to applied mathematicians, and mathematically minded physicists and engineers, it contains much of interest to mathematical analysts. Historical aspects are not overlooked, and it comes as no surprise to find that Dirac's delta function had been extensively used by Oliver Heaviside. A confusing notational mishap occurs on p. 7, where the same symbol, used in different senses, appears in both the Fourier series and Fourier integral.

M. A. JASWON

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(London and New York: Pergamon Press, 1964 and 1965.)

THESE volumes of Russian tables, which are supplied with an English translation of the introductory matter, were printed in Poland. The pages are generally clean, without too many rules, and the old-style fount is pleasant and legible, though the slightly exaggerated "heads and tails" occasionally make for a crowded appearance when the setting is close. Differences are not printed.

Volumes 23 and 27 deal with the error integral for a complex argument in polar form,  $z = \rho \exp(i\theta)$ , in each case giving the real and imaginary parts of the function

at an opening to five decimals. In Volume 23 the range of  $\theta$  is  $45^\circ$  ( $0^\circ.3125$ )  $48^\circ.75$  ( $0^\circ.625$ )  $55^\circ$  ( $1^\circ.25$ )  $65^\circ$  ( $2^\circ.5$ )  $90^\circ$  and the range of  $\rho$  depends on  $\theta$  with a lower limit of 0, 0.5, 1, 1.5, 2, 3.5 or 4 and an upper limit of 3, 4 or 5, at intervals of 0.001 or 0.01. A subsidiary table gives  $F$  for real  $z$  ( $\theta = 0$ ),  $\rho = 0$  (0.001) 10. Volume 27 was compiled in connexion with a problem on the propagation of radio waves, and supplies information for the ranges:

$$\theta = 0^\circ, \rho = 0 \text{ (0.001) } 2 \text{ (0.01) } 10;$$

$$\theta = 0 \text{ (2.5}^\circ) 30^\circ \text{ (1.25}^\circ) 35^\circ \text{ (0.625}^\circ) 45^\circ,$$

where  $\rho$  has an upper limit 5, a lower limit, depending on  $\theta$ , between 0 and 3.5, and intervals 0.01, 0.001 or 0.0002. Schematic representation of the argument range and functional relations provides assistance in reading the tables and in extending the range.

The incomplete elliptic integral of the third kind:

$$\Pi(n, k^2, \varphi) = \int_0^\varphi (1+n \sin^2 x)^{-1} (1-k^2 \sin^2 x)^{-1/2} dx$$

depends on three parameters and is correspondingly tiresome to compute and to tabulate. If we are prepared to accept a fairly crude interval for  $\varphi$ , then Volume 37 gives good information with seven significant figures for the ranges  $n = -1$  (0.005)  $-0.98$  (0.01)  $-0.94$  (0.02)  $-0.88$  (0.03)  $-0.85$  (0.05) 0,  $k^2 = 0$  (0.01) 1.0,  $\varphi = 0^\circ$  ( $1^\circ$ )  $90^\circ$ .

When  $n = 0$ , this is a table of the incomplete integral of the first kind,  $F(k^2, \varphi)$ . Subsidiary tables give:

$$E(k^2, \varphi) = \int_0^\varphi (1 - k^2 \sin^2 x) dx$$

for  $k^2 = 0$  (0.01) 1,  $\varphi = 0^\circ$  ( $1^\circ$ )  $90^\circ$ , and also various elementary integrals which occur as coefficients in expansions of the elliptic integrals. The second part of the table (not yet published) is to give similar information about  $\Pi(n, k^2, \varphi)$  for positive  $n$  in the range from 0 to 100, and the same ranges of  $k^2$  and  $\varphi$ .

The Legendre function  $P_n(x)$  with  $n = -\frac{1}{2} + i\tau$  occurs in potential problems when the boundary is a bowl. In Part 1 (Volume 22) tabulation was effected for the range

$$0 \leq \tau \leq 50, -0.9 \leq x \leq 0.9.$$

The present Volume 38 is concerned with the range  $x > 1$ , and in detail gives seven decimal places for:

$$x = 1.1 \text{ (0.1) } 2.0 \text{ (0.2) } 5.0 \text{ (0.5) } 10.0 \text{ (10) } 60,$$

$$\tau = 0 \text{ (0.01) } 50.$$

These volumes are, of course, highly specialized, but they cope with functions the published tabulation of which has hitherto been inadequate or non-existent. The efficient librarian, if he cannot afford the volumes, will at least make the appropriate notes in the Fletcher, Miller, Rosenhead and Comrie *Index*. T. A. A. BROADBENT

## RUSSIAN UNIVERSITY COURSE IN THERMODYNAMICS

### Thermodynamics

By Prof. I. P. Bazarov. Translated by F. Immirzi. Translation edited by A. E. J. Hayes. Pp. xvi+287. (London and New York: Pergamon Press, 1964.) 50s. net.

**THERMODYNAMICS**, which is by the professor of statistical physics at the Moscow State University, is the first part (it is said in the preface) of a course on thermodynamics and statistical physics as given in the physical and physico-mathematical faculties of the Soviet universities. As such it is emphatically a physicists', rather than a chemists' or engineers' thermodynamics, and it is in the fine tradition of physical thermodynamics and statistics exemplified by an earlier Russian text translated into English, the well-known one by Landau and Lifshitz.

As also in that invaluable *Statistical Physics*, the illumination of thermodynamics by use of problems is

one of the most important features of the present volume. There are altogether a hundred and twenty, many of them extremely interesting and ingenious. For example, in the chapter on the Second Law the student has to explain Wien's light valve paradox, and also the Chinese 'ducking toy', both of which seem to imply perpetual motion of the second kind. Problems of this sort—and there are many such—are of great value in making the student think, and that is something of far greater importance than the attainment of a mere dexterity.

However, I believe that some of these problems, in the abbreviated forms in which they are presented, are far too difficult, and also that one or two of the author's solutions (as given in a fifty-four page appendix) are a little tendentious. For example, his solution to Problem 39 involves some special pleading in order to obtain what he regards as a purely thermodynamic answer to Gibbs's paradox.

One other defect in the book (as it seems to me) is the occasional intrusion of political theory. Thus in the introduction it is asserted that the law of conservation of energy "is indissolubly bound with the dialectic-materialist ideology". Again, dealing with the Second Law, certain bourgeois scientists—notably Clausius and Jeans—are severely taken to task for having developed the notion of the universe's heat death. This, it is said, "leads directly to religious superstition—the existence of God".

Yet these ideological bows having been made, the book provides a quite fascinating discussion of physical thermodynamics and it does so moreover with a happy recognition of the subjects' truly international character.

The treatment of the Second Law is very detailed and is based on Carathéodory's method, but also contains much that is original and perceptive. The author further discusses the concept of negative absolute temperatures (and the bearing of this concept on the conventional formulation of the law) arising from Purcell and Pounds's experiments on the reversal of nuclear spins in crystals of lithium fluoride. Other important sections are concerned with the thermodynamics of radiation and plasma, of dielectrics and magnetic substances, of surface phenomena and phase transitions. Nernst's heat theorem is presented and there is also an outline of Onsager's theory and its application to the thermo-electric effect.

The translator has had a difficult task and this has mostly been well achieved. Yet some of the arguments are not so clear as they should be and there are certain ambiguities. To what extent these are due to the original, or to the difficulties of conveying into another language the subtleties of thermodynamic discussion, it is hard to say. Thus, the book in translation is to be recommended not so much for definitive statement as for its power of conveying a real sense of interest in its subject-matter and for its originality. It is certainly a book which I wish to possess.

K. G. DENBIGH

## ULTRASOUND FOR THE CHEMIST AND BIOCHEMIST

### Ultrasound

*Physical, Chemical, and Biological Effects.* By Isaak Efimovich El'piner. Authorized translation from the Russian. Pp. viii + 371. (New York: Consultants Bureau, 1964.) 22.50 dollars.

IT is a major difficulty, for those embarking on any research work in which ultrasound is used as a tool rather than as the subject of study, that there is a dearth of suitable text-books. Until recently, the books available were almost all written from the point of view of the physicist or engineer, or occasionally from the point of view of the specialist in physical medicine.

Now books are beginning to appear which are aimed at the needs of other users of ultrasound, and *Ultrasound:*

*Physical, Chemical, and Biological Effects* supplies a very genuine need. It is translated from the Russian by F. L. Sinclair and is doubly welcome for the large amount of Russian work which it summarizes.

Dr. El'piner has clearly been an important member of a large group of research workers whose work, owing to the curse of Babel, is not normally known in Britain or the United States. However, a warning must be given that the title of the book as it appears in English is misleading.

In a book of more than 300 pages, only nine are devoted to the physics of the subject. This is, of course, a brief summary of the basic principles only. The next twenty pages are devoted to the physical aspects of cavitation and give a good description of shock-wave production. There is then a valuable chapter on the neglected subject of ultrasonic luminescence.

After this uneven coverage of the physical effects, the book launches into nearly 200 pages of description of what is clearly Dr. El'piner's main field of work, the action of ultrasound on organic chemical substances whether synthetic or natural. A very large proportion of the work described is Russian and much of this is Dr. El'piner's work.

It is quite impossible to summarize the contents of these chapters, just as it is impossible to summarize a dictionary. The book is really a work of reference that will prove invaluable to anyone intending to embark on comparable work, but it inevitably is very heavy going as reading matter. The complexity of the subject is due to the large number of factors that influence the action of ultrasound in a single chemical compound. Naturally enough, the normal physical parameters of ultrasound, intensity, frequency and duration, have to be supplemented by temperature and pH that determine most chemical activity. What is less obvious is that the nature of the dissolved gas and the pressure in the irradiation chamber are of even greater importance, while some substances have a catalytic action even in trace amounts.

The possible permutations of all these parameters with the whole range of synthetic and natural organic substances produce an almost limitless experimental field. For good measure, in some cases different workers have obtained different results in what were ostensibly similar circumstances. Unfortunately, no pattern appears from the mass of recorded observations. Sometimes a dissolved gas will accelerate a chemical change, sometimes the same gas will inhibit the change. Sometimes ultrasound produces polymerization, but much more often it depolymerizes. Rarely is the chemical change produced freely enough to tempt the chemical engineer to consider its commercial exploitation—though sometimes small amounts of compounds are obtained by ultrasound that defy other methods of 'obtention' (if one of the translator's favourite words may be borrowed).

The major criticism of this work is the inadequacy of the information regarding the irradiation methods used. In the biological fields the problem of intensity measurement has proved very severe in non-cavitating conditions. Radiation pressure measurements have now become almost universal in the medical field, but they cannot be used in cavitating fields with comparable success. For example, on p. 63 reference is made to irradiation at "frequency 600 kc, intensity 5 W/cm<sup>2</sup> of emitting surface, duration of irradiation 4 h". Lower on the same page it is implied that cavitation occurs in such circumstances. Most reference books quote Keesche as indicating that the threshold of cavitation at this frequency varies from about 50 W/cm<sup>2</sup> if water is not degassed, to about 100 W/cm<sup>2</sup> in degassed water.

Clearly the cavitation phenomenon, which seems to produce temperatures of 10,000° C momentarily as a cavity collapses, is of crucial importance in determining the chemical activity or inactivity of ultrasound. It would have been a much more valuable book had information been given not merely as to whether audible

cavitation was occurring but also what routine was used to check the intensity. It is common knowledge that standing waves in tanks make the level of ultrasonic intensity vary greatly with quite minor differences of depth of liquid. Where irradiation is maintained for hours at high intensity evaporation might well cause the actual intensity to vary over a wide range.

The final sixty pages summarize the published work in ultrasonic surgery and diagnosis. Here the Russian work appears to be much less in relation to the rest of Europe and the United States. Here again it is tantalizing to be shown ultrasonic tomograms produced by El'piner with no indication of the method of scanning by which they were obtained.

The whole effect of the book has been to intensify greatly my long-standing desire to visit the U.S.S.R. and study the work at close quarters. Many other readers will feel a similar need to obtain a fuller account of what is clearly very important work. DOUGLAS GORDON

## GEMMOLOGY

### Gem Testing

Seventh edition. By B. W. Anderson. Pp. 383. (London: Temple Press Books, Ltd., 1964.) 60s. net.

IT is seventy years since Sir Henry Miers, when an assistant in the Department of Mineralogy at the British Museum (Natural History), gave a short series of lectures in London on gemmology, as the scientific study of gemstones is now called, and it is nearly sixty years since the National Association of Goldsmiths set up an Education Committee to arrange lectures on the subject and to organize examinations. This Committee was reconstituted in 1931 as the Gemmological Association of Great Britain.

Since then gemmology has made steady progress in Britain and in many other countries. Great impetus was given to it by the introduction of synthetic rubies made by the Verneuil process in 1904, and it received another fillip by the arrival on the market of cultured pearls in 1921. Since then, many new synthetic stones have been manufactured, and also there are now grown in Japan the new 'Biwa' cultured pearls which have no nucleus of mother-of-pearl but instead only little stripes of pearl-secreting mantle. Fortunately, radiographs serve to distinguish these from natural pearls.

As each new synthetic stone or imitation appears, means must be found of detecting and identifying it. Successive editions of *Gem Testing* have kept pace with this demand. The new edition also announces new discoveries of natural stones—a new source of ruby in Tanganyika, and of emeralds in Rhodesia and Pakistan—as well as new developments in technique and apparatus.

Among synthetics the 'Chatham' emeralds receive more attention than formerly, and new arrivals are the 'Lechleitner emeralds', made in Innsbruck, in which a thin shell of emerald is made to grow on a seed crystal or cabochon of beryl of inferior colour. Here, as in many other cases, the study of the microscopic inclusions in the stones is a great help in identification. B. W. Anderson has also discovered a marked difference in transparency to ultra-violet rays between the 'Chatham' emeralds and natural stones which are opaque to all rays shorter than 3000 Å.

Fluorescence, excited by either long- or short-wave ultra-violet light, has been used for many years as an aid in gem testing. Several fresh examples of its usefulness are given in this edition, especially in the sections on diamond where the variable fluorescence of different kinds or colours of diamond is described. There are developments also in the use of fluorescence excited by X-rays, and this is coming into use for testing cultured pearls and seems to be replacing the endoscope which was much used

for the detection of cultured drilled pearls. Unfortunately Anderson reports that the endoscope is no longer being made.

In the earlier chapters, which deal in detail with apparatus and methods, new sections describe dispersion, the magnetic properties of gemstones, and methods of handling and housing gemstones. The last-mentioned subject is dealt with here for the first time in a new chapter that will prove very welcome to collectors.

Certainly, as Anderson warns in his introduction, the book provides no light reading, but the more one reads it the more one finds that is new and useful.

W. CAMPBELL SMITH

## METAZOAN EVOLUTION

Dynamics of Metazoan Evolution: The Origin of the Coelom and Segments

By R. B. Clark. Pp. x+313. (Oxford: Clarendon Press; London: Oxford University Press, 1964.) 48s. net.

*DYNAMICS of Metazoan Evolution* is concerned with the part which has been played in evolution by the mechanical needs of animals and with the importance of taking such functional considerations into account in the construction of phylogenies. Dr. R. B. Clark considers that the comparative morphology of structures which have been elaborated in solving mechanical problems during evolution has received inadequate attention and intends his book to remedy this. After considering theories which attempt to explain the evolution of the coelom and the segmentation of invertebrates, the variety of methods of movement and their underlying mechanism in acoelomates and coelomates are discussed.

An account of gastropod locomotion occurs in the chapter headed "The Acoelomate Condition" and the changes of *Metridium* and nematode locomotion are discussed in the chapter on "The Coelomate Condition". Such an argument is striking when taken out of context, but such treatment was found to be necessary in support of the author's argument that the solution of the same mechanical problem is frequently arrived at in the same way in widely different animals. This point has been made before, but in the present book it is backed by examples from a variety of phyla and is argued at length. In the next chapter Dr. Clark considers the septate condition and the refinements in the movement and locomotion of animals with hydraulically arranged muscular antagonism which this permits. Undulatory swimming is then considered and a brief account given of this form of locomotion in invertebrates and vertebrates. That segmentation evolved independently in the invertebrates and vertebrates and is not necessary for sinusoidal swimming is shown by the occurrence of such movements in non-segmented invertebrates. But in the chordates the action of the lateral musculature is increased in efficiency by division into segmental blocks. If this section is less satisfactory than its predecessors it probably arises from two causes. First, the author is well known for his researches on polychaetes which are, mechanically, a central group in the investigation of the 'hydraulic organism'. Secondly, in spite of the investigation that has been devoted to the locomotion of fishes, there is no entirely satisfactory analysis of the action of the longitudinally arranged muscles and their relation to the complex and variable pattern of myocommata found in these animals. If the chordates do not play a part in the main theme of the book comparable with that of the annelids, they serve to emphasize the point that the concept of metameric segmentation is imprecise and of little explanatory value.

In the final chapter the present theories of the phylogeny of the metazoa are exposed to criticism based on

the idea that any hypothetical form should be a viable animal conforming to the laws of mechanics. These do not change with the passage of time, however much physical and biotic factors of the environment may have altered during evolutionary history. The case is closely argued and, although the author does not hazard his own suggestion for a family tree, he wisely emphasizes that not only organisms but also phylogenies evolve with increasing knowledge and that all that is known and is relevant must be taken into account when constructing phylogenies rather than selected aspects of the biology of a group. The importance which he attaches to mechanics leads him to question the accepted view that polychaetes are nearer than oligochaetes to the ancestral protoannelid stock. Although this view may not gain immediate acceptance, it will certainly demand a fresh examination of all the evidence bearing on the problem.

An aspect of such major evolutionary steps as the acquisition of a coelom or metameric segmentation which is rarely considered is the selective advantages of an incompletely evolved state. It is easy to see how, once evolved, the coelomate, or pseudocoelomate, marks an advance on the acoelomate. Parenchyma which is slightly more fluid or which has a few cracks in it presumably enabled an ancestral form to move a little faster, to gather a little more food and to leave a few more progeny than one with a more viscous interior. Dr. Clark is well aware of this problem and touches on it here and there, but one would like to see this aspect of evolution carefully examined. In what field could this be better essayed than in that of mechanics? But one can scarcely complain of this as an omission from the present book, which leaves little to be desired in clarity and rigour as an example of functional morphology applied to phylogeny.

G. CHAPMAN

## CANCER RESEARCH

### Advances In Cancer Research

Vol. 8. Edited by Prof. Alexander Haddow and Sidney Weinhouse. Pp. viii+482. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 12s. 6d.

THERE are nine contributors to Volume 8 of *Advances in Cancer Research*. E. L. Wynder and D. Hoffman dedicate their masterpiece of reviews on experimental tobacco carcinogenesis (covering the past decade) to the American Cancer Society. Backed by more than 700 references and occupying about half the volume, we are instructed in the production of cigarettes through stages of tobacco curing, addition of humectants, seasoning and casing material. The tobacco products, fractions, and single components are then thoroughly analysed in a detailed search for carcinogens. A selective summary of experiments on the biological testing of these carcinogens on a variety of animals and birds extends in a concise tabular form occupying 37 pages.

There is no doubt as to the carcinogenic action of tobacco products, particularly smoke condensates, but with rare exceptions, however, passive inhalation of tobacco smoke has not produced true bronchogenic cancer in laboratory animals. This is not surprising as there is no epidemiological evidence that indirect inhalation of tobacco smoke contributes to the development of lung cancer (pathways other than the respiratory tract may convey carcinogens to the lungs).

In interpreting experimental findings the authors suggest that tobacco smoke condensates contain tumour initiators in the form of a variety of polynuclear and heterocyclic aromatic hydrocarbons which would be complete carcinogens if present in large concentrations. However, on chemical findings, smoke condensate contains

more tumour promoters than initiators and the promoting activity of these condensates is regarded as being greater than their initiating activity. Prophylaxis is aimed at reduction of harmful products rather than prevention through abstinence. A table full of hopeful suggestions includes measures aimed at reduction of total smoke condensate, and the reduction of all-important but somewhat neglected cilia-toxic agents.

In another fascinating chapter entitled "Carcinogenesis Related to Foods Contaminated by Processing and Fungal Metabolites", H. F. Kraybill and M. B. Shimkin deal mainly with two topics, trout hepatoma and mycotoxins. A marked increase in hepatomas unaccompanied by cirrhosis has coincided with advances in processing methods which have replaced wet by dry pelleted diets in trout hatcheries. The neutral lipid fractions from tumorigenic rations play the major aetiological part, but the authors cautiously include other possible factors including genetic predisposition, viral, bacterial and parasitic infections, ionizing radiation, defoliants, pesticides and fungal metabolites (some of the last-mentioned two are lipid-solvent soluble).

Mycotoxins are discussed in general and aflatoxins in particular. They are possibly the most potent hepatocarcinogens in a variety of animals and in some are associated with cirrhosis. Aflatoxins are derived from a fungus which may affect groundnuts during growth, gathering, or drying. There are no data available showing that this natural hazard affects man, nor is a screening test for aflatoxin or a metabolite of aflatoxin in the urine yet available. Lactones derived from the metabolites of other fungi and fungal metabolites from rice contaminated with *Penicillium islandicum* are carcinogenic in animals. How many of the quarter of a million or so fungal species in Nature will induce cancer?

In a review of nucleolar chromosomes (mainly in normal cells) M. J. Kopeć and G. M. Matheyko point out that there is no universally accepted interpretation of the structure or function of the nucleolus in the interphase nucleus and there are no clear differences, morphological or biochemical, between normal and malignant nucleoli. Nucleolar chromosomes, their nucleolar organizers, and the origin of nucleolar substance are considered in detail. Variations in puffing patterns in different cells and tissues, both natural and experimentally induced, are also described. The final third of the chapter summarizes present knowledge and contains speculations on the relationship of chromosomes, nucleoli, nuclei and other subcellular structures on the control of cellular differentiation which in turn hinges on the cancer problem.

The nuclear proteins of neoplastic cells are considered by H. Busch and W. J. Steele. The authors emphasize that the understanding of the chemistry of nuclear protein is in its infancy and the techniques for isolating nuclei from cells of tumours or normal tissues are not yet perfect. They describe the difficulties in procedures for isolating nucleoli, chromosomes, nuclear ribosomes and other nuclear components. About half the review concerns the proteins associated with DNA and in particular with the basic histone proteins of the deoxyribonucleoprotein complex the systematics of which are still somewhat confused. Techniques for the fractionation of histones are more advanced than methods for acidic nuclear proteins, but apart from *RP2-L*, a histone fraction in tumours of murine and human origin, there appears to be no evidence that there are differences between the histones of tumours and of other tissues.

In a beautifully illustrated chapter, A. F. Howatson gives a clear and concise account of the structure of tumour viruses of laboratory animals and birds and of those more immediately concerned with man (human papilloma, SV40, adeno-virus type 12). Advances in negative-staining techniques and contrast reversal photography permit direct comparison of electron micrographs with suitably orientated models. Most virus particles of a



particular species conform to a definite structural pattern, but aberrant forms are not unusual and there is still hope (in the case of mouse leukaemia virus) that 'tails' are not entirely artefactual. A scheme of classification based on symmetry, nucleic acid type, presence of envelope, and site of assembly of virus in the cell does not result in any clear-cut separation of oncogenic viruses from non-oncogenic viruses.

This volume maintains the high standard of this series. The summary of contents before each chapter is particularly helpful for rapidly obtaining information. The only adverse criticism is that there are a few spelling mistakes. I hope the word 'homograph' is not a new addition to transplantation terminology! F. C. CHESTERMAN

## SOIL SCIENCE

### Elements of Tropical Soil Science

By Dr. T. Eden. Second edition. Pp. ix+164. (London: Macmillan and Co., Ltd.; New York: St. Martin's Press, Inc., 1964.) 13s. 6d. net.

### Practical Soil Science

(Israel Program for Scientific Translations.) By N. N. Nikol'skii. Translated by Rivka Nadel. Pp. v+240. (Distributed by: Oldbourne Press, London; 1963.) 72s.

### The Physical Chemistry and Mineralogy of Soils

By C. E. Marshall. Vol. 1: Soil Materials. Pp. ix+388. (New York, London and Sydney: John Wiley and Sons, Inc., 1964.) 90s.

DR. EDEN'S *Elements of Tropical Soil Science* is directed particularly to farmers and planters in the tropics rather than to the generality of students, but nevertheless for the greater part comprises chapters on the widely applicable principles of morphology, the physical and chemical properties of soils, and on manuring and the availability of nutrients. The chapters on erosion and the maintenance of soil fertility have a more specific direction to tropical circumstances, but for the rest tropical references are confined to illustrative examples. Because the author has lived his life in the East and in Africa these illustrations are usually very good indeed, as in his treatment of the soil catena and of laterization.

The book is descriptive, so that errors of technology (for example, the definition of the water table is wrong) have no catastrophic consequences.

Dr. Nikol'skii's *Practical Soil Science* is approved in the U.S.S.R. as a text for the faculties of agrochemistry, soil science and agronomy. Nearly three-fifths of the book is a laboratory manual for the quantitative chemical analysis of soil, and another fifth describes physical analyses. Only the remainder concerns itself with the field description and mapping of soil profiles, a subject which Russians have pioneered.

The chemical section is divided into chapters on the analysis of whole soil, including the determinations of organic matter, carbonates and mineral elements; on the determination of exchangeable cations and of the base exchange capacity; on soil acidity; and on the analysis of water soluble material. Every determination is described in detail and the formulae for working out the results are written down. Some disorder is noticeable, matter being placed in the sections somewhat arbitrarily and occasionally in the wrong company. It is in the character of the book that in only one case is the agronomic significance of a determination discussed, and the reader is left to wonder just what is the agronomic relevance of a fusion analysis of whole soil. This part is a welcome addition to laboratory texts for students.

The physical analyses include measurements of soil density and volume weight, moisture content by straight-

forward gravimetric methods, shrinkage, stability of aggregation, and mechanical analysis in some detail. The author follows the American usage in identifying, by definition, texture and mechanical composition, which is at best an impoverishment of the language and at worst a source of confusion. Selected soil states for the measurement of moisture content are rather arbitrary, and soil permeability is covered without mention of Darcy's law. All this is conventional descriptive soil physics which has little relation to the application of the laws of physics to soil.

The translation of the sections on soil morphology and mapping has evidently been difficult and some interpretation is still left to the reader. The elements of profile description are familiar enough, but here the wealth of illustrative examples drawn from a wide experience of soils representative of the great world groups is quite fascinating. The description of soil structure is very well illustrated, although not everyone would limit the definition of structure to the shapes and sizes of aggregates. If this part is more appropriate to a general textbook of pedology than to a practical manual, the balance is restored by the chapter on field survey procedure and mapping.

For more than thirty years Dr. Marshall has been one of the leading contributors to our understanding of the physical chemistry of colloidal materials in the soil. *The Physical Chemistry and Mineralogy of Soils* distils the essence of his experience of that field and its appearance is a notable event.

The various chapters summarize the basic physico-chemical concepts and methods applicable to soil constituents; discuss in some detail the crystal structures of minerals which characterize the clay fraction and the coarser fractions; but necessarily are reduced to more empirical descriptions of organic matter. They go on to describe the molecular adsorption of gases and liquids, principally water; the electrochemistry of clays and the closely allied subjects of the exchange and fixation of ions from extraneous solutions; and conclude with a discussion of the electrokinetic and physical properties of cataphoresis, coagulation, viscosity and swelling.

Let it be said at once that this is not a course text-book. Much of the groundwork is taken for granted or the reader is directed to the standard works. Rather little is worked out in detail and fully developed. This is essentially a coverage of a wide range of diverse kinds of experimental work described in just sufficient detail to enable the author to place each item in the general picture which emerges. The wealth of references makes this a source book and a launching pad for further researches.

It is Dr. Marshall's particular difficulty that he has to try to reconcile two concepts which are in some ways mutually incompatible. On the one hand the Stern and Gouy layers, which are at the foundations of modern analysis, are regions of non-uniform distributions of electric potential and ionic concentration. On the other hand, the conventional terminology of ionic activities, degree of dissociation, membrane potential, Donnan equilibrium and so on imply uniformity of these quantities. Success in his task is not aided by some hasty composition, as when he tells us that "the arithmetical average of the electrostatic potentials becomes the logarithmic average of the ionic activities".

The outlines are again blurred late in the book when the author contrasts what he alleges to be a surface tension model of clay swelling with the Gouy layer picture. The latter does, of course, account physically for a swelling pressure, while the former proves to throw no light whatever on the mechanism of swelling.

These are small criticisms to put against the great service that the author has rendered in putting a little order into this heterogeneous field and in particular for reproducing for a wider public some truly magnificent electron photomicrographs.

E. C. CHILDS



**Handbook of Chemistry and Physics**

A Ready-Reference Book of Chemical and Physical Data. Edited by Robert C. Weast, Samuel M. Selby and Charles D. Hodgman. Forty-fifth edition. Pp. xxi+1470. (Cleveland, Ohio: The Chemical Rubber Co.; Oxford: Blackwell Scientific Publications, Ltd., 1964.) 147s.

OVER the past fifty years the *Handbook of Chemistry and Physics* has built for itself a prominent place on the bookshelves of all chemists and physicists, and in many cases a prominent niche on the desk or laboratory bench.

The latest edition, the forty-fifth, which appears under the joint editorship of Dr. R. C. Weast (editor-in-chief), Dr. S. M. Selby and Prof. C. D. Hodgman, embodies a number of major changes, not the least of which is the change of format. The increase in material presented can be realized from the fact that not only has the page size been doubled, but also the volume has maintained the same thickness as the forty-fourth edition.

Concomitant with the increase in page size are the greatly enlarged tables of physical constants of organic compounds and of inorganic compounds, and it is claimed that data for approximately 5,000 more compounds have been added to this edition. The table dealing with isotopes has been completely revised and brought up to date, and the section on the elements, by C. R. Hammond, has been greatly extended. While the format of certain tables has been completely changed, some of them are now presented in the form of two pages of the old size side by side on the new page. It is to be hoped that in later editions all tables will be presented in the new style, as it is a great aid to clarity.

The old system of presenting the material in ten major sections has been completely revised, and it now appears in six: (A) Mathematical Tables; (B) Elements and Inorganic Compounds; (C) Organic Compounds; (D) General Chemical; (E) General Physical Constants; (F) Miscellaneous. All in all, this, along with the binominal code system, leads to considerable ease in references from the index or contents.

The *Handbook of Chemistry and Physics* in its latest edition should be within easy reach of all scientists, whether they work in Government, industrial, academic or private institutions, and whatever their discipline or nationality. Dr. Weast and his colleagues, along with the publishers, the Chemical Rubber Co., are to be congratulated on this production. 147s. is a small price to pay for so much information so readily available.

**Encyclopedia of Chemical Technology**

By Kirk-Othmer. Vol. 5: Chlorine to Colors for Foods, Drugs and Cosmetics. Second completely revised edition. Pp. xiv+884. (New York and London: Interscience Publishers, a division of John Wiley and Sons, Inc., 1964.) 338s.

THE publication of each volume of the second edition of 'Kirk-Othmer' is eagerly awaited by every user of the *Encyclopedia of Chemical Technology*. Once again the editors are to be congratulated on their presentation of a further series of reviews, of the expected high standard, covering subjects ranging from "Chlorine" to "Colors FD&C".

It is worthy of note that the articles on "Chlorocarbons and Chlorohydrocarbons", "Chocolate and Cocoa", and "Coal" are reviewed by experts working in the United Kingdom. It is to be appreciated that these articles are intended for use mainly by American technologists. The articles on "Chlorocarbons and Chlorohydrocarbons" are particularly informative and contain many references to synthetic processes, chemical reactions, uses and toxicity. The quoted threshold limit values (TLV), also referred to as maximum allowable (or permissible) concentrations (MAC or MPC) for chlorinated hydrocarbons, are up to date, and, although the fact is not mentioned, are generally accepted as international standards. The broad

spectrum cyclodiene insecticides of the aldrin, dieldrin, endrin series are adequately reviewed from a technological point of view, but in light of their high toxicity some emphasis should be placed on their restrictive uses in practice (that is, to tolerance limits of residues in foods).

For food technologists there are informative articles covering "Chocolate and Cocoa", "Coffee" and "Colors FD&C". References to other food additives are found under articles on "Chlorophyll", "Cinnamaldehyde" and "Citric Acid". As one compares the lists of permissible and non-permissible food colours accepted by the United States with those accepted by other countries, one realizes that international standardization on this topic is long overdue.

In the medical field, interesting articles deal with "Choline" and with "Coagulants and Anticoagulants".

Other subjects reviewed include "Clays", important to so many industries; "Coal", where we are reassured that during the next generation there will be many developments in coal production and utilization; and "Coated Fabrics", an article dealing with the use of plastics in industry.

For the technologist seeking information on analytical techniques, there are adequate reviews on "Chromatography" and "Colorimetry and Fluorimetry". Under "Chromatography", more emphasis could be given to the technique of thin-layer chromatography. This article closes with a glossary of terms and symbols encountered in chromatography.

Finally, I must mention the comprehensive review on "Color Photography", an article that includes many references to the chemical processes and materials involved in this subject. The article closes with special references of colour photography in colour television and in infra-red aero applications.

K. FIELD

**Current Aeronautical Fatigue Problems**

Proceedings of a Symposium held in Rome, 23-25 April, 1963. Edited by J. Schijve, J. R. Heath-Smith and E. R. Walbourne. Pp. viii+499. (New York and London: Pergamon Press, 1964.) 120s.

THIS symposium was the third of a series concerned exclusively with fatigue problems, organized jointly by the International Committee on Aeronautical Fatigue and the Advisory Group for Aeronautical Research and Development—organizations respectively and more familiarly known as I.C.A.F. and A.G.A.R.D. A total of seventeen papers is presented, representing the United Kingdom, the United States, France, Germany, Italy and the Netherlands.

The papers are arranged in three sections, dealing with (i) elevated temperature effects (6 papers); (ii) crack propagation and residual static strength (7 papers); and (iii) structural fatigue testing (4 papers). Each section is helpfully introduced by a short technical preface; while, according to the custom for such records of proceedings, the discussions which followed the individual papers are fully reported. The contributions are very variable in regard to bibliographic references; but in the volume as a whole more than 170 references are listed—in the main to very recent work.

To review a symposium of papers is in many ways an unsatisfactory task; but on this occasion the work is eased by the careful standardization of the form of the reports. This is an authoritative volume that will be indispensable to all concerned with the elimination of fatigue failures, for much of the work reported is applicable to other fields than the aeronautical industry alone. It is no reflexion on the other two sections to single out for special mention the section on crack propagation: this records a remarkably detailed and increasingly accurate understanding of the basic mechanics of the phenomenon and a number of elegant experimental procedures are reported.

B. N. COLE

# A NEW CLASS OF FAULTS AND THEIR BEARING ON CONTINENTAL DRIFT

By PROF. J. TUZO WILSON, O.B.E.

Institute of Earth Sciences, University of Toronto

**TRANSFORMS and half-shears.** Many geologists<sup>1</sup> have maintained that movements of the Earth's crust are concentrated in mobile belts, which may take the form of mountains, mid-ocean ridges or major faults with large horizontal movements. These features and the seismic activity along them often appear to end abruptly, which is puzzling. The problem has been difficult to investigate because most terminations lie in ocean basins.

This article suggests that these features are not isolated, that few come to dead ends, but that they are connected into a continuous network of mobile belts about the Earth which divide the surface into several large rigid plates (Fig. 1). Any feature at its apparent termination may be transformed into another feature of one of the other two types. For example, a fault may be transformed into a mid-ocean ridge as illustrated in Fig. 2a. At the point of transformation the horizontal shear motion along the fault ends abruptly by being changed into an expanding tensional motion across the ridge or rift with a change in seismicity.

A junction where one feature changes into another is here called a transform. This type and two others illustrated in Figs. 2b and c may also be termed half-shears (a name suggested in conversation by Prof. J. D. Bernal). Twice as many types of half-shears involve mountains as ridges, because mountains are asymmetrical whereas ridges have bilateral symmetry. This way of abruptly ending large horizontal shear motions is offered as an explanation of what has long been recognized as a puzzling feature of large faults like the San Andreas.

Another type of transform whereby a mountain is transformed into a mid-ocean ridge was suggested by S. W. Carey<sup>2</sup> when he proposed that the Pyrenees Mountains were compressed because of the rifting open of the Bay of Biscay (presumably by the formation of a mid-ocean ridge along its axis). The types illustrated are all dextral, but equivalent sinistral types exist.

In this article the term 'ridge' will be used to mean mid-ocean ridge and also rise (where that term has been used meaning mid-ocean ridge, as by Menard<sup>3</sup> in the Pacific basin). The terms mountains and mountain system may include island arcs. An arc is described as being convex or concave depending on which face is first reached when proceeding in the direction indicated by an arrow depicting relative motion (Figs. 2 and 3). The word fault may mean a system of several closely related faults.

**Transform faults.** Faults in which the displacement suddenly stops or changes form and direction are not true transcurrent faults. It is proposed that a separate class of horizontal shear faults exists which terminate abruptly at both ends, but which nevertheless may show great displacements. Each may be thought of as a pair of half-shears joined end to end. Any combination of pairs of the three dextral half-shears may be joined giving rise to the six types illustrated in Fig. 3. Another six sinistral forms can also exist. The name transform fault is proposed for the class, and members may be described in terms of the features which they connect (for example, dextral transform fault, ridge-convex arc type).

The distinctions between types might appear trivial until the variation in the habits of growth of the different types is considered as is shown in Fig. 4. These distinctions are that ridges expand to produce new crust, thus leaving residual inactive traces in the topography of their former positions. On the other hand oceanic crust moves down under island arcs absorbing old crust so that they leave no traces of past positions. The convex sides of arcs thus advance. For these reasons transform faults of types a, b and d in Fig. 4 grow in total width, type f diminishes and the behaviour of types c and e is indeterminate. It is significant that the direction of motion on transform faults of the type shown in Fig. 3a is the reverse of that required to offset the ridge. This is a fundamental difference between transform and transcurrent faulting.

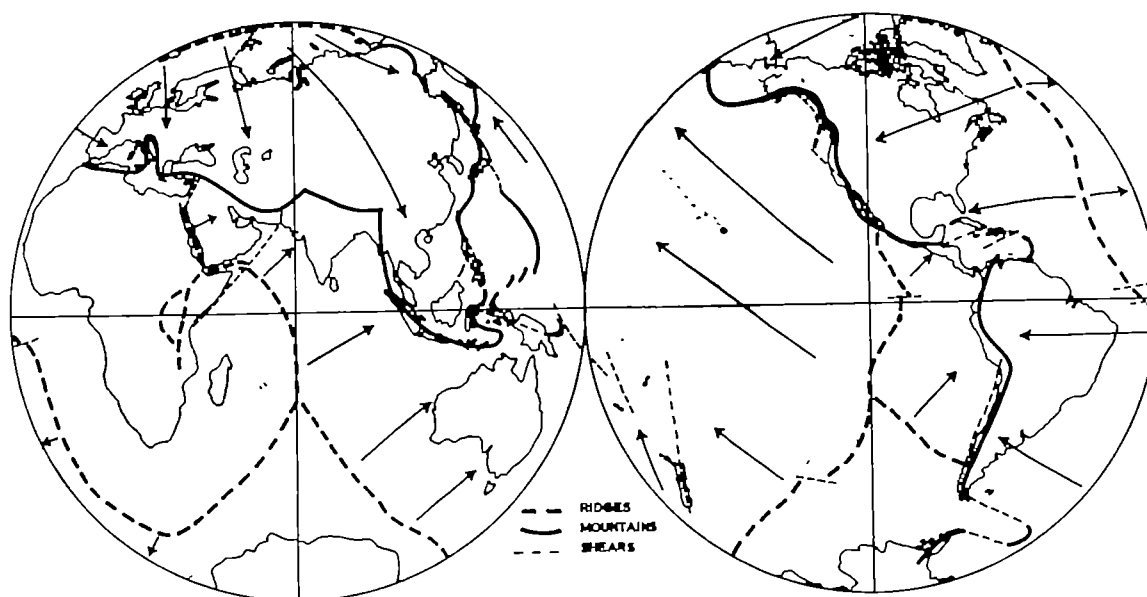


Fig. 1. Sketch map illustrating the present network of mobile belts, comprising the active primary mountains and island arcs in compression (solid lines), active transform faults in horizontal shear (light dashed lines) and active mid-ocean ridges in tension (heavy dashed lines).

Many examples of these faults have been reported and their properties are known and will be shown to fit those required by the constructions above. If the class as a whole has not heretofore been recognized and defined, it is because all discussions of faulting, such as those of E. M. Anderson, have tacitly assumed that the faulted medium is continuous and conserved. If continents drift this assumption is not true. Large areas of crust must be swallowed up in front of an advancing continent and re-created in its wake. Transform faults cannot exist unless there is crustal displacement, and their existence would provide a powerful argument in favour of continental drift and a guide to the nature of the displacements involved. These proposals owe much to the ideas of S. W. Carey, but differ in that I suggest that the plates between mobile belts are not readily deformed except at their edges.

The data on which the ensuing accounts are based have largely been taken from papers in two recent symposia<sup>4,5</sup> and in several recent books<sup>6,7</sup> in which many additional references may be found.

**North Atlantic ridge termination.** If Europe and North America have moved apart, an explanation is required of how so large a rift as the Atlantic Ocean can come to a relatively abrupt and complete end in the cul-de-sac of the Arctic Sea. Fig. 5 illustrates one possible explanation.

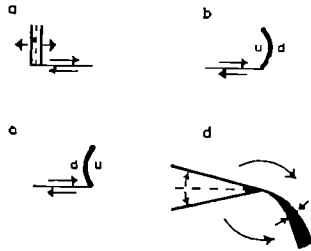


Fig. 2. Diagram illustrating the four possible right-hand transforms. a, Ridge to dextral half-shear; b, dextral half-shear to concave arc; c, dextral half-shear to convex arc; d, ridge to right-hand arc.

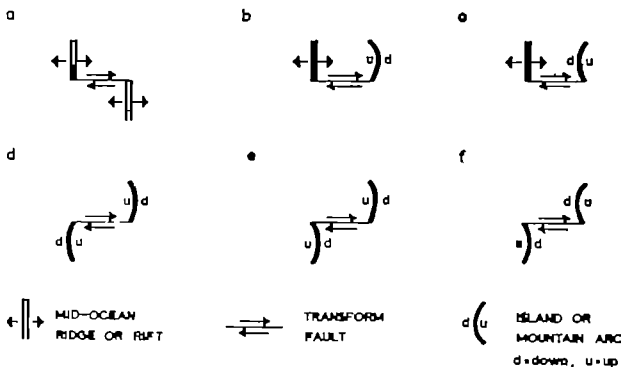


Fig. 3. Diagram illustrating the six possible types of dextral transform faults. a, Ridge to ridge type; b, ridge to concave arc; c, ridge to convex arc; d, concave arc to concave arc; e, concave arc to convex arc; f, convex arc to convex arc. Note that the direction of motion in a is the reverse of that required to offset the ridge.

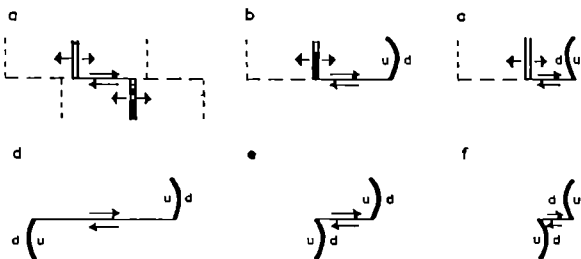


Fig. 4. Diagram illustrating the appearance of the six types of dextral transform faults shown in Fig. 3 after a period of growth. Traces of former positions now inactive, but still expressed in the topography, are shown by dashed lines.

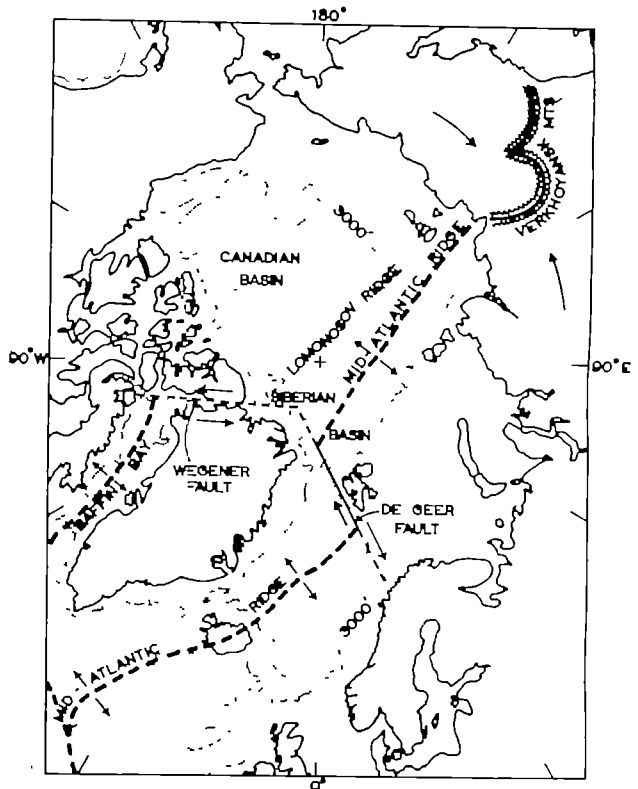
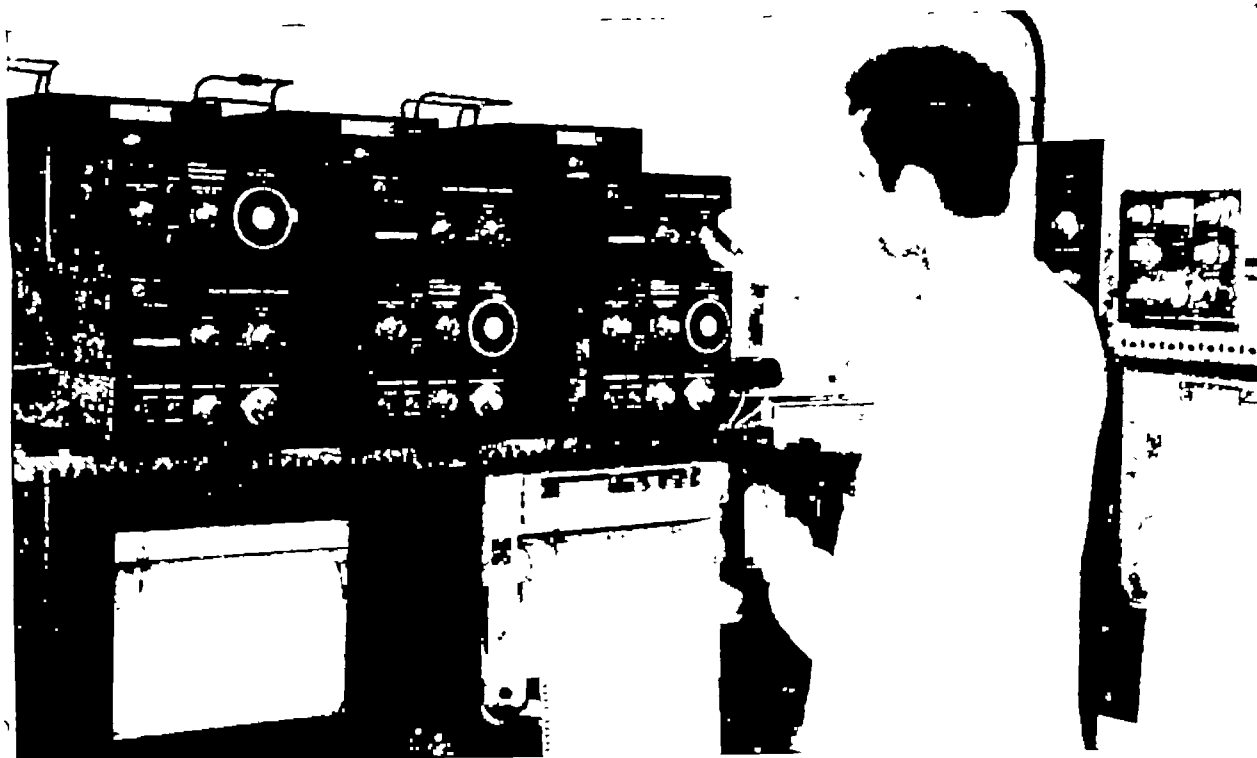


Fig. 5. Sketch map of the termination of the Mid-Atlantic ridge by two large transform faults (Wegener and De Geer faults) and by transformation into the Verkhoyansk Mountains.

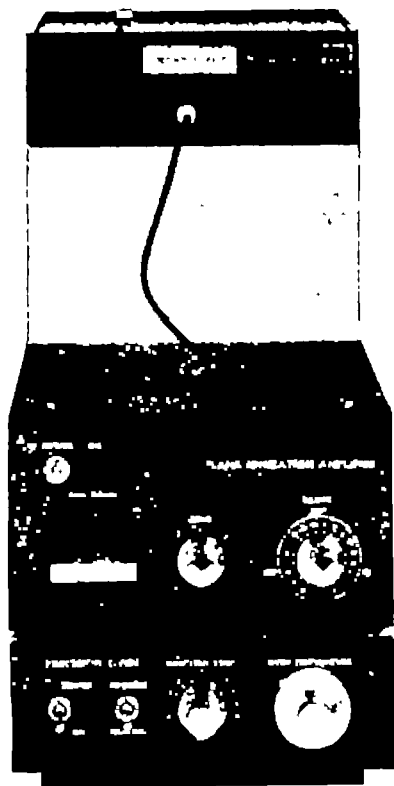
Wegener<sup>8</sup> suggested that the strait between Greenland and Ellesmere Island was formed by a fault, here postulated to be a sinistral transform fault (ridge-ridge type). Wegmann<sup>9</sup> named another between Norway, Spitsbergen and Greenland, the De Geer line, which is here regarded as a dextral transform fault (ridge-ridge type). The extension of the Mid-Atlantic ridge across the Siberian basin was traced by Heezen and Ewing<sup>10</sup>, while Wilson<sup>11</sup> proposed its transform into the Verkhoyansk Mountains by rotation about a fulcrum in the New Siberian Islands. In accordance with the expectations from Fig. 4a earthquakes have been reported along the full line of the De Geer fault in Fig. 5, but not along the dashed older traces between Norway and Bear Island and to the north of Greenland. The Baffin Bay ridge and Wegener fault are at present quiescent. W. B. Harland<sup>12</sup> and Canadian geologists have commented on the similarities of Spitsbergen and Ellesmere Island.

**Equatorial Atlantic fracture zones.** If a continent in which there exist faults or lines of weakness splits into two parts (Fig. 6), the new tension fractures may trail and be affected by the existing faults.

The dextral transform faults (ridge-ridge type) such as AA' which would result from such a period of rifting can be seen to have peculiar features. The parts AB and B'A' are older than the rifting. DD' is young and is the only part now active. The offset of the ridge which it represents is not an ordinary faulted displacement such as a transcurrent fault would produce. It is independent of the distance through which the continents have moved. It is confusing, but true, that the direction of motion along DD' is in the reverse direction to that required to produce the apparent offset. The offset is merely a reflexion of the shape of the initial break between the continental blocks. The sections BD and D'B' of the fault are not now active, but are intermediate in age and are represented by fracture zones showing the path of former faulting.



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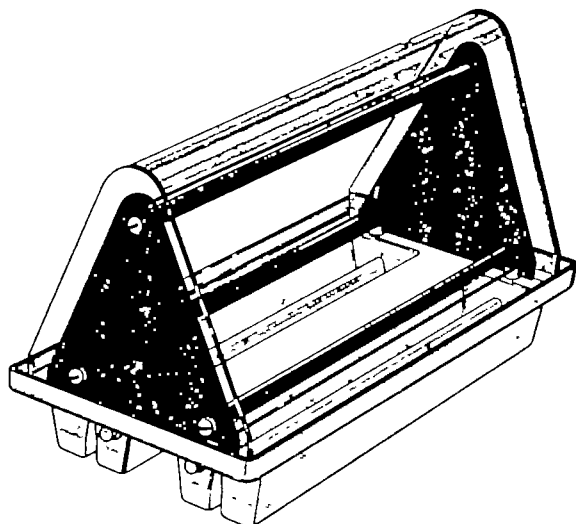
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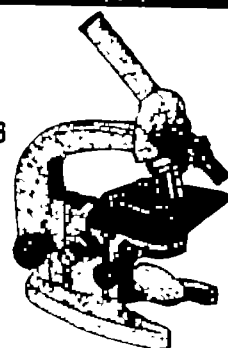


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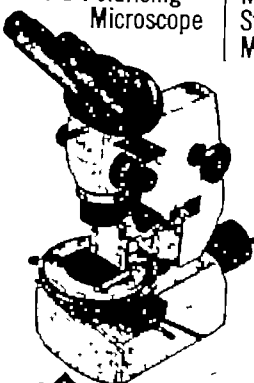
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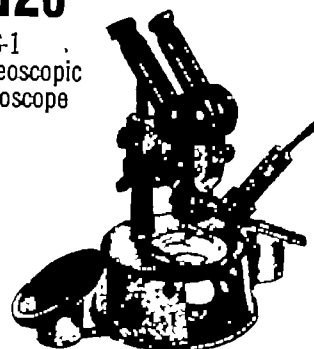
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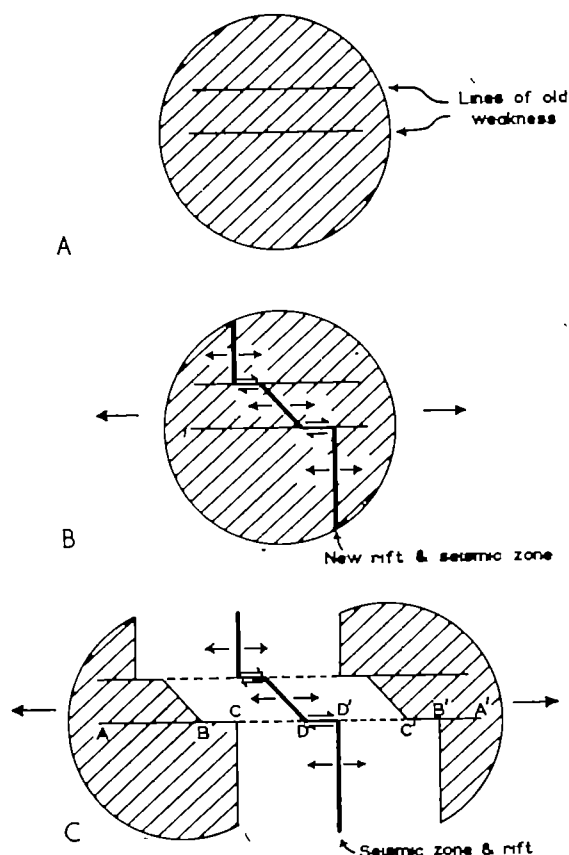


Fig. 6. Diagram illustrating three stages in the rifting of a continent into two parts (for example, South America and Africa). There will be seismic activity along the heavy lines only

Fig. 7 shows that the Mid-Atlantic ridge and the fracture zones in the equatorial Atlantic may well be a more complex example of this kind. If so the apparent offsets on the ridge are not faulted offsets, but inherited from the shape of the break that first formed between the coasts of Africa and the Americas. Fig. 7 is traced from Heezen, Bunce, Hersey and Tharp<sup>12</sup> with additions to the north from Krause<sup>13</sup>. The fracture zones are here held to be right-hand transform faults and not left-hand transcurrent faults as previously stated. If the fracture zones can be traced across the Atlantic and are of the type postulated, then the points where they intersect the opposite coasts are conjugate points which would have been together before rifting.

It seems possible that the old fault in Pennsylvania and the offset of the Atlantic Coast described by Drake and Woodward<sup>14</sup> are of the same nature, although it is suggested that it is not usual for a fracture zone to follow a line of seamounts, and that the fracture zone may extend eastward, not south-east.

A possible explanation of the termination of the Carlsberg ridge. Another type of transform fault is found in the Indian Ocean and Arabian Gulf opened during the Mesozoic and Cenozoic eras by the northward movement of India, new ocean floor must have been generated by spreading of the Carlsberg ridge. This ends abruptly in a transcurrent fault postulated by Gregory<sup>15</sup> off the east coast of Africa. A parallel fault has been found by Matthews<sup>16</sup> as an offset across the Carlsberg ridge and traced by him to the coast immediately west of Karachi. Here it joins the Ornaoh-Nal and other faults<sup>17</sup> which extend into Afghanistan and, according to such descriptions as I can find, probably merge with the western end of the Hindu Kush. This whole fault is thus an example of a sinistral transform fault (ridge-convex arc type).

At a later date, probably about Oligocene time according to papers quoted by Drake and Girdler<sup>18</sup>, the ridge was extended up the Red Sea and again terminated in a sinistral transform fault (ridge-convex arc type) that forms the Jordan Valley<sup>19</sup> and terminates by joining a large thrust fault in south-eastern Turkey (Z. Ternek, private communication). The East African rift valleys are a still later extension formed in Upper Miocene time according to B. H. Baker (private communication).

The many offsets in the Gulf of Aden described by Laughton<sup>20</sup> provide another example of transform faults adjusting a rift to the shape of the adjacent coasts.

Possible relationships between active faults off the west coast of North America. This tendency of mid-ocean ridges to be offset parallel to adjacent coasts is thought to be evident again in the termination of the East Pacific ridge illustrated in Fig. 9. The San Andreas fault is here postulated to be a dextral transform fault (ridge-ridge type) and not a transcurrent fault. It connects the termination of the East Pacific ridge proper with another short length of ridge for which Menard<sup>21</sup> has found evidence off Vancouver Island. His explanation of the connexion—that the mid-ocean ridge connects across western United States—does not seem to be compatible with the view that the African rift valleys are also incipient mid-ocean ridges. The other end of the ridge off Vancouver Island appears to end in a second great submarine fault off British Columbia described by Benioff<sup>22</sup> as having dextral horizontal motion.

In Alaska are several large faults described by St. Amand<sup>23</sup>. Of the relations between them and those off

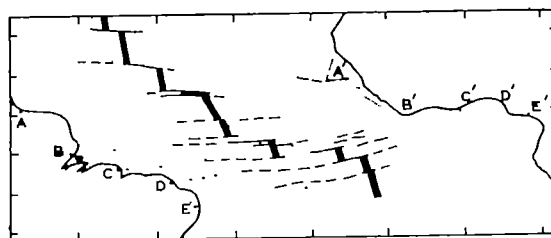


Fig. 7. Sketch (after Krause and Heezen *et al.*) showing how the Mid-Atlantic ridge is offset to the left by active transform faults which have dextral motions if the rift is expanding (see Fig. 4a). —, Mid-ocean ridge; —, active fault; ---, inactive fault trace; . . . , hypothetical extension of fault

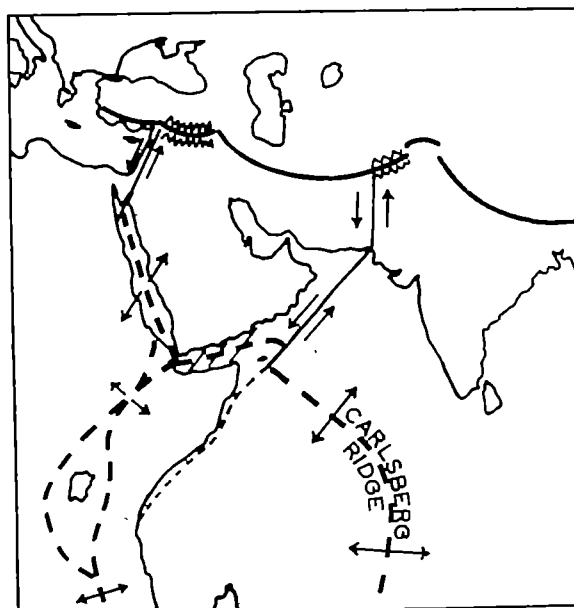


Fig. 8. Sketch illustrating the end of the Carlsberg mid-ocean ridge by a large transform fault (ridge-convex arc type) extending to the Hindu Kush, the end of the rift up the Red Sea by a similar transform fault extending into Turkey and the still younger East African rifts

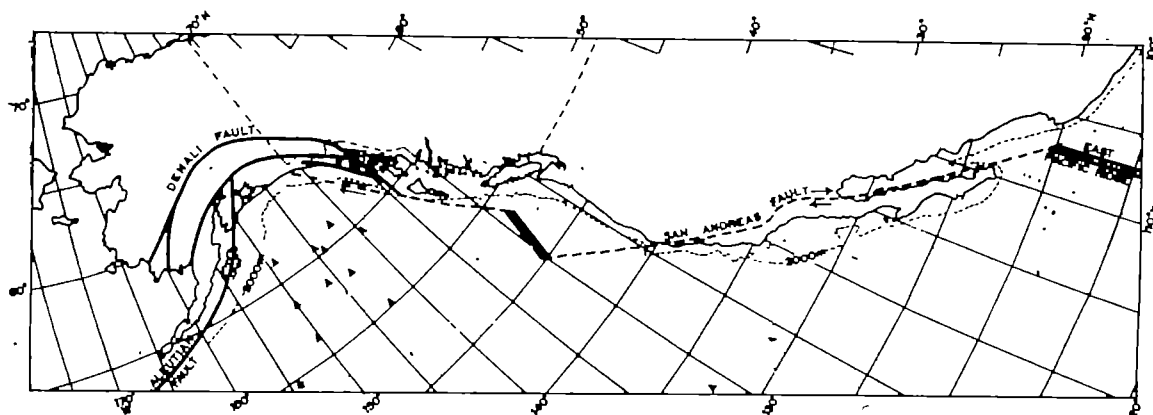


Fig. 9. Sketch map of the west coast of North America showing the approximate location of a submarine thrust fault along the Aleutian trench, the Denali faults (after St. Amand), the San Andreas and another large transform fault (after Benioff) and part of the East Pacific ridge and another mid-ocean ridge (after Menard)

the coast he writes: "If the two systems represent one consistent system, some interesting possibilities arise. One that the San Andreas and Alaska Complex is a gigantic tear fault, along which the Pacific Basin is being slid, relatively speaking under the Alaska Mainland, and the Bering Sea. On the other hand, if the whole system is a strike-slip fault having consistent right-lateral offset, then the whole of the western north Pacific Basin must be undergoing rotation".

St. Amand was uncertain, but preferred the latter alternative, whereas this interpretation would favour the former one. Thus the Denali system is considered to be predominantly a thrust, while the fault off British Columbia is a dextral transform fault.

At a first glance at Fig. 9 it might be held that the transform fault off British Columbia was of ridge-concave arc type and that it connects with the Denali system of thrust faults, but if the Pacific floor is sliding under Alaska, the submarine fault along the Aleutian arc that extends to Anchorage is more significant. In that case the Denali faults are part of a secondary arc system and the main fault is of ridge-convex arc type.

*Further examples from the Eastern Pacific.* If the examples given from the North and Equatorial Atlantic Ocean, Arabian Sea, Gulf of Aden and North-west Pacific are any guide, offsets of mid-ocean ridges along fracture zones are not faulted displacements, but are an inheritance from the shape of the original fracture. The fracture zones that cross the East Pacific ridge<sup>11</sup> are similar in that their seismicity is confined to the offset parts between ridges. An extension of this suggests that the offsets in the magnetic displacements observed in the aseismic fracture zones off California may not be fault displacements as has usually been supposed, but that they reflect the shape of a contemporary rift in the Pacific Ocean. More complex variants of the kind postulated here seem to offer a better chance of explaining the different offsets noted by Vacquier<sup>12</sup> along different lengths of the Murray fracture zone than does transcurrent faulting. If the California fracture zones are of this character and are related to the Darwin rise as postulated by Hess, then the Darwin rise should be offset in a similar pattern.

The southern Andes appear to provide an example of compression combined with shearing. The compressional features are obvious. The existence of dextral shearing is also well known<sup>13</sup>. It is suggested that the latter may be due to the transformation of the West Chile ridge into a dextral transform fault (ridge-convex arc type) along the Andes which terminates at the northern end by thrusting under the Peruvian Andes (Fig. 10).

The observation that there is little seismicity and hence little movement south of the point where the West Chile ridge intersects the Andes can be explained if it is realized that the ridge system forms an almost complete ring about

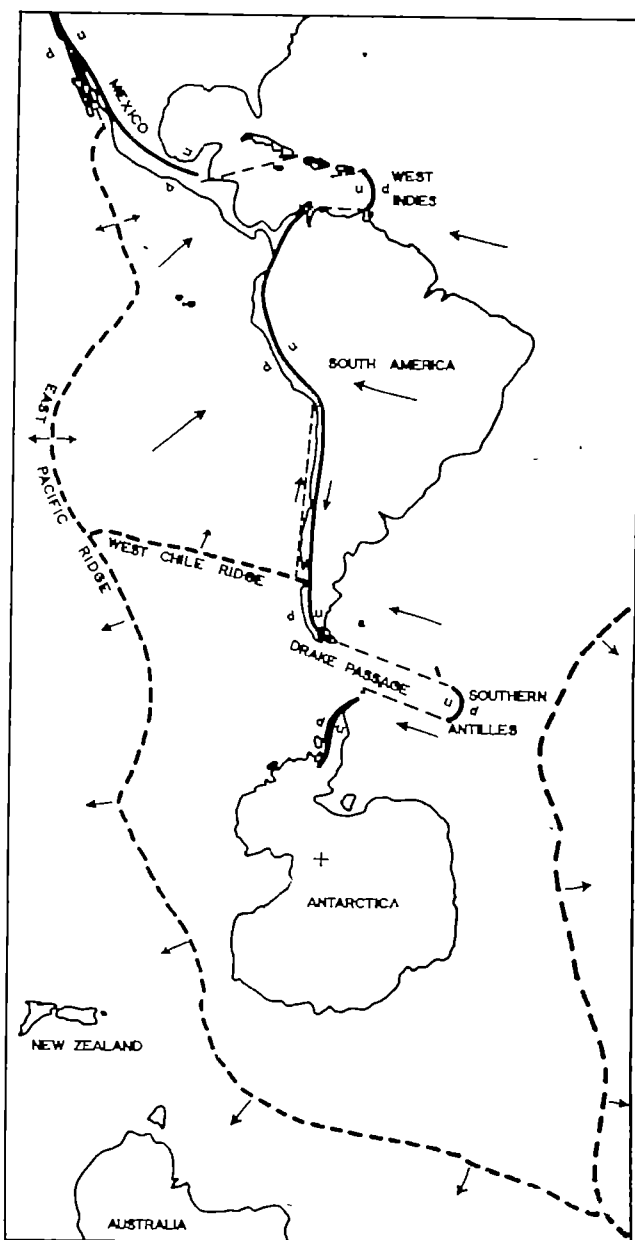


Fig. 10. Sketch map of Mexico, South America, Antarctica and part of the mid-ocean ridge system (heavy dashed lines) illustrating that the great loop of the ridge about Antarctica can only grow by increasing in diameter. Transform faults are shown by light dashed lines



Antarctica, from which expansion must everywhere be directed northwards. This may explain the absence of an isthmus across Drake Passage.

It would also appear that the faults at the two ends of the South Antilles and West Indies arcs are examples of dextral and sinistral pairs of transform faults (concave-concave arc types). According to Fig. 4 both these arcs should be advancing into the Atlantic and inactive east-west faults should not be found beyond the arcs.

This article began by suggesting that some aspects of faulting well known to be anomalous according to traditional concepts of transcurrent faults could be explained by defining a new class of transform faults of which twelve varieties were shown to be possible.

The demonstration by a few examples that at least six of the twelve types do appear to exist with the properties predicted justifies investigating the validity of this concept further.

It is particularly important to do this because transform faults can only exist if there is crustal displacement and proof of their existence would go far towards establishing the reality of continental drift and showing the nature of the displacements involved.

I thank the Departments of Geodesy and Geophysics and of Geology and Churchill College, University of Cambridge, for the opportunity to write this article, those whose data I have used, and colleagues—especially Sir Edward Bullard, W. B. Harland, H. H. Hees, D. H. Matthews and F. J. Vine—for advice; and Sue Chappell and Sue Vine for assistance. This is a contribution to

the Vela Uniform programme and to the Canadian Upper Mantle Project.

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## ACTIVATION ANALYSIS

SINCE the first international meeting on activation analysis, held in Vienna six years ago, the technique has been vigorously developed for practical use in many areas of science and technology. Comprehensive reviews of recent progress and current research were provided at two large gatherings which were held in April 1965. More than sixty papers were read at the Second International Conference on Modern Trends in Activation Analysis, sponsored by the Texas Agricultural and Mechanical University, the United States Atomic Energy Commission, Euratom and the International Atomic Energy Agency. This meeting, which was held at College Station, Texas, during April 19–22, attracted 500 participants. The Advanced Seminar on Activation Analysis, organized by the General Atomic Division of the General Dynamics Corporation, met in San Diego during April 26–28 and was attended by 230 research workers. The programme comprised 45 invited papers, with emphasis on practical applications. In the report which follows, an asterisk is used to identify papers given at the San Diego meeting.

Activation analysis is basically a simple process, involving the examination and assay of the radioactivity induced in a sample after bombardment by neutrons or other missiles. Its potentialities are limited by the imagination of the experimenter, who may not find it easy to devise or interpret an experiment making full use of the extraordinary sensitivity of a method which, for some elements, reaches 1,000 or even a million times beyond the limit of achievement by other means.

Sensitivity is not all-important, even in the estimation of trace impurities. A. A. Smales (Atomic Energy Research Establishment, Harwell) emphasized the superiority of emission spectroscopy and spark source mass spectrometry where (as is often the case) a general survey of all possible impurities is desired. If the offending trace element can be nominated, activation analysis and mass

spectrometric isotope dilution analysis are preferable. At the ultimate limit of sensitivity, activation analysis has the important advantage of freedom from reagent contamination errors; even so, fluorescent X-ray spectrometry will be chosen when it is necessary to discover the location of a small quantity of impurity within a specimen.

Neutrons are commonly used for the activation of the sample to be analysed, partly because they are readily available at low cost, but also because of their indifference to the potential barriers which limit the access of charged particles to the target nuclei. The possibilities offered by thermal neutrons and by 14-MeV neutrons (from a deuterium-tritium discharge tube) have been fairly thoroughly explored, but interesting work remains to be done at intermediate energy levels, as E. L. Steele (General Atomic, San Diego) explained.

By careful choice of neutron energy, the selectivity of fast neutron activation analysis may be greatly increased. It is, for example, difficult to distinguish oxygen from fluorine when 14-MeV neutrons are used, since both elements yield the same product, by the reactions  $^{19}\text{F}(n,\alpha)^{16}\text{N}$  and  $^{18}\text{O}(n,p)^{18}\text{N}$ . The first of these processes has a threshold (for neutron energy) at 1.5 MeV and the second at 10 MeV. For energies between these limits, only fluorine will form  $^{16}\text{N}$ . Threshold differences may be exploited for the estimation of magnesium in the presence of aluminium, since the reaction  $^{24}\text{Mg}(n,p)^{24}\text{Na}$  has a threshold at 4.7 MeV, while the interfering process  $^{27}\text{Al}(n,\alpha)^{24}\text{Na}$  has a threshold at 3.1 MeV. Selection of neutron energy also permits the estimation of silicon in the presence of phosphorus, chromium in the presence of manganese and sulphur in the presence of chlorine.

Steel uses a 3-MeV Cockcroft-Walton generator to accelerate positive ions ( $p, d, ^3\text{He}$  or  $\alpha$ ) which are used to bombard a target; Li, Be, B, C, Al, V and others are being investigated. By appropriate choice of particle,

accelerating potential and target material, approximately monoenergetic neutrons are produced in useful quantities and with energies in the range from a few keV to 20 MeV, allowing many reaction thresholds and resonance absorptions to be utilized.

For the estimation of elements with  $Z < 10$ , thermal neutrons are of little value. Fast neutrons are more serviceable, but interferences from neighbouring elements are often troublesome. Activation analysis with  $^3\text{He}$  ions is attractive; because of the large binding energy, many useful reactions occur at relatively low incident energies, from 12 MeV downward. E. Ricci (Oak Ridge National Laboratory) reported a sensitivity of  $3 \times 10^{-11}$  g in the determination of oxygen by the reaction  $^{16}\text{O}(^3\text{He}, p)^{14}\text{F}$ , using a 25- $\mu\text{amp}$  beam of  $^3\text{He}^{++}$  ions. E. L. Steele\* discussed the difficulty associated with the poor penetration of heavy ions, and suggested that vaporization of the sample under bombardment (in a suitable container) might be tolerated. He quoted excellent sensitivities for the detection of several light elements, but pointed out that the products are mostly positron emitters all producing annihilation radiation of energy 0.51 MeV. Consequently, identification of the desired activity must be done by resolution of complex decay curves rather than by pulse-height analysis.

O. Engelmann (Centre d'Etudes Nucleaires de Saclay) used photons, protons and  $\alpha$ -particles at energies up to 40 MeV for the determination of light elements (C, N, O, F) in reactor materials, by non-destructive methods with sensitivities better than  $10^{-7}$  g.

Although thermal neutrons are not effective in the activation analysis of light elements, secondary processes occurring in a nuclear reactor may sometimes be pressed into service. H. J. Born (Institut für Radiochemie der Technischen Hochschule, Munich) explained how the successive reactions  $^6\text{Li}(n, \alpha)^3\text{H}$  and  $^{16}\text{O}(t, n)^{14}\text{F}$  may be used for the determination of oxygen or lithium in a system containing both elements. Knowledge of the oxygen content of lithiumbutyl (a polymerization catalyst) is significant in the manufacture of plastics and the exact measurement of the lithium content of many substances is important both for geochemical problems and for checking the purity of reactor materials.

Most of the samples submitted to activation analysis display a complex pattern of induced radioactivity after neutron bombardment. In rare instances the desired activity can be separated by simple instrumental means, as, for example, in the estimation of oxygen in steel, where the  $(n, p)$  reaction on  $^{16}\text{O}$  gives  $^{16}\text{N}$ . The  $\gamma$ -radiation emitted by this isotope is of such high energy (6.13 and 7.13 MeV) that it can be detected without interference from any of the other activities induced in the steel specimen.

More often a detailed separation must be attempted, either by chemical means (after irradiation, when contamination from reagents is no longer a hazard) or by fairly elaborate  $\gamma$ -ray spectrum analysis. Chemical separation is necessary to achieve the ultimate sensitivity, but instrumental techniques are sometimes convenient. M. D. Cohan (Armed Forces Radiobiology Research Institute, National Naval Medical Center, Bethesda) described a counting system incorporating an electronic computer to provide immediate calculation of analytical results, with appropriate graphical or digital presentation to the user, during the course of the experiment. Several laboratories record complex  $\gamma$ -ray spectra on magnetic tape for subsequent resolution with the help of electronic computers.

Present applications of activation analysis fall into four main categories. In science the main interest is in geochemistry and in cosmochemistry, inferred from analysis of meteorites. Knowledge of trace element concentrations both in the Earth's crust and in extra-terrestrial material has been radically altered during the past ten years by the use of activation analysis. The determination of

major constituents in meteorites by fast neutron activation analysis is of growing importance for the study of the rarer meteorites, where the limited supply of material makes non-destructive analysis particularly attractive. J. R. Vogt (University of Kentucky) described methods for the rapid estimation of oxygen and silicon in meteorites. An IBM '1410' computer is used to handle the experiment data and to calculate the results, allowing complete analyses to be performed at the rate of 60/h.

The most widely useful industrial application of activation analysis is the estimation of oxygen in steel. With automatic equipment now available, a complete analysis can be made in 2 min or less, with accuracy better than 2 per cent. D. E. Wood (Kaman Nuclear, Colorado Springs) reported a careful comparison with the commonly used vacuum fusion method, concluding that activation analysis with 14 MeV in the reaction  $^{16}\text{O}(n, p)^{16}\text{N}$  is more reliable, since it accounts for the whole of the oxygen in the sample, regardless of chemical form; in vacuum fusion the oxygen may not be fully extracted.

Technical advances made possible by the development of relatively cheap and simple neutron generators were typified in the nuclear analysis system for coal, described by T. O. Martin (Texas Nuclear Corporation, Austin). This equipment measures the carbon, oxygen, silicon and aluminium content of coal on a conveyor belt, using a neutron generator and monitor, two scintillation spectrometers and a four-channel system for data analysis. The carbon and oxygen contents are measured by the inelastic scattering of fast neutrons, using a  $\gamma$ -ray spectrometer positioned near the generator. Aluminium and silicon are estimated by activation analysis, with the help of a second spectrometer placed about 40 sec downstream. The calorific value of the coal can be estimated with reasonable accuracy from the carbon content. The percentage of moisture is related to the oxygen content, especially in wet coals, and the proportions of aluminium and silicon are a guide to the ash content.

The compactness of fast-neutron activation analysis equipment has already been exploited in building apparatus which, when delivery can be effected, will help to determine the composition of the lunar surface. Additional information can be gained from the direct or indirect examination of various capture and scattering reactions, not necessarily associated with the induction of radioactivity.

An ingenious example of this approach was discussed by R. L. Caldwell\* (Socony Mobil Oil Co., Dallas, Texas) in relation to the analysis of oil-bearing rocks. A small generator, with suitable detection equipment, is lowered into a borehole, where a 20–200- $\mu\text{sec}$  pulse of 14-MeV neutrons is delivered. The aftermath of this pulse is revealed by measuring the thermal neutron flux (using a boron trifluoride counter) or the promptly-emitted  $\gamma$ -rays from various nuclei after neutron activation.

The production of thermal neutrons depends on slowing-down by collision, mainly in oil or water. The rate at which the burst of thermal neutrons dies away is influenced by the presence of elements such as sodium and chlorine, with large capture cross-sections. The die-away measurement technique can be made to yield a remarkable amount of information on the presence of oil, fresh water or salt water and has the advantage of being able to work effectively through the iron pipe that normally lines the borehole.

In clinical science the principal application of activation analysis is in the determination of trace elements. The experimenter's task is often made difficult by the presence of interfering elements. Sodium is the most significant, because of its abundance in biological material and of its large neutron capture cross-section. Ion exchange techniques are effective in the removal of sodium, but may also remove, in whole or in part, the trace elements which

it is desired to measure. M. P. Menon and R. E. Wainardi (Texas Agricultural and Mechanical University) described a simple procedure based on the precipitation of carrier and active sodium by a mixture of *n*-butanol and hydrochloric acid, removing the  $^{24}\text{Na}$  (and, incidentally, most of the chlorine activity) but having little effect on the remaining trace elements in the irradiated specimen.

The metabolism of manganese offers many problems illustrating the potentialities—and pitfalls—of activation analysis. Manganese-levels in biological fluids (blood serum, cerebro-spinal fluid, bile, saliva) are seldom above 1  $\mu\text{g}/100\text{ ml}$ . Attempts to remove sodium by dialysis before neutron irradiation have been criticized with some justification, because of contamination with manganese from the buffer solution.

To estimate the error involved in this procedure, E. Kanabrocki\* (Veterans Administration Hospital, Hines, Illinois) exposed buffer solution to neutron bombardment before dialysis of unirradiated samples of various biological fluids. Corrections of up to 25 per cent were found to be necessary. In the estimation of manganese in urine, the contribution made by the buffer solution to the final result was found to be as high as 72 per cent; some workers would put this figure at 100 per cent, since it has been asserted that the mammalian kidney does not excrete manganese at all.

Determination of tissue concentrations is the first step towards elucidating the physiological or biochemical role of an element. Discussing the homeostasis of manganese, G. Cotzias\* (Brookhaven National Laboratory, Upton, New York) reported animal experiments showing that dietary manganese-levels can be increased by a factor of 180–200 before the tissue-levels will be doubled. The mechanism for this very effective control is not known, but there is evidence—as yet inconclusive—that the adrenal hormone influences excretion of manganese.

Another unsolved problem was illuminated by E. D. Bird (University of Florida) in an investigation of trace elements in urine. Hypertrophic cartilage from rachitic rats can be mineralized *in vitro* by incubation in urine from patients actively forming kidney stones, but not in urine from normal patients. Minute amounts of zinc, manganese or cadmium are found to inhibit mineralization *in vitro*. Bird reported, however, that patients with kidney stones generally excrete abnormally large amounts of zinc. In most cases, calcium-levels in urine were also elevated. It was noted that zinc excretion increased when the dietary intake of calcium was increased. Since most of the zinc in the body is incorporated in bone, it is not surprising that the metabolism of zinc is closely involved with that of calcium, but further work is needed to obtain a convincing picture of the relationship.

The intact human subject has been used in activation analysis, by exposing the body to a broad beam of fast neutrons and studying the induced activity with a whole-body counter. J. M. A. Lenihan\* (Western Regional Hospital Board, Glasgow) suggested that the total quantity of iodine in the thyroid gland (a matter of great interest to the physician) might be determined by irradiating the organ with thermal neutrons and assaying the induced activity due to  $^{131}\text{I}$ , by external scintillation counting. Sodium and chlorine, the main interfering activities, are rapidly cleared, but iodine is trapped in the thyroid—though some loss might occur as a result of the Sklar-Chalmers process. A total dose of  $10^{10}\text{ n/cm}^2$ , corresponding to 10 rads, should be adequate.

Forensic science offers scope to the activation analyst, since the investigation of material traces is frequently decisive in linking the suspect with the crime—or, what is just as satisfying, in dissociating him from it. Small samples of paint may be examined for evidence of common origin by estimation of trace elements (such as manganese, indium or copper) which are present at such low levels as to be of no obvious importance to the user and are there-

fore not controlled in the manufacturing process. For this reason, the concentrations usually vary considerably from one manufacturer (or even one batch) to another. V. P. Quinn (General Atomic, San Diego) suggested that gunpowder might be deliberately labelled with minute amounts of elements such as dysprosium and europium, readily determined by activation analysis. Collaboration among manufacturers would make it possible to determine not only whether a suspect has recently fired a gun (from the presence of powder traces on the hand or cheek) but also to identify the brand and calibre of the ammunition used.

M. J. Pro\* (U.S. Internal Revenue Service) reported that activation analysis had helped to fortify the revenue in more than 100 cases investigated during the past eighteen months. Approval is awaited from the Food and Drugs Administration for a scheme to label all narcotics produced in the United States; 16 elements will be available as markers and three will be used for each drug, allowing 560 combinations. Trace element analysis has been used successfully to identify whisky from an illicit still. Age claims (up to 100 years) in a consignment of imported brandy were refuted without the need for activation analysis by estimating the tritium content of the liquor.

Several groups are now examining the possibility of personal identification by trace element analysis of hair. W. S. Lyon\* (Oak Ridge National Laboratory, Tennessee) considered that positive identification was not yet possible, mainly because of differences in chemical composition among samples from the same head—or even from the same hair. R. F. Coleman\* (Atomic Weapons Research Establishment, Aldermaston) gave a preliminary report of investigations now being made to assess these difficulties and to develop a statistically reliable criterion for the identity of origin of two samples. J. P. W. Houtman\* (Reactor Institute, Delft) discussed the influence of diet on the composition of hair. In samples from 16 Trappist monks, for whom diet and environment were strictly controlled, concentrations of bromine, gold and zinc in the hair showed little variation from one individual to another, but considerable differences were found in the levels of arsenic, chromium and mercury.

Houtman also described work on activation analysis of pigments as an aid to the detection of false attribution of painting. White lead is a suitable material for investigations of this sort, since it has been used by painters for more than five centuries. Its major constituents are not appreciably activated by thermal neutrons, but a number of impurities, including silver, copper, antimony, zinc and bromine, are readily made radioactive. Estimation of these and other trace elements gives useful information as to the period in which the pigment was manufactured.

These two meetings gave evidence of the rapid spread of ideas and practical collaboration from the relatively small number of laboratories now engaged in activation analysis. The technique has not penetrated effectively into veterinary science or agricultural chemistry and is clearly capable of greater usefulness in biochemistry, both in the examination of trace element metabolism and in the determination of oxygen and nitrogen. Industrial applications, depending on the rapid analysis of raw materials or manufactured products, are also likely to grow with the increasing availability of modestly-priced neutron generators and pulse-height analysers.

The methods of activation analysis will not be fully exploited in scientific research and in industry until the subject finds a place in the undergraduate curriculum. The experimental principles are well within the grasp of the able student and can be taught in a relatively short time with simple equipment. There is also an evident need for a greater amount of systematic instruction at the postgraduate level, to meet the needs of research workers and teachers.

J. M. A. LENIHAN

## THE LONDON MATHEMATICAL SOCIETY

By PROF. H. DAVENPORT, F.R.S.

University of Cambridge

THIS year the London Mathematical Society completes one hundred years of existence, and the occasion was marked by a Centenary Meeting during July 13-15. There were seven lectures by eminent mathematicians, pure and applied, including two of the honorary members of the Society: Prof. Cartan of Paris and Prof. Nevanlinna of Helsinki. The lectures were delivered at University College, London, and a reception was also held in the rooms of the Royal Society on the evening of July 13 and a dinner at Goldsmiths' Hall on July 15.

It was particularly appropriate that the Centenary Meeting should have been held at University College, London, for it was there that the Society came into being. The proposal to form a mathematical society was first put forward by a small group of students there, and in the circular letter which invited attendance at a preliminary meeting on November 7, 1864, it was called the University College Mathematical Society. One of the students was George de Morgan, a son of Augustus de Morgan, professor of mathematics at the College during 1828-1831 and 1836-1866. Prof. de Morgan took a keen interest in the proposed Society, and it was due largely to his influence that it became a national learned society and not a College society. He was a mathematician with a broad range of interests, though not in the first rank as a discoverer, and was widely known for his writings (especially his *Budget of Paradoxes*), for his long connexion with the Royal Astronomical Society, and for his public service. (He was a prominent member of the Government Committee which reported on decimal coinage in 1863 in much the same terms as a similar Committee in 1963.) De Morgan's role in the foundation of the Society is commemorated by the De Morgan Medal, awarded every third year for distinguished contributions to mathematical knowledge.

The inaugural meeting of the London Mathematical Society, with twenty-seven members, was held on January 16, 1865, with Prof. de Morgan, the first president, in the chair. His address can still be read with interest. After stating the aims of the Society and giving his views with regard to the best policy by which to pursue them, he went on to urge the importance of a deeper study of the relationship between mathematics and logic. He also attacked (with good reason) the Cambridge examination system of the time.

The first vice-president was Dr. Thomas Hirst, who had studied at Marburg, Göttingen and Berlin, and was therefore, for a British mathematician of that period, unusually familiar with current mathematical developments on the continent. He was then professor of mathematical physics at University College, but succeeded de Morgan as professor of mathematics after the latter had resigned in 1866, in protest against an appointment made by the College Council under the influence (as he believed) of sectarian considerations.

The speed with which the Society attracted the support of leading mathematicians was remarkable. It must be attributed partly to the influence of de Morgan and Hirst and partly to the fact that the new society met a real need. The next two presidents after de Morgan were Sylvester and Cayley, the two greatest British pure mathematicians of the century, and a few years later H. J. S. Smith of Oxford served as president, and was followed by Lord Rayleigh. Rayleigh (then the Hon. J. W. Strutt) had joined the Society as a young man in its early days, and served for a long time on its Council. Another young mathematician of great originality, who

joined the Society while still a Cambridge undergraduate, was W. K. Clifford. Among other active members in the 1870's were Clerk Maxwell and William Thomson (later Lord Kelvin). There was a good deal of overlap between the memberships of the London Mathematical Society and of the Royal Astronomical Society, and some (for instance, Cayley) were active in both\*.

The aim of the Society has been constant from the day of its foundation: "the promotion and extension of mathematical knowledge". This aim has been mainly pursued by the publication of original papers; the Society has now published 104 volumes of the *Proceedings* and 89 volumes of the *Journal*. In addition there have always been monthly meetings, held nowadays in the rooms of the Royal Astronomical Society at Burlington House (except for one meeting each year, held in a university city other than London). The meetings served a somewhat different purpose in the early days of the Society than they do at present. They attracted men who were pursuing mathematical research, often of real originality, in isolation, while earning a living in some profession; and brought them into contact with the leading mathematicians of the country and with one another. A good deal of informal discussion occurred at the meetings, not always related to the papers that had been read. In recent times the meeting is usually given over to a lecture, often by a visiting mathematician from abroad, though occasionally there is a group of short talks on related topics instead.

The Society's publications have naturally reflected, in the character of their contents, the changing interests of the mathematicians of the country, and these have generally corresponded (though sometimes after a delay) with the interests of the mathematical world as a whole. Until the 1890's, there was a preponderance of papers on algebra and geometry. Then began the reign of analysis, which may be said to have continued, with variations of emphasis, until after the Second World War. In the 1930's and 1940's there were also many papers on number theory, and in particular on the geometry of numbers. In recent years, topology has loomed large, and algebra, in its various abstract forms, perhaps even larger. This is to speak only of pure mathematics; in applied mathematics the Society's publications, although containing many important papers, cannot now be considered to be fully representative of the tendencies of current research. This is mainly a consequence of the gradual evolution of various branches of applied mathematics into independent sciences with their own journals.

The scale of publication has had to be increased steadily over the years, and even now, with about 1,600 pages a year in the *Proceedings* and *Journal*, it barely keeps pace with the flow of good papers. The editing, printing and publishing of so much difficult mathematical material each year make heavy demands on the time and energy of the secretaries and editors, particularly as each paper involves correspondence with one or more referees, and consideration of referees' reports by the Council or the Journal Committee. The Society has never had any full-time paid staff.

Indeed, the Society has always been run with financial resources which were small in relation to its potential commitments, and has depended heavily on the devoted services of its officers. Dr. Glaisher (president 1884-86) recalled in his reminiscences on the occasion of the

\* More about the history of the Society will be found in Sir Edward Collingwood's article in *New Scientist*, 23, 94 (1965).

sixtieth anniversary of the Society (*Journal*, 1) that there was serious financial stringency in the early 1870's, and that a reduction in the scale of publication was then averted by a generous benefaction of £1,000 from Lord Rayleigh. "I do not think," wrote Glaisher, "that any gift was ever more opportune, or that any money was ever better spent."

One of the most eminent, and most devoted, members of the Society was G. H. Hardy, who was twice president (1926-28 and 1939-41) and twice secretary. He took on the secretaryship again immediately after his second term as president, at a time when war conditions made it difficult to carry on the Society's work. In his presidential address of 1928 he said: "The Society has always meant much more to me than any other Society to which I have belonged". He took a deep interest in the Society's affairs for more than forty years. It was mainly at his suggestion that the *Journal* should contain shorter (and possibly more readable) papers than the *Proceedings*. An editorial committee appointed by the Society is at present producing Hardy's *Collected Mathematical Papers*, and it is hoped that the first of the seven volumes will be published by the Clarendon Press this year. The Society has just published an extra volume of *Proceedings* in honour of the eightieth birthday of J. E. Littlewood, whose long and extremely fruitful collaboration with Hardy is without precedent in the history of mathematics.

The Society received its greatest benefaction to date under the will of Hardy's sister, Miss G. E. Hardy, who

survived him and died in 1963. This, like Rayleigh's benefaction, has been of great value, in that it has encouraged the Society again to expand its publications and to embark with greater confidence on further activities in pursuance of its aims. One of these is the organizing of instructional conferences of two- or three-week duration, at which courses of lectures on some important branch of mathematics in which current research is active are given by leading experts from Britain and from overseas. So far there has been one such conference on functional analysis and one on probability theory, and the third, on algebraic number theory, is to be held at the University of Sussex in September. Other recent developments are the foundation (jointly with other Societies) of the *Journal of Applied Probability*, and the publication (with support from the Science Research Council) of *Russian Mathematical Surveys*.

The London Mathematical Society of to-day, with its eight hundred members, is on a larger scale than could have been anticipated by its founders, but it is still animated by their spirit. The desire to help one another in the advancement of mathematical knowledge is still strong. This applies not only to the officers but also to the many members who share in the work (which is often onerous) of refereeing papers. There can be few British mathematicians who have not, at some time, profited greatly from the comments and criticisms of referees of the London Mathematical Society. In this way, as in others, the Society plays a part of vital importance in higher education and research.

## OBITUARIES

### Dr. J. W. S. Marr

JAMES WILLIAM SLESSER MARR, who died on April 29 in his sixty-third year, made outstanding contributions to polar exploration and marine biology. While reading classics at Aberdeen he was chosen as a Scout to take part in the Shackleton-Rowett Expedition of 1921-22 in the *Quest*. After the death of Sir Ernest Shackleton in South Georgia, the expedition failed to achieve its main objective, but much was accomplished over a wide scientific field, notably on the islands of South Georgia, Elephant, Gough and Tristan da Cunha. He returned to Aberdeen to complete his studies and was awarded the M.A. degree in classics in 1924, and the B.Sc. degree in zoology in 1925.

Marr then took part in the British Arctic Expedition with T. A. Worsley and G. Algarsson, and described the plants of West Spitzbergen, Northeast Land and Franz Joseph Land, and the results of dredging in 78°-82° N. After a year as Carnegie Scholar at the Marine Laboratory in Aberdeen, he joined the *Discovery Investigations*. During 1928-29 he worked in the R.R.S. *William Scoresby*, mainly on oceanographic surveys of the South Georgia whaling grounds but also on a winter trawling survey of the Patagonian shelf within a few days steaming of the Falkland Islands. His next voyage (1929-30) was in the R.R.S. *Discovery* with the British, Australian and New Zealand Antarctic Expedition, doing extensive work in Kerguelen, Heard Island and off Enderby Land. In 1931-33 he was in the R.R.S. *Discovery II* working on a programme which included oceanographic observations round the winter ice-edge, and a survey of the South Orkney Islands. An oceanographic survey of the Ross Sea was one of the outstanding events of his next voyage, in 1935-37; but the main feature of the work, much of it in the drift from the Weddell Sea, was his concentration on the study of krill (*Euphausia superba*) on which the baleen whales feed. He experimented with new methods of sampling, fishing deeper and deeper nets to catch the

eggs and early larval stages, and doing his best to measure the extent to which adult krill avoid the ship and towed nets in daylight, and to find a practical method for quantitative sampling which would overcome the patchiness of the krill, which occurs in shoals or swarms.

He deals with these questions in his *Natural History and Geography of the Antarctic Krill*. He shows that the krill population is maintained principally by spawning on or near the Antarctic coastal shelf, where the current follows the prevailing east wind. The west-wind zone farther north has relatively few krill except where there is a marked northerly outflow of water from high latitudes, the great exception being in the Atlantic sector, where the west-wind zone, being fed by a massive outflow from the Weddell Sea, is the area where krill is most abundant. It is there that they are largest and most numerous, the whales fattest and the oil of highest quality. But this area of great abundance is not a successful spawning area. Female krill ready to spawn are taken in the nets and found in the stomachs of whales, and krill eggs can be collected by keeping the gravid females alive in a tank until they spawn, but very few eggs, nothing like enough to support a population, are taken from the sea. Study of the vertical distribution of krill larvae in the ocean shows that krill eggs hatch at great depths, probably below 2,000 m if the water is deep enough, and the hatched larvae rise stage-by-stage, reaching the surface as first Calyptopis larvae. This developmental ascent is seen to take place successfully near the Antarctic shelf, but not in the west-wind drift, and the rich population east of South Georgia is seen to be maintained not by eggs hatched locally but by larvae rising from the Weddell drift which brings them from successful hatching regions farther south. What happens to the eggs laid in the rich east Atlantic sector—whether they are carried back to the south, or lost to the north—remains a mystery.

It was to be expected that such remarkable conclusions would cause a stir, and Marr was extremely careful about putting forward all points of view, and very thorough in

reasoning those which seemed to him best to fit the evidence. His conclusions are based on an unprecedented number of observations, an enormous collection of all stages in the life-history, copious field notes on habits and behaviour, counts, measurements, sex determinations, and information on developmental conditions to which most *Discovery* scientists had contributed over 25 years. It has a very wide coverage in place, time and depth, though not so critically distributed as it could be now that the work is done. No one is likely to collect as much again; but we can now see what to do next, where to look and what to look for. To have made this possible is a very great achievement since the krill occupies a supreme position in the economy of the Antarctic seas.

Marr wrote a fascinating account of the history of the South Orkney Islands, and of the scientific work done during the *Discovery's* survey of the group, taking great pains to give a clear account of what earlier explorers had done. His collecting was done with tremendous enthusiasm and he spared no effort in recording and preserving the material. He wrote on the preservation of marine animals and was so outstandingly successful in collecting and keeping them in good condition that visitors to the ship, whether eminent scientists or gracious Commonwealth Governors, were always charmed with what they saw. His last major work was on the natural history and distribution of the unstalked crinoids of the Antarctic shelf. He always wrote with a zest for his subject that makes it a pleasure to read.

During the Second World War he served in Iceland, the Far East and South Africa, and in 1943 he was given the special task of organizing and commanding land bases in the Grahamland region of Antarctica. He was three times awarded the Polar Medal, and he also received the Bruce Memorial Prize of the Royal Scottish Geographical Society and the Back Grant of the Royal Geographical Society.

Worsley, writing of the voyage in 1925, said Marr was the strongest man on board and their champion at stowing any sail, square or fore and aft. He was a great man. However hard and unpleasant the work, he never spared himself, and his failing health during the past few years was undoubtedly due to the prolonged and exceptional conditions of his work at sea and in the Antarctic. His *Natural History of the Antarctic Krill* is a history and text-book of marine exploration and science as well as a remarkable sum of knowledge and experience of Antarctic biology: an American reviewer has described it as natural history at its best.

G. E. R. DRACON

<sup>1</sup> *Discovery Reports*, 22, 23 (1962).

### Prof. V. F. Hess

THE work during the years 1911 and 1912 of Prof. Victor Hess, who died at the age of eighty-one on December 18, 1964, has been recognized as that which clearly established the extraterrestrial origin of the cosmic radiation, and it was for these investigations that he received the Nobel Prize for Physics in 1936.

Born in Waldstein and educated in Graz, where he took his doctorate in 1910, he worked for some ten years at the Radium Research Institute in Vienna before returning as professor to Graz. In 1931 he was appointed professor and director of the newly founded Institute of Radiology at Innsbruck, and there founded the famous Hafelekar Cosmic Ray Observatory at an altitude of 2,300 m, one of the earliest stations for the continuous observations of cosmic-ray intensity. In 1938, moving to the United States, he took a professorship at Fordham University, which he held until his retirement to an emeritus professorship in 1956. He was a member of the Academy of Sciences at Vienna, of the Pontifical Academy of Sciences in Rome and a Fellow of the American Physical Society.

Before Hess undertook his classical experiments, considerable attention had already been directed to the 'residual ionization' which was always found to be present in the gas of an ionization chamber, and speculation had already turned to the possibility of an extraterrestrial origin. The 'residual ionization' varied remarkably little from one place to another, on the surface and under it, over land and on water, and the absorption recorded when the chamber was surrounded by lead shields was unexpectedly slight. Early attempts to move away from the influence of the condensed Earth by ascending the Eiffel Tower and in the first balloon experiments were inconclusive—partly since the effect is not well marked at the heights of ascent which were reached because of the real attenuation of terrestrial radiation, partly because of inadequate instrumentation.

Hess based his work on a detailed investigation of the absorption of  $\gamma$ -rays in air and, for he was a man of conspicuous experimental ability, on greatly improved ionization chamber techniques using hermetically sealed chambers. In 1911 and 1912 he made a number of balloon ascents, finally reaching a height of 5,300 m. He established that while there was a decrease of ionization with increasing altitude up to about 1,000 m, at greater altitudes the observed intensity of ionization increased so that at 5,000 m it was twice that at the ground. Later work was to exhibit a much more striking increase at still greater heights. Since there was no significant difference in the observations by day and by night, nor, on one occasion, during the short period of a solar eclipse, Hess concluded that the postulated extraterrestrial radiation was not of solar origin.

Although this work and the conclusion which Hess drew from it were to become recognized as sound, they did not at first go unchallenged. This was particularly so because of the confusion which surrounded apparently direct absorption measurements in, for example, water, at a time before there was any understanding of transition effects, and it was not until about 1926 that all doubts about an extraterrestrial origin of the penetrating radiation were resolved.

During his work at Vienna, Hess had already become aware of apparent fluctuations of intensity, and over the succeeding years these were to be the subject of many series of observations by different groups of workers, specific features of which only too often were not supported by subsequent work. The Hafelekar Observatory was set up in 1931, and it played a leading part for many years of sustained and reliable intensity monitoring. In addition to important work in the interpretation of the atmospheric effects (including the identification of the negative temperature effect for which an explanation only became possible some years later), the true solar diurnal variation was established. During this period also, Hess considered that an extremely small sidereal variation had been detected, which might be linked with the Compton-Getting analysis of galactic rotation.

In recent years the main advances in the physics of the cosmic radiation have come from high-altitude and extraterrestrial observations, on one hand, and from an ever-widening range of continuous monitoring systems, on the other. Toward both these areas of investigation Hess was responsible for pioneer work of conspicuous excellence, and his death breaks one of the last links with the earliest observations and the identification of the cosmic radiation.

J. G. WILSON

### Mrs. L. W. Jones

Mrs. L. W. JONES, known to her family and friends as 'Joan', died after a sudden two-day illness at Addenbrooke's Hospital, Cambridge, on June 6. The wife of Mr. Lawrence W. Jones (who is a long-serving executive and director of Pye, Ltd., of Cambridge), she came in



close contact with the radio and electronics industry in the late 1920's. At the outbreak of war in 1939, Mrs. Jones started an engineering shop in the garden of her home as her contribution to the national War effort. She led this operation tirelessly throughout the War years, finding additional premises, plant and personnel to keep pace with Britain's desperate need for radio and radar equipment. 'Labgear' was the name ultimately chosen for this business, because of the nature of its products. When the War was over, Mrs. Jones had to make one of her most difficult decisions, whether to terminate the activity or to continue as a manufacturing business for peace-time requirements. Her sense of adventure and loyalty to the staff who had helped her make such a success of the war-time endeavour soon led her to decide to continue.

The immediate post-war years were difficult ones; it was a period of change. Many new companies were

formed; some succeeded, many failed. However, under the guidance of Mrs. Jones, as its founder-director, Labgear, Ltd., prospered and, from its humble beginnings, to-day employs more than four hundred people and is now a member of the Pye Group of Companies.

Mrs. Jones was loved and respected by her colleagues and every employee of the Company. No one did more to ensure a happy relationship between management and work-people, and she was regarded as a specialist in personnel and production management. Apart from her main business responsibilities she did much to organize welfare and social activities in which she personally participated.

Some five years ago Mr. and Mrs. Jones moved to a country residence and but for Mrs. Jones's untimely death she would have retired from business life this year to enjoy the rewards of her full and active career. Mrs. Jones leaves a widower and two sons who are directors of engineering businesses.

## NEWS and VIEWS

Lord Florey, O.M., F.R.S.

As announced from Buckingham Palace, H.M. the Queen has graciously awarded the Right Hon. Lord Florey the Order of Merit. Lord Florey is president of the Royal Society and provost of Queen's College, Oxford. It has also been announced that Lord Florey is to receive the 1965 John Scott Award from Philadelphia, awarded to "ingenious men who make useful inventions". The award consists of £700, a medal and a citation.

Royal Institution: Sir Lawrence Bragg, O.B.E., F.R.S.

SIR LAWRENCE BRAGG will shortly be retiring from his position as director of the Royal Institution—a post which includes the titles of Fullerton professor of chemistry (held by Sir Lawrence since 1953) and resident professor and director of the Davy Faraday Laboratory (since January 1954). This year also he celebrates the jubilee of the award of the 1915 Nobel Prize for Physics to his father, Sir Henry William Bragg, and himself jointly, for their work on X-rays and crystal structure. Only a few months previously they had been jointly awarded the Bernard Gold Medal of the U.S. National Academy of Sciences, an award made only once in five years (previous recipients having been Ernest Rutherford, William Ramsay and Wilhelm Röntgen). It is interesting to note that W. L. Bragg was only twenty-five years old when these awards were made and that he had studied no physics at school and only a very little in the Honours Mathematics course at the University of Adelaide. His serious study of physics only began when he was twenty and in his second year at the University of Cambridge. Two years later, while still studying, he began that fruitful collaboration in research (mainly in vacations) with his father, which effectively founded the science of the structural analysis of solids. After several years in the Armed Forces during the First World War, he was elected a Fellow of the Royal Society in 1921 and was awarded the Hughes and Royal Medals in 1931 and 1946, respectively. He had been made a Fellow and lecturer in natural sciences at Trinity College, Cambridge, in 1914. After demobilization in 1919, he was appointed Langworthy professor of physics, Victoria University, Manchester, where he built up a famous research school concerned with fundamental problems of X-ray diffraction by crystals, and in particular with the structure of the silicates. In 1937 he became director of the National Physical Laboratory, but in 1938 he left Government service to become for fifteen years Cavendish professor of experimental physics in the University of Cambridge.

Here he resumed his earlier researches into the structure, texture and properties of metals and he became especially interested in the determination of the structure of protein molecules; both these interests he has maintained in directing the work of the Davy Faraday Research Laboratories.

As resident professor of the Royal Institution he has introduced several brilliantly successful innovations. The popularity of the Christmas Lectures for children, a set of which he himself had given in 1934–35, persuaded him that fourth-, fifth- and sixth-form scholars would benefit by having similar courses throughout the year. These could be aimed at a more homogeneous audience and illustrated by demonstrations involving apparatus too large or too costly for school resources. Many thousands of young people have now been interested and inspired by such Royal Institution lectures. Other schemes have involved science teachers and classically trained Civil Servants. While the subjects of the Friday Evening Discourses have been broadened somewhat in interest, the social side of the Royal Institution has also been extended, a responsibility very fully shared by his distinguished wife, and by the presidents of the Royal Institution under whom he has served, the late Lord Brabazon and the present president, Lord Fleck.

Prof. G. Porter, F.R.S.

PROF. G. PORTER has been appointed to the directorship of the Royal Institution in succession to Sir Lawrence Bragg. After graduation from the University of Leeds, where he was Aikroyd Scholar, Prof. Porter served in the Royal Navy in the Western Approaches. He then went to Cambridge, and pioneered the technique of flash photolysis for which he received his Ph.D. in 1949. He then became a demonstrator in physical chemistry in the University of Cambridge, and was made an assistant director of research and a Fellow of Emmanuel College in 1952. In 1954 he joined the British Rayon Research Association as assistant director. He was appointed to the newly created chair of physical chemistry in the University of Sheffield in 1955, and became Firth professor and head of the Department of Chemistry in 1963. Prof. Porter has received world-wide recognition for his researches into free radical spectroscopy, chemical kinetics, energy transfer, and the physical and chemical properties of aromatic molecules in triplet states. He was awarded the Corday-Morgan Medal in 1955, was Tilden Lecturer of the Chemical Society in 1958–59, was elected to the Royal Society in 1960, was Peter C. Reilly



lecturer of the University of Notre Dame in 1961, and delivered the Remsen Memorial Lecture of the American Chemical Society in 1962. Prof. Porter has a deep interest in scientific education as well as in scientific research, and is well known for his lectures on chemistry to school children. The professorship in chemistry at the Royal Institution was revived for him in 1963, and he has given many lectures in this capacity, both to scientists and non-scientists, while also leading the Department of Chemistry in Sheffield. It is to be hoped that this new appointment will give him the maximum opportunity for the advancement of scientific research and education.

#### National Institute of Agricultural Botany:

##### Establishment of Systematic Botany Branch

A new Branch has been established within the National Institute of Agricultural Botany. This will be known as the 'Systematic Botany Branch' and will consolidate work on the description and identification of plant varieties, which is at present undertaken by Trials and Seed Production Branches. Another function of the new Branch will be to undertake tests on new plant varieties which are required under the Plant Varieties and Seeds Act, 1964. The Branch will form part of the Variety Classification Unit of the Plant Variety Rights Office and will provide information on varieties which have been submitted either for a grant of rights or for inclusion on the Index of Varieties. The Branch will also supply the rest of the Institute with information about the identification of varieties. This information will be particularly valuable in the planning of future trials to test the merits of varieties, and later for the purpose of the various seed certification and seed crop approval schemes.

##### Mr. A. F. Kelly

The first head of the new Branch will be Mr. A. F. Kelly, who is transferring from his present post of head of Seed Production Branch. Mr. Kelly has been at the Institute since June 1945, when he assisted with the establishment and recording of the renewed programme of variety trials after the Second World War. Later he was in charge of the Institute's seed multiplication work and the production of basic seed of varieties from the official Plant Breeding Stations from 1947 until 1954. In 1954 he was seconded to the Organization for European Economic Co-operation for two years to assist in the development of variety trials of grasses and clovers, and of an international agreement on seed certification methods.

##### Mr. G. Finch

Mr. G. Finch, who has been appointed acting head of Seed Production Branch, is probably better known to vegetable growers than to farmers in general. He joined the National Institute of Agricultural Botany in 1948 and has been specially concerned with the development of trials on varieties of vegetables throughout England and Wales. In his earlier years at the Institute he was responsible for the seed production side of the work as well as the variety trials and is consequently well known to most producers of root and vegetable seeds.

#### The Shirley Institute

After forty years at Shirley Institute, Mr. L. H. C. Tippett and Mr. W. A. Richardson retired at the end of June. Both joined the staff on the same day and have seen the Institute grow from small beginnings to an organization with world-wide reputation.

##### Mr. L. H. C. Tippett

Mr. L. H. C. Tippett, assistant director in charge of physics and mechanical processing since 1951, has become internationally known for his work on operational research and statistics. He was a pioneer in the statistical investi-

gation of process operations in the textile industry. He has served on numerous committees concerned with statistics and allied topics. He was the first chairman of the Committee of Statistics in Industry and Technology of the International Statistical Institute and also the first chairman of the Textile Institute's Quality Control Group. He is now president of the Royal Statistical Society. During the course of his career he has received a number of awards for his work, including the Guy Medal in Silver of the Royal Statistical Society and the Textile Institute's Warner Medal. In 1962 the American Society of Quality Control presented him with its Shewhart Medal for "his outstanding leadership in the field of modern quality control".

##### Mr. W. A. Richardson

The other member of staff retiring is Mr. W. A. Richardson, whose work as a senior finishing liaison officer since 1953 has brought him into contact with many people in the industry. During the first twenty years of his Institute career his research work on starch resulted in a notable contribution to the technology of warp sizing. From 1943 until 1958 he was head of the Rayon Department and became concerned in the ever-widening field of man-made fibre processing. He has served on several international and Textile Institute committees concerned with the standardization of chemical methods to analysis of fibre mixtures.

#### Royal Society Research Appointments

THE Royal Society has announced the following appointments to fellowships, dated from October 1, for two years in the first instance: *A Mr. and Mrs. John Jaffé Donation research fellowship*, to Dr. R. F. Griffin to enable him to continue his work on (i) direct photoelectric measurement of stellar radial velocities, (ii) photometric surveys of stellar spectra at high resolution at the Observatories, University of Cambridge; *The Mackinnon research studentship*, to Dr. P. E. Baker to enable him to carry out studies on volcanological and petrological investigations on island-arc and mid-ocean ridges at the Department of Geology and Mineralogy, University of Oxford; *A Stohert research fellowship*, to Dr. C. L. Nobbs to enable him to continue his work on the determination of the mode of attachment of oxygen to myoglobin of the sperm whale at the Medical Research Council Laboratory of Molecular Biology, Cambridge.

The first Bruno Mendel travelling fellowship has been awarded to Dr. R. Whittam, of the Department of Biochemistry, University of Oxford, to enable him to work in the Department of Biophysics of the Weizmann Institute, Rehovoth, during the academic year 1965-66. This appointment has been established with a gift from Mrs. Hertha Mendel to enable a travelling fellowship to be awarded in successive years to British, Dutch and Israeli candidates to enable them to work abroad in the United Kingdom, The Netherlands or Israel.

#### Medical Research Council

THE Secretary of State for Education and Science, Mr. A. Croxall, in consultation with the Medical Research Council and the President of the Royal Society, has appointed the following to the Medical Research Council from October 1: Prof. D. G. Evans, Department of Bacteriology and Immunology, London School of Hygiene and Tropical Medicine; Prof. W. Melville Arnott, Department of Medicine, University of Birmingham; Prof. J. L. Gowans, Henry Dale professor of the Royal Society, Department of Pathology, University of Oxford. These members have been appointed in succession to Prof. M. M. Swann, who resigned from the membership of the Council on his appointment to the Council for Scientific Policy, and to Prof. Wilson Smith and Prof. M. L. Rosenheim, who are retiring on completion of their term of office.

## Advisory Committee for Scientific and Technical Information

THE Secretary of State for Education and Science has appointed the following to the Advisory Committee for Scientific and Technical Information, the setting up of which he announced in the House of Commons on April 27 (*Nature*, 206, 685, 1965; 207, 234, 1965): Sir James Cook, Vice-Chancellor, University of Exeter (chairman); Dr. N. F. Astbury, director, British Ceramic Research Association; Dr. J. W. Barrett, research director, Monsanto Chemicals, Ltd.; Prof. J. D. Bernal, professor of physics and of crystallography, Birkbeck College, University of London; Dr. G. M. Dyson, consultant; Prof. S. Gill, professor of computing science, Imperial College of Science and Technology, London; Prof. S. P. Hutton, head of Mechanical Engineering Department, University College of South Wales and Monmouthshire, Cardiff; Dr. R. M. Lodge, research manager, Imperial Chemical Industries Fabrics, Ltd.; Prof. G. A. Smart, professor of medicine, University of Newcastle upon Tyne; Mr. B. C. Vickery, librarian, Manchester College of Science and Technology.

## Mond Chemical Products

THE name 'Mond' has become so closely associated with nickel as to be practically synonymous. We think of Mond nickel in terms of various types of nickel steel production, nickel crucibles, tubes, modern coinage, 'nichrome' electric resistance furnaces and heaters, etc., but the diverse applications of this metal in the form of oxides and salts are perhaps not so generally appreciated and do not necessarily recall the name 'Mond' in this particular context. If such omission has indeed existed, it has now been well and truly repaired by the recent issue of a booklet entitled *Mond Chemical Products* (Pp. 10. London: International Nickel Co. (Mond), Ltd., 1965). Apart from nickel oxide and its salts, the 'Mond' range of compounds summarized in this publication includes cobalt oxides and salts, selenium compounds and tellurium. In an accompanying Press release note it is stated that: "Mond chemicals . . . are used in one form or another in such differing places as the kitchen and the paint pot, the machine shop and the farm, the plating shop and the pottery". This great range of uses obviously implies a common factor, namely, a high degree of uniformity and purity in the several chemicals themselves. In this booklet each of the commonly available compounds of nickel, cobalt, selenium, and the tellurium product, are described under the headings: product and formula, approximate content of nickel, cobalt, etc., appearance (form and colour), trade in which used, and purpose of such use; it is in the last category that the wide use of these chemicals is realized. Nickel compounds find uses as alloy additions, active materials for storage batteries, catalysts, colours, fungicides, plating solutions, etc.; cobalt oxides and salts are used as catalysts, colours, for correcting cobalt deficiency in farming, driers, moisture indicators and mordants; selenium compounds are used as colours, catalysts, decolorizers, electrical rectifiers, oxidizing agents, stainless steel, etc., tellurium finds its chief use in the metal industries as an addition element for machinability or, in the case of cast iron, as a carbide stabilizer. A short list of publications dealing with the use of these 'Mond' chemical products, and a list covering world-wide distributors of them, complete the useful data to be found in this booklet.

## The Albright and Wilson Group

THE name of Albright and Wilson has long been associated with the manufacture and marketing of phosphates on a world-wide scale, but in the past few years there have been some major changes in product range, brought about partly by the absorption of other manufacturing companies concerned with both fine and heavy chemicals. To-day, the Albright and Wilson Group

embraces more than thirty-five companies operating predominantly in the chemical field; it manufactures in eleven countries, employs about 11,000 personnel, commands assets now exceeding £45 million, and has a sales turnover of more than £80 million. To bring the public up to date with the present and expanding activities of the Group, there has recently been published a small illustrated brochure which provides a succinct account of its contemporary activities (Pp. 28. London: Albright and Wilson, Ltd., 1965). Detergent and shampoo raw materials have, according to this booklet, been the greatest single factor in the growth of Albright and Wilson during the past fifteen years, because heavy-duty powder detergents contain at least a third by weight of sodium tripolyphosphate as an essential water-softening and dirt-disposal agent. Marchon Products, Ltd., joined the Group in 1955 and this led to further contributions to detergents, notably surface-active agents such as alkyl aryl sulphates, fatty alcohol sulphates, and other specialized ancillaries such as hydrotropes and foam stabilizers. The Group is one of the leading world suppliers of shampoo raw materials for hair-washing, car and carpet shampoos. In the field of fertilizers, insecticides and herbicides, the Group is strongly established; a plant at Port Maitland, Ontario, has an output of 275,000 tons of single and triple superphosphate, phosphatic solutions and feed-grade dicalcium phosphate. Thiophosphates, long made by Albright and Wilson for insecticides, continue in demand, but with the recent addition to the Group of Stafford Allen and Sons, Ltd., the range is now supplemented with the important pyrethrum and derris extracts. Sodium chlorate continues as a weed killer, but the tendency is now towards the use of organic herbicides such as ammonium sulphamate; for fungicides and bactericides, organo-tin chemicals manufactured by the Group (for example, tributyl tin oxide) are of increasing importance. Another important field concerns flavours and fruit extracts, involving production of essences, essential oils, juice compounds, and powders; spices are included. Fruit juices, extracts and concentrates are produced by the Group in eight countries, the most important being derived from lemons and oranges. Other interests lie in metal-finishing chemicals and processes, manufacture of perfumery and cosmetic products, pharmaceutical products, plastics and paint chemicals, silicones, sodium chlorate and paper products. The brochure concludes with brief notes on other products of the Group, on sales and marketing organizations of the member companies, technical service, research and development, and on the work of its engineering departments responsible for design and construction of most of the new plants and factories required.

## City of Gloucester Museums

THE report of the City of Gloucester Museums for the year ended March 31, 1964, issued in an attractive format, gives some idea of the extremely munificent bequest received under the will of the late Mr. Samuel Stanley Marling (Pp. 16+4 plates. Gloucester: The Museums, 1965). It comprised eighteenth-century furniture, English domestic silver, Bristol and Nailsea glass, period barometers, clocks and watches, early trees, English gold coins, and eleven oil-paintings attributed to Richard Wilson (1713-82). Outstanding objects include an ivory-cased stick barometer by Daniel Quare (1648-1724), a month long-case clock by Christopher Pinchbeck (1670-1732), and an exceptionally fine silver-gilt inkstand in the form of a celestial globe, made by John Robbins in 1792. A token exhibition of the bequest was staged in 1963, but the remainder must await the building of two additional galleries as extensions to the City Museum. These will be constructed over the present single-storey section of the Brunswick Road buildings. Six further cabinets have been constructed to house the herbarium, and the Upton Collection of land and freshwater molluscs has been

re-catalogued. Ten type specimens of fossil brachiopoda and a crushed specimen of *Liparoceras cheltonae* from the lower Lias of Battledown Brickworks, Cheltenham, have been transferred to the British Museum (Natural History).

#### City Museum, Sheffield

THE annual report of this progressive Museum for 1963-64 records that work has commenced on the long-term project of compiling a card-index of all published references to the natural history of the Sheffield region (Pp. 23+8 plates. Sheffield: The City Museum, 1965). Even in its early stages it is proving valuable to the Museum staff in dealing with enquiries, and eventually it should prove equally valuable to research workers and members of local societies. An unusual feature in an annual report is a chronological survey of table cutlery. The compendium covers the typology of cutlery, especially knives, from the tenth to the twentieth century. It is fully illustrated and the dating will be of interest for its own sake and for the light which it may shed on the date of associated items or structures. Sometimes, an estimate of the date of manufacture of a knife which is featured in a painting or drawing may assist in determining the age of the picture. Likewise, the representations of knives sometimes found carved on wood or stone, engraved on metal, painted on ceramics or even embroidered on textiles, may help to date the objects themselves.

#### Serotine Bats

THE Earl of Cranbrook has reported that, during late June, serotine bats were observed to be moving in a more or less discrete group up and down a two-mile stretch of road and feeding on summer chafers (*Transactions of the Suffolk Naturalists' Society*, 13, Part 1; February 1965). A small number were captured in mist nets. When in pursuit of their prey the bats showed a remarkable facility in avoiding the relatively dense motor traffic along the road; no casualties were observed. All the bats captured were lactating females, although they were identifiable as juveniles by weight and tooth wear. A few noctules flew with the serotines in pursuit of the same prey. The serotines seemed to be affected more by the weather than were the noctules.

#### Fruit and Vegetable Dehydration

Among the methods of preserving and processing food, that of dehydration occupies an important position. It turned out to be particularly valuable during the Second World War, and there was an outburst of technical and scientific activity to cope with the demands at that time. After a post-war recession of interest in dehydrated products, they are now one of the most rapidly developing sections in the field of food technology. A good deal of this is due to the pioneering work carried out at the Ministry of Agriculture, Fisheries and Food factory at Aberdeen, especially in developing methods of freeze-drying of foods. The publication of *The Dehydration of Fruits and Vegetables—A Review of Methods*, by L. P. Hall, is to be welcomed; this is published as *Technical Bulletin* No. 9 by the Fruit and Vegetable Canning and Quick-Freezing Research Association, Chipping Campden. (The word "Dehydration" could now well be included in their name, or perhaps "Processing" could be substituted for Canning, Quick-Freezing, and Dehydration.) This is a short and competently written survey of existing methods of dehydration. It is sufficiently up to date and comprehensive to include tumbling freeze-drying, as well as fluid-bed and belt-trough drying; and explosion puffing as a means of pre-heating foods to speed up the actual drying process. The excellent descriptions in the booklet (Pp. 16) might have been shortened and improved by a number of diagrams showing the principles of the various processes. There are useful sections on bacteriological and nutritional aspects of dehydrated fruits and vegetables. The review as a whole is a model of brevity and clarity, and will be a

useful short guide to the subject even after a fuller and more comprehensive account of dehydration methods has been produced.

#### Descriptions of Pathogenic Fungi and Bacteria

THE Commonwealth Mycological Institute, Kew, began in 1964 to issue a series of loose-leaf sheets "to provide standard descriptions of pathogens for use by pathologists, particularly those isolated workers with restricted library facilities". These have now reached a very substantial volume of six sets of ten sheets each (price 5s. per set, post free) and it is obvious that they fill a long-felt want not particularly related to restricted library facilities. The first set, moreover, dealt with ten rust diseases of warm region crops, but subsequent sets have described pathogens found over a much wider range of climate. Set 2 considered ten bacterial diseases; Set 3, *Fusarium* spp.; Set 4, *Phytophthora* and *Pythium* spp.; Set 5, another ten bacteria; and Set 6, rust diseases of temperate and tropical crops. Workers at the Institute seem to have selected for description pathogens which present some difficulty in practice, and their treatment is invariably concise yet comprehensive. Each pathogen is described in detail, with cultural information where applicable, with host range, symptoms, geographical distribution, references to literature and most useful miscellaneous notes. Descriptions of fungi are illustrated by at least two half-tone illustrations which are excellent for the purpose. These sheets save much time in the day-to-day diagnosis of plant pathogens and have a remarkably wide application.

#### Development and Genetics in Higher Plants

THE success of modern research is very dependent on the choice of a suitable experimental material, especially in the field of development and genetics in higher plants. In this respect the small annual cruciferous plant *Arabidopsis thaliana* (L.) Heynh. has many advantages, for example, high variability in natural habitats, little space requirement, low demands in culture, rapid generation succession, abundant seed production, readiness for experimental hybridization and autogamy, a polymorphic spectrum of induced mutations, vigorous growth in sterile culture, availability of clear phenotypic marker genes and low chromosome number. Therefore in the past decade *Arabidopsis* has gained special attention as a model plant in laboratories throughout the world. This fact was confirmed at the first international symposium on "Arabidopsis Research", held in Göttingen during April 21-24. The symposium was arranged in five sessions: (1) taxonomy and variation (including ecological distribution, interspecific hybridization, polyploidy and embryology); (2) development (genetic control of differentiation and morphogenesis, seed dormancy, vernalization and flowering); (3) genetics (genetic basis of segregation, linkage, non-meiotic recombination and plastom mutation); (4) mutant analysis (isolation and analysis of biochemical mutants, chlorophyll formation and plastid fine structure in leaf colour mutants); (5) mutagenesis (X-ray-induced embryonic malformations, chemical structure of  $M_1$ -plants, heterologous tritiated-DNA- and thymidine-analogue incorporation, induction of recessive lethals and action of various chemical mutagens). The versatility of *Arabidopsis* was thus well demonstrated by this broad spectrum of subjects as well as by some particularly outstanding experimental results, such as the first proof of mitotic recombination in higher plants, or a detailed insight into the mechanisms of vernalization or the pathway of thiamine biosynthesis by means of nutritional mutants. The symposium was especially valuable for the exchange of methods and general experiences dealing with the design of experiments with this 'botanical *Drosophila*'. This was implicit in the twenty-nine papers and the discussions that followed them from the thirty-five

research workers who took part in the symposium. Thus the *Proceedings* of this symposium should serve as a good introductory guide for the utilization of *Arabidopsis* in scientific experiments (they are available from the editor, Dr. G. Röbbelen, Institut für Pflanzenbau und Pflanzenzüchtung, Universität Göttingen, von Sieboldstr. 8, Deutschland).

### Clean Air

THE thirty-first annual Clean Air Conference, sponsored by the National Society for Clean Air, was held at Harrogate during October 20-23, 1964, and the *Proceedings* have recently been published (Pp. 132. London: National Society for Clean Air, 1965. 25s.). The opening address was given by the Rt. Hon. Lord Sheffield, until recently chairman of the Atomic Energy Authority; his benediction was appropriate: "If you want to have cheap power with clean air, go nuclear". Dr. Albert Parker delivered his presidential address, which preceded the opening in the Exhibition Hall of the largest "Clean Air, Fuel Efficiency and Domestic Heating Exhibition" in the Society's history. The subsequent proceedings covered smoke-control area reports from various parts of Britain; papers on the rarely discussed topic of air pollution and town planning; on smokeless fuels—the future supply position. Dr. J. S. Carter gave the Des Vœux Memorial Lecture, entitled "A Century of Achievement, reference the Alkali Act". Another subject discussed was road vehicle pollution, dealing particularly with black smoke, some causes and possible methods of control. The important document concerning the menace of sulphur dioxide, the *Sulphur Dioxide Report*, the work of a special technical committee, as presented by Dr. J. S. G. Burnett, is available as a separate publication; although not included in this volume, the discussion on it followed that on "Road Vehicle Pollution", and is given in detail.

### Announcements

THE fifth International Seaweed Symposium will be held in Dalhousie University during August 25-28. Further information can be obtained from Dr. E. Gordon Young, National Research Council, 1411 Oxford Street, Halifax, Nova Scotia.

THE fourteenth North American Clay Minerals Conference will be held in the University of California during August 23-26. Further information can be obtained from Clay Minerals Conference, Engineering Extension, University of California, Berkeley, California.

THE seventh international conference on "Phenomena in Ionized Gases" will be held in Belgrade during August 22-27. Further information can be obtained from the Seventh International Conference on Phenomena in Ionized Gases, P.O. Box 699, 16/0/IV, Studentski trg, Beograd, Yugoslavia.

THE thirty-eighth congress of the Australian and New Zealand Association for the Advancement of Science will be held in Hobart, Tasmania, during August 16-20. Further information can be obtained from Mr. E. L. Freeman, Science House, 157 Gloucester Street, Sydney, New South Wales.

A SYMPOSIUM on "Insect Behaviour", arranged by the Royal Entomological Society of London, will be held at the Imperial College of Science and Technology during September 23-24. Further information can be obtained from the Registrar, Royal Entomological Society of London, 41 Queen's Gate, London, S.W.7.

AN international symposium on "Microchemical Techniques", organized by the American Microchemical Society, will be held at the Pennsylvania State University during August 22-27. Further information can be obtained from Mr. H. J. Francis, jun., Pennsett Chemicals Corp., 900 First Avenue, King of Prussia, Pennsylvania.

AN international conference on "Luminescence", sponsored by the International Union of Pure and Applied Physics, will be held in Budapest during August 23-30. Further information can be obtained from Dr. G. Szigeti, Research Institute for Technical Physics of the Hungarian Academy of Sciences, Budapest, POB: Ujpest 1.

THE annual meeting of the Plant Phenolics Group of North America will be held in Albany, California, during August 23-25, and will include a symposium on "Phenolic Compounds as Metabolic Regulators". Further information can be obtained from Dr. V. C. Rimeckles, Imperial Tobacco Company of Canada, Ltd., P.O. Box 6500, Montreal, Quebec.

AN international conference on "Electron Diffraction and Crystal Defects", sponsored by the Australian Academy of Sciences, the International Union of Crystallography and the International Union of Pure and Applied Physics, will be held in Melbourne during August 16-21. Further information can be obtained from Dr. R. I. Garrod, Aeronautical Research Laboratories, Box 4331, G.P.O., Melbourne, Victoria.

## THE NIGHT SKY IN AUGUST

All times are in Universal Time

### MOON

New Moon 20d 10h  
Full Moon 12d 06h

### CONJUNCTIONS WITH THE MOON

Venus 20d 06h 4° S.  
Mars 2d 06h 5° S.; 31d 03h 4° S.  
(1.86° N. of Spica 8d 16h)  
Jupiter 22d 07h 2° S.  
Saturn 14d 10h 3° N.

### PLANETS

Times of Rising (R) and Setting (S) during the month

Name	R/S	Beginning	Middle	End	Mag.	D <sub>g</sub> (10 <sup>4</sup> miles)	Zodiacal position
Mercury	R	Unfavourably placed		1h 30m		124	
Venus	S	21h 00m	20h 25m	19h 50m	-3.4	163	Virgo
Mars	S	22h 00m	21h 10m	20h 30m	+1.2	513	Taurus
Jupiter	R	0h 30m	22h 50m	23h 00m	-1.7	811	Aquarius
Saturn	R	21h 00m	20h 10m	19h 30m	+0.9		

D<sub>g</sub> is the distance of planet from the Earth on the 15th of the month.

### OCCULTATIONS OF STARS BRIGHTER THAN MAGNITUDE +6 AT GREENWICH

Star	R/D	Time	Mag.
33 Ari	R	19d 00h 54.6m	5.2
* Gem	D	22d 03h 24.2m	3.2
* Gem	R	23d 03h 57.8m	3.2

(D, disappearance; R, reappearance)

### METEORS

Name	Active period	Date of maximum	Radiant	Remarks
δ Aquarid	July 15-Aug. 10	July 29	239° R.A. -17° Dec.	Observation favourable
Perseid	July 29-Aug. 17	Aug. 12	46° R.A. +53° Dec.	Observation unfavourable at maximum due to full moon

OTHER PHENOMENA: None

## GAS CHROMATOGRAPHY

AN informal symposium organized by the Ministry of Technology was held on May 31 at the Paisley College of Technology, Paisley, with Mr. A. F. Williams in the chair. The participants were welcomed on behalf of the College by the principal, H. N. Henry.

The first paper was given by Mr. G. R. Jamieson (Paisley College of Technology) on "Gas-Solid Chromatography using Adsorbents with Modified Surfaces". He first pointed out that the advances in gas-solid chromatography had been much less spectacular than those in gas-liquid chromatography but, during the past two years, an increasing number of investigations using gas-solid chromatography had been reported in the literature. Possibly one of the main reasons for this renewal of interest was that with the advent of sensitive ionization detectors, there was now no major difficulty in operating in the linear range of the adsorption isotherm. He then went on to say that although gas-liquid chromatography was, at the moment, more versatile than gas-solid chromatography, the latter method had two potential advantages: (1) the great potential selectivity of the adsorption process; (2) the sharper resolution that should be attainable and thus the greater speed of separations. Various illustrations of these advantages were given: (1) separation of methylpyridines on alkali metal nitrate columns; (2) separations on various inorganic salt columns operated at 88° C; (3) separations on porous layer glass beads-metal oxide columns; (4) separations of quaterphenyls and hexaphenyls on lithium chloride and caesium chloride columns.

Mr. Jamieson next described some separations which he had carried out on various alumina columns. He showed how the retention indices of benzene and alkenes varied depending on the pretreatment temperature of the alumina, and also how, using small sample sizes, well-shaped peaks were easily obtainable. The effect on the retention indices of the foregoing compounds of modifying the surface of the alumina with different sodium halides was described, and he showed that column characteristics could be altered by changing the anion, by either slurring the alumina with the halide or by mixing dry, and by varying the pretreatment temperature of the alumina before modifying the surface with the halide. The effects of using silica instead of alumina were next described and also the results obtained by butylating the silica.

The final point made by Mr. Jamieson was that the use of liquid crystals as stationary phases offered a field of investigation. Liquid crystals behave mechanically as liquids, but they preserve some of the order of crystalline solids. Taking *p*-azoxyanisole as an example, he said that this compound in the nematic state would be expected to show a selective affinity for linear molecules and on a 4 ft. x 0.25 in. column of *p*-azoxyanisole on 'Celite' he was able to separate the isomeric bromotoluenes, the expected order of emergence, *ortho*, *meta*, *para*, being realized.

Mr. Jamieson then took the chair for the second paper, which was given by Mr. A. F. Williams (Imperial Chemical Industries, Ltd., Nobel Division) on "Techniques for Quantitative Gas Chromatography; the Analysis of Cellulose Ethers and Nitroglycerine". Mr. Williams first discussed quantitative gas chromatography in terms of sample addition, the detector and the use of peak heights or peak areas for calculation of results. The katharometer and flame ionization detectors were considered to be the most widely used detectors for quantitative work in the general field of organic analysis. When the katharometer was used for detection, careful control of the amount of sample going on to the column was essential because the detector is linear only over a limited range of concentration. This degree of control was much more important when

nitrogen was used as carrier gas than when helium or hydrogen was used and also varied with the composition of the sample and its type. It was therefore usually necessary to carry out two determinations if accurate results are required, although this depends to some extent on the nature of the sample. When the flame ionization detector was used, considerably more latitude concerning the amount of load may be taken, although the amount of sample used for analysis is the order of a factor of 100-1,000 times less than that used in conjunction with katharometers. No evidence was obtained to show that the use of peak areas instead of peak heights led to more accurate results. A detailed examination of the results obtained using both types of detector for the analysis of a simple benzene, toluene and xylene mixture has been carried out and the effects of load, using a splitting procedure and changes in electrode gap, were illustrated. It was concluded that the flame ionization detector was more useful than the katharometer for quantitative work.

Mr. Williams concluded his paper by illustrating the application of the flame ionization detector to the analysis of mixtures of nitroglycerine, ethylene glycol dinitrate and nitrotoluenes, and also to the rapid determination of alkoxy groups both in simple ethers and also in cellulose ethers.

A considerable amount of discussion followed Mr. Williams's paper about the relative merits of the use of 'peak heights' and 'peak areas' for quantitative analysis and of the problems which arise in the quantitative analysis of mixtures of biochemical origin when the components can be identified, but no pure standards are available for quantitative calibration purposes.

The third paper was given by Dr. F. L. Mitchell (Royal Infirmary, Edinburgh) on "Gas Chromatography of Radioactive Substances". He said that with the increasing use of radioactive isotopes in chemistry and biochemistry, it is important that such a powerful analytical tool as gas chromatography should incorporate a sensitive device for the detection and measurement of radioactivity. Various techniques have been tried, including scintillation counting of continuously collected zones from the column, and gas-flow proportional counting, working at both column and room temperature.

A technique based on that of James and Piper (1961) has been developed commercially and has proved very satisfactory for the counting of carbon-14 and tritium. Before passing through a gas-flow counter at room temperature, the column effluent is first subjected to oxidation and reduction by passing the gas at 800° over cupric oxide followed by iron filings. The argon then contains only hydrogen and carbon dioxide from the organic material originally present, and 4-5 per cent of dry carbon dioxide is added to the stream to produce a suitable gas for proportional counting. The gas-flow counter consists of a metal tube of approximately 10 ml. capacity with a fine stainless-steel wire stretched longitudinally from end to end. After amplification, the output from the counter is fed to a ratemeter which displays on the recorder either the rate at which the pulses are arriving, or the integral of their total. By using the integrator 5 mpc. can easily be detected and measured. The gas stream from the column may be split, one part passing through the normally used detector while the other is directed through the counter, or the whole may be passed through the counter after passing through a conventional closed detector such as that using argon ionization.

Separation by gas chromatography, with simultaneous detection of the zones by both normal techniques and radioactive measurement, has proved useful to the biochemist in the examination of biosynthetic pathways, to the isotope manufacturer for establishing the purity of

labelled compounds, and to the chemist whenever information on the fate of a particular compound during a complex chemical reaction is required.

Mr. J. Wight (University of Strathclyde) read a paper on "The Examination of Food Volatiles by Gas Chromatography". He said that what we term 'flavour' is a combination of taste, smell or aroma, and the 'feel' of the food in the mouth. Of these the aroma is by far the most important and, since this depends on the presence—usually in minute amounts—of certain volatile compounds, the advantages of gas chromatography in aroma investigations become apparent. There are few gas-chromatographic techniques special to these investigations, and the main difficulties lie with the collection and concentration of the volatiles and with the identification of the components. Where large quantities of food have to be stripped of their volatiles, for example, by distillation, steam or nitrogen stripping, care must be taken to avoid loss or change; high-temperature treatment will almost certainly result in the production of artefacts.

Separation and identification are complicated by the large number of components present, the presence of chemically dissimilar classes of compounds and the wide range of retention times. Use must be made of techniques other than gas chromatography, and recently the mass spectrometer has proved to be a most powerful aid to identification.

There are many distinct applications of gas chromatography, for example, the general study of aroma volatiles, the production of aroma by cooking or processing, the investigation of flavour defects of stored foods and the quality control of materials such as essential oils.

It must be remembered that the results obtained by gas chromatography are essentially analytical and that these results have still to be interpreted in terms of

aroma. In the absence of sufficient data on threshold values both of pure substances and of mixtures, it is important that the analytical work be supported by flavour panels. The aroma as sensed by the nose is, after all, the final criterion. Mr. Wight concluded his paper by showing chromatograms obtained from the volatiles of various common foodstuffs.

The final paper was read by Mr. J. Craig Higgins (National Coal Board) on "Developments in the Technique of Mine Air Analysis by Gas Chromatography". Mr. Higgins first of all pointed out that for the special problems involved in mine air analysis, no commercial gas chromatograph had been found suitable and a gas chromatograph had been designed and constructed at the laboratories of the National Coal Board.

He then went on to give a very full description of this gas chromatograph, which was a dual-column instrument but not in the usual sense of the meaning of dual column. Two different column packings were used, 'Molecular Sieve 5X' and silica gel, and detection was by thermal conductivity cells. By means of a special valve system, samples of the gas to be analysed were put on to both columns simultaneously and flow rates were adjusted so that the components from one column were recorded before the components started to emerge from the second column. In using this system a complete analysis of the mine gas could be recorded on the one chromatogram.

Mr. Higgins ended by showing how these chromatographs were used in the National Coal Board's mobile laboratories, and he pointed out that wherever possible duplication of equipment was essential to obviate any breakdown or failure of components in these mobile laboratories.

The symposium concluded with an address by Mr. A. C. Low of the Ministry of Technology. G. R. JAMIESON

## CNIDARIA AND THEIR EVOLUTION

THE Zoological Society of London organized a symposium during March 3-4, at which evolution in the Cnidaria as well as many diverse aspects of this plastic group were reviewed and discussed.

Opening the symposium, Prof. C. F. A. Pantin (Cambridge) gave a stimulating address on "Homology, Analogy and Chemical Identity". To the first two, he added "chemical identity", that is, resemblances which can be traced to the absolute similarity of each set of isotopes in the Periodic Table and of the molecules built from them. He indicated that chemical identity can give rise to packing phenomena, as in crystals, creating well-defined morphological features, far above the atomic level, which are, or partially only, the result of adaptive selection. The independent appearance of particular classes of molecule such as are found in pigments and other 'chemical building stones' arises from chemical identity. Much caution should therefore be adopted in using features of this kind as evidence of evolutionary relationship.

Prof. Pantin indicated that analogy arises because natural selection operates by setting the organism engineering specifications for each part that must be realized for survival. He regarded the longitudinal musculature of cnidarian polyps as an example of a weak specification allowing many solutions. On the other hand, rigid and detailed specifications like those responsible for close similarities in complex eyes are not evident in the Cnidaria.

Homologies of parts account for the most characteristic resemblances between animals, these homologies depending on a plan or archetype to which the structure of all species in the class may be referred. This archetype (which is not an ancestor) and its homologies he considered as a network of tissue relationships. Further, he thought the system as a whole met an engineering requirement and

that natural selection acted strongly against any serious departure from the network of tissue relationships. However, this network could be distorted to meet the needs of adaptive radiation, and as examples he quoted the different mesenteric patterns of the Anthozoa.

Dr. L. E. R. Picken and Dr. R. J. Skaer (Cambridge) gave a lucid review of researches on nematocytes and dealt comprehensively with the subject. Among other interesting items, the authors discussed a new aspect of nematocyst chemistry—phosphatases, 5-nucleotidase and cholinesterase. Several amino-acid analyses of nematocysts isolated in bulk are now available and something is known of certain other chemical components.

There was also a very interesting demonstration, from electron microscope investigations, that the wall of the undischarged thread in isorhizas of *Corynactis viridis* is pleated to form a triple screw and that the undischarged capsules have a 'notional' osmotic pressure up to approximately 140 atmospheres.

The authors believe that cnidoblasts may still be regarded as independent effectors although the excitation-threshold of the cnidocil-cnidoblast unit may be under nervous control in some instances, depending on the physiological condition of the animal. It also emerged that taxonomic investigations on the different kinds of nematocysts, based on the light microscope, are not enough and that further electron-microscope examinations are essential.

Dr. David Chapman (Halifax, Nova Scotia) developed some interesting ideas regarding the origins of the Scyphozoa from an examination of the anatomy of the scyphistoma of *Aurelia*. He was inclined to regard the fossil conulariids as ancestral to this group, basing his conclusions on certain puzzling features in the anatomy of the scyphistoma which could be explained by adopting



this evolutionary concept. A new notion that mesoderm is found in polyps in the form of a surface epithelium was linked with the formation of podocysts and like structures. Dr. Chapman concluded from the behavioural investigation that the polypoid preceded the medusoid stage in the evolution of the Scyphozoa and further suggested that the scyphopolyp was the simplest cnidarian polyp. The development of the strobila was regarded as an aeration device for developing gametes. Dr. Chapman concluded his paper with a discussion of the modifications of the scyphistoma in the class, in which he regarded the rhizostome scyphistoma as a very simple cnidarian polyp possessing a complex medusa. This would, in effect, be an example of mosaic evolution although Dr. Chapman did not actually call it so.

From a review of the five orders of Scyphozoa, Dr. Hjalmar Thiel (Hamburg) concluded that the scyphozoan stem form was a tetra-radial polyp with four tentacles, four peristomial pits and four septa, which ancestrally also possessed four or eight gonads on the septa. He derived the metagenetic development of the medusa from the transverse fission of the scyphistoma in which the products of the fission were originally daughter polyps evolving by way of a planktonic life into medusae. The Stauromedusae, a group that combines polypoid and medusoid characteristics, he regarded as having originated through the fusion of the two generations (scyphopolyp and medusa), or in other words an arrested metagenetic development.

The interstitial fauna of sandy beaches and sub-littoral sand contains some very interesting forms and among them some minute Hydrozoa. Since Remane described two actinula-like forms which he called *Halammohydra* in 1927, a further two and a new genus, *Otohydra*, have been investigated at Roscoff by Dr. B. Swedmark and Prof. G. Teissier (Kristineberg and Roscoff). In their contribution to this symposium they reviewed these strange forms (combining the characteristics of polyp and medusa, as well as possessing larval features). These they have placed in a new order, the Actinulida. In their comprehensive survey of these aberrant forms, Dr. Swedmark and Prof. Teissier consider that the Actinulida have arisen from an ancestral actinula which colonized the interstitial environment very early on and there acquired statocysts—a type of organ very common in many different organisms in this environment. In other words, vague suggestions made over the years that the Actinulida are reduced Narcomedusae that have adopted an interstitial habitat are rejected, but, in the discussion that followed, Dr. B. Werner indicated his adherence to the older concept. This paper was followed by a film of *Halammohydra* made at Roscoff.

The evolution of the Actiniaria was discussed in a controversial and stimulating paper by Prof. Cadet Hand (California), who was led from an investigation of the puzzling pattern of development of mesenteries, first as couples and later as pairs, to consider the origins of sea anemones. He pointed out that coupling has an adaptive value in preserving symmetry round the mouth axis but that the pairing of mesenteries does not have this significance. However, in contrast to the situation in the madrepores, the pairing of mesenteries was demonstrated to be an adaptive and functional character. Accordingly, Dr. Hand suggested that the Actiniaria have been derived from corals and, furthermore, that the so-called primitive anemones (Athenaria) may be better considered as secondarily derived forms—thus reversing present ideas. From this new hypothesis the thesarian anemones would be the most generalized and most closely related to corals, and from these a course of evolution to the acanthate anemones and to the athenarian forms was traced.

Prof. Garth Chapman (London) reviewed the structure, function and what is known of the origin of the mesogloea. In many species the mesogloea is of a fibrous nature, and, from its amino-acid composition and

X-ray diffraction pattern, may be called a collagen. It was pointed out that the scyphomedusan mesogloea would probably be represented as a simple polymeric gel, whereas the mechanical properties of the actinians may be best represented as a parallel cross-linked and a non-cross-linked polymeric system. It emerged that the functions of the mesogloea are diverse, being neither clear-cut nor uniform, but include acting as antagonist to muscle, flotation and as a possible nutritional reserve.

Dr. Maxwell Braverman (Pittsburgh) reviewed his work with Dr. R. S. Schrandt on the development of the athecate hydroid *Podocoryne cornuta* by culture from single individuals on microscope slides and gave a fascinating account of an attempt (in collaboration with Dr. Robert G. Schrandt, of Los Alamos Scientific Laboratory) to simulate this development on an electronic computer. He demonstrated how the computer generates a relatively complex pattern as a mathematical model of the growing system by the iteration of a set of simple rules of growth. This growth, which consists of a connected network of points in a two-dimensional net, can be followed generation by generation and selected parameters of the generated pattern compared with those of real colonies. Dr. Braverman also suggested that the ability of simple recursive rules to generate complex patterns may mean that genetic instructions of developing systems may be partly of a similar nature. From the discussion that followed it also emerged that the production of reproductive hydranths is stimulated by an increase in the carbon dioxide in the stolons that give rise to them.

The various theories concerning the origin and evolution of the Hydrozoa were discussed by Dr. W. J. Rees (London), who attempted to provide an explanation of the origin of metagenesis (the alternation of a fixed benthic hydroid phase with a free-swimming medusa phase). Many early authorities were disposed to regard the tetra-radial polyp as a basic cnidarian, but this is too specialized an organism. It was suggested that the proto-coelenterate was a gastrula and that the ancestral cnidarian was a fertile actinula. The actinula possesses all the basic features which through radiation could give rise to all three classes. In this way, the adoption of a pelagic habit probably led to the evolution of Trachymedusae with a direct life-cycle (egg-planulae-adult medusa) and to the Narcomedusae also with a direct life cycle. In the latter, however, some forms such as *Peyronia* exhibit the beginnings of a metagenesis, while the Hydromedusae living to-day possess a well-developed metagenesis, even in the most primitive family, the Moerisiidae. In the hydroids and medusae the trend is towards the elaboration of the hydroid and the gradual suppression of the medusa. This trend would not appear to be the other way round, as Huxley would have us believe in his Turbellaria theory of the origin of the Cnidaria.

The mushroom corals (Fungiidae), both recent and fossil, were reviewed by Prof. J. W. Wells (New York), who discussed their present status from the stratigraphic, biogeographic and systematic aspects. It was shown that they well illustrate an evolutionary trend (which is common to most living and extinct zoantharian corals), a tendency towards colonial or polycentric forms from the basic monostomatous condition found in *Fungia*. Prof. Wells, who also provided a key and synopsis of genera (including several new ones), considered that this evolutionary trend in fungiids is in full spate at the present time, whereas in most scleractinian groups it has already reached its full development. *Cycloseris* appeared in the early Tertiary, and by Miocene times the central form *Fungia* had evolved from it. Radiation in *Fungia* gave rise to several sub-genera which by the late Miocene had given rise to simple polystomatous types, and Prof. Wells thought it probable that by the Pleistocene times all eight of the so-called colonial genera had probably



developed, although three of these are so far known only from the Recent.

In his account of pathways of excitation in Otenophora, Dr. G. A. Horridge (St. Andrews) analysed the conducting systems principally in *Beroë* and *Cestum*, referring also to other species he had investigated. He recognized four conducting systems and, without going into details here, concluded that separate conducting systems arise and multiply in primitive nervous systems independently and without the establishment of functional contacts with the neurones of other pathways. This he regarded as a necessary formula for the development of a central nervous system.

M. Yves Bouligand gave a comprehensive review of all known ectoparasitic and endoparasitic copepods associated with Anthozoa. Most of these are ectoparasitic, belonging to the families Asterocheridae and Lichomolgidae and to the Xarifiidae. This third family has numerous species associated with Madreporaria.

All the endoparasitic copepods belong to the family Lamippidae—a family in which the sexes exhibit a marked degree of dimorphism. There is a considerable amount of deformity, but the characteristics of the integument and of the musculature enable the metameric plan to be worked out. The ultrastructures of the cuticle seem to show that this is involved in nutritional functions in several species. Various aspects of host-parasite relations were also discussed.

A contribution from Dr. J. H. Barnes (Cairns, North Queensland) dealt with three species of venomous Cubomedusae; these were: a small and as yet unidentified carybdeid (the cause of the Irukandji sting), *Chiropsalmus quadrigatus* Haeckel and *Chironex fleckeri* Southcott; the last of these being identified as the only lethal form and the probable cause of at least fifty deaths in North Australian waters. *Chironex fleckeri*, probably the most venomous marine invertebrate known, may kill bathers, in cases of massive envenomation, in 3 minutes or less. Dr. Barnes's lucid and comprehensive paper may be regarded as an interim report on present progress in identifying the organisms, on treatment and on investigations in train.

Dr. Elaine Robson (Cambridge) reviewed the evolution of the swimming habit in the Actinaria—a process which she believes originated independently in the Goniactinidae, the Boloceroididae and the Actinostolidae. In all, the swimming reflects the temporary excitation of pacemaker elements in the nervous system, and its evolution in at least three separate groups may partly be attributed to the fundamental properties of the coelenterate neuromuscular system. Dr. Robson suggested that pre-adaptive features such as the presence of well-developed ectodermal neuromuscular elements and weak basilar musculature, in forms that use the tentacles, and of well-developed perieto-basilar muscles in those that bend the column, may be significant. It is further suggested that comparatively minor quantitative changes in neuromuscular

and receptor properties may have far-reaching effects in actinarian behaviour.

Prof. Demarest Davenport (California) reviewed recent progress in the study of cnidarian symbioses, giving a lively account of work on the behaviour, *inter alia*, of the sea anemone *Calliactis* in relation to its normal substratum (shells occupied by hermit crabs). In particular, he discussed the unique sensory discriminatory power of the tentacles of the anemone to recognise the periostracum of the shell. This work (with which Prof. D. M. Ross is also associated) was illustrated towards the end of the meeting by some remarkably revealing colour films of *Calliactis* behaviour and also of the swimming behaviour of *Stomphia* exhibited by Prof. Ross.

The phylogenetic and evolutionary value of skeletal formations in athecate hydroids was discussed by Dr. W. Vervoort (Leiden), who was concerned with the family Solanderiidae. It was demonstrated that the idea of a mesogloal skeleton of the Solanderiidae was no longer tenable and that as in other capitate hydroids the skeleton was ectodermal in origin. Dr. Vervoort also demonstrated that the genus *Rosalinda* should be placed in this family, being able to accept neither Picard's placing of this genus in the Zancleidae nor his concept of "Pteronematoidae" as a super-family. It was also his opinion that too much stress should not be laid on the value of nematocytes in classification without adequate support from morphological evidence.

Growth in the hydroid *Tubularia crocea* L. Agassiz was discussed by Dr. G. O. Mackie (Edmonton, Alberta). This species was cultured for ten weeks at Villefranche in clean sea-water at 14° C; a diet of *Artemia* was found satisfactory. Continuous growth was recorded in both stolons and hydrocauli along with the production of many new hydranths. Dr. Mackie recorded the patterns of growth for an established colony and for settled actinulae. It appears that hydranths retain full regenerative ability, and although shedding of hydranths was seen, most of them were never shed during the period of investigation. Contrary to some prevailing assumptions, there is no evidence that hydranths become senescent, requiring to be replaced periodically. Hydrocauline growth does not depend on such replacements and it was suggested that hydranth shedding is associated with poor water conditions, and that, given favourable ones, hydranths probably live indefinitely. Dr. Mackie's results thus question earlier conclusions that periodic hydranth shedding is a normal feature of the life of *Tubularia*.

M. Jacques Theodor (Banyuls) exhibited a remarkable series of clear underwater photographs of cnidaria which he had photographed *in situ* in the Mediterranean and in the Pacific. There was also a demonstration of living *Stephanoscyphus* polyps by Dr. B. Werner (Heligoland), who had recently returned from a cruise in the German research vessel *Meteor*. W. J. REES

## MECHANISM OF POPULATION INVERSION AT 6149 Å IN THE MERCURY ION LASER

By DR. D. J. DYSON

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**F**OLLOWING the first report of the mercury ion laser by Bell<sup>1</sup>, several further reports have been published<sup>2-4</sup>. Apart from some tentative suggestions by Bell *et al.*<sup>2</sup> no definite conclusions as to the mechanisms of selective population of the upper laser levels have been put forward. This article describes some results of the observation of the time-variation of the spontaneous emission from the helium-mercury discharge which appear to clarify the

mechanism responsible for excitation of the 6149 Å laser transition in this system.

Fig. 1 shows the intensity, as a function of time, of some spontaneous emission lines from a helium-mercury vapour mixture, following the discharge through it of a condenser of a few hundred picofarads charged to 5–10 kV. These excitation conditions are favourable for laser action at 6149 Å. The 5460 Å line showed a time variation

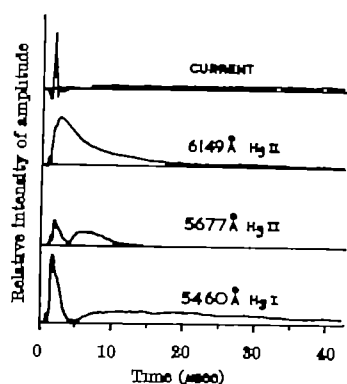


Fig. 1. Spontaneous emission pulse shapes from a helium-mercury discharge compared with the discharge-current pulse

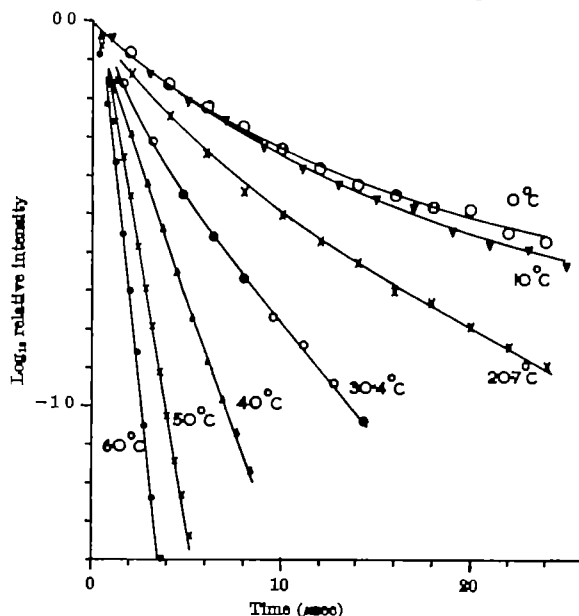


Fig. 2. Decay curves of the logarithm of the 6149 Å emission intensity for a series of temperatures

typical of most of the HgI lines, and the 5677 Å line, which is also a laser line under suitable conditions, showed a time variation typical of most HgII lines. The 6149 Å line, and lines cascading from this, were unique in showing a smooth decay following an initial rapid rise during the relatively short current pulse.

The decay rate characterized by the decaying edge of the 6149 Å emission pulse was found to be sensitive to the discharge tube temperature, controlling the mercury vapour pressure, rather than to the helium pressure. Fig. 2 shows plots of the logarithm of the emission intensity as a function of time for a series of temperatures, the curves having been normalized to the same initial intensity. These measurements were taken with a discharge tube of 2.3 cm internal diameter and of approximately 1 m in length. The discharge condenser was 1,000 picofarads and the charging voltage 8 kV. The helium pressure was about 5 torrs. A mercury pool, with a continuous arc running to a nearby auxiliary anode, formed the cathode. In making the measurements care was taken to observe only emission from the near-end of the discharge tube so as to avoid distortion effects due to the finite optical gain.

The curves shown in Fig. 2 are characteristic of what would be observed if the 6149 Å emission resulted from excitation of the mercury ground-state atom by collision with some excited species formed during the discharge. Writing the concentration of this excited species as  $x$ , and the concentration of the mercury ground-state atoms as  $y$ , then the intensity of the emission at any instant is,

neglecting the small effect of the temperature on the diffusion velocities, proportional to  $xy$ , the product of the concentrations. If  $y$  is predominant, it can be considered to be approximately constant throughout the emission time, and the emission intensity is proportional to  $x$ . The rate of decay of the logarithm of the intensity is therefore

$$\frac{d}{dt} \log_{10} x = \frac{1}{2.303} \frac{1}{x} \cdot \frac{dx}{dt}$$

$$\text{If:} \quad \frac{dx}{dt} = -(k_1 xy + k_2 x)$$

where  $k_1 xy$  represents the rate of disappearance of  $x$  due to collisions with mercury atoms, and  $k_2 x$  the rate of disappearance of  $x$  in the absence of mercury atoms, then:

$$\frac{d}{dt} \log_{10} x = \frac{-1}{2.303} (k_1 y + k_2) \quad (1)$$

The curves A and B of Fig. 3 are plots of the slopes of the curves in Fig. 2 against the corresponding mercury atom concentration  $y$ , taken at times of 2 μsec and 15 μsec respectively. They are approximately in agreement with equation (1) if  $k_2$  is made time-dependent.

If the initial value of  $x$  were independent of  $y$ , then it would also be expected that the initial intensity of the emission would be proportional to  $y$ . Fig. 4 shows a logarithmic plot of the initial intensity against  $y$ . Approximate proportionality is obtained for the lower values of  $y$  where the effect of this on the initial value of  $x$  would be least.

The curve C of Fig. 3 was obtained in the same way as curve A, but using a discharge capacity of 500 picofarads

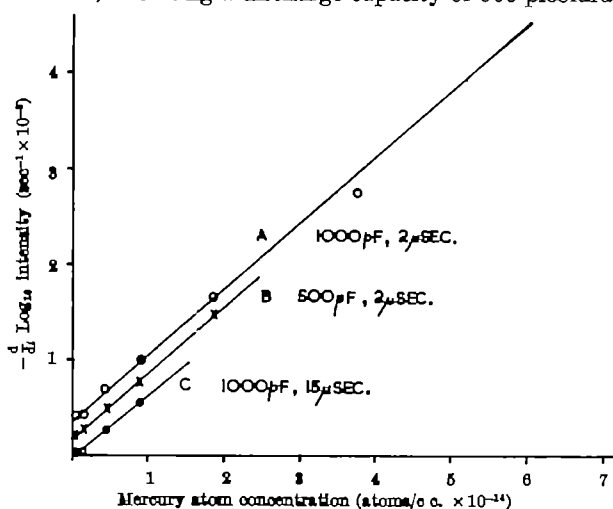


Fig. 3. Decay rate of the 6149 Å emission as a function of the mercury atom concentration

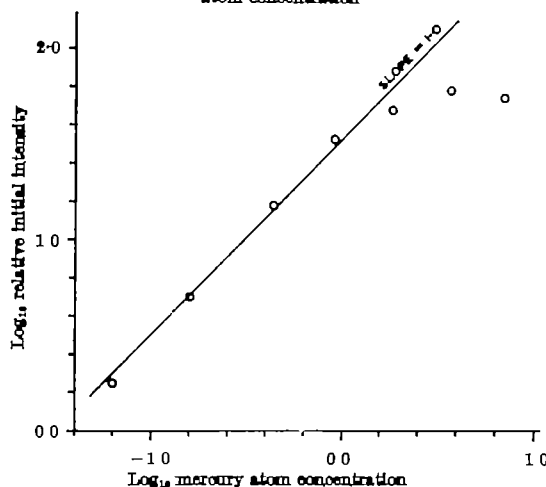
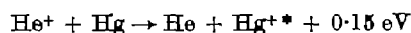


Fig. 4. Initial intensity of the 6149 Å emission as a function of the mercury atom concentration

rather than 1,000. The slope is the same as that of curve A, which shows that the destruction rate of  $\alpha$  due to the mercury vapour is not dependent on the discharge energy, and is therefore not associated with some excited state of the mercury atom, such as a metastable level or the ion, formed by the discharge.

The only excited species capable of raising the ground-state mercury atom to the upper level of the 6149 Å transition is the helium ion. This interpretation is also consistent with the observed time-dependence of  $k_2$  of equation (1), since the rate of loss by ambipolar diffusion is a function of the electron temperature, and the rate of loss by ion-recombination is a function both of the electron temperature and of the electron density.

The laser action at 6149 Å must, therefore, be due to the process:



where  $\text{Hg}^{+*}$  denotes the  $5d^{10}(1S) 7p3/2$  level of  $\text{HgII}$ , the upper level of the laser transition. The cross-section for this process (derived from the slope of the curves in Fig. 3) is  $1.3 \times 10^{-14} \text{ cm}^2$ .

This work was supported by the Ministry of Defence.

<sup>1</sup> Bell, W. B., *App. Phys. Letters*, **4**, No. 2, 24 (1964).

<sup>2</sup> Bloom, A. L., Bell, W. B., and Lopez, F. O., *Phys. Rev.*, **125**, No. 2A, 578 (1964).

<sup>3</sup> Heard, H. G., and Peterson, J., *Proc. IEEE*, **52**, No. 9, 1049 (1964).

<sup>4</sup> Gerdtien, H. J., and Gooderford, P. V., *J. App. Phys.*, **35**, 3060 (1964).

## EFFECT OF ROTATION ON THE STABILITY OF VERY MASSIVE STARS

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IT is now well established that a spherically symmetric massive star, of order  $10^4 - 10^5 M_\odot$ , becomes unstable before the central temperature has risen to the  $6 \times 10^7$  °K needed for nuclear reactions to provide the energy which is radiated away. An approximate analysis of Fowler's<sup>1</sup> gives the same results as detailed investigations by Chandrasekhar<sup>2</sup>, so I shall here follow the approximate method of Fowler to examine the effect of rotation on the stability of massive objects.

With masses of order  $10^4 - 10^5 M_\odot$ , radiation pressure is much greater than gas pressure, the star is convectively unstable, and the structure of the star is that of a polytrope of index  $n$ , where  $n$  approaches 3 as the ratio of specific heats,  $\Gamma$ , approaches  $4/3$ . The virial theorem applied to such a system gives:

$$3(\Gamma - 1)U - \Omega = 0 \quad (1)$$

where  $U$  is the internal energy and  $\Omega$  the gravitational binding energy, reckoned as a positive quantity. With radiation pressure dominant we have<sup>3</sup>:

$$\Gamma = \frac{4}{3} + \frac{\beta}{6} \quad (2)$$

where  $\beta$  is the ratio of gas pressure to total pressure. The total energy of the star is then:

$$E = U - \Omega = -\frac{\beta}{2} U \simeq -\frac{\beta}{2} \Omega \quad (3)$$

As  $\beta \rightarrow 0$ ,  $E \rightarrow 0$ , and the classical massive star approaches neutral equilibrium. For massive object  $\beta$  is given by Eddington's equation<sup>4</sup> which approximates to:

$$\beta = 8.6 \left( \frac{M_\odot}{M} \right)^{1/3} \quad (4)$$

When general relativistic effects are included equation (3) for the total energy is replaced by:

$$E = -\frac{38}{4} \frac{GM^2}{R} + 5.1 \frac{G^2 M^3}{R^2 c^2} \quad (5)$$

where we have substituted for  $\Omega$  the value for a polytrope of index 3, and the extra term is the leading relativistic correction as given by Fowler<sup>1</sup>. As the star contracts the energy will ultimately become positive and the star will then be unstable. In fact, the instability occurs as soon as  $\partial E / \partial R < 0$  as the star is then unstable against contraction; instability will therefore occur for any radius less than:

$$R_I = 5.1 \left( \frac{2GM}{c^2} \right) \frac{4}{3\beta} \simeq 0.8 R_g \left( \frac{M}{M_\odot} \right)^{1/3} \quad (6)$$

where  $R_g$  is the Schwarzschild radius. (This formula is only valid for  $M \gg M_\odot$ .)

If we neglect relativistic effects for the moment, the star would contract, drawing on its internal energy to provide the luminosity, until the central temperature reaches  $6 \times 10^7$  °K when nuclear reactions will provide the energy. The radius of the star can then be calculated from stellar structure theory, and is<sup>4</sup>:

$$R_{MS} \simeq 10^{11} \left( \frac{M}{M_\odot} \right)^{1/3} \simeq 5 \times 10^4 R_g \left( \frac{M_\odot}{M} \right)^{1/3} \quad (7)$$

The star will only reach this hydrogen burning stage if  $R_{MS} > R_I$  and this gives:

$$\left( \frac{M}{M_\odot} \right) < 6 \times 10^4 \quad (8)$$

None of the massive objects with  $M \gg 10^4 M_\odot$  will achieve an equilibrium configuration where hydrogen burning at the centre balances the energy loss by radiation.

During the stable contraction phase, the luminosity of the star is given by the surface boundary condition as<sup>4</sup>:

$$L = 2 \times 10^{34} \left( \frac{M}{M_\odot} \right) \text{ ergs sec}^{-1} \quad (9)$$

This energy loss is balanced by the change of the total energy of the star. If the time required for the star to contract from a very large radius to the radius of instability is  $\tau$ , we have:

$$L\tau = -E = \frac{38GM^2}{8R_I} \sim 2 \times M_\odot c^2 \quad (10)$$

giving a contraction time:

$$\tau = 2 \times 10^{14} \left( \frac{M_\odot}{M} \right) \text{ sec} \quad (11)$$

This contraction time is only of order 10 years for  $M = 10^5 M_\odot$ , and is much shorter than the estimated life-time of the optical phase of radio stars which is some  $10^4 - 10^6$  years<sup>5</sup>.

The estimated life-time of the optical phase of radio stars is of the same order as the nuclear time-scale of hydrogen burning, and if we could find an effect which would stabilize the star we could call on the theory of massive stars, as proposed by Hoyle and Fowler<sup>6</sup>, to explain this optical emission. One such effect is rotation.

The primary effect of rotation is that the classical polytrope  $n = 3$  is stable when rotation is included. This is quite easy to see. The virial theorem for a gas with motion, but in a steady state, is:

$$3(\Gamma - 1)U - \Omega + 2T = 0 \quad (12)$$

\* On leave of absence from the Department of Mathematics, King's College, University of London.

where  $T$  is the kinetic energy,  $\Omega$  the gravitational energy (reckoned positive) and  $U$  the internal energy. For  $\Gamma = 4/3$ , a polytrope of index 3, the total energy is:

$$E = U - \Omega + T = -T < 0 \quad (13)$$

The system is therefore stable. With radiation pressure dominant we have:

$$\Gamma = \beta + \frac{(4 - 3\beta)(\gamma - 1)}{\beta + 12(\gamma - 1)(1 - \beta)} \simeq \frac{4}{3} + \frac{\beta}{6} \quad (14)$$

since  $\gamma = 5/3$  for stars. The total energy is then:

$$E = -\frac{\beta}{2} U - T < 0 \quad (15)$$

The reason that the total energy is now negative definite, even when  $\beta \rightarrow 0$ , is that the contribution of the rotational distortion terms to the gravitational energy, and the decrease of internal energy due to reduced pressure force, more than compensate for the added kinetic energy term.

So far as order of magnitude estimates are concerned since  $\beta$  is small, the  $U$  in equation (15) can be replaced by the unperturbed value,  $U_0$ , and the binding energy is then:

$$E = -\frac{3\beta}{4} \frac{GM^2}{R} - \frac{1}{2} I \omega^2 \quad (16)$$

where  $I$  is the moment of inertia and  $\omega$  the angular velocity. The moment of inertia can be evaluated from the theory of rotating polytropes, and for uniformly rotating polytrope of index 3, rotating with maximum possible speed (centrifugal force balancing gravity at the surface) we have:

$$E = -\frac{3\beta}{4} \frac{GM^2}{R} - 0.02 \frac{GM^2}{R} \quad (17)$$

where the number of 0.02 comes from a detailed investigation of rotating polytropes<sup>1</sup>. For massive stars  $\beta \sim 10^{-3}$  and the energy is decreased by a factor of order 30.

The stability of the objects when general relativity is included is now changed. As the rotation will only be a slight perturbation over most of the star, we can neglect rotational effects in the relativistic correction, so that the total energy is:

$$E = -\frac{3\beta}{4} \frac{GM^2}{R} - 0.02 \frac{GM^2}{R} + 5.1 \frac{GM^2}{R} \left( \frac{GM}{Rc^2} \right) \quad (18)$$

The massive star will become unstable when  $\partial E / \partial R = 0$  which gives:

$$R_I \simeq 250 R_g \quad (19)$$

independent of the mass of the object provided  $\beta$  is small.

Now the radius of main sequence massive objects is given from the structure of the star as<sup>2</sup>:

$$R_{MS} = 1.5 \times 10^{11} \left( \frac{M}{M_\odot} \right)^{1/3} \text{ cm} \quad (20)$$

This is slightly larger than the non-rotating value due to the expansion of the equatorial regions by the centrifugal force<sup>3</sup>. In terms of the Schwarzschild radius, this is:

$$R_{MS} = 7.5 \times 10^3 R_g \left( \frac{M}{M_\odot} \right)^{-1/3} \quad (21)$$

Hence we have:

$$\frac{R_{MS}}{R_I} = 3 \times 10^3 \left( \frac{M}{M_\odot} \right)^{-1/3} \quad (22)$$

This brings the instability right into the interesting region where  $M \simeq 10^3 M_\odot$ . Stars smaller than this can reach the main sequence configuration, burn hydrogen and then collapse.

The rotation will also affect the time-scale of the Hayashi type evolution prior to hydrogen burning or gravitational collapse. With a luminosity:

$$L \sim 5 \times 10^4 \left( \frac{M}{M_\odot} \right) L_\odot \quad (23)$$

which draws on the internal energy, the time required to contract to the radius of instability,  $\tau$ , satisfies:

$$L\tau = -E = \frac{1}{100} \frac{GM^2}{R_I} \quad (24)$$

which gives:

$$\tau \simeq 10^4 \text{ years} \quad (25)$$

for any mass. This estimate is not accurate, as we have neglected the energy carried away by mass loss during contraction. This will reduce the life-time by a factor of two<sup>4</sup>.

If the star is rotating non-uniformly, with the central regions rotating faster than the surface layers, this will aid stability. An inward increase in  $\omega$  by a factor of 8 would permit a star of  $10^{10} M_\odot$  to reach the hydrogen-burning phase. Such an inward increase may be possible in a convective star with very large turbulent velocities where each turbulent eddy is conserving its angular momentum. This possibility will be considered elsewhere<sup>5</sup>, as it requires an examination of the convective energy transport in massive objects and is outside the scope of the present article.

It should be noted that the stabilizing effect of rotation is due to the presence of  $2T$  in the virial theorem. Consequently any other form of kinetic energy, such as that in the turbulent convection, could also exert a stabilizing influence.

This investigation arose out of discussions with Prof. W. Fowler at the Second Texas Symposium on Relativistic Astrophysics held at the University of Texas, Austin.

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## COOLING OF NEUTRON STARS

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THE discovery of discrete sources of X-rays in the sky has led to much speculation as to the mechanisms responsible for the X-ray emission<sup>1-4</sup>. Among these suggestions has been the thermal emission of X-rays from the hot surfaces of neutron star remnants of supernova explosions<sup>1-3</sup>. Calculations of the cooling of such a neutron star following its formation have seemed to indicate that

the temperature would still be several million degrees at an age of several thousand years. Such cooling calculations have been based on estimates of photon emission from the surface and of neutrino-antineutrino pair emission by the plasma process from the interior. There have recently been new suggestions regarding other possible mechanisms of neutrino emission from the interior<sup>5</sup>, and this has led

to the impression that neutron stars might cool too rapidly to be observable as celestial X-ray objects<sup>4</sup>. We believe this view to be too pessimistic, and we give here a brief summary of some of our cooling calculations for neutron stars.

The neutron star models we have used in our calculations have been based on composite equations of state with nuclear forces included. The details of the construction of the equations of state have been described in a thesis<sup>5</sup>, and will be published in due course. At the lowest densities, but still at substantially high temperatures, the composition was assumed to be iron. As the density increases, the main contribution to the pressure comes from the degenerate electrons, and the mean molecular weight per electron gradually increases as the high Fermi level of the electrons forces the nuclear composition of the material to change toward higher mass number. At densities between  $10^{11}$  and  $10^{14}$  g/cm<sup>3</sup> the heavy nuclei gradually dissolve into neutrons. The system then consists of degenerate neutrons, protons and electrons. Near and above  $10^{13}$  g/cm<sup>3</sup>, many other particles appear in the mixture, commencing with the  $\mu$  meson and the  $\Sigma$ -hyperon. In constructing our equations of state, the degeneracy pressures of all the individual fermions were added, and nuclear force terms were also included.

Nuclear forces are generally attractive at large internucleon distances and repulsive at small internucleon distances. In this work we chose forms of the potential interactions between neutrons suggested by Levinger and Simmons<sup>6</sup>. Their potentials  $V_\beta$  and  $V_\gamma$  were utilized. Both these potentials are attractive at low densities, although  $V_\gamma$  is somewhat more attractive than  $V_\beta$ . At high densities  $V_\gamma$  rapidly turns repulsive, while  $V_\beta$  only slowly turns repulsive. For our neutron star equations of state, we have applied these potentials between the baryons without distinction as to the type of baryon. At densities less than, or equal to, nuclear densities, the character of nuclear forces is reasonably well known, and is given to a rough approximation by either of these potentials; and the composition of the matter is mostly neutrons, for which the potentials were originally constructed. At much greater than ordinary nuclear densities many different types of baryon are present, and the

rapidity with which nuclear forces turn repulsive is very speculative. Hence the two potentials  $V_\beta$  and  $V_\gamma$  tend to span a range of possible behaviour of the nuclear forces at high densities, and the differences in the neutron star models which result from the adoption of one or the other potential will give an indication of the uncertainty due to lack of knowledge in this area of physics.

In the case of a perfect fluid there is a general relativistic limitation on the pressure such that it cannot exceed one-third of the proper energy density<sup>7</sup>. In a fluid with suitable anisotropic properties, this condition may be violated; but the ultimate relativistic condition still remains that the pressure cannot exceed the proper energy density<sup>11</sup>. If this were to be violated the speed of sound would exceed the speed of light in the medium. Accordingly, the composite equation of state constructed as already described was cut off with one of these two pressure saturation conditions.

The general relativistic equations of hydrostatic equilibrium were derived by Oppenheimer and Volkoff<sup>11</sup>. For a given assumption about central density, the numerical integration of the differential equations of hydrostatic equilibrium gives models with uniquely determined masses. The gravitational and proper masses determined in this way as a function of central density for the two composite equations of state are shown in Fig. 1. Several interesting comments follow from this figure. It is evident that the details of the structure of the neutron star models are very sensitive to the uncertainties in the rates at which nuclear repulsive forces enter in the baryon mixture at high densities. For each equation of state the mass rises with increasing central density toward a principal peak, beyond which it falls and then oscillates. It is interesting to note that the gravitational mass is less than the proper mass over the entire range of neutron star models, thus showing that such models are gravitationally bound. This is contrary to the behaviour of models constructed with non-interacting neutron gases. A more detailed discussion of the stability of the models beyond the main peak will be given separately<sup>12,13</sup>. It may also be noted that pressure saturation does not set in until the vicinity of the principal peak has been reached. Hence uncertainties in the pressure saturation effects play no significant part in the discussion which follows.

In order to calculate representative cooling curves for neutron star models, three models were chosen so that one was of low mass at the low-density base of the principal peak, one was half-way up the peak, and the other was near the top of the peak. To these models were fitted hot atmospheres corresponding to a series of surface temperatures. These atmospheres were composed of both iron and magnesium; the results were nearly the same, and only the neutron stars with iron envelopes are discussed here. It was typically found that the temperature rose by about a factor of 50 between the photosphere of the neutron star and the interior where the high thermal conductivity of the electrons assured a flat temperature distribution. The atmospheres were constructed with the help of opacities calculated from the Los Alamos opacity code of A. N. Cox *et al.* The atmospheric structure was determined by requiring that the luminosity should not change from one layer to the next.

The heat capacity of the neutron star models is a function of their temperature<sup>14</sup>. The presence of nuclear forces in the equation of state will modify the heat capacity by an amount which typically can be of the order of a factor 2, according to rough estimates which we have made. We did not take such modifications into account in making the actual cooling calculations.

The cooling of the neutron stars is due to the combination of neutrino emission from the interior and photon emission from the surface. The construction of the envelope automatically provided us with the photon cooling rates. Three neutrino cooling rates were taken into account, as follows:

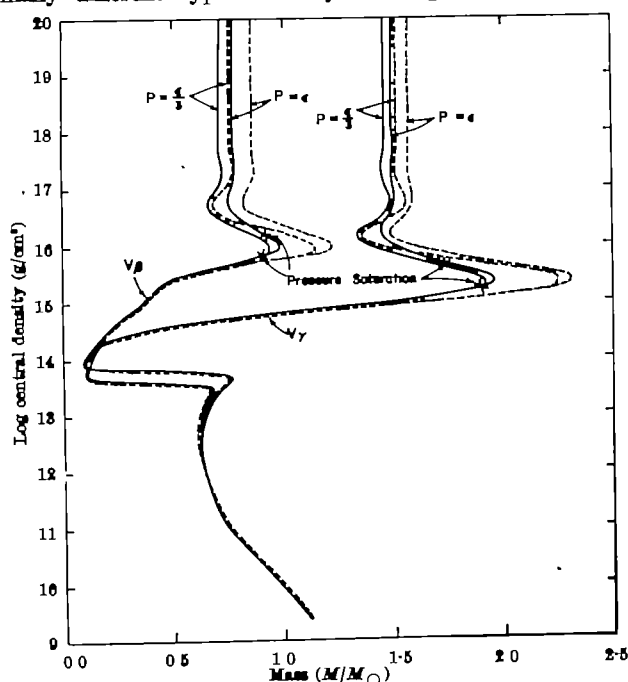


Fig. 1. Gravitational (—) and proper (---) masses of the neutron star models constructed with both composite equations of state and plotted as a function of central density. The different models resulting from the two pressure saturation conditions at the highest densities are separately indicated.

(1) *Neutrino pair emission from the plasma process.* These neutrinos arise from the decay of plasmons in the degenerate electron gas in the interior of the neutron star. The rates have been given by Adams, Ruderman and Woo<sup>18</sup> and by Inman and Ruderman<sup>19</sup>.

(2) *The URCA process.* Bahcall and Wolf have recently given an estimate of neutrino emission from this process<sup>7</sup> in which neutrons decay into protons and protons capture electrons. The rate is somewhat greater than that of the plasma process.

(3) *The neutrino bremsstrahlung process.* The neutrino pairs are emitted when electrons scatter from positive or negative baryons in the interior of the neutron star. M. A. Ruderman and G. Festa (private communication) have kindly provided us with the following approximate preliminary expression for this process:

$$q \left( \frac{\text{ergs}}{\text{g-sec}} \right) = 10^8 Z^2 \frac{n_s}{n} (T_s)^4$$

for  $E_F \gg mc^2$

where  $Z$  is the effective charge of the electron scattering centres,  $n_s$  is the number density of such centres, and  $n$  we take here to be the baryon number density. Ruderman and Festa have suggested that there may be proton clustering in neutron star interiors in the presence of very large numbers of neutrons, so that the effective charge of scattering centre might be 2. However, it may well be that under conditions of interest in the interiors of neutron stars there will also be a large number of  $\Sigma^-$  hyperons, which may hinder the clustering process. Consequently, we have chosen to take the effective charge of a scattering centre equal to unity and to count as the scattering centres both the protons and the  $\Sigma^-$  hyperons. This process is less important than the URCA process at high temperatures, but it is more important at low temperatures.

The cooling curves for the six chosen neutron star models as a function of age are shown in Fig. 2. It may be seen that the rate of cooling has a significant dependence on the mass of the star. The low-mass stars cool quite rapidly, but the medium and heavy mass stars still have temperatures exceeding  $2 \times 10^8$  °K for times of the order of  $10^4$  or  $10^5$  years. Hence it is evident that thermal emission of X-rays from neutron star surfaces should continue to be regarded as candidates for identification with some of the X-ray sources which are being found in the sky.

Bahcall and Wolf<sup>7</sup> have raised the question of neutrino cooling from pion decays in neutron star interiors. Such pion decays can occur only if pions should have a small effective mass in the presence of a largely neutron gas. Both Bahcall and Ruderman have indicated to us (private communications) their expectations that, under the conditions in which pions may be present in a neutron star, there will be a predominantly repulsive interaction between the pions and the neutrons. This would raise, rather than lower, the effective mass of the pions, and make it very unlikely that pions will be present in the interiors of neutron stars on the low-density side of the principal peak.

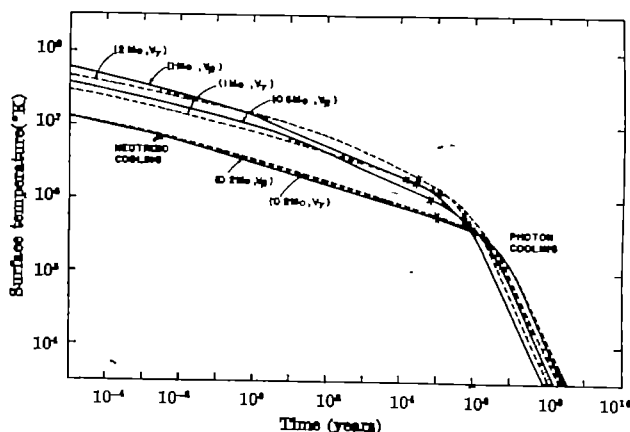


Fig. 2. The cooling curves for selected neutron star models fitted with atmospheres of iron, with three models for each composite equation of state. In the portions of the curves to the left of the crosses cooling occurs predominantly by neutrino emission from the interior; to the right of the crosses photon cooling from the surface predominates. —,  $V_p$ ; ---,  $V_n$ ; x, cooling rates equal.

The remarkable NRL rocket experiment carried out during a lunar occultation of the Crab Nebula has shown that the X-ray source associated with the Crab Nebula has dimensions very much larger than those of a neutron star. One of us has suggested that these X-rays may be due to the synchrotron process from high energy electrons accelerated in the magnetosphere of a vibrating neutron star<sup>4</sup>. Similar non-thermal processes may well be associated with other X-ray sources, if they are neutron stars, and hence non-thermal components to X-ray spectra may be common. Hence we believe that many more highly refined experiments will be necessary before the true nature of the celestial X-ray sources will be determined.

We thank Dr. M. A. Ruderman and Mr. G. Festa for communicating to us in advance of publication their preliminary results on neutrino emission by the bremsstrahlung process.

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## INTERSTELLAR EXTINCTION BY GRAPHITE GRAINS

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MANY observational data have recently accumulated on the law of interstellar extinction in the galaxy<sup>1-3</sup>. The general trend of the new results appears to be a considerable variability in the extinction law. The 'unique extinction law' hitherto believed to be generally valid now appears to hold only in limited regions of the sky. The results of a recent investigation by Johnson<sup>4</sup> indicate different extinction curves for Cygnus, Orion,

Perseus, Cepheus and NGC 2244 (Fig. 1). Dr. K. Nandy<sup>18</sup> also obtains different extinction curves for Cygnus and Perseus. The observations of Johnson<sup>4</sup> are normalized to give an extinction of 1 mag. at  $\lambda = 5470$  Å.

From a theoretical point of view the present situation is in fact quite satisfactory. To understand a unique extinction law one has to impose very stringent conditions on the sizes of interstellar particles. For a physically

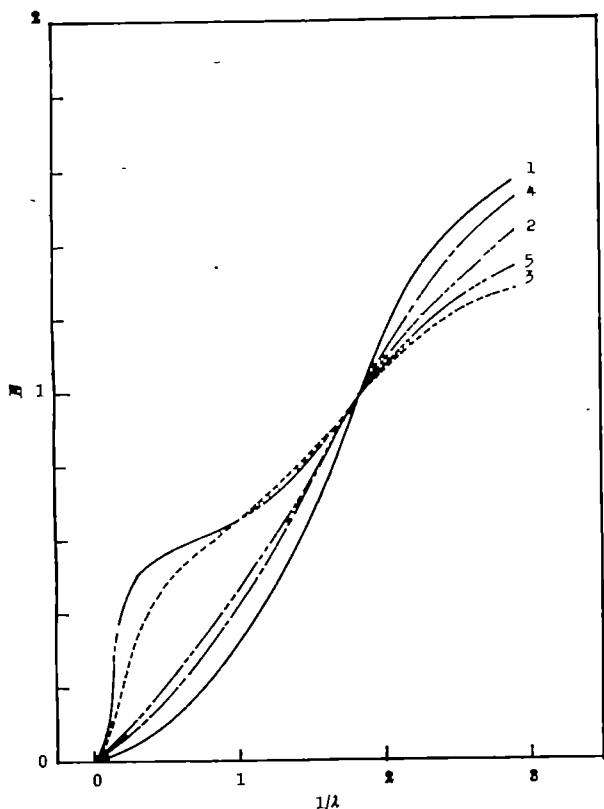


Fig. 1. Regional extinction curves plotted from data of Johnson (ref. 2). 1, Perseus; 2, Orion Belt; 3, NGC 2244; 4, Cygnus; 5, Cepheus

realistic size distribution of ice grains<sup>4</sup> the mean particle radius has to be specified to within a few per cent in order to fit the reddening curve for Cygnus. The physical accident required to produce this precise mean size in every part of the galaxy is very difficult to understand, and is perhaps the most unsatisfactory feature of the traditional explanations.

With graphite grains the situation appeared to be somewhat better. For the original model proposed by Hoyle and Wickramasinghe<sup>5</sup>, the 'unique reddening law' then accepted appeared to arise quite naturally from the extinction formula for very small particles. The reddening law was thus determined essentially by the assumed refractive index of graphite, provided the grains were all sufficiently small.

The data on the refractive index of graphite available at the time of the Hoyle-Wickramasinghe paper were, however, very sparse. A constant conductivity  $\sigma = 1.2 \times 10^{11} \text{ sec}^{-1}$ , and a dielectric constant  $K = 2$ , was assumed on the basis of old reflectivity measurements limited to a few optical wave-lengths. The refractive index  $m$  of graphite was therefore taken to be given by:

$$m^2 = K - 2i\sigma\lambda/c \approx 2 - 8i\lambda \quad (1)$$

with  $\lambda$  in microns, over the whole of the relevant wave-length range.

In a recent paper, Ergun and McCartney<sup>6</sup> reported measurements of the absorptive index at wave-lengths between 2100 Å and 5500 Å, of single graphite crystals. Their observations, which indicated a sharp peak near 2600 Å, appear to be inconsistent with the earlier assumption of a constant conductivity<sup>7</sup>. More recently Carter, Huebner, Hamm and Birkhoff<sup>8</sup> and Taft and Philipp<sup>9</sup> have reported direct measurements of the real and imaginary parts of  $m^2$  from 1100 Å in the ultra-violet into the far infra-red. The results of Taft and Philipp<sup>9</sup> are in general accord with the Ergun-McCartney results for the region of overlap of their investigations.

Let us denote the real and imaginary parts of  $m^2$  by  $\epsilon_1$ ,  $\epsilon_2$ , respectively. For our present investigation we shall

Table 1. OPTICAL DATA FOR GRAPHITE

$\lambda^{-1} (\mu^{-1})$	$\epsilon_1$	$\epsilon_2$	$n$	$k$
0.47	4	21.3	2.583	2.973
0.86	4	11.6	2.582	2.034
1.19	4	8.4	2.579	1.650
1.56	4	7	2.456	1.425
1.73	4	7	2.456	1.425
1.83	4	7	2.456	1.425
1.93	4	7	2.456	1.425
2.26	4	7	2.456	1.425
2.30	4	7	2.456	1.425
2.49	4	7	2.456	1.425
2.68	4	7	2.456	1.425
2.78	3.8	7	2.425	1.443
3.01	2.3	7.5	2.253	1.665
3.85	-3.4	7	1.480	2.865
4.54	-2	2.8	0.840	1.649
5.00	-1	2	0.786	1.273
6.67	1.2	0.6	1.127	0.256

adopt the values of  $\epsilon_1$ ,  $\epsilon_2$  extracted from the graphs of Taft and Philipp (TP<sup>9</sup>) and Carter, Huebner, Hamm and Birkhoff (CHHB<sup>8</sup>). Fig. 3 of CHHB gives  $\epsilon_1$ ,  $\epsilon_2$  as functions of wave-lengths in the range  $1100 \text{ Å} < \lambda < 3000 \text{ Å}$ . For  $\lambda > 3000 \text{ Å}$   $\epsilon_1$  remains approximately constant until the far infra-red;  $\epsilon_2$  remains approximately constant until  $\lambda \approx 7000 \text{ Å}$  and thereafter begins to rise steeply (TP, Fig. 2). For  $\lambda > 7000$  the case of a constant conductivity is realized (TP, Fig. 6) so that  $\epsilon_1 = -2\sigma\lambda/c$ , increases as  $\lambda$ . The values of  $\epsilon_1$ ,  $\epsilon_2$ ,  $n$  and  $k$  emerge as given in Table 1. These data probably represent the best available at the present moment and apply for light polarized with electric vector parallel to the basal planes.

Using these values of  $m = n - ik$ , the efficiency factors for extinction of light by spherical graphite grains may be calculated from the Mie formulae. A detailed account of these computations will be published elsewhere; only a summary of the more important features of our results will be given here.

For grains of a single radius  $a$  in the line of sight of a star, the resulting extinction in magnitudes is related to the efficiency factor  $Q_{\text{ext}}$  for a single grain by:

$$\Delta m(\lambda) = 1.086 N \pi a^2 Q_{\text{ext}}(\lambda) \quad (2)$$

where  $N$  is the number of grains in the line of sight. For comparison with observations a normalized extinction  $E(\lambda)$  is defined by:

$$E(\lambda) = \frac{Q_{\text{ext}}(\lambda)}{Q_{\text{ext}}(\lambda_0)} \quad (3)$$

where  $\lambda_0 = 5470 \text{ Å}$ . The normalization adopted is the same as that of Johnson<sup>2</sup> and is such that  $E(\lambda) = 1 \text{ mag}$  at  $\lambda = \lambda_0$  (Fig. 1).

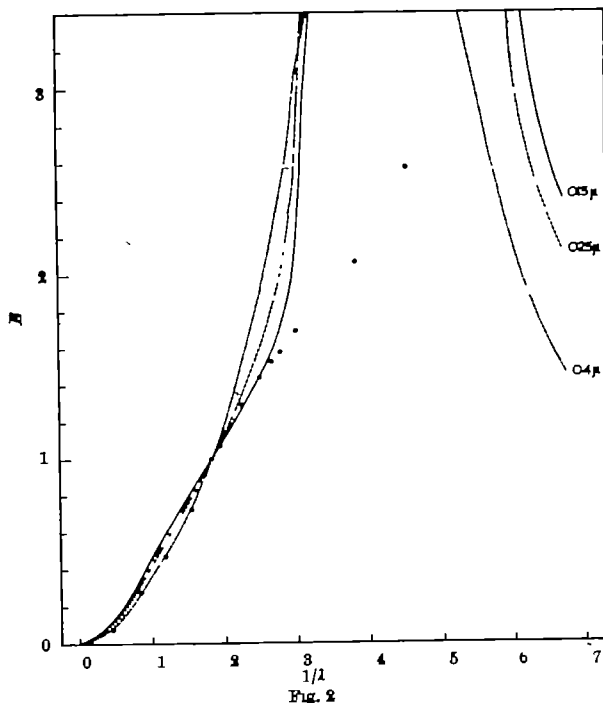


Fig. 2



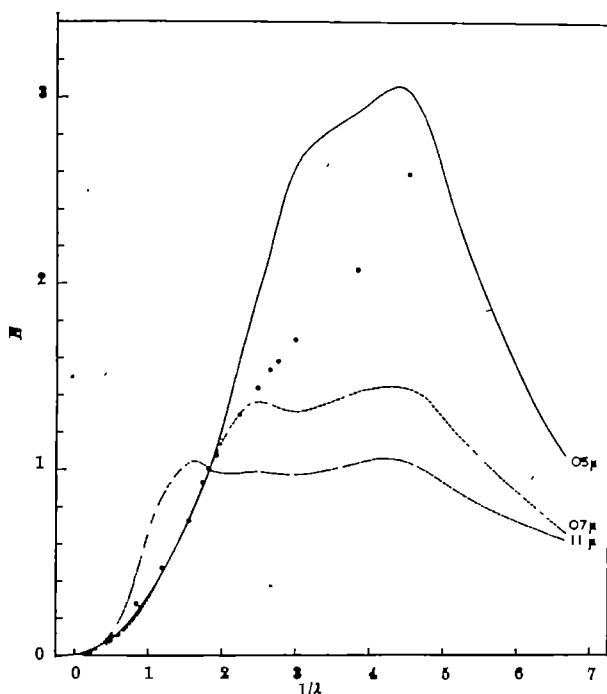


Fig. 3

The theoretical curves of  $E(\lambda)$  for various graphite grain radii are plotted in Figs. 2 and 3. The open circles plotted in Figs. 2 and 3 represent the extinction curve given by Boggess and Borgman<sup>1</sup> including their ultra-violet observations.

It is seen that the shapes of the theoretical curves for  $\lambda^{-1} < 3$  (Figs. 2 and 3) bear a general resemblance to the observational curves in Fig. 1. There seems to be little doubt that any of the observational curves reported by Johnson, with the possible exception of Cepheus and NGC 2244, cannot be reproduced by a suitable mixture of graphite grain sizes. The curves for Cepheus and

NGC 2244 have been plotted from observations of 5 and 3 stars respectively. Data for more stars are required before we can accept the curves reported for these two regions.

The most distinctive feature of graphite absorption, however, occurs in the ultra-violet. Very small graphite particles are able to produce a sharp quenching effect in the ultra-violet near 2200 Å (Fig. 2). For a grain of radius 0.016 μ,  $Q_{\text{ext}}(2200)/Q_{\text{ext}}(5470) \approx 12.3$ ; the extinction thereafter decreases until at 1500 Å,  $Q_{\text{ext}}(1500)/Q_{\text{ext}}(5470) = 2.41$ . The quenching effect decreases in absolute value for larger sizes, but the maximum at 2200 Å and a minimum at 1500 Å remains (Fig. 3). There is a strong possibility that this is in fact the effect observed by Boggess and Borgman<sup>1</sup>. On the basis of graphite absorption one would also expect a fairly sharp drop in the extinction curve towards 1500 Å. This is in fact the remarkable outcome of a preliminary investigation by Dr. J. Borgman<sup>10</sup>. Such a result, if confirmed, would give strong support to the graphite grain theory. On the basis of pure ice grains it does not seem possible to explain the ultra-violet measurements<sup>4</sup>.

Moreover, recent attempts to isolate certain infra-red interstellar absorption bands to be expected on the basis of ice or dielectric absorption have produced negative results<sup>11</sup>. Although the accuracy of the rocket experiments might be questioned, the absence of these bands if further established would provide additional evidence against ice absorption.

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## GENETIC INVESTIGATIONS OF AUTOIMMUNE DISEASE IN MICE

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FOR several years we have had under intensive study the unique mouse strain [NZB/BL], developed by Bielschowsky *et al.*<sup>1</sup>. These mice develop an autoimmune type haemolytic anaemia which, in almost all respects, presents close analogies to the human disease, acquired haemolytic anaemia and, in particular, to the 'warm' antibody type of Dacie. Investigations of the natural history of these mice and of the haematological characters of their disease have been published both from the Otago laboratory in which they were developed<sup>2,3</sup> and from Melbourne<sup>4,5</sup>. One paper on the behaviour of the F1 cross NZB × O3H has been published<sup>6</sup> and a good deal on the NZB × NZW F1 hybrid which has a strikingly high incidence of kidney disease<sup>7,8</sup>.

With the partial completion of studies on NZB × O3H and NZB × AKR F1 and back-crosses and less complete results from NZB × NZW, NZB × O37 BL and NZB × T6 F1's, a considerable amount of material has now been obtained. In this article we wish to discuss the behaviour of the Coomb test as an index of the autoimmune processes in the various F1 hybrids and back-cross populations.

The test involves the detection of incomplete antibody on the surface of the animal's red cells. Technical details

are described elsewhere<sup>9</sup> but, in outline, mice are bled from the tail at monthly intervals, the red cells washed four times in warm saline and then tested for slide agglutination with two dilutions 1:5 and 1:50 of a standard antiserum. The test sera were prepared in rabbits immunized with mouse globulin (almost wholly Ig G) and absorbed before use with normal mouse red cells. In this way, large numbers of results roughly quantitative in character, since the intensity ± to +++ of agglutination for two dilutions was noted, became available. From the raw data arranged according to nominal months (28 days) each mouse was allotted a time, for example, 11.5 'months' as representing the first time it was definitely positive. As it was not always possible to test all mice every 4 weeks, when necessary a value was interpolated. Normally, if the first positive reaction from a mouse showed +++ agglutination with both dilutions of serum and the mouse had not been tested during the previous two months, its conversion was pre-dated one month.

The proportion positive at any month was calculated in terms of the total number of mice previously converted plus those changing during the month plus the number of still negative mice present throughout the period. Once

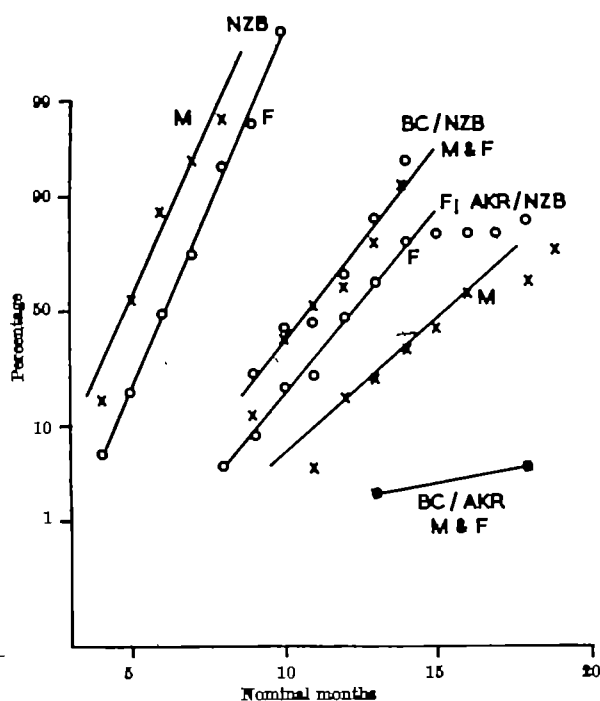


Fig. 1 Times of first change from Coombs-negative to Coombs-positive (Coombs conversion) for  $F_1$  and back-cross hybrids of strains  $NZB$  and  $AKR$ . Cumulative percentage of conversions obtained as described in the text plotted on probability paper.  $F_1$   $NZB/AKR$  (male,  $\times$ , female,  $\circ$ ;  $BC/NZB$  back-cross ( $F_1$   $NZB \times AKR$ )  $\times$   $NZB$ ;  $BC/AKR$  back-cross ( $F_1$   $NZB \times AKR$ )  $\times$   $AKR$ ).

a mouse had given a definite positive reaction ( $DO+$ ) it was counted as positive throughout the calculations irrespective of whether it had reverted to negative or not. This gave a cumulative percentage which could conveniently be plotted on probit paper.

The strains concerned,  $NZB$ ,  $NZW$ ,  $OSH$ ,  $AKR$  and  $CS7 BL$ , are maintained in the Institute by brother-sister mating.  $T6$  males were obtained by the courtesy of Dr. Peter Ilbery. The standard  $F_1$  crosses were made by mating  $NZB$  females with males of the other parent strain, and, for the back crosses,  $F_1$  females were mated with a male of the appropriate parental strain.

Small numbers of each of the parent strains, other than  $NZB$ , have been shown to be Coombs-negative at  $\pm$  one year of age and, in the absence of reports to the contrary, we have assumed that they are uniformly Coombs-negative throughout life. In the case of  $OSH$  and  $AKR$ , this assumption is almost completely validated by the results with the back-crosses described here.

**$NZB \times AKR$  hybrids.** In the  $NZB-AKR$  series the results (Fig. 1) indicate an approximately normal distribution of the times of conversion, but in the  $F_1$ , and particularly in the females, there is a persistent failure to convert a significant proportion. The median times of conversion supply most of the information but, in addition, there are differences in the slope of the curve (corresponding to the size of the standard deviation) and in the proportion of positive reactions which afterwards become negative. These are not shown in Fig. 1, which is concerned only with the time of the first recognized conversion from a negative to a positive direct Coombs test.  $NZB$  mice show a median age of conversion of 160 days for males, 188 for females\* (see Table 1).

The  $F_1$   $AKR \times NZB$  strain shows a marked difference between male and female, but in the opposite direction from  $NZB$ , the female converting earlier and showing a steeper slope than the male.

Except when lymphoid tumours develop, the great majority of  $NZB$  conversions are permanent, but in all the hybrids we have studied there has been some irregularity in the results of successive Coombs tests, and it may

Table 1. MEDIAN AGE FOR COOMBS CONVERSION IN DAYS

	Male	Female
$NZB$	160	188
$NZB \times OSH$ $F_1$	—	540
$F_1 \times NZB$ $BC$	310 $\pm$	310 $\pm$
$F_1 \times OSH$ $BC$	—	—
$NZB \times AKR$ $F_1$	454	342
$F_1 \times NZB$ $BC$	310	310
$F_1 \times AKR$ $BC$	—	—
$NZB \times CS7$ $F_1$	425	425
$NZB \times T6$ $F_1$	730 $\pm$	510
$NZB \times NZW$ $F_1$	—	320

Table 2. IRRREGULARITIES IN COOMBS RESPONSE IN  $NZB \times AKR$   $F_1$  MICE

Type of response	Male	Female
A Positive persisting in all later tests	6 25%	14 54%
B Early irregularities with final stable positive	4 17%	0
C Various irregularities not ending as positive	6 25%	6 23%
D Definite positive for at least 2 months, afterwards negative	5 21%	3 11%
E Always negative	3 12%	3 11%
Total tested	24	26

Table 3. DISTRIBUTION OF COOMBS'S RESPONSES IN RELATION TO THE PRESENCE OF LYMPHOID TUMOURS AND TO PROLONGED SURVIVAL

Type of response	Lymphoid tumour		Survived 800 days	Overall	
	M	F		M	F
Standard $AB$	0	2	0	10	14
Irregular $CD$	3	6	4	11	9
Negative $E$	0	1	3	3	3

be desirable to give some detail of the results with the  $NZB \times AKR$   $F_1$  hybrids. Table 2 shows a division into the several types of response that followed the first appearance of a positive Coombs reaction. Although the proportion giving at least one positive is essentially the same (88–90 per cent) for both sexes, females not only show earlier change but are less prone to show irregular responses, 54 per cent as against 25 per cent in males showing persisting positive reactions once Coombs conversion had occurred.

Of the 50 mice studied in detail and recorded in Table 2, 3 males and 9 females died with unequivocal lymphoid tumours while 7 males and no females survived more than 800 days (Table 3).

It is clear that, as might be expected, the development of a lymphoid tumour was significantly associated with irregularity of the Coombs reaction. It is perhaps no more than an interesting lead that no mouse that developed a persisting Coombs test survived 800 days while the only 3 male mice which were consistently negative from 14 months onward all survived more than 800 days.

The back-cross to  $AKR$  produced a considerable number of cases of lymphoma/leukaemia which will be described elsewhere, and the very low incidence of Coombs conver-

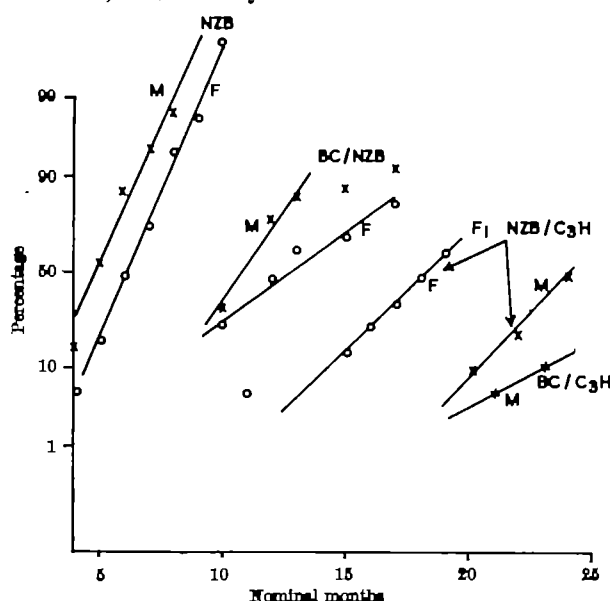


Fig. 2. Times of Coombs conversion for  $NZB$ ,  $OSH$   $F_1$  hybrids and back-crosses.

Legend as Fig. 1.  $BC/NZB$  back-cross ( $F_1$   $NZB \times CSH$ )  $\times$   $NZB$ ;  $BC/OSH$  back-cross ( $F_1$   $NZB \times CSH$ )  $\times$   $OSH$ .

sion may be related in part to early death from leukaemia. However, when tested at 350–400 days, 1/45 males and 1/56 females were  $DC+$ , both reactions persisting, while of tests made between 450 and 550 days when many had died of leukaemia, 2/24 males and 2/19 females were positive. It is not legitimate to give a definite figure for the percentage of Coombs converters, but it is unlikely to exceed about 10–15 per cent.

It is clear from Fig. 1 that in both back-crosses the Coombs behaviour shows no resemblance to what would be obtained from a population of equal numbers of  $F_1$  and parent. The difference between the capacity to allow a positive  $DC+$  test at any time during the life of the mouse and its absence cannot therefore be produced by a single gene change.

**NZB-C3H crosses.** The results of  $F_1$  and of the back-cross to NZB have already been published\*. Fig. 2 is drawn from the same data used in that paper with additional results from 50 mice back-crossed to C3H. It will be seen that there is a greater difference between male and female for the  $F_1$  results than is shown in the NZB  $\times$  AKR series. The back-cross to NZB showed conversion to  $DC+$  at an intermediate period between NZB and  $F_1$ . The number of mice involved was small (male 26, female 23) and, in view of the considerable scatter of the male values, it is not possible to say that there is a significant male-female difference. In the back-cross  $F_1(NZB \times C3H) \times C3H$  there were only two typical  $DC+$  tests, both in old males. Two females out of 25 tested at about one year of age gave a weak positive (but well controlled and definite) test which afterwards became negative. Twelve females surviving two years were all negative.

**Other NZB crosses.** Three other strains have been used in crosses with NZB, namely, NZW C57BL and T6, and in each case about 50 mice were studied by serial Coombs tests. The  $F_1$  results for NZB  $\times$  C57BL and NZB  $\times$  T6 are shown in Fig. 3. The first series differs from the others in showing no significant differences between males and females. The NZB  $\times$  T6  $F_1$  mice show the usual greater activity of females reaching 50 per cent conversion in about 500 days, while there were still 10 males alive beyond 750 days which had never given a positive test.

The chief interest of the NZB  $\times$  NZW cross is the very high mortality from kidney disease and this has the effect of eliminating all females before 400 days and greatly

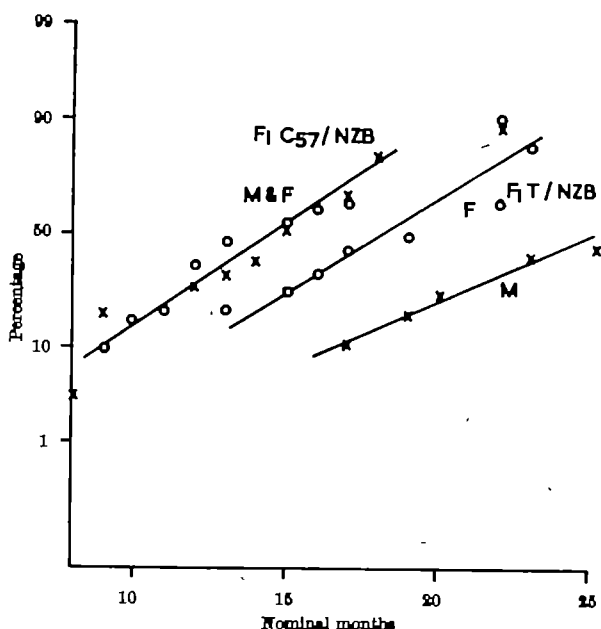


Fig. 3. Times of Coombs conversion for NZB  $\times$  C57BL and NZB  $\times$  T6  $F_1$  hybrids. Legend as Fig. 1

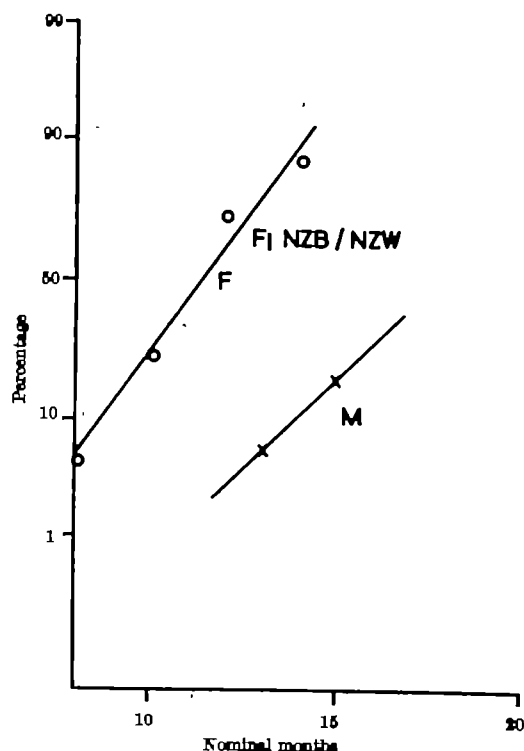


Fig. 4. Times of Coombs conversion for NZB  $\times$  NZW  $F_1$  hybrids. Legend as Fig. 1. Note that, owing to early deaths from kidney disease, the figures for both sexes are based on small numbers.

depleting the numbers of males available for Coombs test at later stages\*. However, the trend was clearly similar to the other crosses and most of the surviving females more than 300 days of age were positive, while there were only two males for whom a late  $DC+$  was recorded (Fig. 4).

### Discussion

For several years we have regarded the 'forbidden clone hypothesis' as the most satisfactory way of co-ordinating the facts of autoimmune disease. The availability of the present results from genetic experiments offers new material for an attempt to disprove or modify the hypothesis. In broad terms, the forbidden clone hypothesis of autoimmune disease is an almost necessary corollary of a clonal selection approach to immunity. It assumes that abnormal auto-antibodies or pathogenic immunocytes are the product of clones which have been allowed to develop by the failure of some homeostatic process to prevent the emergence of lymphoid (immunocyte) stem cells with immune patterns that can react with antigenic determinants present in accessible body components. Formally, the homeostatic process in question might fail either because the process itself was ineffective or because a stem cell resisting the process had appeared by somatic mutation. From such a cell a descendant forbidden clone of immunocytes could arise with pathogenic potentialities. It must be emphasized that the mutation producing a forbidden clone is not in virtue of the appearance of an immune pattern which reacts with a body component via one of its antigenic determinants, but because of its resistance to a homeostatic process, possibly active in the thymus, which should normally destroy all cells with such patterns as they arise.

In the homozygous NZB mice the appearance of a positive direct Coombs test is a convenient indicator that a population of pathogenic cells has developed to a demonstrable level. Appropriate transfer experiments<sup>10,11</sup> have shown that a cell population of adequate size is present only in the spleen of mice already Coombs-positive. Transfer to young isologous mice will regularly produce

a temporary  $DO+$  reaction (sometimes persisting) and, in addition, enlargement of spleen possibly analogous to Simonsen's graft-versus-host reaction<sup>14</sup>. These reactions are absent in young recipient mice given control material and in mice that will not accept  $NZB$  cells, that is, all except isologous animals or  $F1$  mice with one parent  $NZB$ .

If such cells arise by somatic mutation, the regularity of their appearance needs to be accounted for. For one cell to have  $10^5$  descendants, which seems likely to be the order of the number of cells giving antibody capable of coating all the red cells of the body, 20 binary generations are needed. Having regard to the unlikelihood of proliferation at maximal speed of such a clone, we can probably allow 20 days from the emergence of the mutant stem cell to the appearance of a  $DO+$  reaction. The time of emergence of such a mutant cell would then be, for male  $NZB$ , 80–220 days, and for females, 108–248 days, based on a median time for appearance of the  $DO+$  reaction of male 160 days, female 188 days. The general resemblance of this time-scale with that for the emergence of leukaemia in strain  $AKR$  mice<sup>15</sup> suggests that, if somatic mutation is the rate determining process in  $AKR$  leukaemia, it is equally likely to be so for  $NZB$  haemolytic anaemia.

Our approach to an interpretation of the data that have been presented will be to assume, following Burch<sup>14</sup>, that both an appropriate genotype and a process of somatic mutation are necessary for the manifestation of haemolytic anaemia in  $NZB$  mice. In the absence of compelling reasons to the contrary it is simplest to assume that the initiating somatic mutation allows a cell to escape the normal homeostatic process by which immunocytes capable of reacting with accessible body components are destroyed or inhibited, and that this change  $R^+$  to  $R$  may occur in any breed of mice. The emergence of descendants of such a cell in sufficient numbers to produce the phenotypic expression of Coombs conversion will obviously be influenced by many factors. A further somatic mutation or sequence of mutations may be needed and, on Burch's view, would probably be the dominant rate-controlling process. The results we have reported here, however, suggest strongly that phenotypic expression of the (final) somatic mutation both as regards timing and intensity depends on the nature of a gene complex  $B$  which is present in a specially favourable form in male  $NZB$  mice.

The complexity of  $B$  is evidenced by the results of back-crosses shown in Figs. 1 and 2. Back-crosses to the 'normal' parental strain gave less than 10 per cent showing Coombs conversion but in each set there were one or two strongly positive reactors. If we take the simplest possible assumption, this would indicate that 3–5 unlinked genes were included in the complex. Similarly, in the back-crosses to  $NZB$ , the timing of the change to  $DO+$  is

intermediate in character between  $NZB$  and  $F1$  but is not interpretable as a mixture of the two types. In the reconstruction of the  $B$  complex in the various  $F1$ 's and back-crosses there will be possibilities of influence on time of conversion as well as of determining whether conversion can or cannot occur. The influence of sex is clear in nearly all the populations studied and obviously some of the components of the gene complex  $B$  are carried on the  $X$  chromosome. We hope to look for more direct evidence of this by appropriate reciprocal matings. Results, however, may be difficult to interpret since we have shown that in homozygous  $NZB$  mice the earlier and more active change to  $DO+$  is in males, while in most  $F1$ 's the females carrying one  $X$  chromosome derived from  $NZB$  and one foreign  $X$  convert earlier than the males with only the  $NZB X$  in their genome.

The findings are in general accord with the hypothesis of a specific type of somatic mutation the results of which depend on the rest of the genetic environment. They would be equally in accord, however, with an appropriately developed hypothesis that the somatic genome was actively modified by the incorporation of part of a virus genome giving a heritable somatic genetic change. So far there is no evidence from any other direction for the presence of a virus, but, if it is postulated that the virus is transmitted vertically by sperm as well as ovum and can demonstrably affect only animals of a strictly limited range of genotypes, it becomes very difficult to disprove the virus hypothesis experimentally.

In the course of these experiments a large amount of data on mortality with special reference to leukaemia-lymphoma and lethal kidney disease was accumulated. The incidence and type of proliferative lesions in the thymus have also been recorded. These findings and their relationship to the distribution and age incidence of Coombs conversion will be discussed elsewhere.

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## EXAMINATION OF THE MECHANISM OF ANTIBODY FORMATION USING NUCLEIC ACID AND PROTEIN INHIBITORS

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RECENT experiments have demonstrated that antibody production can be considered as a form of protein synthesis which is regulated by nucleic acids and follows metabolic pathways similar to those utilized for other types of protein. It is formed on ribosomes<sup>1</sup> from a pool of free amino-acids<sup>2,3</sup> according to mRNA which is coded from chromosomal DNA<sup>4,5</sup>. However, high antibody titres have been obtained during the anamnestic response to antigen in the presence of nucleic acid inhibitors<sup>6</sup>. These observations suggest that RNA

necessary for antibody synthesis must be capable of being stored, and that this accumulated RNA can be rapidly activated even in the presence of DNA inhibitors. It would appear that the primary exposure to antigen results in an inactivation of RNA coded for antibody synthesis, while a later re-exposure to specific antigen produces a reactivation of the RNA and a rapid synthesis of antibody<sup>7-9</sup>.

In view of these considerations, attempts were made to modify the degree of immunization by altering the

quantity of nucleic acids present in the cells at the time of primary exposure to antigen. Three experimental approaches were used: (a) cells were treated with nucleic acid and protein inhibitors at the time of primary exposure to antigen, and antibody production was measured during the anamnestic response; (b) the inhibitors were added to antigen to determine whether repeated injections would result in a prolonged suppression of immunity; (c) the inhibitors were added to antigen and then injected into immunized animals to ascertain whether a decrease in antibody response would occur.

The effect of antibiotics on the immune response was determined in adult *BDF<sub>1</sub>* mice.

In the first experiment, each of 60 mice was given 2 injections of Freund's adjuvant plus tuberculin (Difco), spaced 10 days apart, in order to produce a localized population of peritoneal inflammatory cells. Four days after the second injection of adjuvant, the animals were given a primary injection of fluid tetanus toxoid (Lederle). Antibiotics were administered intraperitoneally at 12-h intervals for a total of 5 days beginning 12 h prior to the primary antigen injection. In group A, the mice were re-injected with the water-soluble or fluid tetanus toxoid at bi-weekly intervals. In group B, the mice were injected with a precipitated antigen consisting of tetanus toxoid adsorbed on aluminium phosphate (Lederle).

In the second experiment, the antibiotics were combined with fluid tetanus toxoid and administered to 40 mice. A total of 8 weekly intraperitoneal injections of 0.4 ml. each were given. The animals were then allowed to recover for a 3-week period before being given further injections of tetanus toxoid.

In the third experiment, 40 mice were first immunized by 2 intraperitoneal injections of tetanus toxoid incorporated into Freund's adjuvant. They were then given 5 weekly intraperitoneal injections of tetanus toxoid combined with the antibiotics.

The inhibitors used were actinomycin D (Merck, Sharpe and Dohme), chloramphenicol (Parke Davis), methotrexate (Lederle), streptomycin, and puromycin (Nutritional Biochemical). These inhibitors were suspended in pyrogen-free saline and injected into groups of 5 animals as indicated in Tables 1-3.

All mice were bled at weekly intervals and the antitoxin titres determined. 0.1 ml. of blood was removed from the tail vein and immediately diluted with 2 ml. distilled water to lyse the cells. Blood from a pool of 5 animals was serially diluted against equal parts of a standardized tetanus toxin solution. The antitoxin titres were determined by biological assay. Duplicate assays were run after the diluted blood had been frozen and stored for 4 or more weeks. In this manner all the sera from a particular experiment could be re-assayed together and the titres compared under nearly identical conditions. Once antitoxin production was initiated, the titres increased progressively following each succeeding injection of antigen. Therefore, in order to simplify the presentation of the data, only bi-weekly titres are included in Tables 1-3.

The degree of primary immunization obtained after a single injection of a water-soluble antigen was too low to be measured by the assay procedure used. However, following each re-injection of the toxoid, progressive increases in titres were obtained (Table 1A). After the fourth injection, the control animals and the animals injected with puromycin, chloramphenicol, erythromycin, and streptomycin had antitoxin titres varying between 45,000 and 90,000 units/ml. blood. Animals receiving 250 µg methotrexate had antitoxin titres of 10,800 units, and animals injected with 10 µg actinomycin D had titres of only 2,400 units. All animals challenged with aluminium phosphate adsorbed tetanus toxoid (Table 1B) had consistently higher titres than animals challenged with fluid tetanus toxoid. The animals previously treated with methotrexate or actinomycin D had markedly

Table 1. EFFECT OF INHIBITORS ADMINISTERED DURING PRIMARY INJECTION OF TETANUS TOXOID ON THE ANAMNETIC RESPONSE

Total dose of inhibitors*	Antitoxin titres 7 days after antigen injection			Mice surviving
	Second injection	Third injection	Fourth injection	
(A) Fluid tetanus toxoid				
Controls (no inhibitors)	0.5†	5.2	45.0	5/5
1 mg puromycin	0	1.0	45.0	5/5
500 µg chloramphenicol	0.3	2.4	90.0	5/5
500 µg erythromycin	0	2.4	45.0	5/5
500 µg streptomycin	0	5.2	45.0	5/5
250 µg methotrexate	0	5.2	10.8	2/5
2.5 µg actinomycin D	0	0.8	22.0	5/5
10.0 µg actinomycin D	0	0	2.4	3/5
(B) Tetanus toxoid (aluminium phosphate adsorbed)				
Controls (no inhibitors)	10.6	45.0	360.0	5/5
3 mg puromycin	5.2	45.0	180.0	5/5
250 µg methotrexate	0.3	5.2	22.0	2/5
10 µg actinomycin D	5.2	22.0	90.0	5/5
20 µg actinomycin D	0	2.4	10.2	2/5

\* Inhibitors were injected intraperitoneally twice daily for 5 days beginning 12 h before the primary injection of antigen.

† No.  $\times 10^4$  equals antitoxin titres/ml. whole blood.

Table 2. ANTITOXIN TITRES PRODUCED BY WEEKLY INJECTIONS OF FLUID TETANUS TOXOID AND NUCLEIC ACID INHIBITORS

Dose of inhibitor per week†	Antitoxin titres*						Mice surviving
	During treatment with inhibitors			Post treatment‡ without inhibitors			
	Fourth injection	Sixth injection	Eighth injection	Ninth injection	Tenth injection	Eleventh injection	
Controls (no inhibitors)	1.0‡	2.4	45.0	22.0	180.0	180.0	5/5
Methotrexate 10-40 µg	1.0	5.2	45.0	45.0	720.0	>1,000.0	14/15
Actinomycin D							
2.5 µg	2.4	11.0	45.0	22.0	180.0	360.0	5/5
5.0 µg	0.3	0.3	2.4	2.4	10.8	22.0	4/5
10.0 µg	0	0	0	0	1.0	10.8	3/5
20.0 µg	0	0	0	0	0.8	0.8	1/5

\* All blood samples were taken 1 week after injection of toxoid.

† Inhibitors were diluted with saline and added to 0.2 ml. tetanus toxoid, making a total injection volume of 0.4 ml.

‡ Following eight injections of toxoid plus inhibitor, the animals were allowed to recover for three weeks and then re-injected with tetanus toxoid at weekly intervals.

§ Number  $\times 10^4$  equals antitoxin titres/ml. of whole blood.

Table 3. ANTITOXIN TITRES IN HIGHLY IMMUNIZED MICE RECEIVING FIVE WEEKLY INJECTIONS OF FLUID TETANUS TOXOID PLUS NUCLEIC ACID INHIBITORS

Dose of inhibitor injected per week*	Antitoxin titres at bi-weekly intervals after initial injection of antigen plus inhibitor			Mice surviving
	First injection	Third injection	Fifth injection	
Controls (no inhibitors)	22.0†	45.0	>1,000	5/5
Methotrexate 10-40 µg	22.0	90.0	>1,000	14/15
Actinomycin D				
2.5 µg	11.0	45.0	>1,000	5/5
5.0 µg	11.0	45.0	360.0	5/5
10.0 µg	5.2	22.0	180.0	4/5
20.0 µg	2.4	45.0	180.0	5/5

\* Inhibitors were diluted in saline and added to 0.2 ml. tetanus toxoid, making a total injection volume of 0.4 ml.

† Number  $\times 10^4$  equals antitoxin titres/ml. whole blood.

reduced titres, compared with titres of the control animals.

In the second experiment, the nucleic acid inhibitors were added to tetanus toxoid and then injected into mice at weekly intervals for a total of 8 injections. These results are shown in Table 2. It may be seen that during the 8 weeks of injection, the addition of 10-40 µg per week of methotrexate had no significant inhibitory effect on antitoxin titres. On the other hand, weekly doses of 5.0 µg or more of actinomycin D had a marked inhibitory effect on antitoxin titres during the entire 8-week period. When re-injected with the toxoid only, these animals were found to respond by producing antitoxin titres typical of a primary response. The animals treated with methotrexate had consistently higher titres than the controls.

In the third experiment, the nucleic acid inhibitors were added to tetanus toxoid and then injected into highly immunized mice. Table 3 presents the antitoxin titres obtained. It may be seen that all animals showed progressive increases in antitoxin titres in spite of the presence of inhibitors. In the control animals and in animals receiving methotrexate, titres of more than a million units/ml. blood were obtained. Similar results were obtained in animals injected with 2.5 µg of actinomycin D. However, titres of only 180,000 and 360,000

units were obtained in animals injected with 5–20  $\mu$ g actinomycin D.

The data obtained in these experiments indicate that injections of actinomycin D, given at the time of primary injection of antigen, markedly reduce the antitoxin titres obtained during the anamnestic response. Treatment with a total dose of 10  $\mu$ g or 20  $\mu$ g actinomycin D resulted in markedly reduced antitoxin titres when the animals were later subjected to repeated challenging injections of either fluid tetanus toxoid or the aluminium-adsorbed toxoid. Moreover, when 10 or 20  $\mu$ g actinomycin D was combined with antigen and then injected weekly for a period of 8 weeks, no measurable antitoxin titres were obtained, compared with 45,000 units in the control animals (Table 2). When allowed to recover from the actinomycin D poisoning they were found to be capable of becoming immunized. Although actinomycin D did not prevent antibody synthesis in immunized animals, it did produce a reduction of titres when repeatedly injected along with the antigen (Table 3).

These results differed somewhat from results reported by Wust *et al.*<sup>8</sup>, who observed that actinomycin D injections in rats delayed the production of antibody to heterologous red blood cells, but did not inhibit the total amount of antibody produced. In our experiments, the 10  $\mu$ g actinomycin D produced a temporary inhibition of immunity, but the mice were capable of responding to later injections of the fluid toxoid (Table 1). Furthermore, the mice which had complete inhibition of antitoxin, produced by 8 weekly injections of toxoid plus actinomycin D, later showed a primary response to the toxoid when injected without the inhibitor (Table 2). It would therefore appear probable that in the experiments of Wust *et al.* the injected red blood cells remained in the rats and acted as a continuing stimulus after the inhibitory effects of actinomycin D had worn off.

Actinomycin D is a polypeptide antibiotic which blocks the synthesis of RNA by combining specifically with the guanine base of the DNA molecule, without markedly inhibiting DNA synthesis<sup>10</sup>. It would appear that a reduction in RNA synthesis at the time of primary antigen injection diminishes the capacity of the cells to become sensitized to the antigen, since rapid high antitoxin titres, indicative of 'immunological memory', did not occur following a re-injection of antigen. Repeated simultaneous injections of antibiotic and antigen resulted in prolonged immunological unresponsiveness.

Methotrexate is a folic acid antagonist which binds to folic acid reductase, eventually inhibiting nucleic acid synthesis. Its action appears to be primarily on DNA, resulting in irregular chromatin masses, and an inhibition of DNA synthesis<sup>11</sup>. Injections of methotrexate reduced the amount of primary immunization (Table 1), but appeared to have no inhibitory effect on antibody production when repeatedly injected with the antigen (Tables 2 and 3).

In the doses administered puromycin, streptomycin, erythromycin and chloramphenicol had no marked inhibitory effect on primary immunization (Table 1). Since these substances inhibit protein synthesis without preventing the synthesis of RNA<sup>12–15</sup>, it would appear that a blockage of protein synthesis *per se* does not prevent immunization to a primary injection of antigen. This is further substantiated by experiments of Weisberger *et al.*<sup>12</sup>, who demonstrated that doses of chloramphenicol which completely blocked measurable protein synthesis during the primary response did not inhibit the anamnestic response to a later re-exposure.

In the experiments reported, there was a marked loss of weight and a high death rate in animals receiving doses of nucleic acid inhibitors sufficiently high to block antitoxin synthesis to a primary injection of antigen. Since the reduction in the anamnestic response produced by actinomycin D was still apparent long after the toxic effects of the antibiotic had worn off (Table 1), it was

evident that failure of primary immunization rather than temporary inhibition of response must have occurred. Failure to induce an 'immunological memory' does not appear to be due to an inhibition of protein synthesis, since protein inhibitors *per se* do not produce this effect. However, the lack of primary immunization may be due to a reduction in the available RNA involved in protein synthesis. Since actinomycin D poisoning inhibits RNA synthesis, there would be a gradual reduction in messenger, ribosomal and transfer RNA within the cells<sup>4</sup>. The evidence at present available suggests that it is the decrease in amount of free mRNA, or the mRNA which is newly formed and unattached to ribosomes, which results in a failure of antigen to induce immunity during the primary exposure to antigen. This is indicated by the specific manner in which actinomycin D produces its inhibitory effect. Antitoxin production during the anamnestic response was inhibited only if the antibiotic had been given during the primary exposure to antigen (Tables 1 and 2). Since RNA extracts from immunized animals have been used to stimulate antibody synthesis in non-immune cells both *in vivo*<sup>17</sup> and *in vitro*<sup>18</sup>, it appears quite possible that the mRNA synthesized during the primary response was utilized as a template for antibody synthesis during the anamnestic response.

Geller and Speirs<sup>6</sup> treated immune mice with daily injections of actinomycin D and were able to demonstrate a rapid high production of antitoxin to a challenging injection. They concluded that the specifically coded mRNA necessary for antitoxin synthesis must have been present in an inactivated form, since in their animals very little circulating antitoxin was present prior to the challenging injection. Fan *et al.* demonstrated that several substances, including chloramphenicol, can inactivate mRNA without resulting in its breakdown<sup>19</sup>, and it would appear quite possible that some component of the antigen could also combine with specific mRNA and thereby temporarily inactivate it. The inactivated mRNA would then be released and re-activated during the events which follow the challenging injection. It is therefore of interest to consider the relationships between antigen and the inactivation and re-activation of RNA.

It has been demonstrated that following injections of radioactive antigen, components of the antigen persist in cells for long periods<sup>7</sup>. Garvey and Campbell<sup>20</sup> noted that the antigen was complexed with RNA and that the complexes retained the capacity for initiating antibody production. Fishman and Adler<sup>21</sup> were able to initiate antibody production in normal rabbits with a ribonuclease-sensitive material extracted from macrophages treated with antigen *in vitro*. These experiments indicate that some antigenic component complexes with RNA resulting in a change in the status of immunity of the cells. Moreover, antigenic material persisting from a primary injection has been shown to undergo degradation during the anamnestic response<sup>22</sup>. This degradation of antigen would presumably cause a release of the complexed RNA, which in the absence of RNase could become attached to ribosomes and induce specific antibody formation. It would seem possible that the period immediately following release of mRNA from antigen complexes, and prior to attachment to ribosomes, would be a vulnerable period. In this regard, it is interesting to note that injections of RNase administered simultaneously with antigen have been found to be an effective suppressant of immunity<sup>16</sup>.

Cells containing radioactive components of antigen participated in the inflammatory reactions produced by later re-injections of a non-radioactive antigen and appeared to be responsible for the specificity of the reactions<sup>7,23</sup>. Primary exposure to antigen could cause an inactivation of the mRNA merely by binding to it and preventing its attachment to ribosomes. Since re-exposure to antigen results in a degradation of the original antigen<sup>24</sup> followed by rapid antibody production<sup>7</sup>, it must be

presumed that the mRNA is released and attaches to ribosomes thereby initiating antibody synthesis. The failure to detect radioactive amino-acid uptake into antibody during the early stages of the anamnestic response<sup>22</sup> indicates that antibody synthesis does not begin immediately but is delayed for a few days. This delay could be explained by the complexing of the antigen with the mRNA freed during the cellular interactions. When all the antigen within the inflammatory cells becomes neutralized or degraded, the mRNA could then attach to ribosomes and initiate antibody synthesis. Since these reactions do not necessitate new synthesis of mRNA, an explanation is obtained for the failure to find an uptake of RNA precursors into plasma cells<sup>23</sup> or for the failure of actinomycin D to block antibody production during the anamnestic response<sup>4,6</sup>. It would appear that the complexing of antigen with mRNA would account for many perplexing immunological phenomena.

The concept that antigen inactivates mRNA can also be extended to include immunological unresponsiveness, if one assumes that mRNA-antigen complexes cannot accumulate in such cells. The capacity to metabolize completely antigen would be characteristic of tolerant and immature cells<sup>24</sup>. Cell multiplication, accompanied by cellular differentiation and specialization, would result in the formation of new types of mRNA, a loss of capacity to detach antigen from RNA, and a transition from tolerance to intolerance. These considerations are important since most theories of immunity cannot satisfactorily explain auto-immunity, or changes in tolerance and immunity to a particular protein<sup>25</sup>.

This work was supported by the Atomic Energy Commission. I am a career scientist of the Health Research Council of New York. The antitoxin assays were carried out under the supervision of Dr. S. Reid and Mr. E. Gentles.

*Note added in proof.* Additional evidence involving preformed RNA in 'immunological memory' and the anamnestic response has become available since submitting this paper for publication. Askonas and Rhodes

(*Nature*, 205, 470; 1965) noted that following a primary exposure to antigen, both antigen and RNA were present in immunogenic extracts of macrophages. In an examination of the secondary response, Kornguth *et al.* (*Exp. Cell Res.*, 37, 650; 1965) demonstrated that increased protein synthesis was not preceded by an increased synthesis of RNA. Further work in our own laboratory indicated that proper timing of the actinomycin D injection could produce complete inhibition of primary immunization at less toxic doses. The maximum inhibitory effect appears to be 1 and 2 days following the antigen injection.

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## STRUCTURAL (TRANSLOCATION) HETEROZYGOSITY OVER THREE SUBSEQUENT GENERATIONS IN MAN

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CYTOGENETIC studies during the past few years have disclosed various states of balanced or nearly balanced structural heterozygosity in man, leading to unbalanced chromosomal sets in the offspring. Up to now one of the best-known examples is the apparently balanced translocation between one small and one large acrocentric chromosome<sup>1</sup> or between two small acrocentrics<sup>2</sup> and the transmission of this abnormal chromosome to the following generation giving rise to the translocation type of trisomy, No. 21 in Down's syndrome. Edwards *et al.*<sup>3</sup> reported two families with a balanced reciprocal translocation most probably involving the chromosomes No. 4 and No. 9 in one case and No. 1 and No. 6 in the other, both leading to unbalanced karyotypes and abnormal phenotypes in the progeny. Further observations of familial translocation were made by Moorhead *et al.*<sup>4</sup>, Gustavson *et al.*<sup>5</sup>, and recently by Lee *et al.*<sup>6</sup>. The latter authors described a reciprocal translocation in two subsequent generations concerning one chromosome No. 2 and one No. 3 as well as an unbalanced karyotype associated with multiple malformations in the third generation. In all these cases rather large translocated fragments are involved in the balance as well as in the derived unbalanced karyotypes of the offspring.

The report of Schmid<sup>7</sup>, however, on the familial occurrence of a probably translocated additional chromatin piece at a No. 21-22 chromosome, associated with repeated miscarriages, indicates that—depending on the chromosome affected—the exchange of very small fragments can also lead to unbalanced karyotypes with deleterious and lethal effects. Considering that only such chromosomal aberrations as involve clearly identifiable parts of chromosomal material can be detected with our present methods, it may be assumed that structural heterozygosity and its transmission by phenotypically normal carriers, as well as the subsequent origin of unbalanced karyotypes, represents a very important mode of inheritance in man<sup>8</sup>.

Investigating a large family, Walker and Harris<sup>9</sup> provided evidence for the transmission of a translocation between two chromosomes of the No. 13-15 group by phenotypically normal carriers through three subsequent generations. A similar translocation had been described earlier in certain single cases of developmental abnormality<sup>10-12</sup> as well as in a randomly selected male subject<sup>13</sup>. The present report refers to a family with transmission of a structural heterozygosity of a further type through three generations. This condition is present in apparently



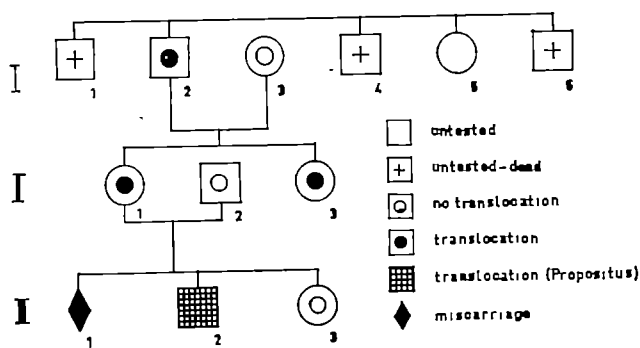


Fig. 1. Pedigree of the family

healthy carriers through two generations and in an affected child in the third generation.

The pedigree of the family studied is represented in Fig. 1. Our interest was drawn to this family by the finding of a chromosomal aberration in cultured lymphocytes of a 3-year-old boy (III, 2) showing features of v. Recklinghausen's disease (neurofibromatosis) and marked kyphoscoliosis. He was the first living child of a 25-year-old mother (II, 1) and a 33-year-old father (II, 2). Previously the mother had had a miscarriage with an abortion in the third month (III, 1). The weight at birth was 3,750 g, the length 57 cm. Kyphoscoliosis was first noted when the boy was 6 months old. Later on this alteration grew worse. There were no signs of rickets. At the age of 15 months many café-au-lait spots of the skin were visible. This suggested the diagnosis of neurofibromatosis (v. Recklinghausen's disease), but nodular neurofibromatous lesions were absent.

The later course was characterized by a marked retardation of mental and static development and by insufficient body-weight. The child appears old compared with others of his age-group. He suffers from repeated infections of the respiratory tract.

Lymphocyte cultures were carried out on two different occasions using a modification<sup>14</sup> of the basic method described by Moorhead *et al.* Chromosome counts (Table 1: III, 2) revealed a modal number of 46 chromosomes. Detailed analysis of metaphases indicate a normal XY sex chromosome complement but in all metaphases two abnormal chromosomes, noted as  $T_1$  and  $T_2$ , are very prominent (Figs. 2 and 3). Replacing No. 3 chromosome there is a submetacentric chromosome ( $T_1$ ), resembling the No. 2 chromosome in size and, to some extent, in the location of the centromere. In the 6-X-12 group from the non-paired autosomes one ( $T_2$ ) seems to be abnormal. It is characterized by a pronounced metacentricity and by a small size, scarcely exceeding that of chromosome No. 16.

The same gross chromosomal anomalies were found in the boy's mother (Table 1 and Fig. 3: II, 1), his aunt (Table 1 and Fig. 3: II, 3) and his maternal grandfather (Table 1 and Fig. 3: I, 2). All three are free of any physical or mental disorder.

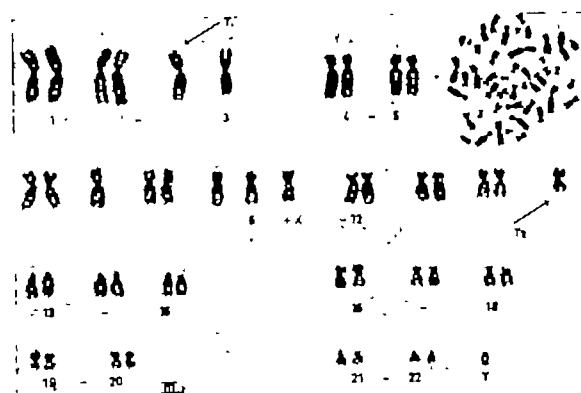
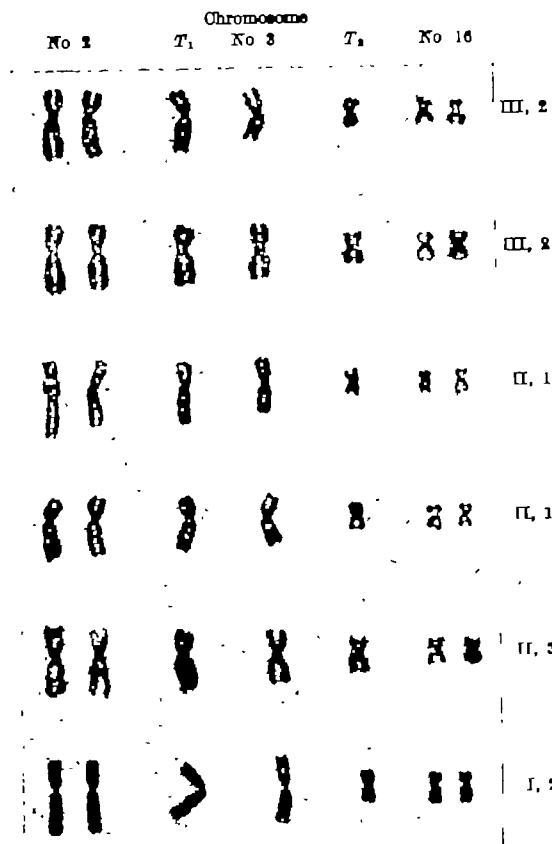
Chromosomal analysis performed on the boy's unaffected younger sister (Table 1 and Fig. 1: III, 3), father (Table 1 and Fig. 1: II, 2) and maternal grandmother (Table 1 and Fig. 1: I, 3) gave normal results. At the present time the mother (II, 1) is in an advanced stage of a so-far uneventful pregnancy.

Both abnormal chromosomes  $T_1$  and  $T_2$  probably originated by reciprocal translocation, possibly involving

a large part of a chromosome of the 6-12 group and a smaller piece of a No. 3 chromosome. The comparison of the abnormal chromosome  $T_1$  with the normal No. 3 chromosome, particularly considering the location of the centromere, may, however, favour the possibility of an even more complicated mode of origin, comprising perhaps not only reciprocal translocation but also pericentric inversion.

The anomaly in this family can be traced back only to the propositus's grandfather (I, 2). The one living sister of this ancestor could not be investigated. His three deceased brothers were presumably free from developmental disorders.

The transmission of structural heterozygosity in the same, apparently balanced and rather stable combination of the abnormal chromosomes  $T_1$  and  $T_2$ , seems remarkable. It can be assumed that the carriers of the anomaly may produce four types of gametes, one of which will be

Fig. 2. Karyotype from cultured lymphocytes of the propositus (III, 2) demonstrating the two abnormal chromosomes  $T_1$  and  $T_2$ .Fig. 3. Abnormal chromosomes  $T_1$  and  $T_2$ . Compare size with the chromosomes Nos. 2, 3 and 16

	Counted metaphases— No of chromosomes				Total	No. of metaphases analysed in detail
	45	46	47	48		
III, 2	1	—	56	—	57	25
III, 3	—	2	53	—	55	15
II, 1	—	—	53	1	54	16
II, 2	—	—	45	—	45	10
II, 3	—	—	31	1	32	11
I, 2	—	—	50	1	51	9
I, 3	3	1	27	—	31	6

normal, one abnormal but genetically balanced, and two unbalanced. It is of interest to notice that in the third generation different patterns occur: abnormal karyotype (III, 2), normal karyotype (III, 3) and an unidentified (III, 1). This family, however, is too small for investigation by segregation methods.

It is doubtful whether the clinical features of the propositus, who by the finding of the chromosomal anomaly stimulated the family study, can be directly related to this cytogenetic disorder. It is conceivable that the balance of the abnormal karyotype in this boy was disturbed by an additional factor, not detectable cytogenetically. But it is also possible that the co-incidence of the cytogenetic abnormalities and the clinical picture of neurofibromatosis and kyphoscoliosis was by chance. Other cases of v. Recklinghausen's disease do not show chromosomal anomalies (among others, two personal observations). There is a certain analogy between the family described here and reported cases of heritable structural anomalies of chromosomes in healthy or randomly selected persons<sup>15-17</sup>.

Balanced autosomal aberrations, either rather stable or occasionally leading to unbalanced karyotypes in the

offspring, may be more frequent in phenotypically normal human groups than has been suspected.

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## SERUM PROTEIN FORMATION OF DONOR TYPE IN RAT-INTO-MOUSE CHIMAERAS

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IT has been shown by various authors that serum proteins of donor origin may appear in the sera of lethally irradiated mice protected by heterologous<sup>1-3</sup> or homologous<sup>4,5</sup> bone marrow. Among the proteins which have been identified are  $\gamma$ -globulin<sup>1-3,5</sup>, transferrin<sup>4</sup> and the *S*<sub>2</sub> substance<sup>4</sup>. Grabar *et al.*<sup>6</sup> demonstrated several other proteins of rat origin in sera from rat-into-mouse chimaeras, but the immunoelectrophoretic patterns were not interpreted with respect to identification of individual proteins.

The work recorded here was undertaken in an effort to identify further the donor serum proteins in such chimaeras and to determine their sites of origin. It had previously been found that haematopoietic and lymphoid tissues of mice<sup>7-9</sup> and rats<sup>9</sup> incorporate <sup>14</sup>C-labelled amino-acids into a few serum proteins including the known immune globulins, transferrin,  $\beta_2$ ,  $\alpha_2$ -macroglobulin and, in the case of haematopoietic tissues, the haptoglobin-haemoglobin complex (HpHb). The technique used to demonstrate such <sup>14</sup>C-amino-acid incorporation into individual serum proteins by tissues *in vitro* involves the combination of immunoelectrophoresis with autoradiography<sup>4-9</sup> as applied to concentrated tissue culture fluids.

Rat-into-mouse chimaeras were produced by intravenous injection of  $2-8 \times 10^7$  Sprague-Dawley rat bone marrow cells into CBA or A mice within 4 h after 750-1,000 r. whole-body X-irradiation. The X-irradiation was administered by a Picker therapeutic unit. The dose rate was 42 r./min, at a distance of 45.5 cm from the centre of the animal body. Other conditions were as previously described<sup>10</sup>. Starting a few days before X-irradiation the mice received neomycin in their drinking water, or aureomycin (6 mg/kg/day) in their food. Blood samples were taken at weekly or bi-weekly intervals from the retro-orbital plexus. Sera were analysed by micro-immunoelectrophoresis<sup>11</sup> using absorbed anti-rat sera as described here.

At intervals of 3-7 weeks after irradiation, mice were killed and their livers and spleens removed aseptically. Approximately 50 mg of each spleen or 100 mg of each liver was cultured for 24-48 h at 37° C in roller tubes with 1-2 ml. of medium as previously described<sup>6,9</sup>. <sup>14</sup>C-labelled

lysine and isoleucine (750  $\mu$ c.-1,000  $\mu$ c./mg, Schwarz Biochem.) were added to a concentration of 1  $\mu$ c./ml. each. After the culture period the culture fluids were dialysed against 0.015 M phosphate buffer, pH 7.2, for 48 h, lyophilized, and redissolved with 0.1 or 0.15 ml. of distilled water.

Identification of the labelled rat proteins was accomplished by the use of unlabelled rat carrier serum, which was added to the antigen well of the immunoelectrophoretic slide prior to the addition of the concentrated culture fluid. The immunoelectrophoretic precipitation pattern was developed with rabbit antisera against rat serum and against rat complement, which had previously been extensively absorbed with lyophilized mouse serum and tissues (liver and spleen), and with fresh mouse serum. The anti-rat complement was prepared by immunization of rabbits with a washed immune complex of bovine serum albumin (BSA) and rabbit anti-BSA, which had been incubated with fresh rat serum and then washed again before mixing with complete Freund's adjuvant. For identification of mouse proteins, a mouse serum carrier was used and the patterns developed with a rabbit anti-mouse serum, similarly absorbed with rat serum and tissue powders.

Anti-rat sera absorbed in this manner did not precipitate any serum proteins from sera of normal or irradiated mice. Furthermore, when these anti-rat sera were used to develop an immunoelectrophoretic pattern of rat serum in the presence of highly labelled culture fluids from normal mouse liver or spleen tissue cultures, no labelling of rat serum proteins was observed with the exception of  $\alpha_2$ -macroglobulin in both liver and spleen, and of HpHb in the spleen. This 'non-specific' labelling of  $\alpha_2$ -macroglobulin has been noted repeatedly in previous studies. It occurs with cultures of virtually all living tissues tested, and in all species examined<sup>1-9</sup>. This observation that  $\alpha_2$ M labelling was seen with mouse cultures even in patterns developed by completely absorbed anti-rat sera, strengthens the previous suggestion that the labelling of  $\alpha_2$ M in many tissues is due to a binding to  $\alpha_2$ M of other tissue products, and not to a real synthesis of this protein

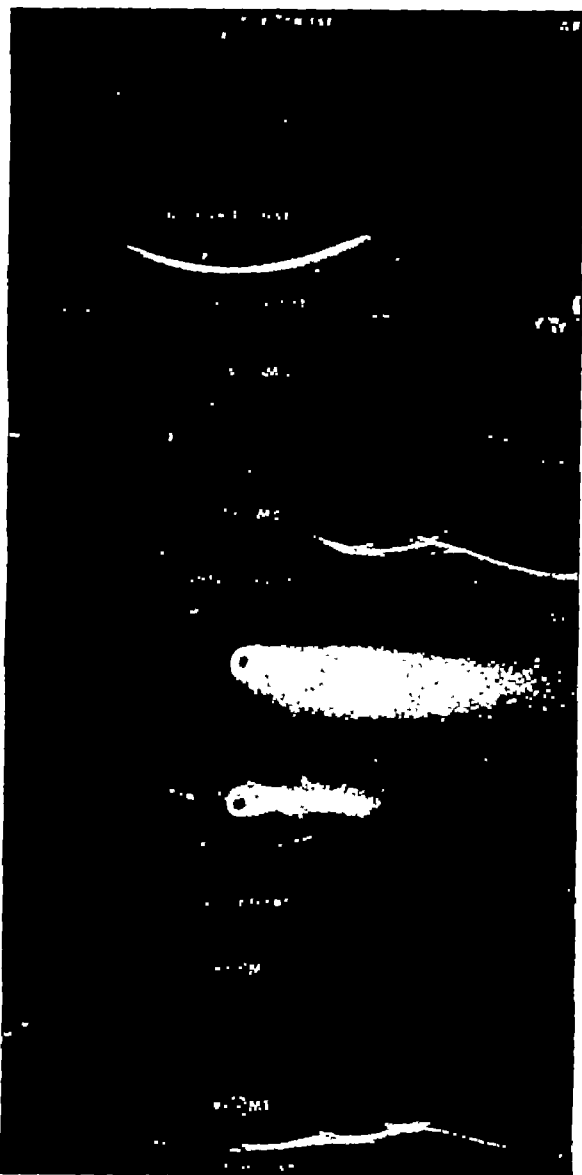


Fig. 1. Immunoelectrophoretic patterns of  $^{59}\text{FeCl}_3$  incubated sera from mice 3 days ( $R \rightarrow M1$ ) and 4 weeks ( $R \rightarrow M2$ ) after irradiation and rat bone marrow protection developed by rabbit anti-mouse and absorbed anti-rat sera. Autoradiographs (AR) show absence of rat transferrin at 3 days and presence of a small amount at 4 weeks. In this case the rat transferrin was not visible on the stained slide. Radioactive mouse transferrin is demonstrated in both sera.

by all these tissues. Labelling of HpHb in this system is very difficult to interpret since rat Hp or Hb may bind mouse Hb or Hp, respectively, and thus carry these labelled mouse proteins into the precipitation lines in spite of the use of absorbed anti-rat sera. No such 'non-specific' labelling was observed for  $\beta_{1C-1D}$ , transferrin, or  $\gamma$ -globulin, which are the three major proteins to be reported here.

No sera from mice obtained during the first week after irradiation and bone marrow transfer showed any evidence of containing rat proteins. During the next week, however, most sera began to show an  $\alpha$  line, which sometimes extended into the  $\beta$ -region, giving the typical double-arc appearance of HpHb. This line showed a positive benzidine reaction<sup>18</sup> and could not be demonstrated by antisera which had been absorbed with rat haemoglobin. When used to develop immunoelectrophoretic patterns of normal rat sera, the anti-rat sera were able to react with rat Hp after absorption with rat Hb, and yet they did not show an HpHb complex in the chimaeric sera. It can, therefore, be concluded that the HpHb line seen in

the chimaeric sera consisted of a complex between mouse Hp and rat Hb. The appearance of this line was variable, probably depending on the level of haptoglobin in the serum and on the degree of haemolysis.

Another rat protein, which appeared within 2-3 weeks after the experimental procedure and which could be readily identified, was transferrin (Fig. 1). Incubation of serum with  $^{59}\text{FeCl}_3$  (2  $\mu\text{c./ml.}$ ) resulted in specific labelling of rat as well as of mouse transferrin in the same serum. The serum of 10 animals was specifically examined for the presence of rat transferrin, and positive results were obtained in each case. No rat transferrin was detected in sera taken a few weeks earlier from the same animals.

The majority (18 of 20) of those chimaeras surviving 4 weeks after irradiation had significant quantities of rat  $\gamma_1$ - and  $\gamma_2$ -globulin in their sera (Fig. 2). No evidence was obtained that  $\beta_{1C-1D}$  of rat origin, the major line shown by the absorbed anti-complement sera, occurred in any of the mouse sera up to 7 weeks after irradiation and bone marrow protection.

During the fourth week after the experimental procedure many of the animals succumbed to secondary disease<sup>19</sup>. Most of the animals surviving the secondary disease past the fourth week were killed for tissue culture examination; four mice at 5 weeks, one at 6 and one at 7. Fig. 3 shows the various labelled proteins observed in spleen culture fluids from chimaeras killed 6-7 weeks after irradiation. Among them are  $\gamma_1$ - and  $\gamma_2$ -globulin, transferrin,  $\beta_{1C-1D}$ ,  $\alpha_2$ -macroglobulin, and HpHb. The latter two will not be considered as rat proteins for the reasons already discussed; but the other four proteins appeared to be of rat origin since none of them was labelled when developed with specific anti-mouse sera and mouse serum as the carrier (Fig. 3). One other protein was detected both in

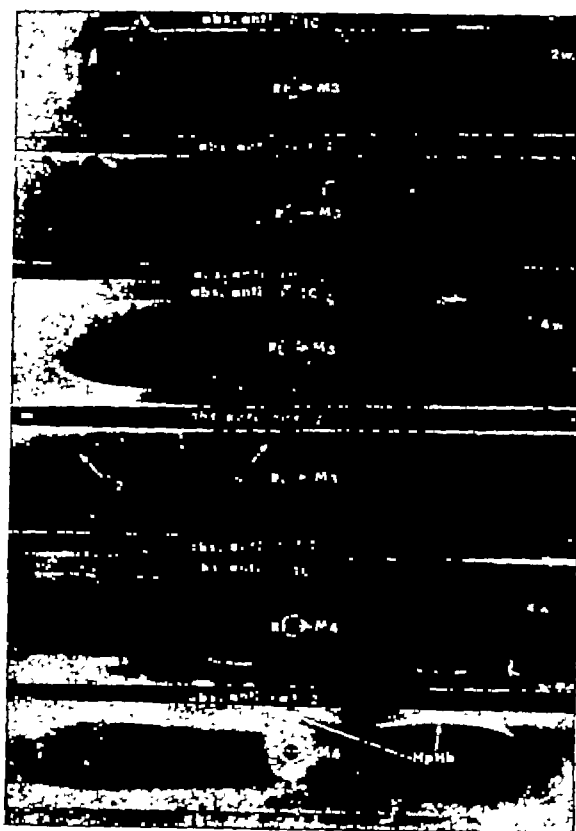


Fig. 2. Immunoelectrophoretic patterns of mouse sera taken 2 ( $R \rightarrow M3$ ) and 4 weeks ( $R \rightarrow M4$  and 4) after irradiation and rat bone marrow protection developed with absorbed anti-rat sera. Note the presence at 2 weeks of some rat  $\gamma$ -globulin, and at 4 weeks of larger amounts of both  $\gamma_1$ - and  $\gamma_2$ -globulins. Rat transferrin can be seen as a small arc in the  $\gamma$ -region (in  $R \rightarrow M3$ , 400). The complex of mouse haptoglobin with rat haemoglobin, most clearly shown by anti-rat serum 2, is seen as a double arc in  $R \rightarrow M4$ .  $\beta_{1C}$  is not present, the line formed by anti- $\beta_{1C}$  is an, as yet, unidentified globulin.

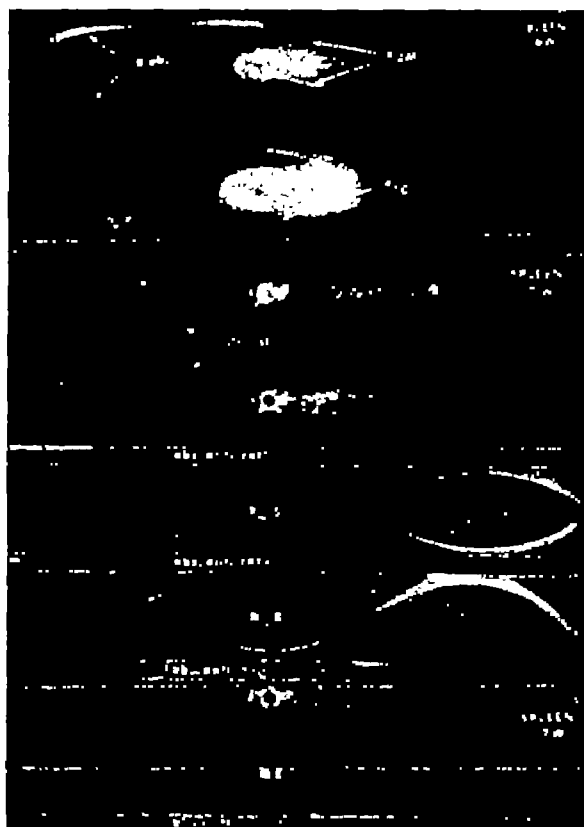


Fig. 8. Autoradiographs from immunoelectrophoretic patterns prepared with rat serum carrier (RS) or mouse serum carrier (MS), and spleen culture fluids from chimaeras taken 6 and 7 weeks after irradiation and rat bone marrow protection. The autoradiograph (A.R., spleen, 7w, bottom) from the specific mouse pattern shows no labelling of any lines, whereas the autoradiographs of the specific rat patterns (A.R., spleen, 6w and 7w, top) show labelling of  $\gamma$ -globulin, transferrin,  $\beta_{10}$  and  $\alpha_{2m}$ .

sera and spleen cultures from several of the chimaeras. This protein cross-reacted with  $\gamma$ -globulin and had a  $\beta_2$  mobility, but needs more precise identification.

The occurrence of rat transferrin and rat  $\gamma$ -globulins in the sera of these chimaeras agrees very well with their production by spleen tissues. These proteins are apparently formed in the mouse spleen by descendants of the transferred rat bone marrow cells. It seems certain that in species such as the mouse and the rat, transferrin is formed by spleen tissue as well as by the liver<sup>4,7,8</sup>, whereas in other species, such as the monkey, human, guinea-pig and sheep, the liver but not bone marrow or spleen incorporates <sup>14</sup>C-amino-acids into transferrin<sup>9,14,15</sup>. Examination of cattle chimaeras also failed to provide evidence of  $\beta$ -globulin (transferrin) production by haematopoietic

cells<sup>16</sup>. Other investigations<sup>9,17</sup> indicate that in the mouse and the rat the synthesis of transferrin by spleen, peritoneal macrophages, and various other tissues, is not correlated with  $\gamma$ -globulin synthesis.

Further work is needed to determine the factors involved in this species difference and the cell type responsible for transferrin production in the spleen.

The specific labelling of rat  $\beta_{10}$  by the spleen cultures agreed with previous findings on the site of  $\beta_{10}$  formation<sup>4,8</sup>, but could not be substantiated by the presence of rat  $\beta_{10-12}$  in the mouse sera. It seems possible that the rate of formation of  $\beta_{10}$  by spleen tissue is too low to lead to detectable serum-levels. Moreover, the presence of secondary disease might have resulted in binding of a portion of newly synthesized  $\beta_{10}$  by antigen-antibody complexes, since  $\beta_{10}$  (O'3) belongs to the complement system<sup>18,19</sup>. The analysis of tissue culture fluids from liver did not reveal the presence of any specific rat serum protein synthesis, whereas various mouse serum proteins, including  $\beta_{10}$ , were actively synthesized by the livers of most chimaeras studied.

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## TURNOVER OF NUCLEAR AND CYTOPLASMIC RIBONUCLEIC ACID AT THE ONSET OF INDUCED AMPHIBIAN METAMORPHOSIS

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**P**ROFOUND and rapid alterations of the structure and function of almost every type of cell occur when amphibian metamorphosis is precociously induced with exogenous thyroid hormones<sup>1</sup>. Artificial induction of metamorphosis therefore lends itself as a convenient system for investigating the sequential steps involved in the regulation of cellular synthetic process during the later stages of embryonic development. The induction or

accelerated synthesis of several proteins, such as adult haemoglobin, serum albumin, hydrolases and urea cycle enzymes following the administration of L-thyroxine or triiodo-L-thyronine ( $T_3$ ) to American bullfrog tadpoles (*Rana catesbeiana*) has now been well established. There is in this species of the frog, however, a lag period of 60–80 h following hormone administration before newly formed protein or morphological changes can be detected.

Finamore and Frieden<sup>8</sup> observed an increased rate of synthesis of total RNA in the liver of *Rana catesbeiana* tadpoles one day after induction of metamorphosis. In view of the distinct roles of the cell nucleus in RNA synthesis, on the one hand, and of ribosomal RNA in protein synthesis, on the other, I have examined separately the turnover of each of the major types of RNA at the onset of induced metamorphosis. It will be shown in this article that an accelerated synthesis of RNA in the liver cell nucleus and the sequential appearance of newly synthesized RNA in the cytoplasmic ribosomes anticipate the morphological and enzymatic changes in *Rana catesbeiana* tadpoles. The appearance of newly synthesized RNA in cytoplasmic ribosomes is accompanied by an increase in the size and number of polyribosomal aggregates and a more tenacious attachment of the latter to the microsomal membranes. We have also found that a breakdown of part of existing ribosomal RNA accompanies or precedes the appearance of new RNA molecules.

Metamorphosis was precociously induced by the injection of 0.2–0.6  $\mu\text{g}$  of  $T_3$  into *Rana catesbeiana* tadpoles, 6–9 cm long, maintained at 18°–20° in batches of eight animals per 2 l. water. The progression of metamorphosis was followed morphologically by recording the increase in hind-leg length and decrease in tail length, and biochemically by the increase or appearance of adult haemoglobin, serum albumin, liver carbamyl phosphate synthetase and cytochrome oxidase. At varying time-intervals after the induction of metamorphosis, livers were pooled and homogenized in a medium containing 0.33 M sucrose, 0.01 M *tris*-HCl, pH 7.5, 0.05 M KCl and 0.0015 M  $\text{MgCl}_2$ . Nuclei were isolated by the method of Widnell and Tate<sup>9</sup>, and mitochondria, microsomes and the soluble fraction separated from the nuclei-free homogenate by differential centrifugation in the same way as for mammalian tissues<sup>4</sup>. Ribosomes were normally obtained by treating the mitochondria-free supernatant with 0.4 per cent sodium deoxycholate before centrifugation, and in some experiments the concentration of the detergent was varied between 0.1 and 0.5 per cent. 35–45 per cent of total DNA was recovered in the nuclear fraction and 65–80 per cent of total protein and RNA in the cytoplasmic fractions. Polyribosome profiles were obtained by centrifugation of ribosomes through a 15–30 per cent sucrose density gradient<sup>5</sup>, layered over a cushion of 60 per cent sucrose and containing 0.02 M *tris*-HCl, pH 7.6, 0.05 M KCl, 0.0015 M  $\text{MgCl}_2$ , in the SW 25 or No. 30 rotor at 22,000 r.p.m. for 100–150 min. Only ribosomes or RNA adhering to microsomal membranes could sediment through the cushion of 60 per cent sucrose.

RNA synthesis and turnover were examined by the incorporation of 10–15  $\mu\text{Ci}$  of uniformly labelled tritiated uridine (1,220 mc./mM), 2  $\mu\text{Ci}$  of orotic acid- $^{14}\text{C}$  (32.6 mc./mM) or 30–60  $\mu\text{Ci}$  of carrier free  $^{32}\text{P}$  into the different subcellular RNA fractions. RNA was extracted from the 0.4 N perchloric acid-insoluble residue with 2 M sodium chloride at 90° after washing with ethanol and ether. It was separated from DNA by alkaline hydrolysis and ethanol extraction and the radioactivity in the ribonucleotides was measured in a Packard 'Tricarb' liquid scintillation spectrometer for tritium, in a Nuclear Chicago gas-flow counter for carbon-14, and with an end-window Geiger counter for phosphorus-32. Chemical estimation of RNA and DNA was performed according to Burton<sup>6</sup> and Ceriotti<sup>7</sup>, protein by the method of Lowry<sup>10</sup> and phosphorus by that of Fiske and Subbarow<sup>11</sup>.

Table 1 shows that the time that elapsed between the administration of  $T_3$  and the first observation of some morphological and biochemical changes which characterize metamorphosis under our experimental conditions is of the order of 65–100 h. A similar lag period preceding the morphological changes or increase in enzyme activity has also been observed by other workers in precocious  $T_3$  or thyroxine-induced metamorphosis in *Rana catesbeiana* tadpoles<sup>1,12</sup>. During this period there was little or

Table 1. LAG PERIOD BETWEEN  $T_3$  INJECTION AND APPEARANCE OF SOME MORPHOLOGICAL CHANGES AND INCREASED SYNTHESIS OF PROTEINS AND ENZYMES

	Lag period (h)
Adult haemoglobin appearance	92 $\pm$ 12
Serum albumin increase	105 $\pm$ 10
Liver carbamyl phosphate synthetase	77 $\pm$ 7
Tail acid phosphatase	65 $\pm$ 6
Tail resorption	68 $\pm$ 8
Hind-leg growth	75 $\pm$ 10
Cytochrome oxidase in liver mitochondria	88 $\pm$ 10

Time that elapsed before a 10 per cent increase or change relative to control values was observed.

no change in the weight, or total protein and DNA content of the liver.

Preliminary investigations with phosphorus-32, tritiated uridine and orotic acid- $^{14}\text{C}$  showed that all these precursors were incorporated more slowly into all types of RNA of tadpole liver than in mammalian tissues. Optimal labelling of the 'rapidly labelled RNA' in the nucleus, with little radioactivity in the cytoplasmic RNA, was obtained only at 70–120 min after the administration of the labelled precursors. Virtually no label was found to be incorporated into liver DNA of either control tadpoles or up to 10 days after the induction of metamorphosis. Fig. 1 shows that the specific radioactivity of 'rapidly labelled' RNA in nucleus was considerably enhanced in those animals in which metamorphosis had been induced 25–30 h before the administration of tritiated uridine. If the animals were killed at later time-intervals after the administration of the radioactive label, RNA of increasing specific radioactivity was recovered in the mitochondrial and microsomal fractions with a fall in the radioactivity of nuclear RNA. The effect of induction of metamorphosis

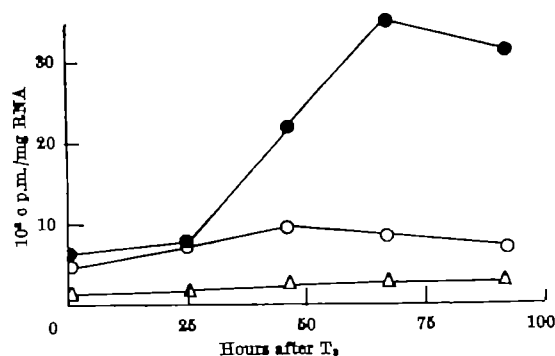


Fig. 1. Specific radioactivity of nuclear, soluble and microsomal RNA in liver of tadpoles killed at different time-intervals after the induction of metamorphosis with  $T_3$ . All animals received 10  $\mu\text{Ci}$  of tritiated uridine 100 min before they were killed. ●, Nuclear RNA; ○, soluble RNA; △, microsomal RNA.

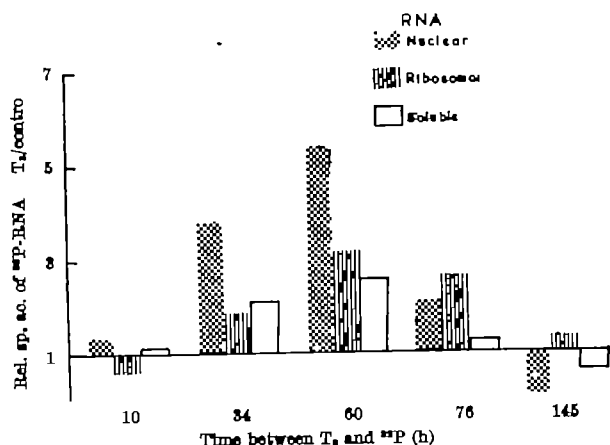


Fig. 2. Ratio of specific radioactivity of liver nuclear, ribosomal and soluble RNAs of induced/uninduced tadpoles at different time-intervals after  $T_3$  administration. All animals were killed 10–5 h after receiving 65  $\mu\text{Ci}$  of  $^{32}\text{P}$  (add 10.5 h to times indicated on abscissa for total time elapsed after the hormone was given).

on the 'slowly labelled' RNA was to enhance the incorporation of the precursor not only into nuclear but also into cytoplasmic RNA. This is illustrated in Fig. 2 for an experiment in which animals at different stages after induction of metamorphosis were killed 10.5 h after phosphorus-32 was administered. The failure to observe the enhanced synthesis of all species of RNA 5 or 6 days after T<sub>3</sub> administration was due to the dilution of phosphorus-32-labelled precursor by the rapid release of nucleotides, etc., from the regressing tail and intestine. Otherwise, all isotope experiments have shown that the first signs of induced metamorphic changes in the liver are preceded by 20-30 h by an enhanced synthesis of nuclear RNA which is soon followed by the appearance of newly formed RNA in the cytoplasm.

There was no substantial net accumulation of nuclear RNA during the first 140 h after metamorphosis was induced and only a slight accumulation of ribosomal RNA after 75 h, thus suggesting an accelerated turnover of RNA in view of the increased specific radioactivity. The following are the actual values: 0.25-0.40 mg nuclear RNA/mg DNA, 1.08-1.62 mg ribosomal RNA/g liver, and an RNA:protein ratio in ribosomes of 0.18-0.24. Pina-more and Frieden<sup>8</sup> had also reported no change in total liver RNA under comparable conditions.

Evidence that the increase in RNA synthesis at the early stages of induced metamorphosis was accompanied by an accelerated breakdown of 'old' ribosomes was provided by an experiment in which ribosomes were labelled with tritiated uridine 5-8 days before induction. Administration of T<sub>3</sub> to tadpoles with pre-labelled ribosomes was followed 24 h later by a 20-30 per cent drop in the radioactivity recovered in ribosomal RNA, compared with a rate of loss of 2-5 per cent of the label/24 h in the uninduced controls. The specific radioactivity was, however, lowered by only 5-10 per cent. The reduction in radioactivity of ribosomal RNA was accompanied by an increase in the specific radioactivity of nuclear RNA, thus suggesting a re-utilization of the degradation products. In fact, the total radioactivity in the tissue remained virtually unchanged during the first 2-3 days after the induction of metamorphosis.

With regard to the nature of newly synthesized RNA appearing in the cytoplasm at the same time as the degradation of 'old' ribosomes, we have separated the 78S ribosomes from the polyribosomal aggregates by sucrose density gradient centrifugation<sup>8</sup> in experiments in which the RNA was labelled after induction of metamorphosis.

The results of a typical experiment (Fig. 3) illustrate the following features: (1) The relative fraction of total ribosomes recovered as polysomes from the livers of metamorphosing tadpoles is about twice that from those of control animals. This finding suggests an increase in messenger RNA content, size or stability during metamorphosis<sup>8,11</sup> (note, however, that tadpole liver preparations contain a higher amount of monomeric 78S ribosomes than mammalian liver preparations<sup>8</sup>). (2) In control animals (Figs. 3A and C) the specific radioactivity of RNA recovered in the polysomes was greater than in the monomers both after short (4.5 h) and long (20 h) periods of labelling, suggesting that prior to metamorphosis messenger RNA may appear more rapidly than ribosomal RNA in the cytoplasm. However, during metamorphosis the specific activity of the monomers and aggregates increased in a parallel fashion, especially as seen after long exposures to tritiated uridine (Fig. 3D). This pattern

was seen over a wide range of time-intervals after induction of metamorphosis, and one explanation of this may lie in the possibility of new ribosomes being 'pre-coded' with messenger RNA before being released into the cytoplasm as has been suggested by Nirenberg<sup>12</sup>. Extraction of RNA from ribosomes and analysis by sucrose density gradient centrifugation gave compatible results, except that a much higher fraction of RNA in all cases was recovered as low molecular weight components (4S-10S) than that reported for mammalian liver<sup>13</sup>. (3) A greater fraction of ribosomes was recovered in the membranes sedimenting through the dense 60 per cent sucrose after induction of metamorphosis, when the same amount of detergent was used per unit RNA to release ribosomes from microsomes. A higher concentration of deoxycholate was necessary to get almost complete release of ribosomes and polysomes in preparations from metamorphosing animals and, after correcting the extinction at 260 mμ for the higher protein content of the membranes, a substantial part of the membrane-bound RNA was newly synthesized. Thus, during metamorphosis there occurs a firmer attachment of ribosomes to the endoplasmic reticulum or an enhanced synthesis of the latter, which is interesting in view of Hiatt's<sup>14</sup> suggestion that the site of cytoplasmic protein synthesis *in vivo* is the polyribosomes attached to the membranes of the endoplasmic reticulum. The accelerated synthesis of new ribosomal RNA at the onset of biological actions of developmental hormones, such as oestrogen, testosterone and thyroid hormones, has also been reported in mammalian systems<sup>15</sup>. It is possible that the formation of new ribosomes may be important for the expression of

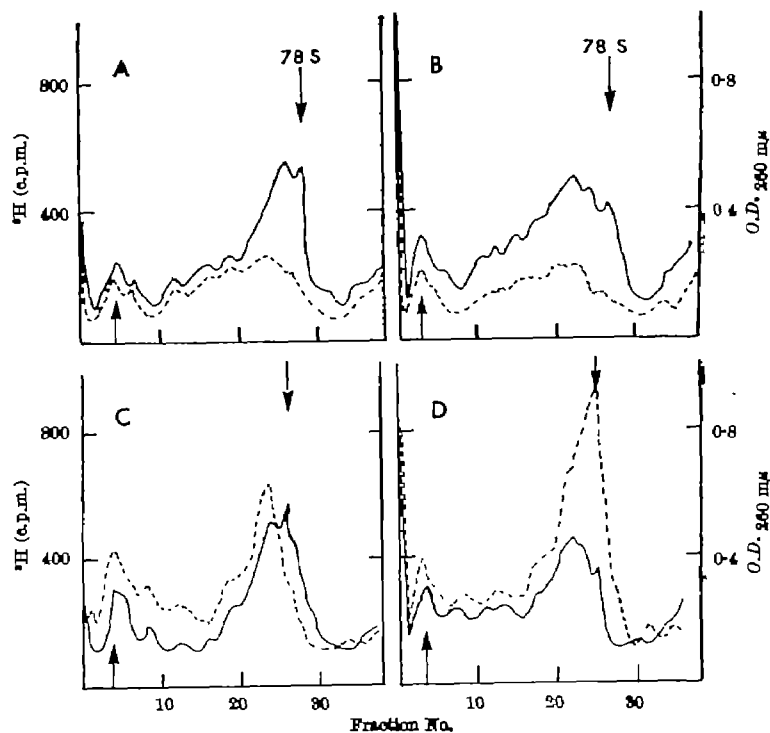


Fig. 3. Distribution of tritium-labelled RNA in monomeric ribosomes (78S) and polyribosomal aggregates in liver ribosomes pooled from 10 tadpoles per group in which metamorphosis was induced (B, D) or not (A, C). Metamorphosis was induced by an injection of 0.5 μg T<sub>3</sub> 116 h before killing; half the animals were injected with 10 μc of tritiated uridine 4.5 h (A, B) and the other half 20 h (C, D) before killing. Ribosomes from 300 to 500 mg liver were layered over a 15-30 per cent sucrose gradient (containing 0.05 M KCl, 0.02 M Tris-HCl buffer, pH 7.6, 0.0015 M MgCl<sub>2</sub>), with a cushion of 60 per cent sucrose at the bottom of the tube, and centrifuged in a No. 30 Spinco rotor at 25,000 r.p.m. for 2.2 h. 1 ml. fractions were collected from which aliquots were taken for the measurement of O.D. at 260 mμ (—) and for the determination of radioactivity present as RNA (---). Direction of sedimentation is from right to left; upward pointing arrows indicate the interface between the 60 and 80 per cent sucrose layers. Note that the 78S ribosomes (downward pointing arrow) have not been clearly separated from the dimeric forms. The pellet at the bottom of the tube, which consisted of membrane-bound RNA, was treated with 1 per cent sodium deoxycholate before determination of optical density and radioactivity in RNA.

newly synthesized messenger RNA necessary for producing the developmental changes that are hormone-dependent.

In conclusion, these experiments have shown that the biochemical and morphological changes of thyroid-hormone induced metamorphosis in *Rana catesbeiana* are anticipated by 24 h by an enhanced synthesis of RNA in the nucleus of the liver which is actively engaged in the synthesis of several new proteins. Activation of nuclei is followed by an accelerated rate of formation of cytoplasmic ribosomal and polyribosomal RNA and a more rapid rate of degradation of ribosomes that were present before the induction of metamorphosis. A firmer binding of ribosomes to the membranes of the endoplasmic reticulum also accompanied the appearance in the cytoplasm of newly synthesized RNA. There is, however, a relatively long lag period of 25-30 h between the time of induction of metamorphosis and the acceleration of RNA synthesis in the nucleus. The mechanisms regulating RNA synthesis and turnover during this initial period, and which respond to the hormonal stimulus for metamorphosis, still remain to be identified.

I thank Mr. A. J. Bell for his assistance.

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## PROPERTIES OF HORSE SERUM GAMMA INHIBITOR

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HORSE serum has been shown to be capable of inhibiting the haemagglutinating action and infectivity of the Asian influenza viruses<sup>1,2</sup>. Attempts have been made to separate the active material ( $\gamma$ -inhibitor) from horse serum, and preparations have been obtained which vary in their electrophoretic mobility and in their sedimentation properties. Thus Křižanová-Laušková *et al.*<sup>3</sup>, by precipitation with 'Neokal' and sulphosalicylic acid and by phenol extraction, obtained two inhibitory preparations, one component moving electrophoretically with the  $\alpha$ -globulins and having an  $S_{20}$  of 3.66 and two components moving just ahead of the  $\gamma$ -globulin and having  $S_{20}$  values of 14.7 and approximately 5.9. Later Křižanová and Lesko<sup>4</sup> reported the isolation of three components moving on electrophoresis with the  $\alpha$ -globulins and with  $S_{20}$  values of 8.6, 3.4 and 2.8. Boretti *et al.*<sup>5</sup>, using a sequence of precipitation methods, also obtained three components with inhibitory activity, and  $S_{20}$  values of 8.7, 17.5-21, and 39.8.

Although none of these sedimentation constants appears to be an extrapolated value, the variation seems sufficiently great for it to be difficult to reconcile all the results so far reported, with the concept that  $\gamma$ -inhibitor is one single substance or group of substances. Recent work in this laboratory<sup>6</sup> makes it clear that the method used in fractionating the serum determines the apparent distribution of inhibitory activity; where relatively mild methods are used the inhibitory activity is confined exclusively to the  $\alpha$ -globulin fraction of the serum. In the present article it is shown that if separation is carried out with due regard to the correct ionic strength and pH, the majority of the inhibitory activity appears to be carried by a single fast-sedimenting component (extrapolated  $S_{20}$  = 18.0) with the electrophoretic mobility of an  $\alpha_2$ -globulin.

Fifty-ml. quantities of freshly clotted horse serum were spun on a Spinco model L centrifuge at 40,000 r.p.m. (40 rotor) for 20 h. The supernatant was removed and the sedimented pellets frozen. The frozen pellets could be easily removed from the 'Lusteroid' tubes by eliciting and were transferred to a dialysis sac and dialysed against 0.15 M barbiturate buffer pH 8.6. The final volume was adjusted to 10 ml., transferred on to an inert cellulose column 42 cm long (LKB column electrophoresis model

3340) and electrophoresis carried out in 0.15 M barbiturate buffer pH 8.6 using a current of 60 m.amp and a potential of 410 V, for 22 h. The column was then eluted with the same buffer and 5-ml. fractions were collected and tested for inhibitory activity against 8 agglutinating doses of influenza A<sub>1</sub> virus by the standard 'pattern' method, and for protein content by measuring extinction at 280 m $\mu$ . Although the column was grossly overloaded by the quantities used, the inhibitor could be well localized. All fractions giving an inhibitor titre > 80 were pooled, dialysed against 0.15 molar saline and concentrated by dialysis against 20 M 'Carbowax'. The final volume of concentrate was adjusted to 8.6 ml., and on sedimentation in a Measuring and Scientific Equipment analytical ultracentrifuge at a total protein content of 0.5 per cent showed two major peaks (Fig. 1), which were calculated to have  $S_{20}$  values of 6.03 and 14.70.

In the next stage, 2 ml. of the concentrate from electrophoresis was layered on to a sucrose density gradient (45 per cent-9 per cent in 0.15 M sodium chloride solution) and centrifuged in a Spinco SW 25 rotor at 25,000 r.p.m.



Fig. 1. Schlieren pattern from analytical ultracentrifugation of electrophoresis preparation 28 min after reaching a speed of 57,140 r.p.m. Angle, 80°. Total protein concentration, 0.5 per cent. Sedimentation towards left.



for 30 h. Two distinct bands (Fig. 2) were produced and 1-ml. fractions were collected by tube puncture and tested for inhibitory activity and protein content. The results (Fig. 3) show that up to 80 per cent of the inhibitory activity recovered on the gradient is carried with the 'fast'-sedimenting band. The fractions corresponding to the two bands were pooled, dialysed against 0.15 M sodium chloride again and re-run in the analytical ultracentrifuge after concentrating with 'Carbowax'. Fig. 4 shows the appearance of the material from the 'fast'-sedimenting band. It will be seen that apart from the expected 'fast'-moving peak (extrapolated  $S_{20,w} = 18.0$ ) there is also a small, slowly sedimenting peak ( $S_{20,w} = 6.33$  at a single concentration of 0.5 per cent total protein). This is probably due to incomplete resolution on the gradient.



Fig. 2. Results of density-gradient run on electrophoresis material showing band separation

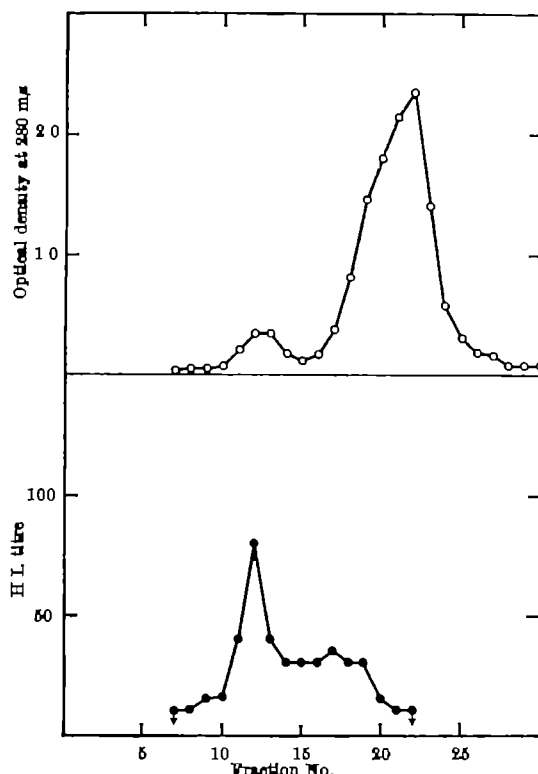


Fig. 3. Distribution of inhibitory activity and protein extinction in density-gradient fractions from centrifugation of electrophoresis material

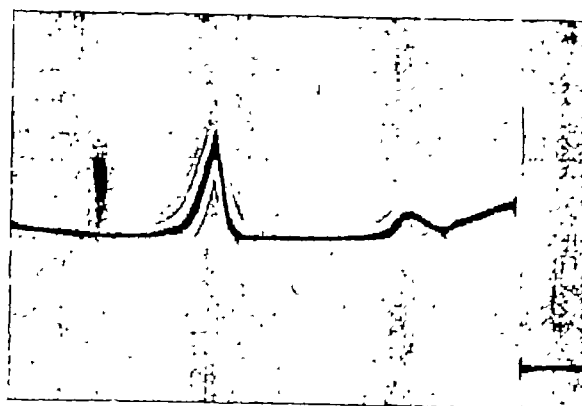


Fig. 4. Schlieren pattern from analytical ultracentrifugation of 'fast' band derived from density gradient, 28 min after reaching speed of 59,820 r.p.m. Angle, 70°. Total protein concentration, 0.33 per cent w/v. Sedimentation towards left

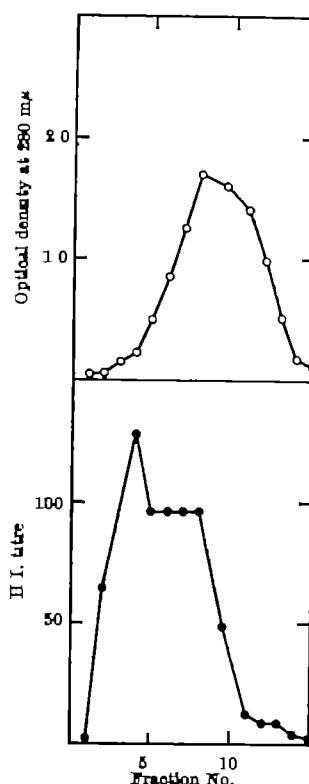


Fig. 5. Distribution of inhibitory activity and protein extinction in density gradient fractions of original horse serum

Table 1 gives the results of one experiment in which the percentage recovery of inhibitory activity at each stage is calculated with reference to the original serum, and also shows the specific activity in terms of haemagglutination inhibition (H.I.) titre per mg of nitrogen. In nine subsequent experiments the recovery of inhibitory activity in the 'fast'-moving band ranged from 55 to 80 per cent of the total activity in the serum.

In connexion with the foregoing, certain points are of great importance. First, the authors quoted here used fairly drastic methods of separation and attention has already been directed to the problems of using such

Table 1. INHIBITORY ACTIVITY RECOVERED AT EACH PREPARATIVE STEP

Material	Specific activity (H.I./mg nitrogen)	Recovery of inhibitory activity (per cent)
Original serum	30	—
Centrifuged concentrate	70	96†
Electrophoresed prep	100	120
'Fast' peak	1,600	87

\* Referred to original serum

† Error of test  $\pm 25$  per cent.

methods. Secondly, previous workers dialysed their products against water at some stage in the procedure. It has been established in this laboratory that if solutions of <0.08 ionic strength are used the inhibitor precipitates out and cannot be completely recovered. Furthermore, there is often a fall in inhibitory activity of the recovered material and this is then very liable to undergo irreversible precipitation. It has also been found that ionic strengths >3 caused a similar effect. In the present work care was taken to work with electrolyte solutions about 0.1–0.15 ionic strength.

It seems likely that the 'slow'-moving components obtained by other workers represent breakdown products of the naturally occurring 18S material, and in our investigations we have seen such a breakdown occurring when potassium tartrate gradients (40–8 per cent) were used. In these cases early denaturation was indicated by a 'fast'-moving band which had intense light-scattering properties and in some of these preparations biological activity was found distributed throughout the length of the gradient.

Thus it appears that the bulk of the  $\gamma$ -inhibitory activity of horse serum is associated with a 'fast'-sedimenting molecule having the electrophoretic mobility of an  $\alpha_2$ -globulin. The subsidiary shoulder of inhibitory activity seen on density gradient centrifugation of the electro-

phoresed portion seems to represent a second molecular species which is present in the original serum and is possibly generated by breakdown of the larger molecule. Density-gradient centrifugation of the original serum before separation of the active components gave the results shown in Fig. 5. The greater part of the inhibitory activity will be seen to be ahead of the main protein band, but there is again a shoulder of activity similar to that seen after separation. It appears that unless operations are carried out in solutions of ionic strength about that of physiological saline such breakdown is considerably accelerated.

We thank the Wellcome Trust and the Fleming Memorial Fund for the purchase of essential equipment. We also thank Mr. F. Clothier (Medical Research Council, Carshalton) for help with the analytical ultracentrifugation, and Measuring and Scientific Equipment, Ltd., for allowing us the free use of their analytical ultracentrifuge.

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## ROLE OF A STRUCTURAL GLYCOPROTEIN OF CORNEAL STROMA IN TRANSPLANTATION IMMUNITY

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**I**SOLATION and characterization of the so-called 'transplantation antigens' are among the major tasks for those studying the problem of histocompatibility. Most effort has been directed towards intracellular proteins isolated from different tissues<sup>1,2</sup>. It appeared interesting to us to explore the possible role of the structural proteins of connective tissue. Cornea was chosen as a model system because of the relatively high percentage of healing ('take') of homo- and even hetero-grafts and because of the abundance of experimental work that has been reported on keratoplasty<sup>3,4</sup>.

We recently isolated a 'structural glycoprotein' from cornea, keratoglycosaminoglycan-I (KGAG-I), which is associated with the insoluble collagenous network of the corneal stroma<sup>5,6</sup>. An immunochemically identical protein is present in the soluble extracts (pro-KGAG) and according to labelled amino-acid incorporation data it behaves as a metabolic precursor of the structurally linked KGAG. Though the isolation procedure first used is a rough one, the preparation obtained from insoluble stroma still behaves as a good antigen and seems to be identical with one at least of the organ- and species-specific antigens of corneal stroma<sup>7-9</sup>.

It could be shown by the immunofluorescent method that this glycoprotein is exclusively localized in the stroma, no detectable amounts being present in the cellular layers of the cornea<sup>5</sup>. Pure KGAG as obtained by the urea extraction procedure<sup>10</sup> gives rise to antisera (in rabbits and chicken) showing sometimes extended cross-reactions between different species<sup>7,8</sup>. The experiments reported here suggest that some parallelism can be established between *in vitro* immunochemical cross-reactions and the *in vivo* reactions observed in pre-immunized animals grafted with homologous and heterologous tissues. They indeed show a parallelism between circulating antibody titres and the intensity of the reaction against the graft. Rabbits were immunized with different

preparations of calf and rabbit cornea as follows. 5 mg of protein was injected with Freund's incomplete adjuvant 3 times a week for 2 weeks. Then 10 mg of protein was injected with the same adjuvant 3 times a week for 2 further weeks. After 1 week of interruption a booster shot was given (10 mg protein). Blood was taken weekly from the ear vein and the anti-KGAG antibody titre established by passive haemagglutination as described<sup>7</sup>. The preparations used for immunization were<sup>9</sup>: (1) pure KGAG prepared from the insoluble corneal stroma by urea extraction, after removal of the collagen by hot TCA<sup>5</sup>; (2) the 'crude soluble collagen' obtained by precipitating it from the calcium extracts of cornea by dialysis; and (3) the soluble proteins of the cornea (OTC-extract), the supernate of the dialysed calcium extract.

One week after the last injection the animals received grafts. All the grafts were of the intralamellar type<sup>4</sup>. The epithelium of the corneal grafts was scraped off in order to eliminate the reaction to the epithelial proteins<sup>5</sup>. The behaviour of the grafts was then closely followed by daily observation using a slit lamp and photographic recordings. Table 1 shows the results obtained.

In the third row of Table 1 are given the circulating antibody titres determined at the end of the immunization period, on blood taken before the day of grafting. The anti-KGAG titres vary according to the antigen used for immunization<sup>7</sup>, the highest titres being obtained when pure KGAG is used as an antigen.

In the control animals the homografts were accepted, without any reaction, within the 8 weeks of observation. The heterografts also gave good results, though in four animals a slight vascularization and transitory opacity was seen during the fifth week after grafting.

In all the animals immunized with calf corneal preparations there was a strong reaction against the heterograft. The time of onset varied from 1 to 3 weeks. Heavy vascularization, pannus formation and severe opacity

Table 1. REACTION OF RABBITS TO CORNEAL HOMO- AND HETERO-GRAFTS (CALF CORNEA)  
Effect of immunisation with corneal extracts. Homograft on left eye and heterograft on right eye

No.	Immunising antigen	No. of anti- male	Circulating antibody titre* $\times 10^{-4}$	Intensity† of reaction to Homograft		Intensity† of reaction to Heterograft	
				Vas- ular reaction	Opac- ity of graft	Vas- ular reaction	Opac- ity of graft
1	No treatment	2	2	0	0	+	+(5)
2	NaCl 0.9%	2	2	0	0	+	+(5)
3	Freund's adjuvant (incomplete)	4	2	0	0	0	0
4	CTC-extract‡ of calf cornea	2	1,600-3,200	0	0	++	++ (3)
5	CSO§ of calf cornea	2	1,600	0	0	++	++ (3)
6	KGAG of calf cornea	3	3,200-12,800	0	± (3)	+++	+++ (5)
7	CSO of rabbit cornea	3	128-1,024	+	+(1)	+	+(2)
8	KGAG of rabbit cornea	2	5,100-10,200	0	± (1)	++	++ (1-3)

\* Determined on blood drawn the day before grafting, with red cells coated with calf-KGAG. The figures represent extremes of haemagglutination-titres found in the different animals.

† The intensity of the vascular-inflammatory reaction and of the opacity is given by the number of plus signs. The figure in parentheses after the sign is the time of onset of the opacity in weeks. The reactions were identical or very similar in intensity in the animals of the same group.

‡ Soluble proteins of the corneal stroma, extracted in Ca-tris-citrate (CTC) buffer.

§ 'Crude soluble collagen' of corneal stroma.

of the heterografts were always observed. The homografts were unaffected except for two of the animals treated with calf-KGAG. In these animals a transient and light opacity of the homograft was observed on the third week after grafting, which disappeared in 5-10 days. This contrasted with the strong and definitive opacity observed in the heterografts.

The rabbits immunized with homologous corneal extracts did not produce antibodies against rabbit-KGAG, except in one case where the titre rose to 1/160. There appeared, however, circulating antibodies detectable with red cells coated with calf-KGAG (see Fig. 1). This phenomenon was observed with all the rabbits immunized with homologous corneal preparations (both crude soluble collagen (CSO) and pure KGAG). In the control animals treated only with Freund's incomplete adjuvant no such reaction could be observed. The haemagglutination gave low but comparable titres with red cells coated with either rabbit or calf KGAG (see Fig. 1).

In agreement with the serological observations (see Table 1) strong reactions were obtained against the calf-grafts in these rabbits treated with rabbit corneal extracts with 'early opacity of the grafts (1-3 weeks).

No reaction, or only a mild and transitory one, could be observed on the cornea that received the homografts. These reactions were somewhat stronger than those observed in the unimmunized control group where transi-

tory inflammatory reactions with opacity could be observed on the first week in some animals, which disappeared on the second or third week.

These results suggest the following interpretation. It is now generally recognized that late clouding of corneal grafts is a result of an immune reaction<sup>10</sup>. Though many antigenic components were demonstrated in cornea<sup>11</sup>, those directly responsible for such reactions have not yet been isolated and identified. Our experiments show that the structural glycoprotein, KGAG, probably has an important role as a mesenchymal antigen in 'immune-recognition-reactions' which occur after keratoplasty. The presence of circulating antibodies against this protein produces a highly accelerated immune reaction against a heterologous tissue implanted even in such an avascular site as the cornea (all the grafts were of the intralamellar type). The highest titres of antibodies and the strongest reactions are obtained when pure KGAG, extracted from the insoluble stroma, is the immunizing antigen. The CTC extract, which has many other soluble proteins beside the precursor-form of KGAG (pro-KGAG), produced antibodies of a low titre and a milder reaction than either the 'crude soluble collagen' fraction (CSO) or the KGAG extracted from insoluble stroma.

The very existence of the phenomenon of late clouding of corneal grafts suggests the importance of insoluble antigenic components. The soluble proteins must have disappeared from the graft by the time the late clouding sets in<sup>3</sup>. A slow solubilization of structurally linked components such as KGAG, however, might well explain the late onset of the immune reaction. When rabbits are immunized with calf or horse cornea preparations the antisera obtained react with rabbit-KGAG also<sup>8</sup>. In spite of this we could not obtain high-titre antisera against rabbit-KGAG by direct immunization of rabbits with homologous corneal preparations. The appearance of antibodies detectable with calf-KGAG-coated red cells can be explained in several ways. A plausible hypothesis would admit that antibody production takes place when immunizing with rabbit cornea, but those antibodies strongly specific to rabbit-KGAG are adsorbed on to identical or cross-reacting structures present in different organs<sup>12</sup>. Only those antibodies with a pronounced specificity to cross-reacting organ-specific sites would remain in the circulation. This explanation is in agreement with the known heterogeneity of the antigenic and antibody sites and with the presence of identical or cross-reacting structures, similar to KGAG in other organs as revealed by immunofluorescence<sup>8</sup>. The slight but definite reaction to homografts and the strong reaction to the heterografts are also in accordance with such an explanation. The homograft would be 'protected' in a way by the presence of cross-reacting (or identical) substances present in other mesenchymatous tissues.

These results, as well as others obtained with pig corneas<sup>11</sup>, suggest the importance of KGAG as a specific antigenic component operating in species-recognition reactions.

The accelerated rejection of intralamellar grafts obtained by immunization with a pure mesenchymal protein suggests that besides the intracellular 'transplantation antigens', connective tissue also carries species-specific antigens. Though the intracellular antigens may be predominant when highly cellular tissues are transplanted, mesenchymal antigens can play an identical part when acellular and avascular tissue, such as corneal stroma, is transplanted. It may be of importance in this respect that KGAG exists in a soluble, 'precursor' form<sup>13,14</sup> and also as an insoluble, structurally linked component of connective tissue. The soluble form may be of major importance in producing sensitization in the host, and the insoluble form would then act as a target for the antibodies and for the sensitized cells. The part of KGAG and of related 'structural glyco-proteins' in immune-recognition reactions in general is now being investigated.

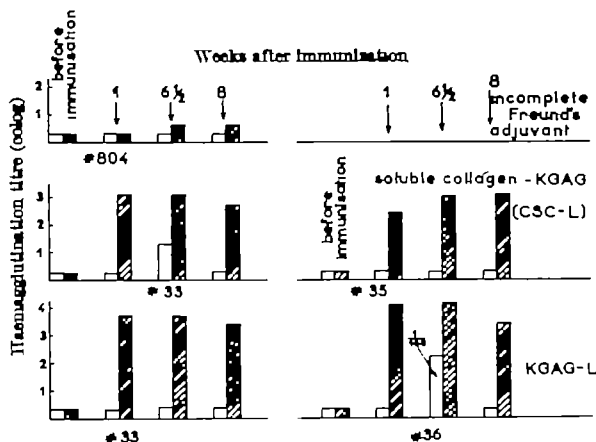


Fig. 1. Anti-KGAG antibodies in the blood of rabbits immunized with rabbit corneal preparations. The immunising antigen is indicated on the right side of the graph. The antibody titres (as obtained by passive haemagglutination) are given as their negative logarithms on the left of the graph. Determinations were done simultaneously with red cells coated with rabbit-KGAG and with calf-KGAG. Unshaded areas, anti-rabbit KGAG antibodies, shaded areas, anti-calf KGAG antibodies

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In a previous report, cleavage of sodium dodecyl sulphate by a bacterial enzyme system was measured by following the accumulation of sulphate ion via the barium chloride procedure<sup>1</sup>. However, the sensitivity of this method was so low that several  $\mu\text{mole/ml}$  of detergent

**Detergent-methylene blue complex method (complex method).** The main procedure of this new method is the spectrophotometric measurement of the amount of methylene blue released from a dye-detergent complex. (Fig. 1)<sup>8</sup>. Advantage is taken of the chloroform solubility of a dye-detergent complex to separate free, water-soluble methylene blue from that portion of the dye still bound to the detergent. During enzymatic splitting of the detergent, an equivalent quantity of dye is released from the complex and the measurement of this released dye constitutes the basic parameter. This is shown schematically as:

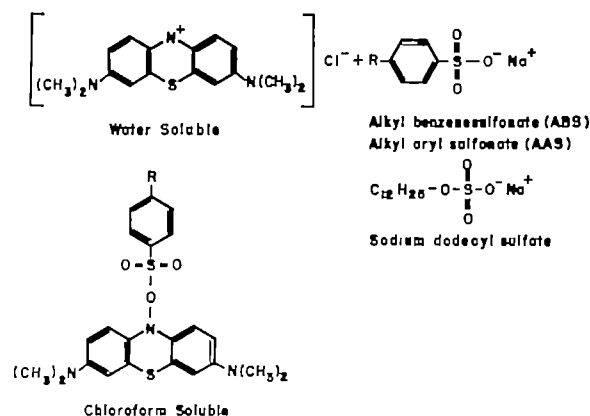
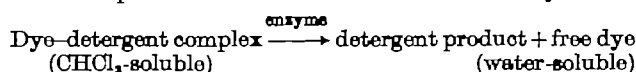
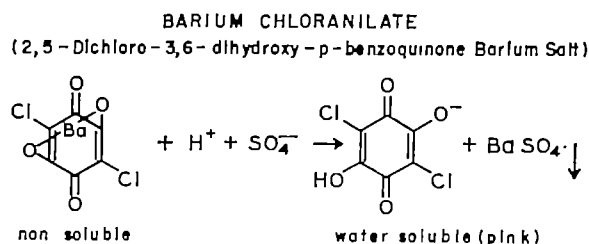


Fig. 1. Methylene blue-detergent complex method. Free water-soluble methylene blue was released from chloroform-methylene blue-detergent complex as the result of enzyme action. To 85 mmole/ml sodium dodecyl sulphate in 0.05 M tri-*n*-hydrochloride (pH 8.0) containing 0.14 M sodium chloride (*trw* 8-9), aqueous  $10^{-4}$  M methylene blue slightly in molar excess was added so that after extraction with one volume of chloroform, the optical density at the aqueous phase was 0.05-0.1. It was important to avoid an excess of sodium dodecyl sulphate to prevent the destruction of the enzyme. The enzyme solution was added to the reaction mixture and extracted at 35° C in a water-bath shaker operated at 100 r.p.m. In order to avoid a possible photodynamic action of methylene blue, the reaction was carried out in the dark. At appropriate intervals, 10 ml. of the reaction mixture were removed and immediately extracted with 10 ml. of chloroform. Multiple extraction with chloroform was not found to be necessary. The colour intensity in the resulting aqueous phase was determined in a spectrophotometer at 560 m $\mu$  using 1.75-in. test-tube. The absorbancy in such a test tube was converted to  $\mu$ mole/ml. of methylene blue concentration by using the factor of 25, since 10  $\mu$ mole/ml. of methylene blue in water have an absorbancy of 0.4.



**Barium chloranilate method.** This method is based on the reaction shown in Fig. 2. Water-insoluble barium chloranilate (2,5-dichloro-3,6-dihydroxy-*p*-benzoquinone barium salt), when combined with free sulphate ions in



**Fig. 2. Barium chloranilate method.** An assay of sulphate released from the detergent as the result of enzyme action. Each 1.5 ml. of the final reaction mixture contains 15  $\mu$ moles of SDS (0.16 ml. of 0.1 M), 0.15 ml. of 5-7 per cent bovine serum albumin dissolved in 0.06 M trihydrochloric acid buffer (pH 8), the enzyme solution and 0.05 M trihydrochloric acid buffer (pH 8) containing 0.14 M sodium chloride (tris 8-8). It is important to mix sodium dodecyl sulphate and bovine serum albumin solution thoroughly before adding the enzyme solution (1). After the enzyme solution was added, the reaction mixture was incubated at 37° C in a shaker. At the end of the incubation period the reaction was terminated by adding 1 ml. of 0.5 M potassium biphenylate and 2.5 ml. of 95 per cent ethanol. The mixture was then heated in a water bath at 70° C for 10 min. The resulting precipitate was eliminated by centrifugation. The supernatant was then transferred to a 125-ml. flask and 20 mg. of barium chloranilate was added. After 15 min. of incubation in a shaker at 37° C, the remaining barium chloranilate and resulting barium sulphate were eliminated by centrifugation. The pink-coloured chloranilate ion was read spectrophotometrically at 510 m $\mu$  of wave-length. Ten  $\mu$ moles/ml. of sulphate ion has 0.85 absorbancy in 1-cm optical path cuvette.

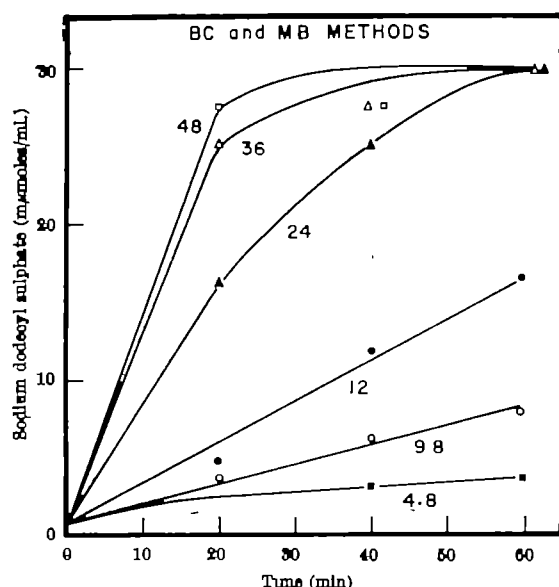


Fig. 3. Comparison of the complex method with the barium chloranilate method. Varied amounts of enzyme previously assayed by the barium chloranilate method were incubated at  $32^{\circ}\text{C}$  with 33 mMoles/ml. of sodium dodecyl sulphate which were mixed with equal molar of methylene blue in 0.06 M Tris 8-8. The figures on each curve represent the partially purified enzyme activity in millunits per ml. assayed by the barium chloranilate method. The enzyme activity measured by the complex method is shown in the ordinate. The intensity of methylene blue colour in water phase after extraction with chloroform was measured at a wave-length of 690 m $\mu$ .

the presence of acid, releases water-soluble chloranilate ions. This results in a solution having a pink colour<sup>4</sup>.

**Definition of unit and specific activity.** One unit of enzyme is defined as: (a) that amount capable of forming 1  $\mu$ mole of sulphate ions by the barium chloranilate method, or (b) the amount which will catalyse the release of 1  $\mu$ mole of methylene blue in 1 h at a temperature of  $32^{\circ}\text{C}$ , and at pH 8 by the complex method.

**Comparison of complex method and barium chloranilate method.** A solution of partially purified enzyme<sup>2</sup> was assayed by the barium chloranilate method and found to contain 60 units/ml. Various dilutions of this enzyme were assayed by the complex method. In a proper dilution of the enzyme solution, the curve showing the amount of dye released with respect to the time was found to be linear. The activity of the enzyme was calculated from the linear portion of the curve. As shown in Fig. 3, 12 millunits per ml. of enzyme assayed by the barium method were observed as against 17 millunits per ml. at 60 min observed by the complex method. The activity assay by the complex method tended to be slightly higher than that measured by the barium chloranilate method. This could be partly due to the high concentration of detergent used in the barium chloranilate method, which tends to destroy the enzyme activity even though the destroying action was counteracted by using bovine serum albumin.

**Molar ratio of methylene blue to sodium dodecyl sulphate in substrate.** The molar ratio of sodium dodecyl sulphate to methylene blue was varied from 1:1 to 2:1 and 3:1. The three mixtures were subjected to constant amounts of the enzyme action. The results shown in Fig. 4 suggest that in the mixture having a ratio of one methylene blue to sodium dodecyl sulphate colour was released into the water phase immediately after reaction with the enzyme. In the mixtures having more sodium dodecyl sulphate the release of the methylene blue colour into the water phase was delayed for a period determined by the quantity of excess detergent. It appears that when excess sodium dodecyl sulphate was present either the enzyme preferred the excess free sodium dodecyl sulphate, or else it digested the sodium dodecyl sulphate complexed with methylene

blue, thereby releasing the latter, which then re-formed additional complexes with unreacted sodium dodecyl sulphate.

Although the new complex method has been used only to assay sodium dodecyl sulphate splitting enzyme and intact cells, it might also be applied to the splitting of other anionic detergents, such as alkyl benzene sulphonate.

The advantages of the complex method over the barium chloranilate method are: (1) the complex method is very much more sensitive than the barium chloranilate method; (2) the contamination of the detergent by sulphate does not appreciably interfere with the complex method; (3) the assay by the complex method takes less time; (4) the increased sensitivity of the method permits the use of a much lower detergent concentration than in the barium chloranilate method, thereby reducing the destruction of the enzyme by the detergent and also reducing the need for protective bovine serum albumin.

**Sodium dodecyl sulphate splitting activity of intact cells.** An experiment assaying the detergent-splitting activity of intact *Pseudomonas* was conducted using the sensitive

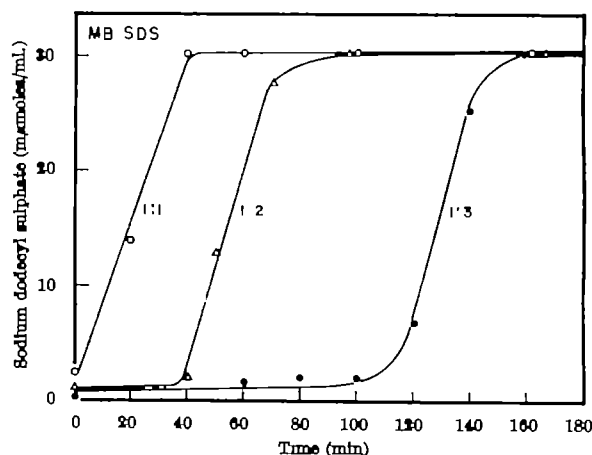


Fig. 4. Varied molar ratio of methylene blue to sodium dodecyl sulphate. Methylene blue was mixed with varied molar ratio of sodium dodecyl sulphate and incubated with constant amount of partially purified enzyme. The amount of methylene blue released from dye-detergent complex was assayed by the complex method.

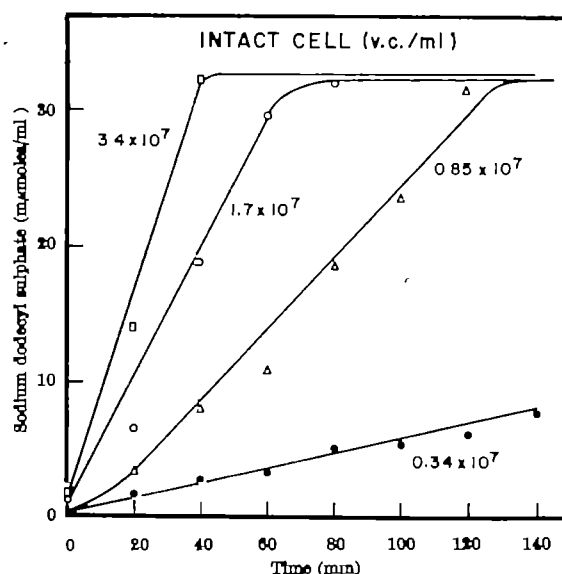


Fig. 5. Cells cultured in the synthetic medium with sodium lauryl sulphate as the sole carbon and sulphur sources were washed by centrifugation and varied amounts of cells assayed for activity by methylene blue-sodium dodecyl sulphate complex method. Viable cells (v.c.) were plated in tryptone-agar and counted after two days.

complex method. The bacteria were cultured overnight in a synthetic minimum medium with sodium lauryl sulphate as the sole carbon and sulphur source with vigorous shaking at 36° C. The bacteria were collected by centrifugation and washed twice with 0.14 M sodium chloride. Portions containing varying numbers of collected and washed bacterial cells were mixed with 0.033 mM of dye-detergent complex in 0.05 M *tris*-hydrochloric acid buffer (pH 8) solution containing 0.14 M sodium chloride (*tris* 8.8). The activity of intact cells can be obtained by dividing the rate in the linear part of the curves in Fig. 5 by the number of cells. It can be seen that 30  $\mu$ moles/ml. sodium dodecyl sulphate were split in 60 min with  $1.7 \times 10^7$  bacterial cells per ml., whereas a concentration of  $0.85 \times 10^7$  bacterial cells required 120 min to accomplish the same degree of detergent degradation. The intact cell activity was found to be about 1.8  $\mu$ moles of sodium dodecyl sulphate degraded per  $10^8$  bacterial cells per hour. This rate may also be expressed as  $1.8 \times 10^{-8}$   $\mu$ moles of detergent split per bacterium per hour under conditions of 32° C and pH 8.0.

With the use of the same technique, a series of separate experiments was performed in order to confirm the estimated amount of sodium dodecyl sulphate split per bacterium per hour at a temperature of 32° C and at pH 8. The results are summarized in Table 1. In the experiment of January 20, the same lot of bacteria was used for the comparison of the rate of reaction at 32° C and 22° C. The rate of reaction at 22° C was approximately half that at 32° C. The average activity from four separate experiments was  $1.4 \times 10^{-8}$   $\mu$ moles per cell per hour at a temperature of 32° C and at pH 8.

Table 1. ACTIVITY OF INTACT CELL BY COMPLEX METHOD

Date	O.D.	Viable count	v.c./O.D.	Units/ml.	u./O.D.	$\mu$ moles/v.c./h	React temp.
Jan. 16	7	$6 \times 10^8$	$0.88 \times 10^8$	7.5	1.1	$1.25 \times 10^{-8}$	32° C
Jan. 20	4.5	$5.1 \times 10^8$	$1.1 \times 10^8$	9.0	2.0	$1.7 \times 10^{-8}$	32° C
	4.5	$5.1 \times 10^8$	$1.1 \times 10^8$	3.9	0.9	$0.76 \times 10^{-8}$	22° C
Jan. 23	6.0	$7.8 \times 10^8$	$1.3 \times 10^8$	12.4	2.1	$1.6 \times 10^{-8}$	32° C
Jan. 28	7.5	$11.0 \times 10^8$	$1.5 \times 10^8$	11.4	1.5	$1.0 \times 10^{-8}$	32° C

Average =  $1.4 \times 10^{-8}$   $\mu$ moles/cell/h (at 32° C, pH 8).

Cells grown overnight in the synthetic medium with sodium lauryl sulphate as sole carbon and sulphur source at 36° C were washed and absorbancy measured. The sodium dodecyl sulphate splitting activity was assayed by the complex method with various amounts of viable cells (v.c.). The activity of intact cells was calculated by the method described in the text and with data from Fig. 5.

**Substrate specificity of intact bacteria.** Varying amounts of sodium dodecyl sulphate and sodium dodecyl benzene sulphonate (DBS) were combined in proportions that maintained a constant concentration of total detergent. The mixtures were mixed with constant amounts of the enzyme extracted from the sodium dodecyl sulphate-adapted bacteria. The results of the reaction are shown

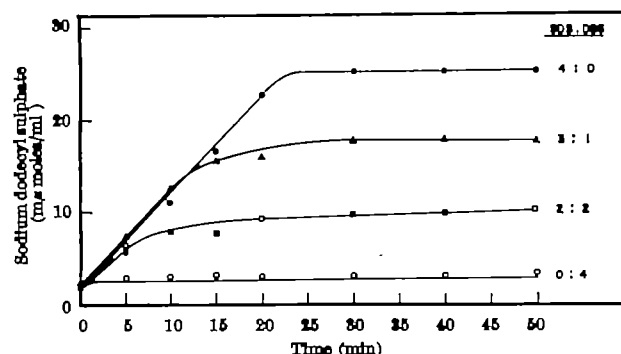


Fig. 6. Substrate specificity of intact cells. Varied molar ratios of sodium dodecyl sulphate to sodium dodecyl benzene sulphonate, having equal total concentrations, were mixed with methylene blue and incubated with constant amount of intact cells. The amount of methylene blue released from dye-detergent complex was measured by the complex method.

in Fig. 6. This experiment shows that the sodium dodecyl sulphate splitting enzyme has no effect on dodecyl benzene sulphonate.

It has been stated by several authors<sup>4,6</sup> that the secondary alkyl sulphates are not biologically degradable. In order to ascertain the specificity of the sodium dodecyl sulphate splitting bacteria on the branched secondary alkyl sulphates, further tests were made. It was found that the sodium dodecyl sulphate splitting bacteria could not utilize the branching secondary alkyl sulphate such as the sodium salt of the sulphate of 2-methyl-7-ethyl-4-undecanol ('Tergitol') as the sole carbon and sulphur source. The enzyme extracted from sodium dodecyl sulphate-adapted bacteria was found unable to release methylene blue into the aqueous phase from the complexed branching secondary alkyl sulphate when assayed by the dye-detergent complex method. Attempts to isolate bacteria which can utilize the branched secondary alkyl sulphates as their sole carbon and sulphur sources so far have not been successful.

The foregoing results permitted some interesting calculations. In order to split one part per million (1 mg/l.) or  $3.5 \times 10^{-3}$   $\mu$ moles/ml. of sodium dodecyl sulphate at 32° C in 1 h, it was found that  $2.5 \times 10^8$  cells/ml. of adapted bacteria are required. At a temperature of 22° C or lower,  $5 \times 10^8$  or more cells per ml. would be required.

As reported previously, the sodium dodecyl sulphate splitting enzyme is adaptive, and the enzyme adapted to dodecyl sulphate is also able to split decyl and tetradecyl sulphate. The micro-organisms must be adapted to one of these alkyl sulphates in order to split the detergent. At present there is no way of knowing whether bacteria in Nature are adapted to sodium dodecyl sulphate. The raw sewage sampled at the influent to the Baltimore Back River Sewage Treatment Plant was noted to contain about  $10^3$ - $10^4$  cells per ml. which could potentially utilize sodium dodecyl sulphate both as carbon and sulphur sources. Knowing the number of detergent-splitting bacteria in Nature, temperature, pH and the rate of detergent-splitting activity per bacterium per hour, one will be able to calculate the theoretical maximum load of detergent that can be destroyed completely within a given treatment period.

From the point of view of degradability, the alkyl sulphates are believed to be superior detergents over alkyl benzene sulphonates. The following three reasons support this view: (1) alkyl sulphate splitting bacteria do exist in Nature; (2) the detergency can be destroyed even in an anaerobic condition by hydrolysing the sulphate ester linkage; (3) the rate of splitting alkyl sulphates by bacteria is fairly rapid.

The primary purpose of this article is to suggest that more detailed knowledge can be obtained on the extent of microbial-specific substrate interaction. It is acknowledged, however, that in Nature the derived rates could vary considerably from the figures calculated from laboratory observations. Nevertheless, as factors controlling the reaction are clarified, a greater understanding will be gained with regard to the failures of conventional waste-treatment processes to degrade detergents biologically. Conceivably, there are several factors that could contribute to the present problem. Some of these are: (1) An adequate number of potential organisms may be present in the water system, but these organisms may not be adapted to the detergent. (2) Adapted organisms may exist, but the time provided is insufficient for complete degradation under prevailing temperature conditions. (3) The detergent loading may be in excess of the amount that can be handled by the existing adapted microbial population. (4) There are large numbers of detergent isomers which antagonize each other and cannot be utilized by micro-organisms. (5) Certain synthesized chemical structures cannot be utilized by bacteria.

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I thank Dr. C. W. Krusé, Dr. L. H. Frank and Dr. R. M. Harriott for their advice, and Dr. K. Kawata and Miss C. Beach for their assistance.

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# MYXOMATOSIS AND THE RABBIT FLEA

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MUIRHEAD-THOMSON (1954, unpublished) established that the rabbit flea (*Spilopsyllus cuniculi* (Dale)) is the major vector of myxomatosis in Great Britain, but very little work has been done since on the relationship of the virus with the rabbit flea.

In Britain over the past 10 years it has been observed that outbreaks of myxomatosis may occur several months after a previous outbreak in the same locality appears to have ended, although there is no evidence that the virus has been re-introduced. We thought that virus might survive on a few infected fleas which lived-on in a burrow after leaving a myxomatous rabbit. This article describes experiments done to test this idea.

It is impossible to keep rabbit fleas under natural conditions and be sure that they have not had access to a host (either a rabbit or another mammal). We therefore devised the following technique. Fleas were put into 3 in. x 1 in. glass tubes together with some rabbit fur, and the tubes were closed with a special polythene stopper (Fig. 1). These tubes were then put on a metal tray strewn with earth, and placed in an artificial burrow in such a way that individual tubes could be removed without disturbing the others.

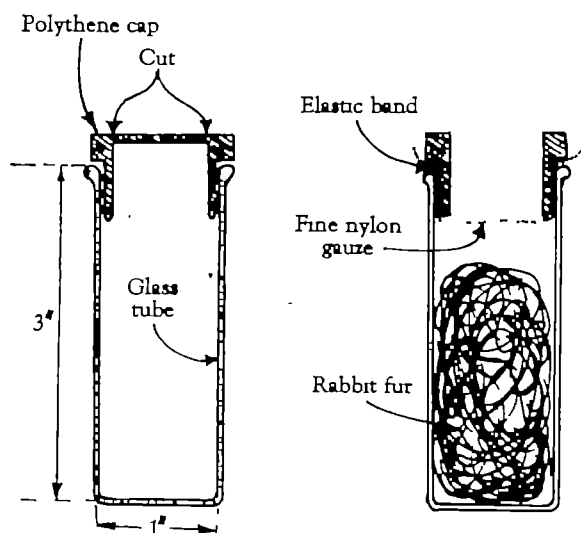


Fig. 1

Two experiments on the survival of fleas underground were carried out. Fleas were fed on a rabbit with advanced myxomatosis, and immediately after feeding were put underground. In the first experiment 9 of the special tubes, each containing 10 fleas, were put underground. As a control, 9 completely stoppered tubes without fur were put in the refrigerator at 4° C, as it has been reported (Allan<sup>1</sup>; Rothschild, personal communication) that fleas may be preserved alive for many months in this way.

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Table 1. SURVIVAL OF FLEAS UNDERGROUND AND AT 4° C

Site of storage	Proportion of fleas still alive when removed after days indicated						
	0	7	14	28	42	56	74
Refrigerator (4° C)	100%	100	90	20	0	0	0
	10/10	10/10	8/10	2/10	0/10	0/10	0/40
Underground	100%	60	100	40	0	0	5
	10/10	6/10	10/10	4/10	0/10	0/10	2/60

Table 1 shows the numbers of fleas surviving in tubes sampled at various times up to 74 days. It can be seen that underground storage preserves the fleas just as well as storage in the refrigerator.

In the second experiment (Table 2) 2 tubes, each containing 25 fleas, were put underground and removed at the end of 84 and 105 days, respectively. There were 5♂ and 2♀ survivors after 84 days and 2♂ and 2♀ survived for 105 days.

Table 2. SURVIVAL OF FLEAS UNDERGROUND

Days underground	No. of fleas examined	♂	No. of fleas alive	Total
84	25	5	2	7
105	25	2	2	4

Having shown that some fleas could survive at least 105 days without feeding, we wished to see whether myxoma virus could also survive. As a preliminary experiment to obtain information on this point, the fleas which had not been put underground or in the refrigerator (that is, the 'time 0' sample of the first experiment) were taken, together with the fleas which had been underground for 74 days. These pools were washed, with the aid of ultrasonics, in tissue culture medium '199' containing 5 per cent calf serum. The fluids were inoculated in 0.1 ml. quantities into marked sites on the shaved back of a healthy rabbit. After about 3 days all the sites showed a specific reaction indicating that virus was associated with some of the fleas. The next experiments were to determine how much virus was to be found on a flea after it had fed on an infected rabbit. A group of 5 fleas that had been allowed to feed on a lesion of a myxomatous rabbit was washed twice in 2.5 ml. tissue culture medium '199' with the aid of ultrasonics. We thought that virus released in this manner represented virus that was on the outside of the flea. The washed fleas were then ground in a pestle and mortar with 1 ml. of medium '199'. The aim in this latter manoeuvre was to try to release virus inside the fleas. The 3 fluids obtained by this technique were titrated using doubling dilutions inoculated in multiple sites on the shaved back of a rabbit. (This is the chessboard technique described by Fenner and Marshall<sup>4</sup>.) Table 3 shows the results obtained.

Table 3. AMOUNT OF VIRUS PRESENT ON FLEAS

Material	Total virus from 5 fleas RID 50*	Average amount of virus/flea RID 50
1st wash with U/S†	80	16
2nd wash with U/S	160	32
Ground-up fleas	1,180	236

\* Rabbit infection dose.

† Ultrasonics.



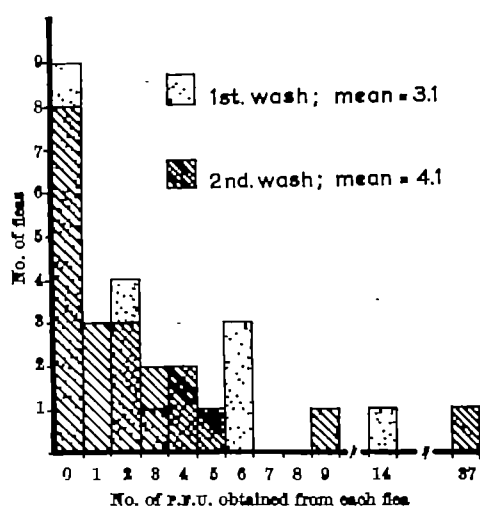


Fig. 2

We concluded that virus can be removed from fleas by washing and that there is a substantial fraction which can only be released by grinding. The next step was to see whether virus could be detected on all fleas removed from an infected rabbit. Two washings were given to each of 16 fleas, using 0.5 ml. quantities of '199' for each wash. To titrate these samples embryonated hens' eggs were chosen which, although not as sensitive as rabbit skin, were much easier to handle and permitted larger numbers of titrations to be done in a given time. Fig. 2 shows the distribution of numbers of pocks obtained from individual fleas. It can be seen that something like 43 per cent of the fleas examined did not have virus which could be detected by our washing followed by titration in egg technique.

It had now been established that virus was present on infective fleas even after 74 days, and there was some indication of how much virus was associated with an individual flea immediately after contamination. The

next step was to see whether virus, on fleas which had survived long periods of starvation, would be transmitted to healthy rabbits by the fleas biting these hosts. In the second survival experiment (Table 2) it was found that some fleas survived for 84 and 106 days. These survivors were put into glass tubes, and by inversion of the tube on to marked sites on the shaved back of a rabbit it was possible to see whether they fed.

Table 4 shows what happened.

Table 4. NUMBER OF FLEAS INFECTIVE AFTER STORAGE UNDERGROUND

Days	Total	No. of live fleas		Infected a rabbit
		Did not feed	Fed	
84	7	4	3	2
106	4	2	2	1

The significant finding in this experiment was that virus can be preserved on, and transmitted by, such surviving fleas.

The indirect evidence of previous workers on the vectors of myxomatosis is that no multiplication of the virus takes place in the vector. This is certainly true in the mosquitoes transmitting myxomatosis in Australia<sup>1</sup>. Andrewes *et al.*<sup>2</sup> in Britain could not detect multiplication of virus in the mosquito *Anopheles maculipennis atroparvus*, and Muirhead-Thomson (unpublished) found no evidence of multiplication of virus in rabbit or squirrel fleas. We are therefore left to conclude that the quite small amount of virus found on the rabbit flea is in a very stable form, probably protected by extraneous protein derived from the myxomatous lesion.

From our experiments we conclude that fleas can survive for very long periods without feeding and that the virus associated with the fleas can be removed by washing them with the aid of ultrasonics or by grinding them up. Virus acquired by feeding on infected rabbits can also survive on the fleas so that they are infective for healthy rabbits.

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## INTERACTIONS IN THE SYSTEM GALENA – POTASSIUM ETHYL XANTHATE – OXYGEN

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PROMPTED by the commercial importance of the flotation process in the recovery of sulphide minerals, numerous investigations have been carried out on the interaction between these minerals and the reagents used, particularly xanthates. Plaksin *et al.*<sup>1</sup> have developed the idea that oxygen plays an important part in the reaction, and this is clearly and strongly supported by the infra-red spectrophotometric studies of Leja and Poling<sup>2,3</sup>. However, the part which oxygen plays is by no means fully understood. Knowledge of the subject at the present time is entirely qualitative, and, indeed, even the fact of the essential nature of the action of oxygen is not universally accepted. Recent publications<sup>4,5</sup> suggest that some adsorption of xanthate takes place on galena even when elaborate precautions are taken to reduce the oxygen content of the system.

We have initiated an intensive programme of research work, aimed at resolving the conflict and providing a quantitative description of the interaction between xanthate, oxygen, and sulphide minerals. The present report describes the results of a preliminary investigation using galena and potassium ethyl xanthate.

The flowing reaction apparatus used was constructed entirely from glass and 'Teflon', and enabled deoxygenated solutions of different reagents to be passed successively through a bed of galena without being exposed to contamination from the atmosphere. The abstraction of xanthate from the solution during its passage through the bed was determined by a spectrophotometric analysis of the effluent. The galena used was prepared from pure lumps of natural galena, and had a specific surface of approximately 350 cm<sup>2</sup>/g. Xanthate solutions were used at

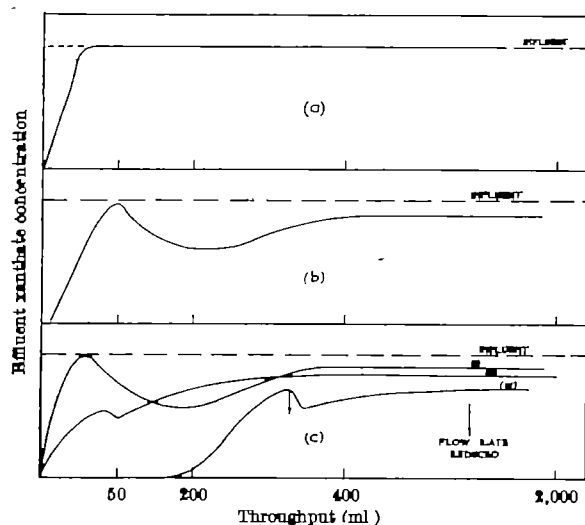


Fig. 1. The effect of additions of oxygen on the xanthate concentration in the effluent solutions from a bed of galena. *a*, No oxygen added; *b*, 0.6 p.p.m. oxygen added to xanthate solution only; *c*, oxygen added to both rinse and xanthate solutions in concentrations of (i) 0.8 p.p.m., (ii) 6.4 p.p.m.

a concentration of  $3.2 \times 10^{-4}$  M and pH 8, and were prepared from repeatedly recrystallized potassium ethyl xanthate.

From the outset, results that differed markedly from those of previous investigators<sup>4,5</sup> were obtained. Those investigators had reported an initially low xanthate concentration in the effluent solution, which increased with throughput until it became equal to the influent concentration; and they had interpreted this as indicating an adsorption of xanthate by the mineral, at first rapid, but then slowing, until finally it ceased. We found that the effluent concentration remained significantly different from that of the head solution for as long as the flow of solution was continued (up to several days), though the cumulative loss of xanthate was equivalent to many hypothetical adsorption monolayers. Furthermore, the effluent concentration was markedly sensitive to the flow-rate of the solution, which suggested that the difference might be due to our use of low flow-rates (0.3–1.5 ml./min).

We learned from Dr. O. Mellgren<sup>6</sup> that considerably higher flow-rates (100–200 ml./min) had in fact been used during his work. This difference in the flow-rates is equivalent to a two-hundred-fold difference in the residence time and hence in the sensitivity of detection of reaction. When our procedure was modified so as to bring the residence time more in line with that obtaining in Mellgren's work, we were able to reproduce quite closely the behaviour that he reported.

We proceeded to examine in greater detail the unexpected phenomenon of the continuing abstraction of xanthate from solution by galena. The introduction of an upward-flow column with its more mobile bed did not eliminate the effect; thus it disposed of the possibility that it might be associated with a mechanical occlusion of part of the mineral surface. Similarly, little improvement resulted when the mineral was pretreated with dithionite, and chemical deoxidants (sodium hypophosphite, sodium phosphite) were dissolved in the rinse and xanthate solutions. The behaviour of the system was significantly changed only when the following procedure for the exclusion of oxygen was adopted. The mineral sample was pretreated with a sodium sulphide solution at a pH between 8 and 9 and then rinsed with water that had been deoxygenated by the passage of nitrogen for several hours. The xanthate solution used had also been thoroughly deoxygenated in the same way. This expedient was effective in reducing the long-term interaction to a rate well below the minimum detectable in the apparatus, namely,  $10^{-6}$  mole  $\text{cm}^{-2} \text{sec}^{-1}$ , and the total detectable long-term abstraction of xanthate to the order of 1 per cent of a hypothetical absorbed monolayer, a level which is also of the same order as the experimental limit. The observations show that no interaction takes place between galena and xanthate solutions in the absence of oxygen. This conclusion is in complete agreement with the findings of Leja and Poling<sup>7,8</sup>.

With the establishment of an effective technique of deoxygenating the system, we were in a position to begin investigating the effect of controlled additions of oxygen. In some experiments these were made to both the rinse and xanthate solutions, but in others, to the xanthate solution alone. The major features of the behaviour observed are illustrated in Fig. 1. In all instances, the

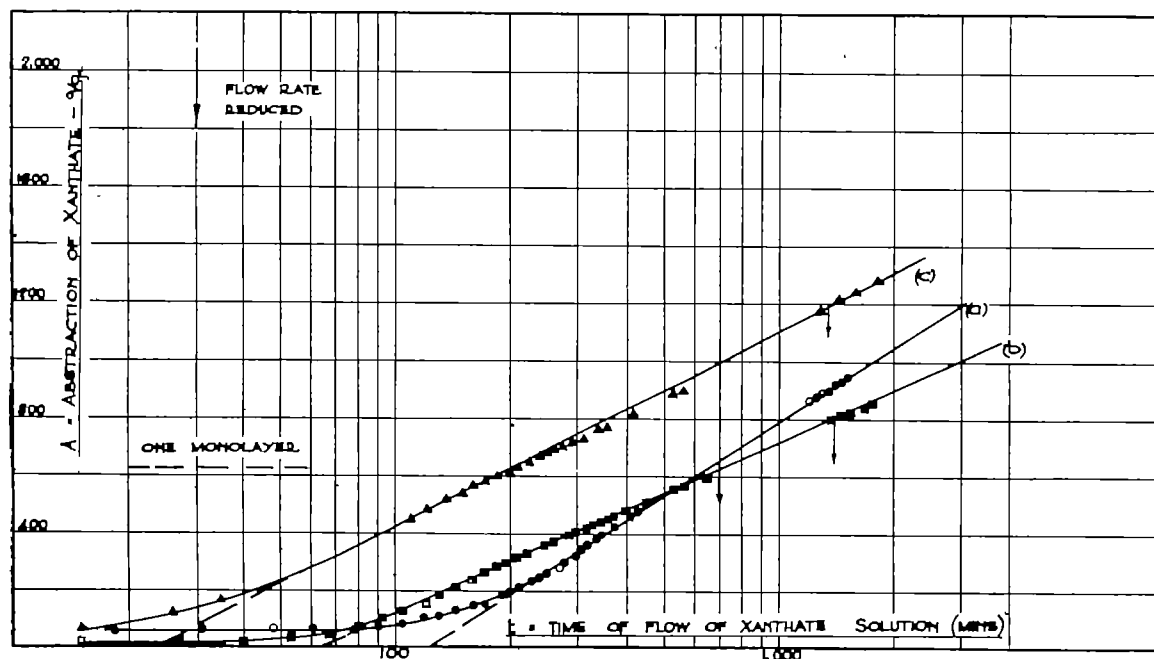


Fig. 2. Relationship between the quantity of xanthate abstracted from solution,  $A$ , and the time of contact with galena,  $t$ . *a*, 0.6 p.p.m. oxygen in xanthate solution only; *b*, 0.8 p.p.m. oxygen in both rinse and xanthate solutions; *c*, 6.4 p.p.m. oxygen in both rinse and xanthate solutions.

system eventually settled down to a continuing abstraction of xanthate from solution at a finite rate, which decreased slowly with time but did not become constant within the period of the experiment. The behaviour in the initial stages of the experiments depended on the oxygenation procedure. When the addition of oxygen was restricted to the xanthate solution, no strong interaction occurred until the mineral and the solution had been in contact for some time (compare Figs. 1a and b). On the other hand, when both the rinse and xanthate solutions contained oxygen, the abstraction of xanthate began immediately the solution was placed in contact with the mineral (Fig. 1c). However, as the elution curves show a distinct maximum in the initial stages, it appears that an induction period is still required before the reaction attains its full rate.

Fig. 2 shows typical graphs obtained by plotting  $A$ , the total quantity abstracted from solution, against the logarithm of  $t$ , the time for which the solution has been in contact with the mineral. After an initial period, during which the slope of the plot of  $A$  versus  $\log t$  increases steadily, the system obeys an equation of the form:

$$A = D \ln t + C \quad (1)$$

where  $C$  and  $D$  are constants. The abstraction value equivalent to a hypothetical adsorbed monolayer is indicated in the figure, as are the times at which changes were made in the solution flow-rates. No change in the behaviour of the system is apparent at these points. The curves are influenced by oxygen concentration; but the relationships are not simple, and it is thought that a more precise control of the oxygen concentration will be necessary before they can be quantitatively defined.

Equation (1) is of the same form as the direct logarithmic equation<sup>7</sup> that has been used to describe the rate of growth

of oxide films in the low-temperature corrosion of metals. It may be reduced to:

$$dA/dt = D \exp(C-A)/D \quad (2)$$

which is of the same form as the Elovich equation<sup>8</sup>, which was developed to describe the chemisorption behaviour of gases on semiconductors. The similarity between the reaction kinetics of metallic and semiconductor surfaces with gaseous reagents and with dissolved reagents is of considerable interest. It suggests that there is a common rate-limiting mechanism that is dependent on the properties of the surface. Both the empirical equations referred to have been justified theoretically<sup>9,10</sup> by assuming a surface-energy barrier in which the rate-limiting step is the transfer of an electron between the adsorbant and the adsorbate.

Continuing research on this topic is aimed at confirming these indications on the mechanism of the interaction and quantitatively determining the effect of oxygen.

This work was performed during the tenure by one of us (N. P. F.) of an appointment as visiting scientist at the Massachusetts Institute of Technology, sponsored by the Government Metallurgical Laboratory, Johannesburg.

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## A COMPUTER PROGRAMME FOR PLOTTING A CRYSTALLOGRAPHIC FOURIER SYNTHESIS

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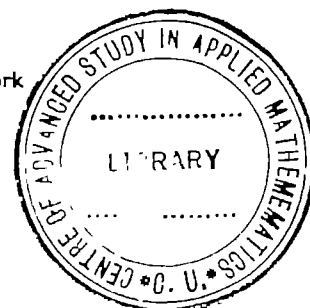
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A PROGRAMME known as plot crystallographic (which will be designated as PC) has been written for the purpose of plotting the results of a Patterson section or a two-dimensional summation, three-dimensional section or a two-dimensional crystallographic Fourier synthesis on the IBM 7090 Data Processing System and the X-Y plotter of the Electronic Associates, Inc., analogue computer. In addition, the positions of the maxima are determined by a parabolic interpolation by the method of Booth<sup>1</sup>.

In view of the availability of computing time and of efficient programmes, it is no longer necessary to do Fourier summations manually. It would also seem advantageous to circumvent the tedious plotting operation by utilising programmes such as PC. There are many similar programmes where the results can be observed on the screen of an oscilloscope, but it is convenient to have the results printed directly on a chart with both the contours

and maxima clearly marked. In addition, PC is suitable for non-orthogonal projections.

This programme has been written in Fortran for the IBM 7090. It can be combined with a programme for carrying out a Fourier synthesis. The results of the Fourier summation are read into the memory directly from the tape. The PC programme examines the array positions in the memory and locates a set of contours of equal electron density and positions of the maxima and plots them on a chart.

The chart may be divided into several parts by a sub-routine; as many as eight Fourier summations can be plotted on one surface. After the chart has been divided, the X-Y plotter is instructed to draw the axes of the unit cell by plotting  $x = 0$ ,  $y = 0$ , if the unit cell is orthogonal. Any other line can be drawn just as easily. The plotter places points along lines representing the axes. These points are separated by a space representing

1/60 of the unit cell edge (or whatever fraction is chosen). The spacing between the points is proportional to the length of the axes, so each co-ordinate of the unit cell is represented by a specific position on the chart.

The computer is directed to plot specific contours. Adjacent positions in the memory are examined for any included contour line. If such a line is found, a linear interpolation is carried out in order to determine its exact location.

If the axes are not orthogonal, the array of contours must be transformed into its natural configuration. If the projection of the two-dimensional Fourier summation is on to the  $x$ - $y$  plane and the angle between  $a$  and  $b$  axes of the unit cell is  $\gamma$ , the new co-ordinates can be defined in terms of the old by means of the relationships:

$$\begin{aligned} x' &= y \cos \gamma + x \\ y' &= y \sin \gamma \end{aligned} \quad (1)$$

The co-ordinates of each contour point are translated into meaningful information for the analogue X-Y plotter. This information is recorded on tape by a sub-routine. Each row of the array is examined in this manner, and the entire process is carried out again on the columns.

The calculated array is then re-examined to find the maximum value of electron density within the contours. The rows are scanned until a specified contour line is encountered. The positions within the contour line are examined. The array position of maximum electron density is determined using a modified version of Booth's method of parabolic interpolation.

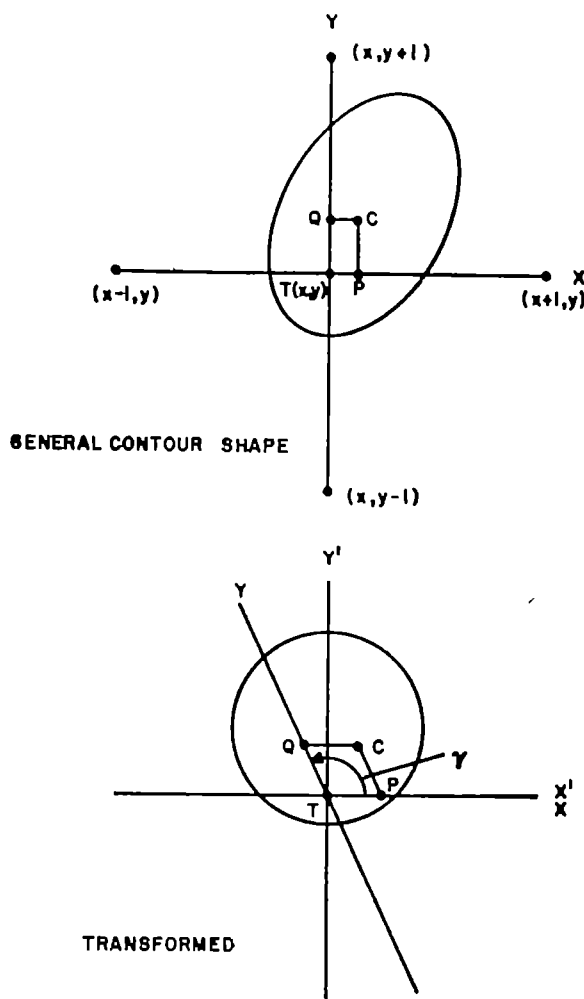


Fig. 1. The general case of a contour and the position of maximum electron density within it

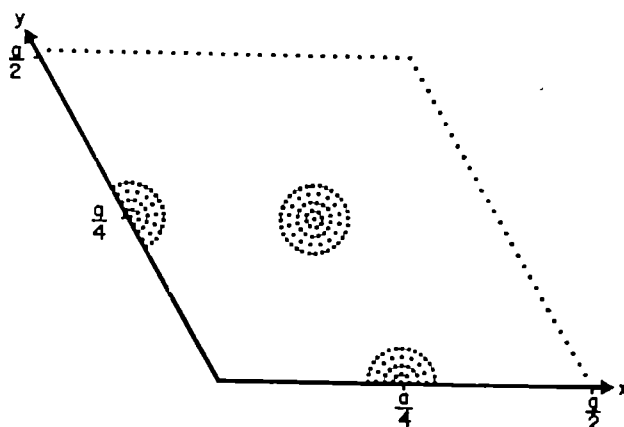


Fig. 2. Electron density of  $\text{NaNO}_3$ , showing the shape and position of the maximum representing the oxygen atoms

Fig. 1 shows the general case of a contour and the position of maximum electron density within it. When the projection is on to a non-orthogonal plane, Fig. 1 (top) is transformed into the figure shown in Fig. 1 (bottom).

The point  $T(x,y)$  is the array position on the tape having the maximum electron density within the contour.  $P$  and  $Q$  are the positions of maximum density along the lines  $x'$  and  $y'$ , respectively. The co-ordinates of  $C$  relative to  $T(x,y)$  can be found by means of equations (2) and (3). Utilizing Fig. 1 (top) and a parabolic interpolation, it can be shown:

$$P = \frac{\rho(x-1,y) - \rho(x+1,y)}{2[\rho(x-1,y) + \rho(x+1,y) - 2\rho(x,y)]} \quad (2)$$

where  $\rho(x,y)$  is the magnitude of the electron density at  $(x,y)$ .  $Q$  can be determined in a similar manner. The position of  $C(x',y')$  can be determined from equation (3):

$$\begin{aligned} C(x) &= P(x) \\ C(y) &= Q(y) \end{aligned} \quad (3)$$

When the unit cell is not orthogonal (Fig. 1, bottom), the position of the maximum must be transformed according to equation (1).

The co-ordinates of the maximum can now be determined with respect to the origin.

$$X'_{\text{max}} = C(x') + T(x') \quad (4)$$

$$Y'_{\text{max}} = C(y') + T(y') \quad (5)$$

The calculated values of  $X'_{\text{max}}$  and  $Y'_{\text{max}}$  are stored on tape to be plotted with the contours. All the elements within the original contour line are set equal to zero so that this maximum will not be encountered again. Scanning by rows is continued until the entire array has been examined.

The result of such a plot is shown in Fig. 2. Each point represents a specifically chosen electron density which is included between two array positions in the memory. Their locations have been determined, stored on tape and plotted. The outermost contour represents  $5 \text{ e}\text{\AA}^{-3}$ . Each inner contour increases by 5 units until the maximum of  $25 \text{ e}\text{\AA}^{-3}$  is reached. Fig. 2 is an illustration of an electron density difference map showing only the oxygen atoms in  $\text{NaNO}_3$ . This section is parallel to the "ac" plane of the hexagonal unit cell. The maxima represent the distribution of the oxygen atoms about a six-fold axis. Hence, the maxima are all of the same magnitude.

The programme is described in more detail in ref. 2.

<sup>1</sup> Booth, A. D., *Fourier Techniques in X-ray Organic Structure Analysis*, 62-65 (Camb. Univ. Press, 1948).

<sup>2</sup> Chorn, P., Madigan, R., and Martin, T., *Conf. Computing*, Yorktown, 1963. IBM No. 62-625-486 (1962).

# LETTERS TO THE EDITOR

## ASTRONOMY

### Barred Spirals and Formation of Spiral Arms by Galaxies

So far the formation of spiral arms, especially regular arms, has not been satisfactorily explained. The investigation of this phenomenon can be facilitated by an analysis of similar, but more peculiar, objects—barred spirals. A characteristic barred galaxy has a straight bar, which at the ends transforms abruptly into spiral arms. The population of bars and arms belongs to type I. For the bar to rotate and conserve its straight-line form it is necessary that the gravitational field within the limits of the bar corresponds to solid body rotation, as, for example, inside a homogeneous sphere. Beyond the ends of the bar rotation is differential, that is, the density should decrease sharply with distance. The density distribution is not determined by the bar but by the old stars of low luminosity. Consequently the old stars in an SB should form either a homogeneous sphere or a disk with a very small concentration toward the centre of the galaxy and their density should suddenly decrease at some distance.

Ordinary spirals and SB were formed during the condensation of gas clouds. For some reason in SB the gas, which had not as yet transformed into stars, was removed to some distance. A sharp boundary can be produced only by a shock wave. It is assumed that powerful explosions, similar to that observed in *M* 82, occurred very frequently at an early stage of evolution of galaxies. Such an explosion sweeps the gas from the central region and concentrates it into a thin spherical shell, the motion of which is retarded by gravitation. If the energy of the explosion exceeds  $10^{47}$ – $10^{48}$  ergs, a shell with  $M \approx 3 \cdot 10^{10} M_{\odot}$  and  $R \approx 10$  kiloparsecs can be formed. If the gas of a galaxy had time to form a flat disk before the explosion, the latter throws out some of the mass along the minor axis. However, its influence on mass distribution in the disk should not be of great importance. Therefore it is assumed that the explosion occurred in a spherical protogalaxy, that is, at the stage of formation of stars of the spherical sub-system. A thin gas shell ( $\Delta R \approx 0.1 R$ ) with  $R = 10$  kiloparsecs can exist for about  $5 \times 10^7$  years. In this time the temperature of the gas should decrease to  $T \leq 5000^{\circ}$ , which is sufficient for fragmentation as the density in the shell is about  $3 \times 10^{-24}$  g cm $^{-3}$ . Clusters and stars with a relatively low angular momentum and high energy are formed. They move along elongated trajectories, and their spatial density is approximately uniform, increasing toward the centre and periphery of the sphere. The concentration toward the centre depends on the angular momentum distribution. The density distribution at the distribution of momentum observed in our Galaxy is given in Fig. 1. The angular velocity  $\omega(r)$  differs only slightly from  $\omega_0$ —the angular velocity at the edge of the sphere.

A constant  $\omega$  is necessary but not sufficient for the straight-line form of the bar. First of all,  $\omega$  is not exactly a constant; secondly, the break at the ends of the bar should be smoothed rapidly by the tension of the lines of force of the spiral arms. Therefore, it is necessary to assume that there are clusters of old stars with  $M \geq 10^6 M_{\odot}$  at the ends of the bar. Inside each cluster there is a gas cloud with  $M \geq 10^6 M_{\odot}$ . At such values the

magnetic forces cannot drag the gas out of the clusters and the deceleration of clusters during  $10^{10}$  years is not very essential. The tension of the magnetic lines between the clusters and the centre straightens the bar and forces it to rotate with a constant angular velocity  $\omega_0$ . The difference  $\omega - \omega_0$  accelerates the gas to the ends of the bar with a velocity of 20–30 km/sec and in the central region moves it toward the centre. Motion to the edge is observed spectroscopically and motion toward the centre explains the presence of gas and hot stars which are usually absent in the centres of ordinary spiral galaxies.

The formation of a bar can be assumed as follows. The gas of the shell, which did not develop into stars, loses energy and forms a disk with circular orbits. The fraction of gas with a low angular momentum concentrates toward the centre together with the tube of magnetic lines frozen in the gas (Fig. 2). The magnetic forces in such an over-tight tube tend to compress the neighbouring parts of the tube. These forces are small in comparison with the gravitational forces and the pressure gradient. However, they serve as small perturbations which develop due to gravitational instability. A condensation is formed at each side of the central thickening and the process is repeated, the magnetic forces stimulating the condensation of gas along the magnetic tube. A magneto-gravitational wave propagates along the magnetic field. Its propagation is facilitated by the general instability of the gas disk. Deviation from axial symmetry leads to the condensation of gas at several radii. The process of condensation of the tube occurs simultaneously with the

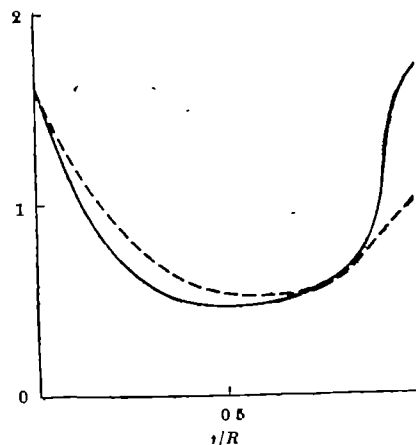


Fig. 1 —  $\omega(r)/\omega_0$ ; ---  $\omega^2(r)/\omega_0^2$

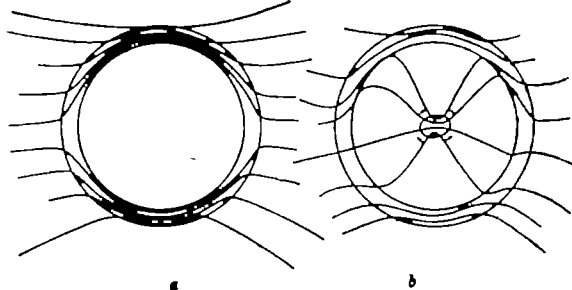


Fig. 2

flattening of the disk. When the wave reaches the remnants of the shell, clusters are formed at the ends of the bar and later spiral arms. The mechanism of formation of ordinary spirals is also connected with the central condensation from which the magneto-gravitational wave originates. Condensation ceases when the magnetic forces, which increase more rapidly than other forces, balance gravitation. The connexion between the central condensation and spiral arms explains the fact that irregular galaxies, which have no central condensation, do not have spiral arms.

SB galaxies often have a ring, the diameter of which is a bar. Besides this ring there are spiral arms extending from the ends of the bar and tangential to the ring. This ring is probably a remnant of the shell, which condensed to the galactic plane. The fraction of gas with a comparatively large angular momentum could, during contraction in the shell and subsequent condensation to a plane, become a gravitationally stable ring, similar to a spiral arm.

The magnetic lines of force extending from the semi-rings and bars are prolonged in the spiral arms. At the places of exit they are compressed by the gas clouds of the clusters (Fig. 3). The splitting of spiral arms into several looks, often observed in ordinary galaxies, is naturally explained: gravitational condensation, which was induced by a single magneto-gravitational wave, could have resulted in the formation of several separate masses, from which separate magneto-gravitational waves originated.

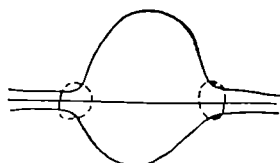


Fig. 3

The relatively high percentage of SB and the short duration of the contraction phase of the spherical subsystem of a galaxy show that the occurrence of powerful explosions was very probable at this phase. Consequently the development of superstars depends on the amount of gas with a low momentum in a galaxy, that is, it is apparently connected with the condensation of massive gas clouds.

The magneto-gravitational wave can explain the bridges and tails of interacting galaxies. The condensation of a galaxy in a large gas cloud facilitates the condensation of the gas along the lines of force of the metagalactic field. When the wave reaches a denser part of a cloud, a second galaxy is formed. The wave propagates further if gas in a state close to gravitational instability is present.

So far the spiral arms have been considered as tubes of parallel magnetic lines. However, if the angle between the magnetic field and the axis of rotation is acute, spherical contraction and subsequent flattening of the gas lead

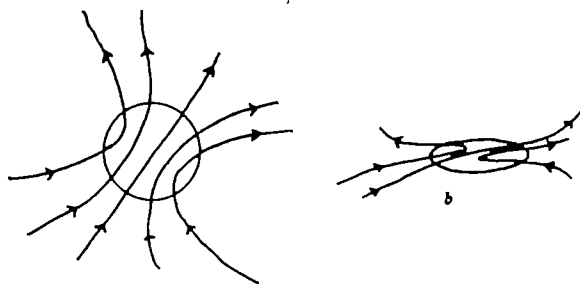


Fig. 4

to a field given in Fig. 4. This explains a recently detected fact: the rotation of the polarization plane of radio sources shows that in the solar vicinity at both sides of the galactic plane the field is of opposite sign. In accordance with Fig. 4 the rotation measured in these two semi-spheres is not equal. In the vicinity of the neutral line the field becomes annihilated and magnetic pressure does not support the gas. Probably here interstellar clouds and associations are formed.

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## ASTROPHYSICS

### Globular Clusters as Remnants of Quasars

In the past two years many theories have been advanced for the explanation of the extraordinarily powerfully emitting cosmic energy sources known as quasistellar radio-sources, or quasars for short. Two particular theories have been brought out in an especially detailed form, namely, the gravitational collapse theory of Hoyle and Fowler<sup>1</sup> and the spherical galaxy formation theory of Field<sup>2</sup>.

It is the purpose of this communication to direct attention to a class of objects, the globular clusters, that may provide tangible evidence for quasar events in the past history of our Galaxy. If this connexion is proved to be true, astronomers will have a unique opportunity of investigating the stellar remnants of actual quasar cores.

Here is outlined qualitatively a model for the formation of globular clusters which draws on some conceptual elements and results from the foregoing two theories, and at the same time some of the consequences of such a model for the explanation of quasar characteristics are discussed.

We identify the supermassive objects of Hoyle and Fowler ( $M/M_\odot = 10^4$ – $10^6$ ) with proto globular clusters, that is, nascent globular clusters. According to the best estimates at present<sup>3</sup> the masses of globular clusters range from roughly  $10^5$  to  $10^7$  solar masses, and it is reasonable to assume that those of the proto globulars are somewhat larger. Representative globular cluster sizes are of the order of 10 parsecs, which incidentally is the estimated lower limit to the size of the HII region of the core of the quasar 3C48, investigated by Greenstein and Schmidt<sup>4</sup>.

I propose that the evolution of the proto globular cluster proceeds along the lines of Hoyle and Fowler's supermassive objects up to the onset of nuclear burning. However, the time for free-fall collapse is so short ( $7.5 \times 10^4$  years for  $M = 10^4 M_\odot$  and 25 years for  $M = 10^6 M_\odot$ , (ref. 5)) and the central temperatures in the later phases of contraction become so high ( $\sim 10^8$  °K) that we are led to postulate the occurrence of the Alpher, Bethe, Gamow non-equilibrium theory of element formation ( $(\alpha, \beta, \gamma)$ -process)<sup>6</sup>. This implies that significant element formation beyond helium does not take place in the proto globular cluster, if the initial material is pure hydrogen.

We then adopt the results of Michel<sup>7</sup> regarding the collapse of the massive star after the exhaustion of nuclear energy. In this theory, the central core ( $M_c = 0.3 M$ ) will collapse much more rapidly than the outer envelope and this results in a separation of the two parts. The suggestion of Hoyle and Fowler is followed that a significant amount of energy is transferred from the core to the envelope, which leads to a rapid expansion of the envelope away from the imploding core. This expansion phase is analogous to the situation envisaged by Field in his 'rebound'-phase. However, Field assumes star-formation to take place before the 'rebound', but we prefer to place

this event after the 'rebound' and to assume that the ( $\alpha$ ,  $\beta$ ,  $\gamma$ )-process of element formation takes place before maximum compression of the envelope. Incidentally the density,  $10^{-10}$  g/cm<sup>3</sup>, for which Field finds a maximum rate of star formation, is equivalent to the smeared-out density of a globular cluster with radius 10 parsecs and a mass of  $10^7$  solar masses.

The reason for my choice of sequence of events rests on the observed element characteristics of the halo globular clusters with their well-documented metal deficiencies. In particular the results of O'Dell, Peimbert and Kinman<sup>8</sup> show that in the halo globular cluster M15 the helium to hydrogen ratio is  $0.18 \pm 0.03$ , while the ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) non-equilibrium value is 0.15. The inference of the operation of the ( $\alpha$ ,  $\beta$ ,  $\gamma$ )-process in proto globular clusters is further strengthened by the fact that the same authors find M15 strongly deficient in oxygen (1/81 of the solar value). Accordingly, I propose that the ( $\alpha$ ,  $\beta$ ,  $\gamma$ )-burning phase of a proto globular cluster represents a quasar or a quasar element.

A common origin in time for the halo globular clusters in our Galaxy is suggestive in viewing their general metal deficiency and comparable old age. Eggen, Lynden-Bell and Sandage<sup>9</sup> from an analysis of the space motions of 221 dwarf stars infer that some  $10^{10}$  years ago a collapse of a spherical gas mass took place rapidly in a time of about  $10^6$  years. This can be identified as the major quasar event in the history of our Galaxy. Thus I propose that the most energetic quasar events represent a stage of multitudinous globular cluster formation very early in the life-time of a galaxy. The distribution of globular clusters in our own Galaxy and nearby ones like M87, NGC4636, NGC4278, etc., indicates an initially spherical mass distribution which is a major requirement for identification with the spherical starlike photographic images of quasars. I postulate that this spherical mass during its collapse will fragment into proto globular clusters, possibly according to Hoyle's hierarchical fragmentation theory<sup>10</sup>.

The energy source of the quasars then resides principally in the nuclear burning of hydrogen into helium, eventually with gravitational energy added by the final implosion of the quasar core. The mass conversion of 0.15  $M$  of hydrogen into helium in the ( $\alpha$ ,  $\beta$ ,  $\gamma$ )-process leads to an energy release of the order of  $10^{48}$  Mev. If subsequent nuclear burning did take place, this would only add  $3 \times 10^{-4}$  Mev to the energy release. With a mass for the proto globular cluster of  $10^7$ – $10^8$  solar masses this yields an energy release of some  $10^{48}$ – $10^{49}$  ergs. The estimated total energy output for a representative quasar is about  $10^{46}$  ergs/sec. Thus the nuclear fuel content in a proto globular cluster lasts some  $10^5$ – $10^6$  years, which is commensurable with estimated life-times for quasars<sup>11,12</sup>.

It appears that the gravitational energy source is not strictly required even for the explanation of the more energetic quasar events, since in our model we admit the co-existence of several supermassive objects. The number of globular clusters in a given galaxy varies widely from possibly none to about one thousand in the giant elliptical galaxy M87 (ref. 13). In our own Galaxy more than 100 globular clusters have been catalogued, but estimates run to a total of 500 (Allen *loc. cit.*, p. 265). This implies that the energy storage requirement of  $10^{46}$  ergs found by Maltby, Matthews and Moffet<sup>14</sup> for some radio sources can be met by the nuclear energy content available in the limit of  $10^2$  globular clusters of mass  $10^6 M_\odot$ . Such energetic sources should be relatively rare occurrences, restricted to giant galaxies like M87.

If a limited number of globular clusters are formed at approximately but not exactly the same time, the observed light variations in quasars over short periods of time may be readily explained (2–10 years for 3C2 and 3C273 respectively). These light variations would merely be a consequence of the implosion of another proto globular cluster with its onset of an ( $\alpha$ ,  $\beta$ ,  $\gamma$ )-process or the termina-

tion of one. Secondary light effects could reside in subsequent star formation and/or supernovae displays.

The globular clusters in our Galaxy show a spread in age from roughly  $10^8$  to  $10^{10}$  years accompanied by a variation in metal content<sup>15</sup>. This may indicate that after the initial massive quasar event involving the formation of a major number of globular clusters, subsequent milder quasar events, that is, the formations of individual or small groups of globular clusters, can occur. The repetitive quality of quasar events has been suggested on other grounds by, for example, Hoyle and Fowler<sup>16</sup> and also by Shklovsky<sup>17</sup>. As a consequence of our model the ( $\alpha$ ,  $\beta$ ,  $\gamma$ )-process will then operate on gradually enriched material, partly from heavy element creation in stellar interiors and partly from previous hydrogen conversion in earlier ( $\alpha$ ,  $\beta$ ,  $\gamma$ )-processings. The metal richer globular clusters should thus also be richer in helium and eventually become hydrogen-deficient. After ten repetitive ( $\alpha$ ,  $\beta$ ,  $\gamma$ )-processings the helium to hydrogen ratio becomes 0.81 if 15 per cent of the remaining hydrogen is always converted into helium and with no helium further processed.

The youngest globular clusters in our Galaxy are about  $10^8$  years old. If we take this as representative of the most recent state of quasar events in our own and neighbouring galaxies, we should have to go out to spatial distances of  $10^8$  light years in order at present to witness quasar events. As we travel further out in space and back in time, the frequency and possibly intensity of quasar events should increase, particularly at distances of about  $10^{10}$  light years away or events  $10^{10}$  years ago, when presumably the most massive quasar event took place in our own Galaxy. Possibly such a time-space-dependent frequency and intensity variation in quasar events could serve as a cosmological discriminant. At present it is inferred, from the red shifts of quasars, that they occur at great distances, invariably farther out than  $10^8$  light years. The nearest quasar, 3C273, is  $1.55 \times 10^8$  light years away according to estimates by Greenstein and Schmidt<sup>18</sup>.

Finally, I wish to reiterate the unique opportunity that our galactic globular clusters afford in the study of quasar remnants. If the present model or some modification thereof holds true, the centre of a globular cluster will contain an imploded quasar core, which represents a significant fraction of the original mass in 'hidden form'<sup>19</sup>. This should affect the stellar spatial and velocity distributions within the globular cluster.

Likewise I wish to emphasize the importance of studying the hydrogen to helium ratio through possible discoveries of planetary nebulae in globular clusters of differing age. The observed hydrogen to helium ratio will have a decisive bearing on whether or not the ( $\alpha$ ,  $\beta$ ,  $\gamma$ )-process has repeatedly taken place in the material of the proto globular clusters.

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## A Search for Low-energy Gamma-rays from the Quasar 3C286

In two experiments on the *Ranger* spacecraft, Metzger *et al.*<sup>1</sup> have observed a flux of low-energy  $\gamma$ -rays at great distances from the Earth. Previous experiments on high-altitude balloons<sup>2,3</sup> and on rockets<sup>4</sup> had looked for, but failed to detect, an isotropic flux of  $\gamma$ -rays incident on the top of the Earth's atmosphere. These experiments used counters with little or no directional sensitivity, and their failure was due to the difficulty in distinguishing between an isotropic primary flux from the background of secondary  $\gamma$ -rays produced by cosmic rays in the atmosphere and in the apparatus itself. However, even in the presence of this background, a counter with directional sensitivity can detect  $\gamma$ -rays from point sources by measuring increases in the total flux in particular directions in the sky. We report here the results of observations made at balloon altitude on the quasar 3C286.

The detector used in this experiment was a 2-in. diam.  $\times$  2-in. high sodium iodide scintillation crystal; it was surrounded by a collimator with an opening angle of  $\pm 5^\circ$ . The telescope was carried to an altitude of 100,000 ft. by balloon on October 28, 1964. During the flight the attitude of the telescope was controlled from the ground by radio-command. Pulse height spectra were recorded from the direction of 3C286 and from neighbouring directions; the spectra were taken frequently, and alternately from the source and from the background, to avoid systematic errors due to changes in the overall gain of the system. A caesium-137 source was carried on the telescope to provide a calibration during the flight. The energy resolution was  $\sim 0.5$  MeV.

The combined spectrum recorded from the direction of 3C286, with the background subtracted, is shown in Fig. 1. The spectrum is not significantly different from zero at any energy.

This result sets upper limits to the flux of  $\gamma$ -rays from several possible processes on 3C286. In each case, the 95 per cent confidence limit is given. No allowance has been made for a possible red-shift of 3C286; the results may need recalculating when the red-shift is eventually measured, but the changes in the limits will probably be small.

(1) *Electron-positron annihilation.* The limit to the flux of 0.51-MeV  $\gamma$ -rays is 0.04 photons/cm<sup>2</sup> sec at the top of the atmosphere.

(2) *Neutron capture in hydrogen.* The limit to the flux of 2.2-MeV  $\gamma$ -rays is 0.13 photons/cm<sup>2</sup> sec.

(3) *Interaction of high-energy electrons with optical photons through the inverse Compton effect.* Ginzburg *et al.*<sup>5</sup> have considered this process for the quasar 3C273, the distance of which is already known. They have

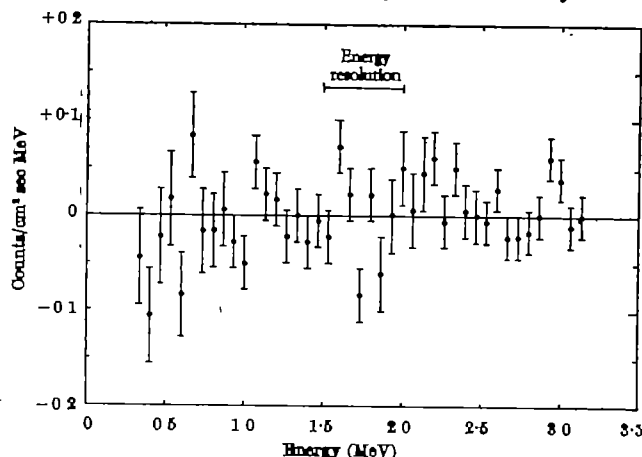


Fig. 1. Pulse height spectrum received from the direction of 3C286 with the background from neighbouring directions subtracted. Errors equal to one standard deviation are shown for each point.

shown that if the optical continuum is due to the synchrotron radiation of high-energy electrons, then the same electrons should produce low-energy  $\gamma$ -rays in collisions with optical photons. If the optical radiation has a spectrum of the form:

$$I(\nu)d\nu = I_0\nu^{-\alpha+1}d\nu$$

then the  $\gamma$ -ray spectrum has the form:

$$F(E)dE = F_0E^{-\alpha}dE$$

The optical spectrum from 3C286 is flat<sup>6</sup>, so the  $\gamma$ -ray spectrum should have  $\alpha = 1$ . The experiment described here sets a limit to  $F_0$  of  $5.5 \times 10^{-8}$  photons/cm<sup>2</sup> sec.

The results of Metzger *et al.* indicate a primary flux with a spectrum of the form:

$$N(E)dE = N_0E^{-1}dE$$

The flux at 1 MeV, integrated over all directions in space, is  $4 \times 10^{-1}$  photons/cm<sup>2</sup> sec MeV. If a similarly shaped spectrum is being emitted by 3C286 the present experiment shows that the limit to the flux at 1 MeV is  $3.6 \times 10^{-4}$  photons/cm<sup>2</sup> sec MeV.

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## GEOPHYSICS

### Continental Drift and the Rotation of Spain

SIR EDWARD BULLARD *et al.*<sup>1</sup> have used a 'least squares' criterion for the geometrical fit of the continents around the Atlantic Ocean. They find that the best fits are obtained when the 500-fathom submarine contours are used to define the continental margins. When all the continents are fitted, it is found on fitting Africa to eastern North America that Africa overlaps the position of southern Spain. To avoid this, Bullard *et al.* rotate Spain, closing up the Bay of Biscay as previously suggested for other reasons by du Toit<sup>2</sup> and Carey<sup>3</sup>. The appropriate part of the computer fit is shown in Fig. 1. It is clearly desirable to look for some independent evidence for the rotation of Spain.

Irving<sup>4</sup> has noted that there is some palaeomagnetic evidence for such a rotation. The argument is that the declinations of the directions of remanent magnetization for rocks of Permian and Triassic ages from Spain are different from those of rocks of the same ages from France, Germany, Britain, Norway and the U.S.S.R. The latter are all consistent, indicating that these regions all formed one unit. With the aid of some new results from the northern Pyrenees, this argument is followed up and the palaeo-declinations for rocks from the north and south of the Pyrenees are compared and then related to the bathymetry of the Bay of Biscay.

Measurements<sup>1</sup> of natural remanent magnetization have been made on some volcanic rocks of late Triassic and early Jurassic ages from southern France. These rocks outcrop in the northern foothills of the Pyrenees in a region extending for about 30 km near latitude  $43^{\circ}$  N., longitude  $1.5^{\circ}$  E. The Upper Triassic rocks are mainly quartz basalts and the lower Jurassic rocks include basalts, tholeiitic basalts and various tuffs. All dip steeply northerly. Eighty orientated samples were measured and unstable components of magnetization removed using alternating fields. After applications of geological corrections the Jurassic rocks have mean declination N.  $16.3^{\circ}$  E. (from means of 8 sites) and the Triassic rocks have mean declination N.  $29.2^{\circ}$  E. (from means of 7 sites).

It is of interest to look at these results in relation to the problem of the rotation of Spain. Fig. 2 shows all the palaeomagnetic results for rocks of Permian, Triassic, and Jurassic ages for Spain, France and the southern part of England which are available at the present time. Some interesting features are noted: first, the declinations (disregarding reversals) for Permian, Triassic and Lower Jurassic rocks from southern England and France are remarkably consistent; secondly, the consistency applies to  $T_1$  and  $J_1$  even though these are close to the Pyrenees; thirdly, the declinations for Permian and Triassic rocks from Spain are different from those from France and England.

In view of the consistency of the declinations, it seems reasonable to take the mean declination for rocks to the north of the Pyrenees and compare it with the mean declination for rocks from the south of the Pyrenees (Table 1). In doing this, one result,  $T_1$ , is omitted as it is considered suspect by the original author<sup>14</sup>. In Table 1, all the declinations are written in the northerly sense, that is, disregarding reversals. The mean declination for rocks from the north of the Pyrenees is found to be N.  $20^{\circ} \pm 7^{\circ}$  and the mean declination for rocks from the south of the Pyrenees is found to be N.  $34.2^{\circ} \pm 9^{\circ}$ . The difference is therefore  $38^{\circ} \pm 16^{\circ}$ .

Calculation of the difference from the vector means gives a value of  $40^{\circ}$ . The remanent vectors together with the vector means are shown on an equal-area projection in the inset to Fig. 2.

The difference may be explained by assuming a rotation of Spain, and the values are remarkably close to the rotation of  $39^{\circ}$  obtained from Carey's reconstruction. However, the closeness of these estimates seems fortuitous as measurements on the opening of the Bay of Biscay using the hydrographic charts suggest the rotation might be as small as  $30^{\circ}$  or as large as  $40^{\circ}$ . The computer fit of Bullard gives a rotation of  $32^{\circ}$ . All these estimates are within the experimental error of the palaeomagnetic results and it is considered that the available palaeomagnetic data lends considerable support to the rotation of Spain and the computer fit of the continents as proposed by Bullard.



Fig. 1. The fit of the continents of the Atlantic after Bullard *et al.*, 1965. Spain has to be rotated clockwise  $32^{\circ}$  to obtain the best fit

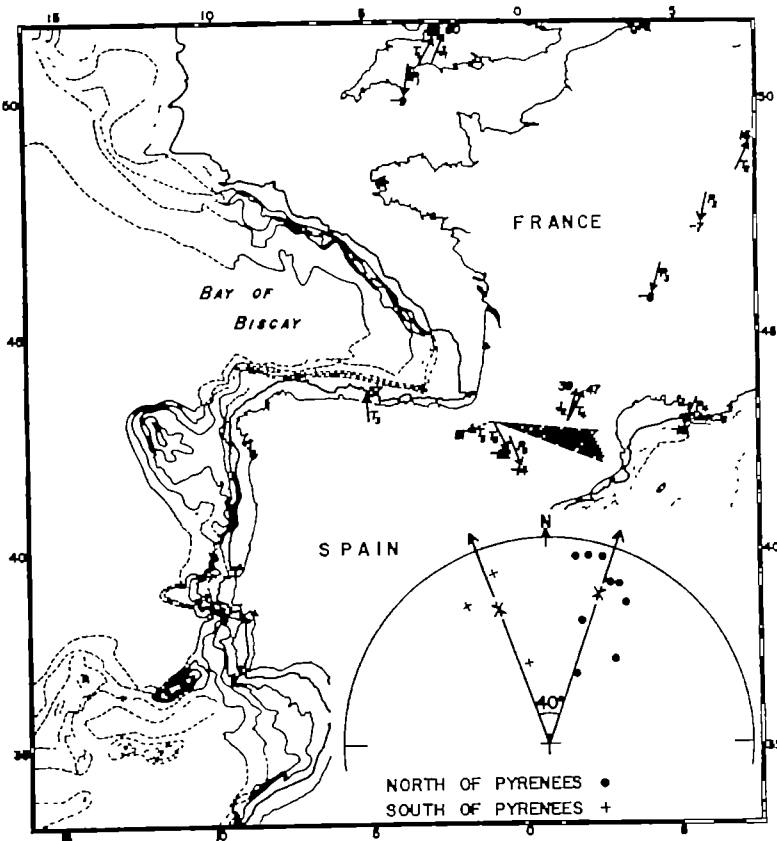


Fig. 2. Map of southern England, France and Spain showing the palaeomagnetic results for Permian, Triassic and Jurassic rocks with palaeo-inclinations. The bathymetry of the Bay of Biscay is taken from Hydrographic Office Chart No. 7650-6 which was kindly provided by the Hydrographer of the Navy. The submarine contours are 100, 500, 1,000, 1,500, 2,000 and 2,500 fathoms. The inset shows the remanent vectors plotted on a Schmidt equal area projection. The vector means are shown (x) and the difference in the mean declinations may be compared with the opening of the Bay of Biscay

Table 1

North of Pyrenees			South of Pyrenees		
No.	Decl.	Ref.	No.	Decl.	Ref.
$J_1$	23	6	$T_1$	353	8
$J_2$	16	5	$T_2$	330	15
$T_1$	30	4	$P_1$	343	16
$T_2$	25	5, 7, 8			
$T_3$	29	5	Mean	343 ± 9	
$P_1$	9	9, 10			
$P_2$	18	11			
$P_3$	17	11			
$P_4$	22	12, 13, 14			
Mean	20 ± 7		Difference	88 ± 16	

If Spain has rotated with respect to France, one might expect to see some effect in the topography of the ocean floor. One such effect may be seen along the continental edge of France at latitude 47° 5' N., longitude 12° W. Here the contours change direction and are at right angles to the general north-westerly direction of the continental edge. This is at the place where the north-west corner of Spain would be if rotated back to its original position.

Clearly, it would be desirable to have more palaeomagnetic results from Spain and to test the hypothesis further, but it should be remembered that there may be an alternative explanation of the palaeomagnetic results. Some geologists working in the Pyrenees find it difficult to reconcile the rotation of Spain with the structural geology of the Pyrenees mountains<sup>17</sup>. It could be that Spain, before continental drift, was not part of the European landmass and it would therefore be valuable to obtain and compare more palaeomagnetic results for Spain with those from North Africa when they become available. If Spain was at some time not part of Europe, this would mean that the agreement between the bathymetry and the presently available palaeomagnetic results from north and south of the Pyrenees is fortuitous, which seems a little unlikely. A further and perhaps better test for the rotation would be to compare some carefully located sea or air total intensity magnetic profiles for the west coast of France and the north coast of Spain. It is hoped to be able to do this in the near future.

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## PHYSICS

### Effective Atomic Numbers of Heterogeneous Materials

For a single element, the three  $\gamma$ -ray processes—photoelectric, Compton and pair production, can be expressed as a function of photon energy  $h\nu$  and the atomic number  $Z$  of the element. At a given photon

energy, the interaction is proportional to  $Z^n$  where  $n$  is between 4 and 5 for the photoelectric effect, 1 for the Compton effect, and 2 for pair production<sup>1,2</sup>. For the purposes of  $\gamma$ -ray attenuation, a heterogeneous material, consisting of a number of elements in varying proportions, can be described as a fictitious element having an effective atomic number  $Z_{eff}$ . In the study of the X-ray energy absorption of certain biological specimens Spiers<sup>3</sup> used the expression  $Z_{eff}^{2.34} = \sum \alpha_i Z_i^{2.34}$ , where  $Z_i$  is the atomic number of the  $i$ th element and  $\alpha_i$  its fractional electronic content. More recent measurements<sup>4</sup> favour a value of 3.1 instead of 2.94 for the exponent for  $Z_{eff}$ . Glasser<sup>5</sup>, in radiological studies, used the expression  $Z_{eff}^3 = \sum p_i Z_i^3 / \sum p_i Z_i$ , where  $p_i$  is the fractional part by weight of the whole mixture occupied by the element the atomic number of which is  $Z_i$ . Extending this idea of the effective atomic number of a material for specific radiation, Hine<sup>6</sup> pointed out that there is a different effective atomic number, for each absorption process in a heterogeneous material, but he could obtain expressions only for the photoelectric and pair production processes as  $Z_{eff}^{2.34} = \sum \alpha_i Z_i^{2.34}$ , and  $Z_{eff} = \sum \alpha_i Z_i$ . It can easily be shown that  $p_i$  and  $\alpha_i$  are very nearly equal<sup>7</sup>. The equations of Spiers and Hine may then be expressed as  $Z_{eff}^n = \sum p_i Z_i^n$ , where  $n$  has values of 4 to 5, 1 and 2, respectively for the photoelectric, Compton, and pair production processes. Hence, an expression for the effective atomic number for the Compton effect could not be obtained. In this communication the validity of these differing expressions is examined and a single effective atomic number is suggested for a heterogeneous material.

In 1 g of a heterogeneous material the number of atoms of the  $i$ th element is  $Np_i/A_i$ , where  $N$  is Avogadro's number,  $p_i$  the fraction by weight of the  $i$ th element in the material and  $A_i$  the atomic weight of the  $i$ th element. The total number of atoms in 1 g of material is  $N(\sum p_i/A_i)$ . The number of electrons of the  $i$ th element in 1 g of material is  $Np_i Z_i/A_i$ , and therefore the total number of electrons in 1 g of material is  $N(\sum p_i Z_i/A_i)$ . Then the effective atomic number and the effective atomic weight of a heterogeneous material are  $Z_{eff} = \sum (p_i Z_i/A_i) / \sum (p_i/A_i)$ , and  $A_{eff} = 1 / \sum (p_i/A_i)$  respectively.

The values of the effective atomic number for seven heterogeneous materials (alloys, compounds and mixtures) are shown in Table 1.

Table 1. EFFECTIVE ATOMIC NUMBERS OF HETEROGENEOUS MATERIALS

	$(\sum p_i Z_i^4 / \sum p_i Z_i)^{1/4}$	$(\sum p_i Z_i^1)^{1/1}$	$(\sum p_i Z_i^2 / \sum p_i Z_i)^{1/2}$	$\sum p_i Z_i$
Plain extruded leaded brass (copper, 80; zinc, 20)	29.21	29.20	29.19	29.20
Phosphor-bronze (copper, 89.75; tin, 10; phosphorus, 0.25)	34.36	32.61	30.10	31.06
Carbon steel (iron, 98.95; carbon, 1.05)	25.98	25.91	25.08	25.79
Monel metal (nickel, 60; copper, 40; iron, 0.5; manganese, 33)	27.22	27.15	27.03	27.07
Concrete (hydrogen, 0.56; oxygen, 49.56; magnesium, 0.24; sodium, 1.71; aluminium, 4.56; silicon, 31.35; sulphur, 0.12; potassium, 1.92; calcium, 8.26; iron, 1.23)	14.76	13.18	9.39	11.52
'Perspex' (carbon, 59.97; oxygen, 31.97; hydrogen, 8.06)	6.94	6.87	3.60	6.24
Sodium iodide (sodium, 15.84; iodine, 84.66)	53.13	50.27	32.01	46.56

The data in Table 1 show that for materials containing elements the atomic numbers of which do not differ greatly, all the expressions give similar values for the effective atomic number. For materials containing very light elements such as hydrogen (for example, 'Perspex') and for materials containing elements of very widely differing atomic numbers (for example, NaI), different values are obtained for the effective atomic number. It is observed that the effective atomic number for the photoelectric effect is always greater than for pair production, in agreement with Hine's suggestion. We conclude that for a heterogeneous material different effective atomic

numbers for different  $\gamma$ -ray processes may be required; they can be approximated to a single effective atomic number  $Z_{eff} = \epsilon(p_i Z_i / A_i) / (\epsilon(p_i / A_i))$ . Since the Compton effect is the predominant process at intermediate energies, this single effective atomic number could be considered to represent the effective atomic number for the Compton effect. The expressions  $Z_{eff} = \epsilon(p_i Z_i / A_i) / (\epsilon(p_i / A_i))$  and  $A_{eff} = 1 / (\epsilon(p_i / A_i))$  for heterogeneous materials are also useful in geophysical studies<sup>1,2</sup>.

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### Reduced Absorption of Light at High Laser Power Densities

In experiments with pulsed lasers, methylene blue has been used frequently as a dye to absorb the energy<sup>1</sup>. In conjunction with heating experiments we measured the laser energy (giant ruby pulse) transmitted through a methylene blue solution and noticed a higher transmission than is derivable from ordinary spectrophotometric data<sup>2</sup>.

To examine this photobleaching more closely we used the 633-m $\mu$  line of a He-Ne-CW-laser and collimated its output to about 0.1 mm<sup>2</sup> along a light path of 10 mm in a quartz cell containing the solution. Convection and diffusion were reduced by enclosing the exposed volume in a cylinder. To assure linear response of the photodetector the power density behind the cell was attenuated. A mechanical shutter between cell and CW-laser initiated the illumination. The following solutions were investigated in some detail: (I)  $2 \times 10^{-3}$  M methylene blue in 0.1 M triethanolamine, pH 7.3; (II)  $4 \times 10^{-3}$  M methylene blue in 0.1 M triethanolamine, pH 7.3; (III)  $4 \times 10^{-3}$  M methylene blue in 0.1 M acetate, pH 5.0; (IV) solution II plus 0.01 M ferrihexacyanide.

The transmissions of these solutions,  $S_1/S_0$ , measured on a Zeiss spectrophotometer at 633 m $\mu$  and directly on the described apparatus at a low light level, were 13.7 per cent for solution I and 3 per cent for the remaining ones. When the shutter is opened at high light level, the signal increases from the initial level  $S_1$  to a new steady value  $S_2$  (Fig. 1). Fig. 2 shows the (final)  $S_2$ -values as a function of  $S_0$ , demonstrating clearly the effects of high-power densities.

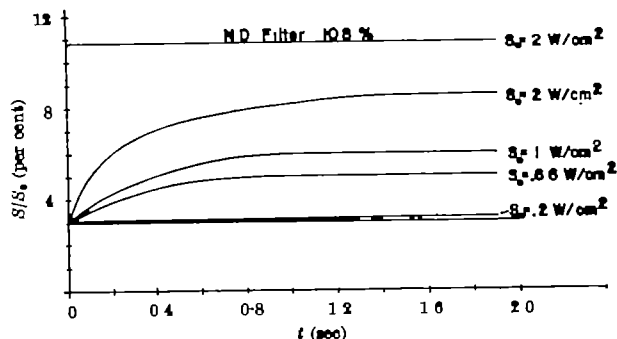


Fig. 1 Percentage transmission as a function of time in experiment with solution II (rise-time 0.3 sec), the shutter is opened at  $t=0$ . The parameter  $S_0$  represents the incident power density. A neutral density filter (10.8 per cent transmission) is inserted alone for calibration and for demonstration of the linear response of the detection system.

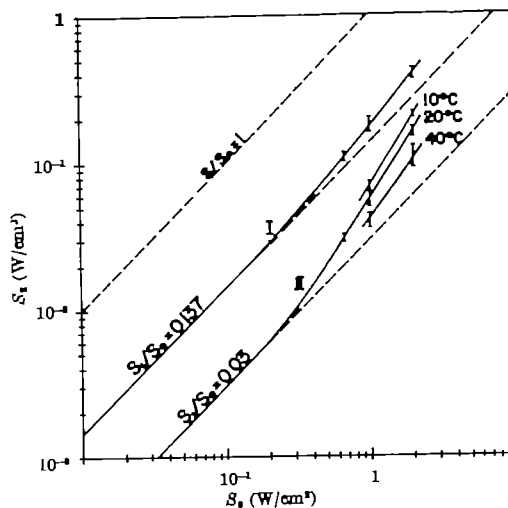


Fig. 2 The transmitted power density finally reached,  $S_2$  (about 2 sec after shutter was opened) as a function of the incident power density,  $S_0$ . Two different solutions are used (I and II).  $S_1/S_0 = 1$  corresponds to 'no methylene blue'.

In a later series of experiments (not included in the graphs of this communication), we increased the power density to about 6 W/cm<sup>2</sup> and obtained for solution I a transmission change of  $S_2/S_1 = 4$  and for solution II one of  $S_2/S_1 = 14$  with rise-times of about 2 sec. When the illuminating power density was switched down to 10 per cent by a neutral density filter, the transmitted signal decayed from 0.1  $S_2$  to a value which reached 0.1  $S_1$  within a factor of  $1.3 \pm 0.2$  (exp. fall-time 1-2 sec). Solutions III and IV showed  $S_2 - S_1 = 0$ , at all power-levels ( $< 6$  W/cm<sup>2</sup>).

The experiments show that triethanolamine is necessary for the appearance of  $S_2 - S_1 \neq 0$ . The experiments seem to indicate the presence of a long-lived complex intermediate, involving triethanolamine and appearing possibly (quenching by ferrocyanide) via singlet-triplet transitions<sup>3-5</sup>.

The appearance of this intermediate complex may be described by a differential equation, consisting of a (first) generating term and a (second) decay term. If  $c_M^0$  represents the analytical (molar) concentration of methylene blue,  $c_X$  the (instantaneous molar) concentration of the intermediary compound X, and  $\tau$  the time constant, with which X decays to the ground state, we may write:

$$\frac{dc_X}{dt} = \frac{10^3 P_0}{N_A h \nu r^2 \pi} (1 - \exp[-\alpha M(c_M^0 - c_X)b]) - \frac{c_X}{\tau} \quad (1)$$

when  $P_0$ , laser input power (W),  $= S_0 r^2 \pi$ ;  $r^2 \pi$ , cross-sectional area of light beam in solution (cm<sup>2</sup>);  $b$ , length of light beam in solution (cm);  $N_A h \nu$ , energy of 1 mole of light quanta in W sec mole<sup>-1</sup>;  $\alpha M$ , natural molar extinction coefficient of methylene blue at 633 m $\mu$  ( $M^{-1}$  cm<sup>-1</sup>). Equation (1) considers only the reversible part of the photolysis, assumes quantum yield of unity, and neglects concentration distribution (as well as the heating and the diffusion out of the constricted volume).

Four limiting cases of equation (1) may be considered: (a)  $dc_X/dt = 0$ , corresponding to the 'steady state'-condition, reached in the horizontal portions of the curves in Fig. 1; (b)  $c_X \ll c_M^0$ , allowing treatment of the 'linearized case', presented in Fig. 1 by the curve with  $S_0 = 0.2$  W/cm<sup>2</sup>; (c)  $c_X = 0$ , equivalent to the 'starting condition', giving the slope (in Fig. 1) of the curve at the intercept with the ordinate; (d)  $P_0 \rightarrow 0$  at the moment of reducing the input power to non-photolytic levels, when the first term vanishes and  $\tau$  is directly obtainable from the decay curve.

Above, a type of photo-stationary method is described, which is new in so far as changes in the population of the singlet ground-state are directly observed. This type is

quite different from that where processes competing with fluorescence emission are observed<sup>4</sup>.

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## METALLURGY

### Influence of Pre-plated Coatings on the Morphology of the Intermetallic Layer in Tinplate

In the manufacture of electrolytic tinplate, a tin coating is electro-deposited directly on to steel. The plated strip is then heated to above the melting point of tin (232° C) and quenched in water. During this treatment cycle a layer of the intermetallic compound FeSn<sub>3</sub> (about 0.15μ thick) is formed at the steel-tin interface<sup>1</sup>.

The intermetallic compound has been shown to have an important influence on the protective value of the coating, particularly with certain canned foods<sup>2,3</sup>. Recent work<sup>4</sup> has also shown that protection is enhanced by more complete coverage of the steel by the intermetallic compound and that the degree of coverage may be associated with the morphology of the FeSn<sub>3</sub> crystallites.

The extent to which complete coverage is achieved depends primarily on the number of nucleation sites for the compound crystallites since, under normal manufacturing conditions, the time available for crystal growth is less than about 3 sec. Under such conditions the crystallites appear as a network of prisms, generally arranged in colonies which correspond epitaxially to the grain directions of the ferrite substrate.

G. G. Kamm *et al.*<sup>5</sup> have suggested that nucleation of compound crystallites is hindered by the presence of surface oxides on the steel prior to plating and have proposed reducing this by pre-plating with tin from an alkaline electrolyte. Complex heat-treatment cycles may also have the effect of modifying the crystal structure of the compound<sup>6-8</sup>.

As part of an investigation in progress at the Tin Research Institute, it has now been found that the



Fig. 1. Intermetallic compound—no undercoat ( $\times 6,000$ )



Fig. 2. Intermetallic compound—steel pre-plated with tin-nickel ( $\times 6,000$ )

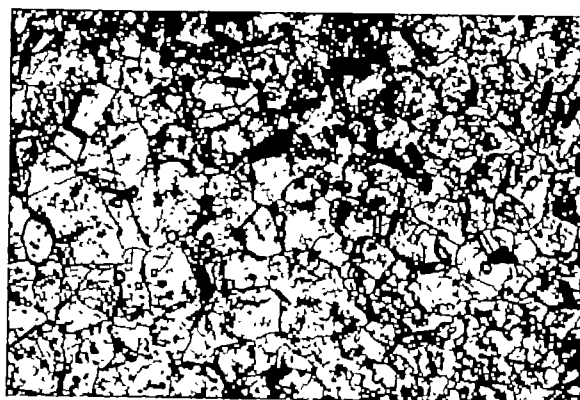


Fig. 3. Intermetallic compound—steel pre-plated with nickel ( $\times 6,000$ )

nucleation characteristics and the crystal habit of the intermetallic compound on tinplate can be markedly influenced by pre-plating the steel with an extremely thin coating of another metal or alloy. The range of undercoating materials studied included iron, lead, tin-nickel, nickel and palladium, but the most significant results were achieved with electro-deposited tin-nickel alloy and nickel.

In contrast to the normal type of compound layer formed when no undercoat is used (Fig. 1), the presence of a tin-nickel or nickel undercoat (0.025μ thick) causes the intermetallic compound to grow in the form of rounded equi-axed crystals (Figs. 2 and 3). The coverage of the base steel seems virtually complete and accelerated corrosion tests appear to indicate a four-fold improvement in corrosion resistance for the materials prepared with tin-nickel or nickel as compared with tinplate prepared without an undercoat. Both nickel and tin-nickel appear to be particularly potent in their influence on the morphology of FeSn<sub>3</sub>.

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### Interaction of Spinel Crystals and Chromium Nitride in Chromium

It has been amply demonstrated that normally brittle metals exhibit substantial ductility at low temperatures when interstitial impurity levels are extremely low. This is particularly true for interstitial nitrogen in chromium<sup>1</sup>. As little as 15 p.p.m. nitrogen has been reported to embrittle chromium<sup>2</sup>.

A rather unexpected result occurs when a spinel-forming oxide such as magnesium oxide is added to chromium. The composite mixture exhibits ductility at room temperature after hot-working for densification<sup>3</sup>.

X-ray experiments confirm that magnesium-chromite spinel forms as magnesium oxide combines with oxygen and chromium in the powder mixture. We believe that the spinel formed in this manner enhances ductility by absorbing nitrogen contained in the chromium.

A composite containing 93.5 per cent electrolytic chromium, 0.5 per cent titanium, and 8 per cent magnesium oxide has been examined in an experiment involving exposure to nitrogen in order to investigate the effect produced by the spinel. The composite mixture was first fabricated by mixing powders of chromium, titanium and magnesium oxide, compacting hydrostatically, sintering, and then extruding.

The extruded composite mixture contains larger amounts of interstitials (120 p.p.m. nitrogen, for example) than the maximum allowable limits normally quoted for ductility. Nevertheless, this material demonstrates ductility in tension and in bending at room temperature. The physical properties of the material, including notch tensile results, have been previously reported<sup>4</sup>.

Sintered and wrought chromium composite material containing magnesium-chromite spinel, as explained here, was exposed at 1,200° C in argon containing 100 p.p.m. nitrogen, quenched in water, and examined metallographically. The first electron micrograph (Fig. 1) shows the structure. Fine nitride needles are present in the matrix. The sample was then aged 1 h at 100° C, resulting in growth of the nitride particles, as seen in Fig. 2. The material was completely embrittled at this stage. It was then held for 4 h at 1,425° C in moderate vacuum. The resulting structure is shown in Fig. 3. The photomicrograph demonstrates that the nitride has completely disappeared from the matrix.

It would seem likely that the spinel crystals absorbed the precipitated nitride. The photomicrograph offers evidence to this effect. A very fine amorphous material has collected around the ceramic grains. This material was removed in the process of replicating the polished and etched specimen for the electron microscope. No

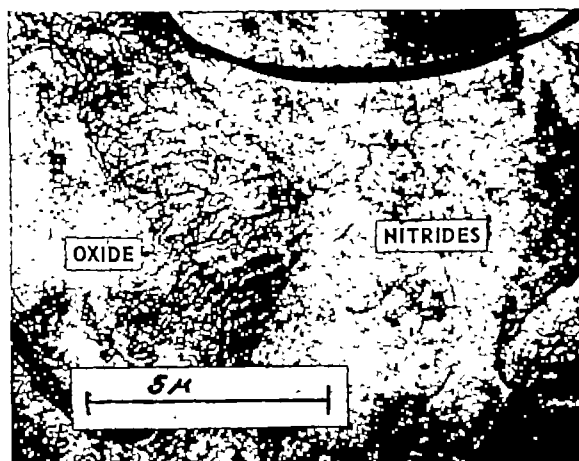


Fig. 1. Chromium nitride needles after quenching (electron micrograph replica)

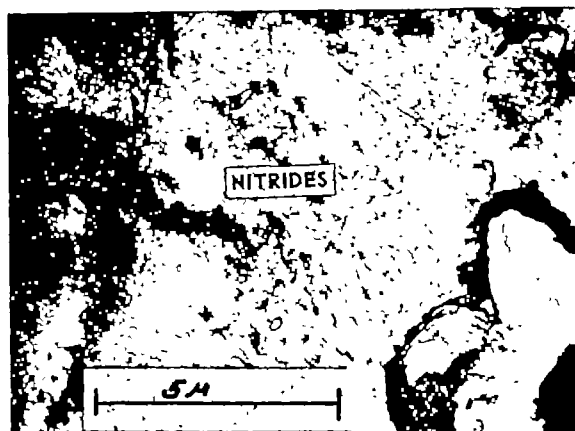


Fig. 2. Chromium nitrides after 100° C ageing (electron micrograph replica)

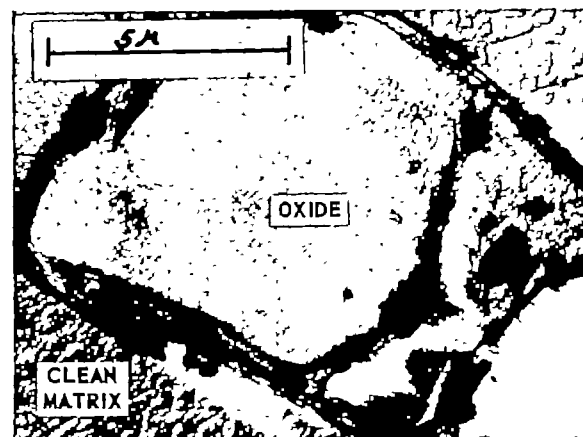


Fig. 3. Reaction product at oxide particle after 1,425° C solution treatment (electron micrograph with extraction technique)

electron diffraction pattern could be obtained from it, indicating that it was amorphous or composed of very small crystals. The composite was ductile and could be bent at room temperature after this heat treatment.

It is possible that the chromium nitride would break down at 1,425° C and go into solution, the nitrogen recombining at the metal surface or simply subliming. Accordingly, a sample of pure electrolytic chromium, containing no magnesium oxide, was fabricated, extruded, and exposed to nitrogen in the same manner. Nitrides were still present in quantity after the 1,425° C heat treatment, including heavily nitrated grain boundaries.

In conclusion, then, it can be said that a dispersed spinel phase exhibits the important property of chemically rendering the matrix free from nitride precipitates. This effect further lowers the amount of nitrogen contained in the matrix to values low enough to promote room temperature ductility.

I thank Mr. Conrad Herald for the electron micrographs.

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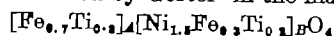
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## CHEMISTRY

Cation Distribution in Ulvöspinel  $\text{Fe}_2\text{TiO}_4$ 

RECENTLY we have considered it of interest to examine several spinel oxides containing iron, using the Mössbauer effect in the isotope iron-57, in order to determine the cationic oxidation state and site distribution<sup>1</sup>. A case in which such information might be valuable is the oxide  $\text{Fe}_2\text{TiO}_4$ . Since the original X-ray intensity investigation of Barth and Poniak<sup>2</sup> this spinel has been considered to possess the inverse arrangement  $\text{Fe}[\text{TiFe}]_2\text{O}_4$ . However, the determination of cation distribution for an oxide with cations of such similar scattering coefficients is not easy. In addition the calculated intensity value for the 135 reflexion given by those authors for the normal arrangement is in error by several orders of magnitude. The inverse arrangement is favoured for a 2-2-4 spinel on the basis of electrostatic considerations<sup>3</sup>, and the known ferrimagnetic properties of  $\text{Fe}_2\text{TiO}_4$  (Curie point 120° K (ref. 4)). The normal arrangement  $\text{Ti}[\text{FeFe}]_2\text{O}_4$  requires  $\text{Ti}^{4+}$  ions on tetrahedral *A* sites. This is uncommon, but has been observed by Gorter<sup>5</sup> in the material:



on the basis of saturation magnetization and *g*-factor determinations.

A sample of  $\text{Fe}_2\text{TiO}_4$  was prepared by calcining a  $\text{TiO}_2$ - $\text{Fe}_2\text{O}_3$  mixture in a CO-CO<sub>2</sub> atmosphere as used by Kunnmann *et al.*<sup>6</sup>. The lattice parameter was determined as  $8.529 \pm 0.003$  Å in good agreement with the figure of 8.53 Å given by Akimoto<sup>4</sup>. The X-ray diagram also indicated the presence of ~1 per cent ilmenite which, by the formation of a proportionate amount of  $\text{Fe}_2\text{O}_3$ , could account for the sample possessing an overall ferromagnetism. (We are grateful to Dr. T. I. Barry for the magnetic measurements.) The room-temperature Mössbauer spectrum of this sample obtained with equipment described elsewhere<sup>1</sup>, and using a source of cobalt-57 in stainless steel, is shown in Fig. 1. The derived isomer shift and quadrupole splitting values are  $1.08 \pm 0.06$  mm sec<sup>-1</sup> and  $1.85 \pm 0.06$  mm sec<sup>-1</sup> respectively. Within the limits of error these values are identical to those obtained earlier for  $\text{FeAl}_2\text{O}_4$  with  $\text{Fe}^{2+}$  on tetrahedral *A* sites<sup>1</sup>. Since the inverse arrangement is favoured for  $\text{Fe}_2\text{TiO}_4$  (possibly with some of the  $\text{Ti}^{4+}$  ions on *A* sites), this result indicates that *A*- and *B*-site  $\text{Fe}^{2+}$  in  $\text{Fe}_2\text{TiO}_4$  gives rise to identical isomer shifts and quadrupole splittings which may limit the use of the effect in similar situations. The presence of  $\text{Fe}^{2+}$  quadrupole interaction in  $\text{Fe}_2\text{TiO}_4$  and  $\text{FeAl}_2\text{O}_4$  but not in  $\text{FeV}_2\text{O}_4$  and  $\text{FeCr}_2\text{O}_4$  indicates that

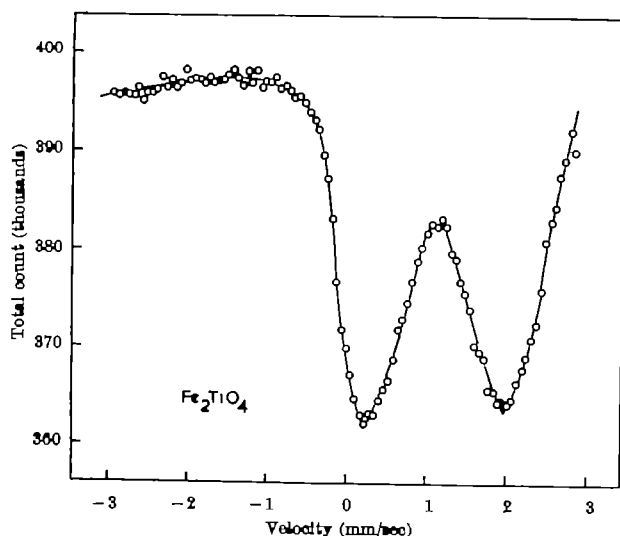


Fig. 1. Room temperature Mössbauer absorption spectrum for  $\text{Fe}_2\text{TiO}_4$ .

the electric field gradient at a nucleus at a given site in a spinel is dependent on the magnetic nature of the cations at neighbouring sites.

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## Superficial Ion-exchange Chromatography

RECENTLY the techniques of gas chromatography have been applied to chromatography in the liquid phase to separate small quantities of mixtures very rapidly. Techniques have been described for adsorption chromatography<sup>1</sup>, partition chromatography<sup>2</sup>, and ion-exchange chromatography<sup>3</sup> where the inner surface of a capillary tube was coated with a liquid ion-exchanger.

In this laboratory, beads of cross-linked polystyrene which bear ion-exchange groups only in a shallow 'surface' layer have been used for superficial ion-exchange chromatography. The ion-exchange groups are not limited to amino-groups, and resins with superficial sulphonic acid groups and with superficial quaternary ammonium groups have been used successfully. The resins are expected to be as stable as the normal polystyrene resins, and columns packed with them should last indefinitely.

In normal ion-exchange chromatography the best results are obtained with very small particles and very low flow rates<sup>4</sup>, partly because the diffusion of ions into and out of the particles is slow. In superficial ion-exchange chromatography the distance the ions have to diffuse into the particles is greatly reduced, and rapid flow rates may be used with beads of convenient size. The beads used to obtain the elution curves shown in Fig. 1 had a mean diameter of 0.24 mm, and were surface-sulphonated to a capacity of 5.3  $\mu\text{equiv./ml}$ . They were packed in a 45-in. column, 6 mm internal diameter, and eluant was pumped through at a rate of 6 ml./min. Molar solutions of metals or 1 per cent solutions of dyes were injected in quantities of 1-10  $\mu\text{l}$ . Curve A shows the elution with 0.3 N hydrochloric acid of a mixture of copper and iron, and the tailing of the iron which occurs in this system. The tailing was reduced by gradient elution (curve B), and the separation was complete in 14 min. These curves should be compared with those obtained for the same metals on 'AG 50X8' by elution with more concentrated acid<sup>5</sup>. Curve A was not appreciably altered by faster or slower flow rates, and the tailing was not improved when a 15-in. column packed with beads of diameter 0.08 mm was used.

In practice, most ion-exchange separations depend less on the efficiency of the column than on the choice of a suitable eluant, which is often a complexing agent<sup>6</sup>. For the separation of metals it should be possible to prepare resins with superficial chelating groups and elute different metals with eluants of different pH. In this way the main drawback of chelating resins, which is the slow diffusion within the particles, could be avoided. However, the chief application of superficial ion-exchange chromatography is likely to be the rapid separation and characterization of organic acids and bases. Curve C shows the separation of two basic dyes, methylene blue (the first peak) and Janus green (the second peak). These dyes could not be eluted with the same eluant (5 N



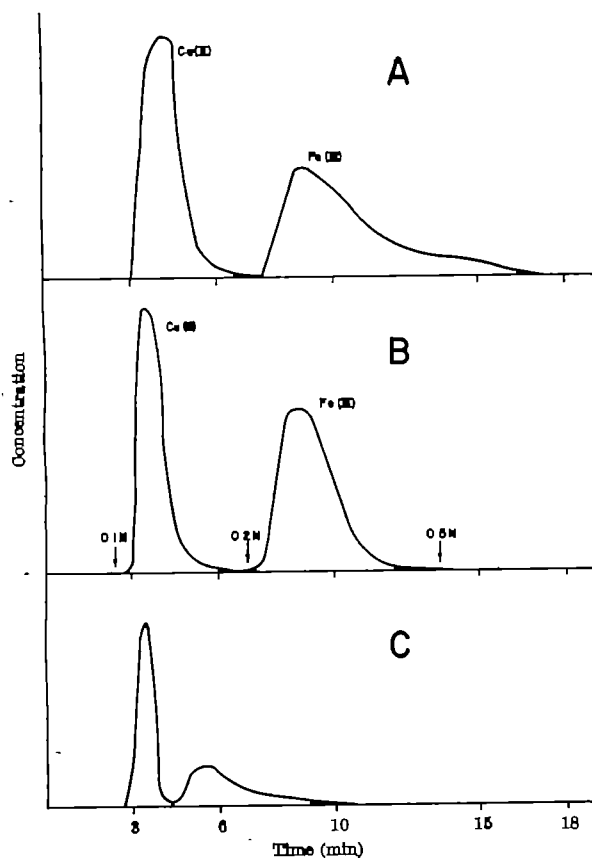


Fig. 1

hydrochloric acid in 6 per cent alcohol) from a normal resin ('AG 50X2'). Similarly, acid dyes have been separated on a superficial anion-exchange resin. For the convenient separation of colourless substances a continuous method of detection would be required. Ultra-violet absorption of the effluent might be measured in many cases, or the type of detector described by Stouffer *et al.*<sup>7</sup> might be suitable if interference from the eluant could be avoided. It is possible to increase the capacity of the beads and use correspondingly larger quantities of the mixture to suit the sensitivity of the detector.

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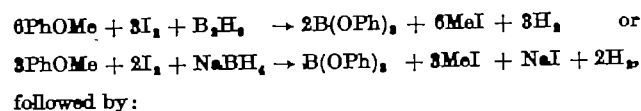
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### Low-temperature Cleavage of Ethers

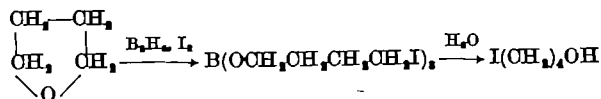
THE cleaving of an ether usually requires the use of a vigorous reagent such as an anhydrous mineral acid, an organic acid halide or anhydride, a Lewis acid, or an organometallic compound. Frequently heating for prolonged periods is necessary.

In examining the chemistry of boron, it has been found that, in conjunction with a halogen such as (by preference) iodine, boranes<sup>1</sup> and metal borohydrides<sup>2</sup> will destroy the

hydroxyl groups of alcohols, which are converted into organic halides. Although diborane on its own does not normally react with ethers near room temperature, except possibly to form a very loose addition compound, in the presence of free halogen a comparable reaction occurs with ethers<sup>1</sup>. An oxygen-carbon link is ruptured, the oxygen attaching itself to the boron while the carbon links up with halogen. Thus anisole gives methyl iodide and triphenyl borate, from which phenol is immediately obtained on subsequent hydrolysis in the cold:



Attention is now directed to this reaction as a means of cleaving ethers under milder conditions than hitherto possible. By it, a rapid, smooth and complete cleavage can be effected, usually at room temperature or even below. The ether must be dry. The relative amount of diborane required is small, since one molecule cleaves six molecules of the ether. The reaction is moreover very widely applicable. The ether may be symmetric, unsymmetric, aliphatic, aromatic or cyclic. With cyclic ethers a boron ester is first produced which on subsequent hydrolysis yields the  $\omega$ -iodo derivative of an alcohol as the ultimate product, for example:



In this way 4-iodobutan-1-ol, was prepared in more than 90 per cent yield from tetrahydrofuran. Its identity was confirmed by C, H and O analysis, both on the original product and on its *p*-nitrobenzoate derivative, which had an observed melting point of 104.5° C.

In general, the cleavage reaction is efficient and rapid, and proceeds quietly to completion within minutes at room temperature. Higher boranes are inclined to react more slowly than diborane. Metal borohydrides are comparable in reactivity to diborane, although sodium borohydride is less reactive than the lithium salt. Bromine and interhalogen compounds such as iodine chloride react more vigorously than iodine, but are in general less convenient. Large excesses of reagents are unnecessary and should be avoided.

The ready availability of metal borohydrides and hence diborane implies that this method could become a useful tool in degradative organic chemistry.

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### Unusual Role of Dimers in Thermal Polymerization of Chloroprene

THE dimerization products of chloroprene have been shown to consist principally of two substituted cyclohexenes and an eight-membered cyclic diene<sup>1</sup>. Carothers *et al.*<sup>2</sup> have noted the presence of a distinct oligomeric fraction in the polymerization products of chloroprene, and more recently Klebenskii *et al.*<sup>3</sup> have shown that this fraction contains both six- and eight-membered cyclic dimers. In previous kinetic studies of the thermal poly-

merization of chloroprene, no special account has been taken of the formation of dimers; it has been tacitly assumed, for example, that the rate and the observed activation energy of the thermal polymerization<sup>4,5</sup> refer to a direct polymerization of the chloroprene monomer.

Our present kinetic experiments, however, lead us to the conclusion that the above assumption is not valid, and that the thermal polymerization proceeds by an unusual mechanism.

The yields of polymer and dimers have been measured as a function of time in three chloroprene systems at 35° C: (i) pure monomer; (ii) pure monomer containing excess inhibitor; (iii) pure monomer containing a small quantity of inhibitor which is consumed during the early part of the reaction. Expressing all rates of reaction in terms of the rate of disappearance of chloroprene monomer, it is found that, within experimental error, the rate of polymerization in (i) is equal to the rate of dimerization in (ii), whereas the rate of polymerization in (ii) is zero.

During the post inhibition period in (iii) the dimer concentration falls rapidly until it reaches a low steady value similar to that observed in (i), whereas the rate of polymerization, initially large, eventually falls to a constant value comparable with that observed in (i).

The simplest explanation of the results is that polymer is actually formed by a process of dimer addition and that little or no monomer is involved in the propagation reaction. Experiments in which small quantities of monomer were added to bulk dimers at 35° C indicate that monomer, or a trace impurity associated with it, plays an essential part in the thermal polymerization reaction, most probably in the initiation process.

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# Inorganic Precipitation of Calcium Carbonate from Sea-water

RECENTLY, Wells and Illing have suggested<sup>1</sup> that the so-called 'whittings' in the Persian Gulf are clouds of calcium carbonate precipitated instantaneously from sea-water that has become supersaturated with respect to calcium carbonate, owing to removal of CO<sub>2</sub> by the photosynthetic action of phytoplankton.

In order to investigate this interesting phenomenon, we have compared the composition of a 'whiting' sample collected by A. J. Wells with that of bottom sediment at a locality near the place where the whiting had formed, and with that of artificial precipitates of CaCO<sub>3</sub>. Artificial precipitation was brought about by removal of carbon dioxide both from Persian Gulf sea-water and from artificial sea-water having the same major-element composition. The mineral composition of the various samples was then determined.

The sea-water sample was placed in the bottom part of a desiccator and was constantly and rapidly stirred by a magnetic stirrer. Supersaturation was obtained either by removal of CO<sub>2</sub> or by addition of Na<sub>2</sub>CO<sub>3</sub>. Removal of CO<sub>2</sub> from the sea-water was brought about by placing a strong sodium hydroxide solution in the upper part of the desiccator. The conditions of the experiments and the approximate time necessary to form a precipitate are presented in Table 1.

Table 1

Experiment	Type of sea-water	Chlorinity (‰)	Proc. obtained by:	Approx time to form a precipitate
1	Doha Bay*	22.5	CO <sub>2</sub> -extraction	3 weeks
2	Doha Bay	22.5	Na <sub>2</sub> CO <sub>3</sub> -addition	2 weeks
3	Artificial	22.5	CO <sub>2</sub> -extraction	2.5 weeks
4	Ditto	22.5	Ditto	2 weeks
5	Ditto	22.5	Ditto	2 weeks

\* Doha Bay is located on the eastern side of the Qatar peninsula.

Table 1 shows that formation of a precipitate is not a matter of minutes but of weeks. Experiment 2 was carried out in order to make sure that the removal of CO<sub>2</sub> from the sea-water by NaOH was not the rate-determining step: a concentrated sodium carbonate solution was added to the water until the pH of the water was 8.98.

In experiment 2 the supersaturation of the water with respect to calcium carbonate is higher than the supersaturation obtained in the other experiments, because the total carbonate content (CO<sub>3</sub><sup>2-</sup> + HCO<sub>3</sub><sup>-</sup> + CO<sub>2</sub>) was greater in experiment 2. In the other experiments the total carbonate content was decreased by extraction of CO<sub>2</sub>. Clearly the CO<sub>3</sub><sup>2-</sup>-concentration reaches a higher value either by Na<sub>2</sub>CO<sub>3</sub>-addition or CO<sub>2</sub>-extraction, since each process brings about the supersaturation with respect to calcium carbonate.

In all experiments the precipitation of calcium carbonate was nothing like fast enough to balance the increasing CO<sub>3</sub><sup>2-</sup>-concentration, and the pH consequently increased. One should therefore expect an increase in pH before whiting formation starts and a drop in pH when precipitation starts. This drop in pH may be difficult to measure if precipitation is slow. However, if a precipitate does form over a large area in a matter of minutes, the drop should be detectable. Field observations, however, gave no evidence of a change in pH during whiting formation.

The precipitates which formed during our experiments were filtered, washed with a small quantity of distilled water and dried at 105° C. The whiting sample, the bottom sediment sample (fraction < 2μ) and the precipitates were examined under the microscope and analysed by X-ray diffraction.

X-ray diffraction analysis was carried out with a Philips X-ray diffractometer, using Cu-Kα radiation. The relative amounts of the various minerals present were estimated by comparing the areas of the peaks in the diffractogram, account being taken of the poor reflective power of aragonite. The values given are only semi-quantitative. As the amount of precipitate recovered in experiments 4 and 5 was too small for diffractometer analysis, Guinier X-ray diffraction photographs of these samples were therefore made. In all precipitates obtained in the laboratory only aragonite was found, except for the precipitate obtained by addition of Na<sub>2</sub>CO<sub>3</sub> to unfiltered Doha Bay water (see Tables 1 and 2). The trace amount of magnesium calcite was found to be originally present in suspension in the water supplied for this experiment. It is clear from Table 2 that the mineral compositions of whiting and bottom sediment are very similar, and that they differ appreciably from the compositions of the artificial precipitates.

Table 2. RESULTS OF X-RAY DIFFRACTION AND EMISSION SPECTROGRAPHIC ANALYSIS

Sample	Description	Sr/Ca ratio (mg/g) of water	X-ray diffraction analysis			
			Ara-gonite (%)	Calcite (%)	Mg-calcite (%)	Quartz (%)
A	Whiting (Wells, 1961)	—	70	10	15	5
B	Bottom sediment (< 2μ)	—	65	10	20	trace
C	Bottom sediment	—	70	15	10	trace
1	Proc. from Doha Bay water made by CO <sub>2</sub> -extr.	22.5	100	—	—	—
2	Ditto by Na <sub>2</sub> CO <sub>3</sub> -add.	22.5	95-100	—	trace	—
3	Proc. from artif. sea w.	65	100	—	—	—
4	Ditto	130	100	—	—	—
5	Ditto	22.5	100	—	—	—

Table 2 shows that pure aragonite was the only type of calcium carbonate to precipitate from sea-water by  $\text{CO}_2$  extraction. This finding has also been made by other workers<sup>2</sup>. Furthermore, the rate of crystallization of aragonite in these experiments was so slow (even at the unnaturally high supersaturation value achieved by adding sodium carbonate to make the solution pH 9) that such crystallization can scarcely be used to explain the rapid formation of whittings seen in the sea off Doha.

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### An Experimental Determination of the Photo-ionization Cross-section of Atomic Hydrogen

ALTHOUGH many theoretical calculations of the photo-ionization cross-section of atomic hydrogen have been performed (see review by Bates<sup>1</sup>), no experimental determination of this quantity has previously been made. An experiment is being carried out here which has yielded a value for the photo-ionization cross-section of atomic hydrogen at 850.6 Å.

Atomic hydrogen is produced by flowing molecular hydrogen through a discharge tube in which an intense radio frequency discharge is run. This dissociates a large fraction of the hydrogen molecules. The mixture of atoms and molecules is then flowed quickly through an all-glass absorption cell which is attached to the exit slit of a one-metre normal-incidence vacuum monochromator. Radiation emerging from the exit slit passes down the absorption cell and is detected by a sensitized photomultiplier placed at the far end of the cell. The light source used to illuminate the entrance slit of the monochromator is similar to that described by Garton<sup>2</sup> and consists essentially of a short-duration high-current spark produced by discharging a capacitor bank. The light source is filled with low-pressure argon gas and the radiation produced by it consists mainly of emission lines of neutral and multiply ionized argon.

The intensity of the radiation emerging from the absorption cell is measured under three conditions: (i) intensity  $I_0$ , with the absorption cell evacuated; (ii)  $I_1$ , with the absorption cell filled with molecular hydrogen at pressure  $p_1$ ; (iii)  $I_2$ , with partially dissociated hydrogen in the absorption cell at total pressure  $p_2$ . The degree of dissociation  $\alpha$  is determined by the Wrede-Harteck method<sup>3</sup>.

From (i) and (ii) the photo-absorption cross-section of molecular hydrogen,  $\sigma_M$ , can be determined from the equation:

$$I_1 = I_0 \exp \left( -N\sigma_M \frac{p_1}{760} \frac{273}{T} l \right) \quad (1)$$

where  $N$  is Loschmidt's number,  $2.69 \times 10^{19}$  particles/c.c.,  $T$  is the absolute temperature of the gas (°K), and  $l$  is the length of the absorption cell (cm).

From a knowledge of  $\alpha$  and  $p_2$ , the partial pressures  $p_A$ ,  $p_M$  of atomic and molecular hydrogen can be calculated. Thus, using (i) and (iii) and the result for  $\sigma_M$  derived from (1), the photo-ionization cross-section of atomic hydrogen,  $\sigma_A$ , can be found from the equation:

$$I_2 = I_0 \exp \left( - \left\{ N\sigma_A \frac{p_A}{760} \frac{273}{T} + N\sigma_M \frac{p_M}{760} \frac{273}{T} \right\} l \right) \quad (2)$$

$p_1$ ,  $p_2$  and  $\alpha$  are measured at each end of the absorption cell and a correction for the concentration gradient along the length of the cell is applied.

Due to the difficulty in obtaining sufficient intensity at the photomultiplier to make reliable measurements, initial results have been confined to a wave-length, 850.6 Å, which corresponds to a strong emission line of the light source and a very small absorption cross-section of molecular hydrogen.

The mean value of the molecular cross-section (together with the probable error) at 850.6 Å is  $(0.213 \pm 0.010) \times 10^{-18} \text{ cm}^2$ , which is not in agreement with Cook and Metzger's<sup>4</sup> result of  $\sim 3.2 \times 10^{-18} \text{ cm}^2$  at this wave-length. This may be due to a difference in the effective resolution of the two systems; as there is no continuous absorption in this region the measured value of  $\sigma_M$  will depend critically on the resolution. Cook and Metzger used a continuum light source in conjunction with a monochromator with a pass band of 0.5 Å. Although our instrument has a similar bandpass, we used a line source and, as the width of the 850.6 Å A IV line is probably of the order of or less than 0.1 Å, our resolution is much better than the bandpass of the instrument.

The mean value of the photo-ionization cross-section of atomic hydrogen at 850.6 Å is:

$$(5.15 \pm 0.18) \times 10^{-18} \text{ cm}^2$$

This is in good agreement with the most recently available theoretical value which is given in graphical form by Bates<sup>1</sup>. At 850.6 Å the theoretical value lies between  $5.1$  and  $5.2 \times 10^{-18} \text{ cm}^2$ .

This work was initiated by Dr. R. B. Cairns and I thank him for his help during the early stages.

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### Structure of Catena-di- $\mu$ -Fluorodifluoro-diaquohafnium(IV) monohydrate ( $\text{HfF}_4 \cdot 3\text{H}_2\text{O}$ )

ALTHOUGH the trihydrate of hafnium tetrafluoride and its dehydration products are stoichiometrically similar to the corresponding compounds of zirconium, it has been shown<sup>1</sup> that they are not isostructural. In general, compounds of the two elements do show identical structure (for example, the anhydrous fluorides<sup>2</sup>), and the fluoride hydrates must be particularly susceptible to differences in bond energy. The structure of  $\text{ZrF}_4 \cdot 3\text{H}_2\text{O}$  has been reported previously<sup>3</sup>, and the hafnium analogue has now been investigated.

Crystals were prepared, as previously described<sup>1</sup>, as an apparently homogeneous sample of colourless needles. Single crystal studies have, however, demonstrated the existence of two distinct species. One of these is monoclinic,  $a = 25.48$ ,  $b = 7.51$ ,  $c = 23.02$  Å,  $\beta = 150^\circ$ , 16 molecules per unit cell. Reflections are systematically absent for all  $hkl$  when  $h + k$  is odd, and for  $hkl$  with  $k$  even, when  $k/2 + 1$  is odd. Reflections with  $k$  odd are weak and are markedly diffused along  $c^*$ , thus suggesting that the structure is at least partially disordered. Our investigation has not proceeded further than the location of the hafnium atom positions.

The other modification is also monoclinic, with  $a = 6.70$ ,  $b = 10.55$ ,  $c = 7.71$  Å,  $\beta = 94^\circ$ , 4 molecules per unit cell, space group  $P2_1/a$ . The structure of these crystals had no such complications and has been solved by conventional methods. The current reliability factor, assuming isotropic thermal motion, is 0.14.

All light atoms were assumed to be fluorine for the purpose of structure factor calculation. The peak electron densities on a subsequent Fourier synthesis were 11.6, 10.7, 9.9, 9.4, 8.6, 6.7 and 6.5 electrons Å<sup>-3</sup>. The smallest

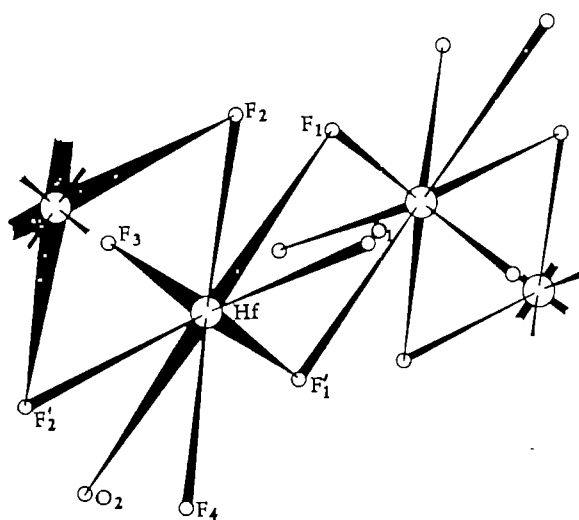


Fig. 1

atom is not directly linked to a hafnium. It makes four tetrahedrally disposed approaches of length 2.68–2.87 Å to other light atoms and can be designated a water molecule. The remaining light atoms are co-ordinated to the hafnium, forming linear chain molecules as shown in Fig. 1. The bridging atoms are the largest peaks and by analogy with similar structure (for example,  $\text{UF}_6$  (ref. 4) with which  $\text{HfF}_6$  is isomorphous;  $\text{ZrF}_6 \cdot 3\text{H}_2\text{O}$  (ref. 3);  $\text{K}_2\text{ZrF}_6$  (ref. 4)) are assumed to be fluorine. The assignment of the remaining atoms, as shown, has been made solely on peak height and can only be regarded as tentative.

The co-ordination polyhedron about each hafnium is a slightly distorted anti-prism, and is very similar to that observed for  $\text{ZrF}_6 \cdot 3\text{H}_2\text{O}$  (ref. 3). The two differ in that the latter is a dimer, with the metal atoms linked by one fluorine double bridge, whereas in this structure each hafnium is linked to two others by fluorine bridges, thus forming a polymeric chain. It is then mere coincidence that the presence of an extra molecule of water of crystallization gives the two compounds identical stoichiometry.

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## BIOCHEMISTRY

### Mechanism of Iron Stimulation of the Enzymatic Activity of Ceruloplasmin

It is well established that certain metal ions stimulate the oxidase activity of the copper-containing protein ceruloplasmin with aromatic diamines and diphenols<sup>1-4</sup>. Using *N,N*-dimethyl-*p*-phenylenediamine as substrate, Curzon<sup>3</sup> observed stimulation of this activity by several transition metal ions, for example,  $\text{Fe}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Co}^{2+}$  and  $\text{Fe}^{3+}$ . By far the most active metal ion was  $\text{Fe}^{3+}$ . Conspicuous by its lack of stimulatory effect was  $\text{Cu}^{2+}$ . Levine and Peisach<sup>4</sup> confirmed Curzon's observation regarding the potent stimulatory effect of  $\text{Fe}^{3+}$  but felt that stimulation due to the other metals can be explained in terms of  $\text{Fe}^{3+}$  contamination. In further investigations of this phenomenon, Peisach and

Levine<sup>5</sup> showed that  $\text{Fe}^{2+}$  stimulated ceruloplasmin activity when the functional groups of the substrate were *para* related (for example, *p*-phenylenediamine (PPD), hydroquinone, but not when the functional groups were *ortho* related (for example, *o*-phenylenediamine, catechol).  $\text{Cu}^{2+}$ , on the other hand, was stimulatory when the functional groups of the substrate were *ortho* related but not *para* related. Peisach and Levine proposed that enzyme copper can form either an active complex with the  $\pi$ -electrons of the aromatic ring<sup>6</sup> or an inactive complex with the functional groups of the substrate. Metals which stimulated the oxidase activity could act by complexing with the functional groups of the substrate, thus preventing the formation of the inactive enzyme-substrate complex.

To test this hypothesis, the effect of  $\text{Fe}^{2+}$  was measured in ceruloplasmin systems using PPD and several of its derivatives as substrates. Experiments were performed spectrophotometrically using a highly purified preparation of pig ceruloplasmin<sup>4</sup> ( $A_{410}/A_{310} = 0.042$ ). The medium contained 0.1 M sodium acetate buffer, pH 5.5, 0.83 mM substrate, 0.023  $\mu\text{M}$  pig ceruloplasmin, and varying amounts of  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_6$ . Enzyme rates were determined by following the increase in absorbancy at 490 m $\mu$  for PPD, at 550 m $\mu$  for *N,N*-diethyl-PPD, and at 610 m $\mu$  for *N,N,N',N'*-tetramethyl-PPD. The results are shown in Fig. 1. For all three substrates, increasing the concentration of  $\text{Fe}^{2+}$  causes an increase in the reaction rate. An optimum  $\text{Fe}^{2+}$  concentration is attained, and further increases in metal ion concentration bring about an inhibitory effect. Inhibition at high concentrations has been observed for most transition metal ions, regardless of whether or not they are stimulatory at low concentrations<sup>4,7</sup>. Alkyl substituents on one amino-group of the substrate, such as in *N,N*-diethyl-PPD, decrease the maximum stimulatory effect of  $\text{Fe}^{2+}$ , while substituents on both amino-groups, such as in *N,N,N',N'*-tetramethyl-PPD, decrease the effect even further. There is less  $\text{Fe}^{2+}$  stimulation of ceruloplasmin oxidase activity in those cases where there is steric-interference of  $\text{Fe}^{2+}$  complex formation. Further evidence for our hypothesis is given in Table 1. A series of experiments were performed similar to the one illustrated in Fig. 1 in which varying amounts of  $\text{Fe}^{2+}$  were added to the reaction mixture. For each experiment a different PPD concentration was used, and the concentration of  $\text{Fe}^{2+}$  required for maximal stimulation was determined in each case. Within the range

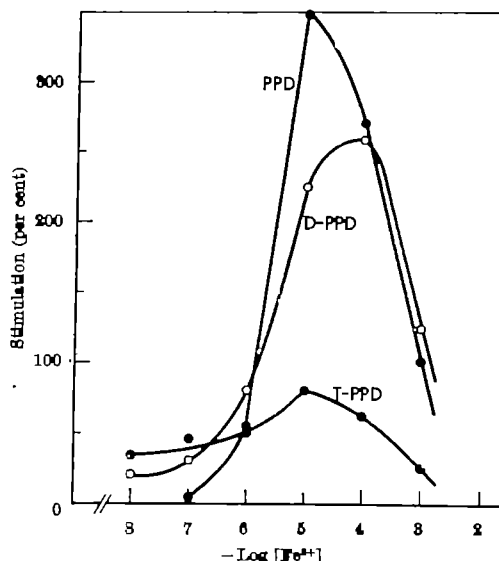


Fig. 1 Effect of  $\text{Fe}^{2+}$  on the ceruloplasmin-catalysed oxidation of aromatic diamines. PPD, *p*-phenylenediamine; D-PPD, *N,N*-diethyl-*p*-phenylenediamine; T-PPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

Table 1. RATIO OF  $\text{Fe}^{2+}$  CONCENTRATION TO PPD CONCENTRATION AT MAXIMAL STIMULATION OF CERULOPLASMIN-CATALYZED OXIDATION

PPD concentration (mM)	( $\text{Fe}^{2+}$ )/(PPD) at maximum stimulation
50	0.08
30	0.017
10	0.01, 0.006
3	0.01

of PPD concentrations reported here, the ratios of  $\text{Fe}^{2+}$  to PPD concentrations were in the same order of magnitude. This strongly implies that a specific  $\text{Fe}^{2+}$ -substrate complex is involved in the stimulation of ceruloplasmin activity. It is postulated that a similar mechanism is operative for  $\text{Cu}^{2+}$  stimulated systems.

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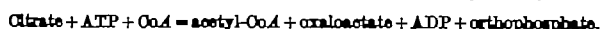
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### Effect of Alloxan-Diabetes on the Activity of Citrate Cleavage Enzyme in Adipose Tissue

It has been known since the work of Stetten and Boxer<sup>1</sup> that glucose is rapidly converted into lipid in tissues of well-fed animals, while lipogenesis is greatly depressed in tissues of diabetic animals. Neither liver slices nor adipose tissue from diabetic rats can convert glucose labelled with carbon-14 into fatty acids<sup>2,3</sup>. The rate of synthesis of long-chain fatty acids from glucose by isolated tissues removed from normal animals is actually more rapid in adipose tissue than in liver, whether expressed on a wet weight or a protein basis<sup>4</sup>. The relatively high rate of lipogenesis in adipose tissue, coupled with its abundance, has suggested that this tissue may be of predominant importance in the *in vivo* synthesis of lipid<sup>4,5</sup>. While there has been extensive work on the enzymatic lesions in fatty acid synthesis in the livers of alloxan-diabetic rats, adipose tissue has not been so widely studied<sup>6,7</sup>.

Recent work has shown that citrate has an important regulatory function in both carbohydrate and fat metabolism<sup>8,9</sup>. Citrate can provide a source of acetyl-coenzyme A (CoA) for fatty acid synthesis in the soluble fraction of the cell by means of the citrate cleavage enzyme:



In the livers of alloxan-diabetic rats this enzyme is decreased to approximately half the control value<sup>8</sup>. In view of these observations and the important part that this enzyme is thought to play in the control of lipogenesis<sup>10</sup>, it seemed of interest to investigate its activity in adipose tissue which is specifically adapted to the formation of fat. An additional advantage in investigations on adipose tissue is the absence of such alternative pathways of metabolism of acetyl-CoA as cholesterol synthesis.

The changes in the activity of citrate cleavage enzyme in the liver and adipose tissue of alloxan-diabetic rats are shown in Fig. 1. In agreement with the results of Kornacker and Lowenstein<sup>8</sup>, it was found that the citrate cleavage enzyme in liver fell in the alloxan-diabetic group to approximately half the control value. The differences were highly significant whether expressed as activity/g liver, as activity/mg protein or as total activity in the

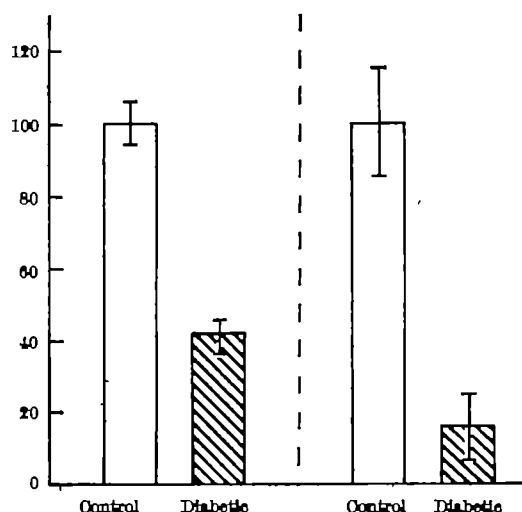


Fig. 1. Changes in the activity of citrate cleavage enzyme in the liver (left) and adipose tissue (right) of alloxan-diabetic rats compared to controls. Adult male albino rats were starved for 48 h and alloxan was given subcutaneously in a dose of 20 mg/100 g body weight. The rats were then given free access to food and water and were maintained on protamine zinc insulin (Boots) 2 units daily for 4 days. Thereafter no further treatment was given and the rats were killed 3 weeks after the last dose of insulin when the blood sugar values of the alloxan-diabetic rats were all greater than 350 mg/100 ml. blood. Controls of the same age and body weight were used. All rats were allowed food *ad lib*. The activity of the citrate cleavage enzyme was estimated in liver and paired epididymal fat pads by the method of Kornacker and Lowenstein<sup>8</sup>. The results, as  $\mu\text{moles acetyl-CoA formed/mg liver protein/h}$  or as  $\mu\text{moles acetyl-CoA formed/mg adipose protein/h}$ , for alloxan-diabetic rats are given as percentage change with respect to the control values. Open columns represent control values, hatched columns diabetic values. The control values for liver and adipose tissue based on the tissue protein content are respectively  $0.7 \pm 0.04$  and  $4.1 \pm 0.7$ . These activities are equivalent to a rate of acetyl-CoA formation from citrate of 137  $\mu\text{moles/g liver/h}$  and 120  $\mu\text{moles/paired epididymal fat pads/h}$  (average weight of paired fat pads of control rats  $2.01 \pm 0.25$  g).

whole organ. The changes in adipose tissue were, however, even more striking. In the alloxan-diabetic rats the enzyme activity fell to only about 18 per cent of the control value. Similar results were obtained when the data were expressed as total activity in the two epididymal fat pads or as activity/mg tissue protein. In view of the very striking changes in the weight of the adipose tissue due to the depletion of the fat content in diabetes, the results are probably not meaningful when expressed as activity/g tissue.

The present observation that, in diabetes, citrate cleavage enzyme of adipose tissue falls more strikingly than that of liver may be considered in the light of the different functions of these tissues. While the main function of adipose tissue would seem to be the formation, storage and release of fatty acids, liver has many functions in which acetyl-CoA is required. Among these functions requiring acetyl-CoA the formation of cholesterol is of importance and it has been shown that the rate of formation of this is not diminished in alloxan-diabetic rats<sup>11</sup>. Consistent with this observation is that direct measurements have shown that acetyl-CoA content of liver is almost unchanged in diabetic rats<sup>11</sup>. Further, in the present experiments, parallel measurements of enzymes of the pentose phosphate pathway (glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase) in liver and adipose tissue have shown a more marked fall in the latter tissue; again this may be correlated with the greater decline in reductive synthetic processes. The fall in citrate cleavage enzyme could be an important factor in the accumulation of citrate within the liver which is known to occur in alloxan-diabetes and starvation<sup>8</sup>. Accumulation of citrate within the cell can have a complex action on the control and direction of both fatty acid synthesis and carbohydrate metabolism. Citrate, in addition to being a precursor for acetyl-CoA, can also activate acetyl-CoA carboxylase, the rate-limiting step in fatty acid synthesis from acetyl-CoA

(ref. 12). In diabetes this stimulatory effect is, presumably, counteracted by the fall in activity of acetyl-CoA carboxylase and fatty acid synthetase and by the inhibitory action of long-chain acyl-CoA derivatives<sup>4</sup>. These effects could also be related to the known action of citrate in inhibiting phosphofructokinase<sup>14</sup>. The inhibition of this enzyme, which is an important control point in the glycolytic pathway, could lead to a decrease in the rate of formation of  $\alpha$ -glycerophosphate and to the esterification of fatty acids. Howard and Lowenstein<sup>14</sup> and Tzur, Tal and Shapiro<sup>15</sup> have pointed out the key role of  $\alpha$ -glycerophosphate in the regulation of fatty acid synthesis.

Many of these control mechanisms have been demonstrated only in liver. The very marked fall in citrate cleavage enzyme in adipose tissue suggests that a similar sequence of events may also occur in this tissue.

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### Effect of Anionic Polyelectrolytes on the Formation and Composition of Plasma Euglobulins

It is historically interesting that euglobulin, the fraction spontaneously precipitating from diluted acidified serum, was the first serum protein fraction to be investigated<sup>1</sup>. There is evidence that these fractions contain insoluble complexes resulting from electrostatic interaction between chondromucoprotein<sup>2</sup>, negatively charged above pH 2 due to dissociated carboxyl and sulphate groups on the chondroitin sulphate moiety, and proteins positively charged at plasma pH values below their isoelectric points, dissociation into soluble components occurring at pH values above those at which they were precipitated<sup>3,4</sup>. The amount of euglobulins precipitated depends on plasma chondromucoprotein concentration, while their composition and biological activities, notably their content of plasminogen and peroxidase-active methaemalbumin<sup>5</sup>, are a function of plasma pH.

Nucleic acids and other anionic polyelectrolytes also interact electrostatically with cationic plasma proteins<sup>6</sup>, and euglobulins precipitated at low ionic strength at certain pH values contain protein-protein complexes formed, for example, following interaction between basic  $\gamma$ -globulins and acidic  $\alpha$ - and  $\beta$ -globulins<sup>7</sup>. Chondromucoprotein, however, is of special interest since, unlike other plasma proteins, it is strongly anionic over a wide pH range. It is also the naturally occurring form of connective tissue chondroitin sulphate, the blood and urine

concentration of which is raised in certain diseases and in rabbits rendered chondrolytic by papain and plasminogen injections<sup>8</sup>.

However, protein fractions precipitating from plasma following dialysis or salting-out procedures are also termed 'euglobulins', and the following experiments were carried out to determine whether the composition of these fractions was also affected by chondromucoprotein.

**Euglobulins precipitated by dialysis of plasma against water at neutrality.** Euglobulins precipitated from normal plasma by this method contain globulins insoluble at low ionic strength near their isoelectric points<sup>9</sup>, in addition to complexes formed by interaction of  $\gamma$ -globulins, positively charged above pH 6.5, with acidic  $\alpha$ - and  $\beta$ -globulins<sup>7</sup>.

Human plasma (2 ml.) and 0.9 per cent sodium chloride (1 ml.) were measured into two tubes (A and B). Into tube A was added 0.9 per cent sodium chloride (0.67 ml.) and into tube B was added chondromucoprotein dissolved in 0.9 per cent sodium chloride (0.67 ml., prepared as described elsewhere<sup>4</sup>, and containing 1.9 mg of chondromucoprotein expressed as chondroitin sulphate). Each mixture was dialysed in air-tight vessels for 5 days against 15 changes of distilled water from which dissolved atmospheric carbon dioxide had previously been expelled by vigorous boiling. The final plasma pH was 7.0. The precipitated euglobulins were measured turbidimetrically at 400 m $\mu$  in a Unicam spectrophotometer (SP 500) in cells of 1 cm light path, extinction values for mixtures A and B being 0.294 and 0.070 respectively.

These results indicate diminished euglobulin precipitation at high chondromucoprotein concentration. The reason for this is obscure, but may be related to a protective colloid effect exerted by chondromucoprotein, a property shared by other mucosubstances<sup>10</sup>.

**Euglobulins precipitated by dialysis of plasma against water containing dissolved atmospheric carbon dioxide.** Plasma was dialysed against distilled water from which dissolved atmospheric carbon dioxide had not been removed by boiling. Under these conditions the plasma pH fell to 5.3. Euglobulin formation, assessed by measurements of protein, lipoproteins (as cholesterol), clottable fibrinogen and sialoproteins (as sialic acid) in the precipitates, was increased in the presence of added chondromucoprotein as shown in Fig. 1, an effect observed previ-

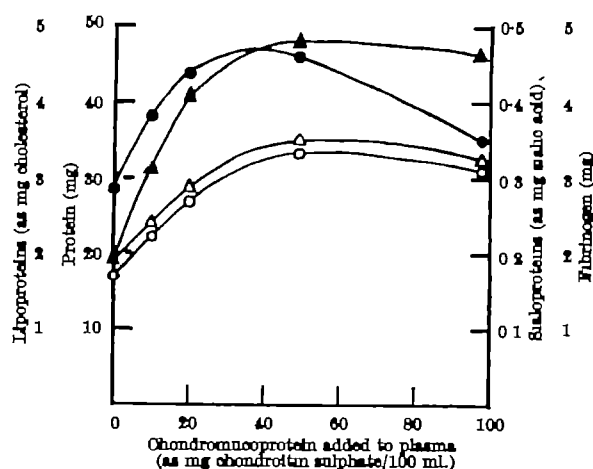


Fig. 1. Effect of chondromucoprotein on the spontaneous precipitation of euglobulins from dialysed plasma. Portions of human plasma (1.8 ml.) were measured into five tubes (1-5). To tube 1 was added 1.3 ml. of 0.9 per cent sodium chloride. To tubes 2-5 were added varying amounts of chondromucoprotein (0.17-1.7 mg, expressed as chondroitin sulphate) dissolved in 1.3 ml. of 0.9 per cent sodium chloride, the resulting plasma chondromucoprotein concentrations, in terms of chondroitin sulphate, ranging from 9.4 to 94 mg/100 ml. Each mixture was dialysed against 15 changes of distilled water at 4° for 5 days, after which time the plasma pH had fallen to 5.3. The precipitated euglobulins were centrifuged down, washed with acetate buffer (0.1 M, pH 5.3), dissolved in 2 ml. of phosphate buffer (0.1 M, pH 8.0) and the euglobulin solutions estimated for protein, sialic acid, clottable fibrinogen and cholesterol. These values are indicated by  $\circ$ ,  $\bullet$ ,  $\Delta$  and  $\blacktriangle$ , respectively.

ously in euglobulins precipitated from diluted plasma by the direct addition of acid<sup>2-4</sup>.

**Euglobulins precipitated from plasma by neutral salts.** Euglobulins were precipitated from plasma by adding sodium chloride or ammonium sulphate to 100 or 33 per cent saturation respectively. The amount and composition of these fractions, which contained fibrinogen and  $\gamma$ -globulins<sup>5</sup>, were unaffected by variable plasma chondromucoprotein concentration.

It is postulated that fractions precipitated from acidified plasma, whether by direct addition of acid or by dialysis of plasma against distilled water containing dissolved atmospheric carbon dioxide, contain insoluble complexes formed by electrostatic interaction between anionic chondromucoprotein and those plasma proteins cationic at pH values below their isoelectric points, their composition and biological activities depending on the precipitating pH. In conformity with these conclusions, euglobulins precipitated on dilution and acidification of plasma collected from rabbits after papain injection, when the plasma chondromucoprotein concentration as a result of chondrolysis can be raised some 100-fold, are considerably bulkier than those precipitated from normal rabbit plasma<sup>4</sup>.

Euglobulins precipitated from neutral plasma, on the other hand, whether by salt fractionation or by dialysis against distilled water free of carbon dioxide, were not increased in amount in the presence of added chondromucoprotein. This result was expected since at neutrality most of the plasma proteins were negatively charged and therefore unavailable for electrostatic interaction with chondromucoprotein<sup>2,4</sup>.

It is evident that, in addition to the complex mechanisms known to be involved in the aetiology of euglobulins precipitated from diluted acidified plasma, electrostatic interactions must be added, involving chondromucoprotein, the presence of which profoundly affects the amount precipitated, their composition and their biological activities.

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## Labelling Rates and Detection of Intermediates in Mitochondrial Phosphorylations and other Sequential Reactions

Use of isotopes frequently provides a sensitive tool for detection of small concentrations of intermediates in metabolic sequences. Valuable information about the possible participation of an intermediate may often be obtained by measurement of rates of appearance of isotopic tracer in a possible intermediate and in a subsequent metabolic product. Such approaches have recently been applied to the assessment of possible phosphorylated intermediates in the formation of ATP by mitochondria, but important differences in the interpretation of the results have arisen<sup>1-3</sup>.

Slater *et al.*<sup>1</sup> expressed the view that the rate of labelling of any intermediate must equal or exceed the maximal rate of labelling of the product. In contrast, Bieber *et al.*<sup>2</sup>

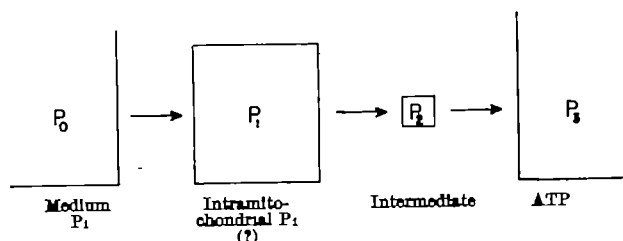


Fig. 1

felt that such a criterion was not applicable to many plausible metabolic situations. Later, Slater and Kemp<sup>3</sup> objected to the interpretations of Bieber *et al.*<sup>2</sup>. Although other items were also presented by Slater and Kemp<sup>3</sup>, they appropriately emphasized that: "The point under discussion is the validity of kinetic criteria for distinguishing between possible reaction pathways in mitochondria." It is toward this point that this communication is directed, in particular to a further assessment of the criterion previously expressed<sup>1</sup> and recently re-stated<sup>3</sup> as: "We wish to reaffirm our view that the criterion that a given compound is an intermediate in oxidative phosphorylation should be that the initial rate of its labelling from <sup>32</sup>P<sub>i</sub> should not be less than the maximal rate of labelling of the product." They assume that the initial rate will be the maximal rate of labelling of an intermediate, an assumption which will hold only under certain conditions. As will be demonstrated here, however, the criterion suggested by Slater *et al.*<sup>1,3</sup> is invalid because either the initial or the maximal rate of labelling of an intermediate in metabolically plausible sequences may be far less than the maximal rate of labelling of the product. Clarification of this point is desirable because an understanding of the essential features of possible labelling patterns of metabolically active intermediates present in low concentration has considerable applicability not only to mitochondrial phosphorylations but also to many other sequential reactions.

As an appropriate system to demonstrate the lack of applicability of the criterion suggested by Slater and Kemp<sup>3</sup> to a reaction intermediate, a sequence of reactions which might logically occur in mitochondrial phosphorylations may be considered. This sequence is one in which <sup>32</sup>P<sub>i</sub> from the reaction medium first enters a pool of intramitochondrial P<sub>i</sub>, thence passes through an intermediate, present in much smaller amount than the intramitochondrial P<sub>i</sub>, to form ATP. Such a system is indicated schematically in Fig. 1. Only unidirectional reactions need be considered because even such a simple system suffices for mathematical demonstration that the initial or the maximal rate of labelling of an intermediate may be considerably less than maximal rate of labelling of the product.

The point to be demonstrated for the scheme depicted in Fig. 1 is that the rate of appearance of tracer, added as P<sub>0</sub>, in the intermediate, P<sub>2</sub>, at any time will be considerably less than the maximal rate of appearance of tracer in the product, P<sub>3</sub>. For derivation of the relationships governing such a system, let the amount of radioactivity present in the various pools be C<sub>0</sub>, C<sub>1</sub>, C<sub>2</sub>, and C<sub>3</sub>, respectively, and the rate of formation of P<sub>3</sub> = r. Assume that synthesis of P<sub>3</sub> is proceeding at a constant rate, and that a trace of isotope is added to P<sub>0</sub>, with a resultant specific activity of C<sub>0</sub>/P<sub>0</sub>. Then for appearance of isotope in C<sub>1</sub>, the appropriate relationship is:

$$\frac{dC_1}{dt} = \frac{C_0 r}{P_0} - \frac{r C_1}{P_1} \quad (1)$$

which on integration gives:

$$C_1 = \frac{C_0 P_1}{P_0} (1 - e^{-rt/P_1}) \quad (2)$$

For the appearance of radioactivity in C<sub>2</sub>:

$$\frac{dC_2}{dt} = \frac{C_1 r}{P_1} - \frac{r C_2}{P_2} \quad (3)$$



Substituting from equation (2) into (3) and integrating gives:

$$C_2 = \frac{C_0 P_2}{P_1} \left( \frac{1 - P_1 e^{-rt/P_1} - P_2 e^{-rt/P_2}}{P_1 - P_2} \right) \quad (4)$$

For the appearance of radioactivity in  $C_2$ :

$$\frac{dC_2}{dt} = \frac{C_2 r}{P_2} \quad (5)$$

Substituting from equation (4) into (5) and integrating gives:

$$C_2 = \frac{C_0}{P_1} \left( rt + \frac{P_1^2 e^{-rt/P_1} - P_1^2 e^{-rt/P_2}}{P_1 - P_2} - P_2 - P_1 \right) \quad (6)$$

Alternatively, in a system in which the  $P_1$  pool is lacking, so that the sequence is simply  $P_0 \rightarrow P_2 \rightarrow P_3$ , the appearance of label in  $P_2$  would be given by the relationship:

$$C_2 = \frac{C_0 P_2}{P_0} (1 - e^{-rt/P_2}) \quad (7)$$

and the appearance in  $P_3$  by the relationship:

$$C_3 = \frac{C_0}{P_0} (rt - P_2 + P_2 e^{-rt/P_2}) \quad (8)$$

To demonstrate the relative labelling rates which might ensue in such a system, assume that  $r = 1$  (for example, 1  $\mu$ mole of ATP being formed per second),  $P_1 = 10 \mu$ moles,  $P_2 = 0.1 \mu$ mole, and  $C_0/P_0$ , after addition of isotope = 1 (for example, 1  $\mu$ curie of  $^{32}$ P per  $\mu$ mole). Calculations from equations (2), (4), and (8) give labelling patterns as illustrated in Fig. 2A. Note that the curve for  $C_2$  is multiplied by 50, and thus that the maximal rate of appearance in isotope into  $P_2$  far exceeds the maximal rate of appearance into the intermediate,  $P_3$ . Only at the very outset, where the rates and the extent of labelling of both  $P_2$  and  $P_3$  are very small, and thus would not be experimentally rate of appearance of label in the intermediate exceed the rate of appearance into product.

If the pool  $P_1$  were not present, the labelling of  $P_2$  and  $P_3$ , as calculated from equations (6) and (7), would be as measurable in most circumstances, does the actual illustrated in Fig. 2B. As contrasted to the situation when  $P_1$  participates, of particular importance is the expected rapid initial rate of labelling of  $P_2$  and the very small lag in the labelling rate of  $P_3$ . Such a system, in which  $P_2$  is formed directly from  $P_0$ , and  $P_3$  does not equilibrate with any other pool, fits the labelling pattern visualized by Slater and Kemp<sup>3</sup>, namely, the initial rate of labelling of the intermediate as the maximal rate and equal to the maximal rate of labelling of product.

In both systems depicted by Fig. 2A and Fig. 2B, the labelling of  $P_2$  meets a quite separate criterion previously

suggested by Bieber *et al.*<sup>2</sup> for an intermediate, namely, that an intermediate should approach maximal labelling as soon as or before the maximal rate of labelling of ATP occurs. Plots of percentage of maximal labelling, contrary to the suggestion of Slater and Kemp<sup>3</sup>, can thus be quite useful.

The disparity in labelling rates for  $P_2$  and  $P_3$  in a system as depicted by Fig. 1 can be readily quantitated for the values used in calculating the curves of Fig. 2A. The rate of labelling of  $C_2$  is given by the derivatives of equation (4), which is:

$$\frac{dC_2}{dt} = \frac{C_0 P_2 r}{P_1 (P_1 - P_2)} (e^{-rt/P_2} - e^{-rt/P_1}) \quad (9)$$

The time at which maximal rate of labelling of  $C_2$  occurs corresponds to that at which the second derivative of equation 4 equals 0. The appropriate relationship is:

$$\frac{d^2 C_2}{dt^2} = \frac{C_0 P_2 r^2}{P_1 (P_1 - P_2)} \left( \frac{e^{-rt/P_2}}{P_2} - \frac{e^{-rt/P_1}}{P_1} \right) \quad (10)$$

For conditions as in Fig. 2A, this derivative equals zero at  $t = 0.48$  sec, and the maximal labelling rate of  $C_2$  may be calculated from equation (8) to be 0.0096 units of radioactivity per second, whereas the maximal rate of  $P_2$  (ATP) labelling is  $r C_0/P_0$ , or 1. Thus even though  $P_2$  is an intermediate in  $P_3$  formation, the maximal rate of labelling of  $P_2$  would be only 0.96 per cent that of  $P_3$  in the reasonable model under discussion. Slater, Kemp, and Tager<sup>1</sup> observed that the maximal rate of labelling of bound phosphohistidine in mitochondria was 1.5 per cent of the rate of labelling of ATP. Clearly on this basis alone and as pointed out by Bieber *et al.*<sup>2</sup>, bound phosphohistidine cannot be excluded as an intermediate in formation of most of the mitochondrial ATP.

The important contributing factors to a slow labelling of the intermediate  $P_2$ , as compared to the labelling of  $P_3$  (ATP) from  $^{32}$ P<sub>i</sub> in the scheme of Fig. 1, are the participation of the relatively large pool,  $P_1$ , as a precursor to  $P_2$ , and the small size and rapid turnover of  $P_1$ . The latter features are quite plausible for any intermediate in oxidative phosphorylation. The occurrence of a lag in the labelling of ATP, well-demonstrated by the results of Slater *et al.*<sup>1</sup> and of Bieber *et al.*<sup>2</sup>, gives strong evidence for the existence of a pool of considerable size through which  $^{32}$ P<sub>i</sub> must pass or with which  $P_1$  may equilibrate during the synthesis of ATP. The extent of any lag is an obvious function of the size of this pool. This is illustrated by the very small lag in ATP labelling exhibited when the synthesis of ATP is considered to involve only the small amount of  $P_2$  as an intermediate (Fig. 2B), as compared with the participation of an appreciable pool (Fig. 2A). As mentioned previously, lag in  $P_2$  (ATP) labelling and a slow rate of  $P_3$  labelling would also result if  $P_1$  were a pool which rapidly equilibrated with  $P_2$ , rather than serving as a precursor. In such a case, no lag would be observed with labelling of the intermediate.

The model system used in this communication to illustrate the limitations of the labelling data considers only a net flux of reactants in one direction, homogeneity of the reactant pools, and lack of other pools which the reactants equilibrate with or pass through. Much more complex relationships might exist in mitochondria, and labelling patterns with flux in both directions could be assessed by tracer methodology as elegantly discussed by Sheppard<sup>4</sup>. Increasing complexity of systems would not relieve the limitations of the simple model, and could introduce additional limitations.

The discussion of labelling patterns of intermediates is an outgrowth of experiments on

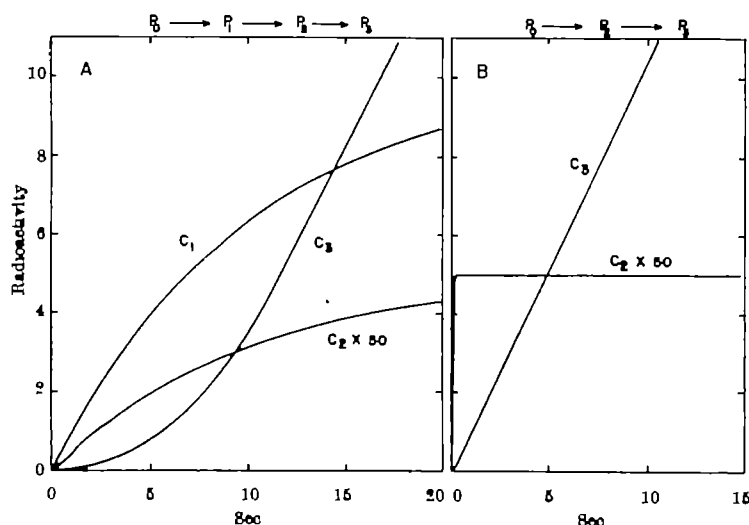


Fig. 2

the metabolic role of bound phosphohistidine<sup>1,2</sup>, and earlier suggestions from this laboratory that phosphohistidine appeared to be an intermediate in oxidative phosphorylation. The most definitive information about the metabolic role of this substance in mitochondria has come, not from measurements of labelling rates, but from observations of the close association of bound phosphohistidine with succinate thiokinase<sup>3,4</sup>. Much or all of the bound phosphohistidine of mitochondria may participate in the succinate thiokinase reaction, but participation of some of the phosphohistidine in other mitochondrial processes remains a distinct possibility<sup>5</sup>.

In summary, in sequential reactions from an initial reactant to a product, with addition of isotopic tracer to the reactant, a compulsory intermediate may show a much smaller maximal rate of labelling than the product. This is the case when the intermediate is present in small concentrations, has a rapid turnover, and is formed from a pool or substance of higher concentration initially labelled by the reactant. Similar considerations would hold if the intermediate were formed directly from the reactant but equilibrated with the other pool or substance before forming product. As applied to mitochondrial oxidative phosphorylations, this means that a phosphorylated intermediate might show a much slower rate of labelling from <sup>32</sup>Pi than does ATP.

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BASED on his observation that mitochondrial protein-bound phosphohistidine becomes maximally labelled with added radioactive inorganic phosphate before maximum labelling of the mitochondrial ATP is reached, Prof. Boyer concluded in 1963 that protein-bound phosphohistidine is an intermediate in respiratory-chain phosphorylation<sup>1</sup>. By the summer of 1964, it was generally accepted that phosphohistidine is not on the main pathway of synthesis of mitochondrial ATP, but on the relatively slow side-path catalysed by succinyl-CoA synthetase and nucleosidediphosphate kinase. We had already pointed out early in 1964 that the kinetics of the incorporation were inconsistent with Boyer's theory and supported phosphohistidine as an intermediate on a slow side-path<sup>2</sup>.

What are the lessons to be drawn from this experience? Our conclusion is that the criterion for an intermediate of respiratory-chain phosphorylation used by Prof. Boyer is insufficient and that the more rigorous criterion emphasized in our recent communication<sup>3</sup> should be adopted. This is that the initial rate of labelling of the proposed intermediate should not be less than the maximal rate of labelling of the ATP. Prof. Boyer now states that there are circumstances in which the maximal rate of labelling of the intermediate could be very much less than the maximal rate of labelling of the ATP.

I accept that, under the conditions specified by Prof. Boyer, this would be the case. However, I do not agree that these conditions are likely to apply. In the model  $P_0 \rightarrow P_1 \rightarrow P_2 \rightarrow P_3$ , the transformation  $P_0 \rightarrow P_1$  represents the mixing of the medium inorganic phosphate with intramitochondrial inorganic phosphate, which is of

quite a different character from the chemical transformations  $P_1 \rightarrow P_2$  and  $P_2 \rightarrow P_3$ . There is an important implication buried in the assumption that, immediately after adding radioactive inorganic phosphate, the rates of all transformations would be the same. This would only be the case if the rate-limiting step of oxidative phosphorylation (or of the inorganic phosphate-ATP exchange reaction) were the penetration of added inorganic phosphate into a mitochondrial pool of inorganic phosphate. Is this likely? Is it not more likely that the rate of the transformation  $P_0 \rightarrow P_1$  will be greater than that of the other transformations, and that  $P_2$ , and therefore  $C_2$ , will rapidly decrease immediately after adding the radioactive phosphate? If this is the case, the mathematical treatments leading to equations 1, 2, 4 and 6 would be invalid.

The linear kinetics of labelling of phosphohistidine up to almost maximum labelling, previously reported (Fig. 2, ref. 2), shows that this labelling does not follow the model proposed by Prof. Boyer. The initial rate of labelling may be accurately calculated. Thus, the simpler assumption, which we made, namely that the mixing of added inorganic phosphate with a pool of mitochondrial phosphate is not rate-limiting in phosphohistidine labelling, would appear to be justified.

Prof. Boyer<sup>4</sup> argues that the lag in ATP labelling which we found shows the existence of a pool of considerable size through which radioactive phosphate must pass during the synthesis of ATP. Whether or not this is the correct explanation of the lag, it would not cause an over-estimate of the ATP labelling and would not, therefore, affect our conclusion that the rate of labelling of the ATP was much greater than that of the phosphohistidine.

Everyone agrees that there is now no evidence that phosphohistidine is an intermediate of respiratory-chain phosphorylation. The only question at issue is: what criterion should we apply to the next claim, that a labelled phosphate component of mitochondria is the long-sought 'X~P' of oxidative phosphorylation? Prof. Boyer reiterates that his criterion is sufficient, and that a slow rate of labelling of the proposed X~P can be accounted for by the considerations which he brings forward. I fear that adopting this criterion alone might lead once again to an unfounded claim. I suggest that, instead of explaining away a slow rate of labelling, conditions be sought in which these considerations do not apply, as is the case with phosphohistidine. For example, under some conditions there is no measurable lag in measuring the incorporation of radioactive phosphate into ATP, so that a "pool of considerable size" is not always present.

I agree that there are circumstances in which a phosphorylated intermediate might show a much slower rate of labelling from radioactive phosphate than does ATP. An example where we had to take this possibility into consideration comes from some recent work of Mr. A. Kemp, Jun., and Dr. R. B. Tobin in this laboratory. They found that, in the same experiment, the relative rates of labelling of phosphohistidine, Beyer's phosphoprotein<sup>4</sup> and ATP were 1.8:0.14:100. Since we have already excluded phosphohistidine as an intermediate, it might appear easy to exclude the still more slowly labelled Beyer phosphoprotein. However, there is a special feature of the labelling pattern in this case which precludes us from applying the same argument which we applied to the case of phosphohistidine. When the percentage of maximum labelling is plotted against time (Prof. Boyer's procedure), the curves for ATP and the Beyer protein fall on the same line. It is, in this case, possible that the rate of the reversible reaction  $X \sim P + ADP \rightleftharpoons X + ATP$  is so much faster than the rate of formation of X~P that the measured rate of incorporation of radioactive phosphate into X~P represents only the rate at which ATP reaches isotopic equilibrium, and nothing can be said about the rate of incorporation of inorganic phosphate into X~P. Thus, although Beyer's phosphoprotein fulfils Prof. Boyer's

criterion, I am not yet prepared to accept it as an intermediate of respiratory-chain phosphorylation. However, although it does not fulfil my criterion, a special circumstance does not allow us as yet to exclude it as an intermediate. Further experiments are required. Incidentally, there was no time lag of labelling of ATP in this experiment.

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### Carbamyl Phosphate as an Essential Component of the Flattening Factor for Cells in Culture

A COMPOUND of low molecular weight which causes attachment and flattening of cells in culture was prepared from the growth-promoting  $\alpha$ -globulin<sup>1</sup>. The protein from which this low-molecular compound was isolated was prepared by chromatography on 'DEAE'-cellulose<sup>2</sup>; in further experiments it was found that this protein could be inactivated by filtration through 'Sephadex G-25'. Reactivation occurred after incubation of this protein with  $\text{NaH}_2\text{PO}_4$  or with  $\text{NaHCO}_3$ ; if not incubated with salts, the protein caused flattening of cells in 4–8 h, so that the process of flattening was prolonged.

These findings suggested that the active factor contained a compound of low molecular weight, capable of binding an inorganic salt. For this reason, the active protein was chromatographed in a butanol-acetic acid-water mixture (4:1:5) without deproteinization. Staining with bromocresol green disclosed two components,  $R_F$  0.08 yellow and  $R_F$  0.18 blue; this active system also contained a protein which remained at the starting spot. On chromatograms treated with ninhydrin one-colour reaction only was obtained. The same  $R_F$ -values and the same reactions were obtained with carbamyl phosphate prepared according to the method of Spector, Jones and Lippman<sup>3</sup>.

To investigate the significance of carbamyl phosphate for cultivation, HeLa cells were used. The cells were incubated in a synthetic medium<sup>4</sup> containing carbamyl phosphate in a concentration of 20–200  $\mu\text{g}$  per ml. At 37° C the cells attached to glass within 1 h and within 20 h they were all flattened, but they did not flatten as well as in a medium with  $\alpha$ -globulin. It was further observed that on supplementation of this synthetic medium with insulin the cells flattened well.

These results were not unexpected, because the crystalline carbamyl phosphate prepared by direct crystallization from the active protein behaves in the same way. In contrast to the crystalline carbamyl phosphate, a low-molecular compound prepared from the same protein after incubation with erythrocytes, and causing a flattening of cells to the same extent as the growth-promoting  $\alpha$ -globulin, contained at least ten amino-acids in acid hydrolysate<sup>4</sup>.

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### Decomposition of Benzhydrol catalysed by Boric Acid

In a recent investigation of the secondary decomposition mechanisms in the pyrolysis of esters, it was discovered that the borate of benzyl alcohol decomposes to anthracene and resinous substances<sup>1</sup>. This result supports the contention of Chapman and Borden<sup>2</sup> that the elimination involves an ionic mechanism.

In an attempt to extend the synthetic capabilities of this reaction, the pyrolysis of benzhydrol was undertaken. The pyrolysis was conducted in the usual manner: the alcohol (1.84 g, 0.01 m) and boric acid (0.62 g, 0.01 m) were mixed in a 'Pyrex' tube and slowly heated to 140°. After 2 h the temperature of the heating bath was slowly raised to 280°–290°. Decomposition was complete after 1 h. The crude products were then dissolved in benzene and chromatographed on alumina. Elution with benzene-petroleum ether gave three products; benzophenone (0.133 g), diphenylmethane (0.085 g) and *s*-tetraphenylethane (0.248 g). Neither 9,10-diphenylanthracene nor other fluorescent derivatives were located.

To explain the different pyrolytic routes of benzhydrol and benzyl alcohol, it was suggested that benzhydrol may have undergone ether formation rather than the expected borate ester formation as in the case of benzyl alcohol. This explanation is reasonable, since benzhydrol ether does give rise to these products under thermal conditions<sup>3,4</sup>, and, theoretically, the rate of ether formation in the benzhydrol reaction would be expected to be faster than benzyl ether formation, since the activation energy required for the formation of diphenylmethyl carbonium ion would be lower than the energy required for the benzyl cation. In an attempt to support this concept, the pyrolysis was repeated; however, in this experiment the degradation was conducted only at the lower temperature for 2 h. At the end of this time, the contents of the tube were cooled and then extracted with ether. On evaporation of the solvent, crystals of benzhydrol ether (1.50 g, 85 per cent) were deposited. The compound was identified by infra-red analysis and mixed melting point determinations.

Under these conditions, therefore, the pyrolysis of benzhydrol appears to involve the formation of benzhydrol ether, and the final products are produced from the decomposition of this intermediate.

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### Pregnant Mare Serum Gonadotrophin Potency: Effect of Single and Multiple Injections

It has been demonstrated that a single subcutaneous injection of pregnant mare serum gonadotrophin (PMSG) is as effective as multiple injections of the same amount of PMSG on the weight increase of the immature rat ovary<sup>1,2</sup>. However, it was later shown that multiple injections of PMSG were more effective in producing an increase in testis weight of three-day-old cockerels than a single injection of the gonadotrophin<sup>3</sup>. Because of these opposing views, it was of interest to re-investigate the chick testis response with a more sensitive assay method.

An assay was developed that made use of the incorporation of inorganic phosphorus-32 into testes of two-day-old cockerels as the end-point for gonadotrophin activity<sup>4</sup>. The phosphorus-32 end-point was more sensitive than gonad weight change, and the incorporation of this isotope

Table 1. UPTAKE OF PHOSPHORUS-32 BY CHICK TESTES AFTER SINGLE AND MULTIPLE PMSG INJECTIONS

Table 1. UPTAKE OF PHOSPHORUS-32 BY CHICK LIVERS AFTER SINGLE AND TRIPLE INJECTIONS OF PMSG						
Treatment with PMSG (I.U.)	One injection		Three injections			
	c.p.m./mg $\pm$ S.D.	Increase (%)	c.p.m./mg $\pm$ S.D.	Increase (%)		
0.025	43.48 $\pm$ 13.88	43	34.83 $\pm$ 6.25	15		
1.25	54.02 $\pm$ 18.21	78	45.94 $\pm$ 8.03	51		
2.50	62.16 $\pm$ 15.23	106	56.54 $\pm$ 12.26	86		
	Low to 0.025	Middle to 1.25	High to 2.50	Low to 0.025	Middle to 1.25	High to 2.50
Dosage of PMSG (I.U.)						
Sum (c.p.m./mg)	782.74	973.28	1,118.95	626.99	823.90	1,017.44
Factorial analysis						
	Sums	Mean squares	F			
$S_y^2$	26,062.70	843.58	1.45*			
Standard vs. unknown	1,499.61	1,499.61	8.91†			
Combined slope	7,337.86	7,337.86	43.00†			
Lack of parallel	41.16	41.16	0.24			
Combined curvature	12.54	12.54	0.07			
Opposed curvature	5.26	5.26	0.03			
Error	17,166.28	166.80				
Relative potency						
$M' = \frac{447\%}{37\%} = 59.95$ per cent						

f for groups, 18; N for sample, 108. Control c.p.m./mg, 30.36. Each chick received 5.5  $\mu$ c. phosphorus-32. Slope, 33.54. †, 0.3867.  
\* Significant at greater than 0.05. † Significant at greater than 0.01.

Table 2. UPTAKE OF PHOSPHORUS-32 BY CHICK TESTES AFTER SINGLE AND MULTIPLE PMSG INJECTIONS

Table 2. UPTAKE OF PHOSPHORUS BY CHICK LIVERS AFTER SINGLE AND MULTIPLE INJECTIONS					
Treatment PMSG (I.U.)	One injection		Three injections		
	c.p.m./mg $\pm$ S.D.	Increase %	c.p.m./mg $\pm$ S.D.	Increase %	
1.25	37.78 $\pm$ 5.12	60	37.35 $\pm$ 4.33	16	
2.50	49.10 $\pm$ 9.78	108	37.95 $\pm$ 6.18	60	
5.00	56.83 $\pm$ 6.06	140	47.76 $\pm$ 5.79	102	
	Lowest	Middle	Highest	Lowest	Middle
	1.25	2.50	5.00	1.25	2.50
Dosage of PMSG (I.U.)					
Sums (c.p.m./mg)	755.70	963.80	1,126.53	546.97	768.97
Factorial analysis					
	Sums	Mean squares		F	
$S_y^2$	15,714.77	132.06		3.18*	
Standard vs. unknown	3,145.83	3,145.83		75.82*	
Combined slope	7,781.90	7,781.90		187.66*	
Lack of parallel	9.37	9.37		0.23	
Combined curvature	33.88	33.88		0.82	
Opposed curvature	14.80	14.80		0.34	
Error	4,720.40	41.40			
Relative potency					
$M' = \frac{447\%}{37\%} = 48.7$ per cent					

f for groups, 20; N for sample, 120. Control c.p.m./mg, 23.64. Each chick received 3.0  $\mu$ c. phosphorus-32. Slope, 33.77. †, 0.1965.  
\* Significant at greater than 0.01.

was linear for the log-dose response line with  $\mu$ g quantities of follicle-stimulating hormone and interstitial-cell-stimulating hormone. In addition, it has been shown that the uptake of phosphorus-32 is also linear with the log-dose of PMSG treatment between 0.025 and 20.00 International Units (I.U.)<sup>6</sup>. The PMSG-chick testis response was re-investigated by the phosphorus-32 assay method, rather than gonad weight change, because of the greater sensitivity of the isotopic end-point.

In Tables 1 and 2 the uptakes of phosphorus-32 by testes in chicks which received a single hormone injection 36 h before autopsy are compared with the uptakes by testes in chicks which received the hormone in 3 injections 36, 24, and 12 h before autopsy. Both experiments indicated that greater testicular stimulation (measured by incorporation of phosphorus-32) occurred with the single PMSG injection. The two experiments cannot truly be compared because the quantity of phosphorus-32 injected into the assay chicks was not the same for both experiments; however, it can be noted that increasing amounts of PMSG caused an increased uptake of phosphorus-32. The phosphorus-32 incorporation was linear when related to the log-dose of PMSG for both experiments.

The data in Table 1 indicate that small quantities of serum gonadotrophin (0.025 I.U.) could be assayed with this phosphorus-32 method. The slope of the phosphorus-32 c.p.m./mg log-dose response line was 33.54, and the percentage increases showed good differential responses with the various treatments. With all treatments, the effectiveness of the PMSG, measured by the incorporation of phosphorus-32, was much greater when administered as a single injection. These data were analysed by a balanced factorial analysis<sup>7</sup>. The results indicated that the standard treatments (single injection) differed significantly from the unknown treatments (multiple injections), but both log-dose response lines were parallel. This response

indicated that these preparations differed only in potency, and therefore the relative potency of the unknown treatments was calculated. It was found that PMSG, administered in three injections, was only 59.9 per cent as potent as the same amount of hormone given in a single injection.

To determine if this type of response could be duplicated, another experiment, using slightly greater concentrations of PMSG, was performed. These data are presented in Table 2, and the results are similar to those of the previous experiment. The single PMSG injection caused a much greater incorporation of phosphorus-32 than did the same amount of gonadotrophin administered in three injections. The relative potency was calculated, and it was found that the multiple injections of PMSG were only 48.7 per cent as potent as the single gonadotrophin injections.

These results support the earlier observations which indicated that a single PMSG injection was as effective as were multiple injections. The explanation for this phenomenon is that the molecular size of PMSG, 68,000 (ref. 7), is too large to be cleared by the kidney, and consequently, it remains in the circulation until utilized or degraded. This explanation would seem at present to be the best one for this phenomenon.

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### Creatine Phosphokinase Isoenzymes

CREATINE phosphokinase (adenosine triphosphate: creatine phosphotransferase) has been shown by electrophoretic separation on agar gel and starch gel to exist in multiple molecular forms (isoenzymes)<sup>1-3</sup>. This communication describes a staining technique for creatine phosphokinase (CPK) and its application to the demonstration of CPK isoenzymes separated on cellulose acetate membranes.

Tissue homogenates and sera (5  $\mu$ l.) are separated on cellulose acetate membranes<sup>4</sup> in barbitone buffer pH 8.6, using a constant current of 0.5 m. amp/cm for 1.25 h. A staining solution the composition of which is based on Oliver's method for CPK determination<sup>5,6</sup> is prepared as follows:

Adenosine-5-diphosphate sodium salt (C. F. Boehringer and Son), 2 mg; hexokinase (C. F. Boehringer and Son), 10  $\mu$ l. (2.8 i.u.); glucose-6-phosphate dehydrogenase (C. F. Boehringer and Son), 10  $\mu$ l. (1.4 i.u.); glucose, 2 mg; magnesium sulphate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ), 7 mg; nicotinamide adenine dinucleotide phosphate (NADP, or TPN; C. F. Boehringer and Son), 3 mg; MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide; (George T. Gurr, Ltd.), 1.6 mg 0.05 M tris buffer pH 7.5, 3 ml.

This solution is divided into equal parts, and 5 mg creatine phosphate (C. F. Boehringer and Son) added to half to constitute the test solution, the remaining half serving as a control solution. Immediately prior to use, 80  $\mu$ l. of phenazine methosulphate (*N*-methyl-phenazonium methosulphate; British Drug Houses, Ltd.), freshly dissolved in distilled water at a concentration of 1 mg/ml., are added to both test and control.

Following electrophoretic separation, the cellulose acetate is divided longitudinally, and one half-strip is stained for CPK activity using the test solution. The remaining half-strip is stained with the control solution. For staining, a layering technique is used<sup>7</sup>, the half-strip being layered on to an additional cellulose acetate strip which has been allowed to soak up staining solution. Both strips are incubated in a moist chamber for 1 h at 37° C.

Fig. 1 illustrates the results obtained by the examination of adult human muscle homogenates. Muscle was homogenized at a concentration of 50–100 mg wet weight of tissue per ml. in distilled water, using a Potter-Elvehjem homogenizer. The homogenate was lightly centrifuged (2,000 r.p.m.) for 10 min, and the supernatant diluted with distilled water to yield a CPK activity of approximately 2,000–4,000 i.u. per litre by Hughes's method<sup>8</sup>. Two sera of activities 5,500 and 3,900 i.u. per litre, from patients with muscular dystrophy of Duchenne type (kindly supplied by Dr. B. P. Hughes of the National Hospital, London), were also examined.

Cardiac muscle showed a major staining band with a mobility intermediate between that of serum  $\beta$ - and  $\gamma$ -globulins. A minor component with a mobility of that of an  $\alpha$ -2 globulin was also demonstrable. No staining of

the control strips was observed. A similar isoenzyme pattern was obtained with three specimens of skeletal muscle shown by histochemical and biochemical means to be composed chiefly of 'red' muscle fibres<sup>9</sup>. Three specimens of skeletal muscle similarly shown to be composed chiefly of 'white' muscle fibres showed only the major isoenzyme band. The sera from the two patients with muscular dystrophy showed only the major component.

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### Effect of Urea on the Cold Precipitation of Protein in the Lens of the Dogfish

WHEN lenses of young rats are cooled below 10° C they become reversibly opaque due to the precipitation of a cold-precipitable protein. This fraction is prepared by homogenizing rat lenses in several volumes of water and cooling the 105,000g supernatant to 0° C. Repeated solubilization at 20° C and precipitation at 0° C serves as a means of purification<sup>1</sup>. The cold-precipitation phenomenon is thought to be caused by changes in protein conformation due to a disturbance of the balance between hydrogen and hydrophobic bonding of protein chains at lower temperatures.

Purified rat lens cold-precipitable protein was found to be homogeneous in the analytical ultracentrifuge (with a 4s sedimentation rate at 20° C), but was shown to be heterogeneous by means of acrylamide gel electrophoresis, agar-plate immunodiffusion, and 'Sephadex G-100' gel filtration investigations<sup>2</sup>. Cold-precipitable protein was found to contain species similar to the  $\alpha$ -,  $\beta$ - and  $\gamma$ -crystallin protein fractions of the lens. Cold precipitation was dependent on the concentration of protein, the ionic strength and the pH of the solution containing cold-precipitable protein.

Further work indicated that a urea level of 0.25–0.3 M tended to inhibit the phenomenon of cold precipitation<sup>3</sup>. This observation led to the present investigation of the effect of the relatively high tissue concentration of urea (0.25 M) on dogfish lens cold-precipitable protein.

Initial investigations demonstrated that intact dogfish (*Mustelus canis*) lenses did not become opaque on cooling. Preparation of the dogfish lens cold precipitable protein fraction therefore required extensive dialysis of the 105,000g supernatant against water in order to remove urea and thus to allow cold precipitation to occur. When 0.25 M urea was added back to a preparation of cold-precipitable protein, cold precipitation was prevented.

Dogfish lens cold-precipitable protein exhibits characteristics similar to that of the rat lens. It decreases in concentration as the animal ages from a level of 17 per cent of the total soluble protein in the young dogfish and 35 per cent in the foetal rat lens to 5–7 per cent in the lenses of older animals. In both species the cold-precipitable protein fraction contains 1.5 per cent (by weight) RNA. Ultracentrifugal analysis of the dogfish or rat lens cold-precipitable protein at 20° C (pH 8.0) reveals a single peak with a sedimentation rate of 4S as compared to the

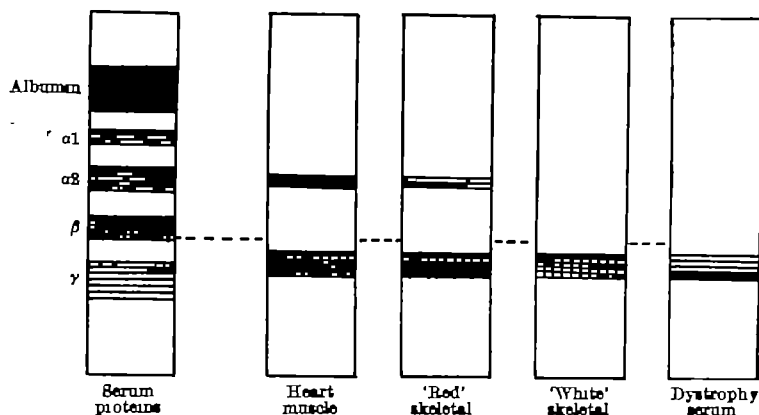


Fig. 1. Creatine phosphokinase isoenzymes of muscle

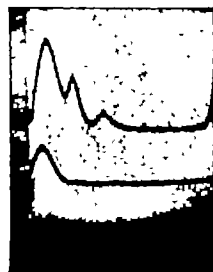


Fig. 1



Fig. 2

Fig. 1. Schlieren patterns obtained with the Beckman-Spinco model B analytical ultracentrifuge for 1.5 per cent cold-soluble dogfish lens protein fraction (above) and for 1 per cent cold-precipitable protein (below) in water. The print was made 20 min after rotation at 56,100 r.p.m. at 20° C. The upper pattern represents the sedimentation of  $\alpha$ -,  $\beta$ - and  $\gamma$ -crystallins with  $S$  values of 19, 13 and 8. The peak in the lower pattern had a 4S rate.

Fig. 2. Effect of acid (pH 3.0) and lowered temperatures (4° C) on the ultracentrifugal pattern of 1 per cent cold-precipitable protein of the dogfish lens. The print was made 20 min after rotation at 56,100 r.p.m.  $S$  values of 13 and 8 were obtained for the first and second peaks respectively.

19S, 13S and 8S values of both rat and dogfish lens  $\alpha$ -,  $\beta$ - and  $\gamma$ -crystallins (Fig. 1). At 4° C and pH 3.0 the single peak is replaced by a minor 8S and a major 13S peak for the dogfish (Fig. 2) as compared with a 8S and 17S peak for the rat preparation.

Gel filtration of purified dogfish lens cold-precipitable protein on 'Sephadex G-100' columns in 0.025 M *tris* buffer (pH 7.4) separates two components as shown in Fig. 3. The first contains two species of lens protein similar in sedimentation rate to that of  $\alpha$ - and  $\beta$ -crystallins; the second peak contains a single species with a sedimentation rate similar to that of  $\gamma$ -crystallin. (The separation of beef lens soluble protein by 'Sephadex' gel filtration was first reported by Bjork<sup>4</sup>.) When the  $\alpha$ -crystallin-type components of the first peak were separated from the  $\beta$ -crystallin-type components (by isoelectric precipitation of the  $\alpha$ -crystallin at pH 5.0) and protein estimations (Lowry procedure) were done on the three sub-fractions,  $\alpha$ - and  $\beta$ -crystallins contributed approximately 20 per cent each and  $\gamma$ -crystallin the remainder.

The experiments so far show that the dogfish lens contains a cold-precipitable protein fraction similar in physical and chemical properties to that of mammalian lenses. They indicate further that 0.25–0.3 M urea can in some way affect the interactions between lens proteins in solution. Finally, it appears that the relatively high concentration of urea in the dogfish lens is a factor in maintaining the clarity of the lens at environmental temperatures below 10° C.

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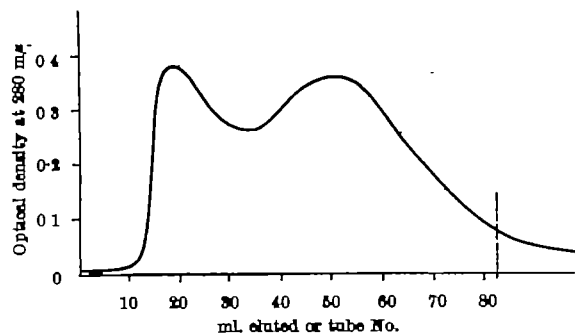


Fig. 3. Elution pattern obtained by passing 20 mg of cold-precipitable protein in 0.5 ml. of 0.025 M *tris* (pH 7.4) buffer through a column of Sephadex G-100 (20 cm x 1.5 cm) suspended in 0.025 M *tris* (pH 7.4) buffer. Each fraction collected contained 1 ml. of elution fluid. The first peak represents  $\alpha$  and  $\beta$ -crystallin components, whereas the second represents  $\gamma$ -crystallin components of the cold-precipitable protein.

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## PHYSIOLOGY

### Evaporative Cooling in the Pig

ALTHOUGH the pig has well-developed structures in the skin which conform morphologically with apocrine sweat glands, the animal does not appear to sweat. Measurements made by Moritz and Henriques<sup>1</sup> and Ingram<sup>2</sup> showed that at temperatures below the critical temperature, cutaneous water-loss over the general body surface, but excluding the snout, is similar to that in man and other animals. At high environment temperatures, however, even when body temperature is elevated, water-loss from pig skin is only of the order of 80 g/m<sup>2</sup> h and can be accounted for by the increased vapour pressure gradient consequent on the increase in skin temperature which occurs after vasodilatation. Confirmation of a low evaporative heat loss also derives from observation made on the new-born pig by Mount<sup>3</sup>, who estimated that the increased evaporative loss at high ambient temperatures could be accounted for by losses from the respiratory tract alone.

Under very humid conditions water passes into the skin of pigs from the atmosphere against the vapour pressure gradient<sup>4</sup>, as it does in man<sup>5,6</sup>, but while in man, who sweats, there is a net loss of water, in the pig there is a net gain<sup>4</sup>. As a result of this low evaporative loss, the pig is only slightly affected by changes in humidity and is not tolerant of high ambient temperatures<sup>4</sup>.

Under natural conditions, however, pigs wallow in any available mud or even their own urine and therefore achieve a greater evaporative loss than the above measurement would suggest. In order to obtain a measure of the effectiveness of this means of cooling, observations were made on the evaporative loss from the flank of the pig smeared with mud or made thoroughly wet with water. The rate of water-loss was measured by means of the ventilated capsule<sup>7</sup> used previously on the pig<sup>3,4</sup>.

The results of an experiment in which a pig was exposed to 35° C dry bulb and 21° C wet bulb, and water-loss was recorded continuously on a chart are displayed in Fig. 1. At first the capsule sampled room air (zero water-loss). It was then strapped on to the animal (A) to register an evaporative rate of 30–40 g/m<sup>2</sup> h. When mud was smeared over the flank of the animal (B) the evaporation rate increased rapidly to about 800 g/m<sup>2</sup> h, and remained at this level for more than 1 h. The dotted line in Fig. 1 indicates the rise in evaporation rate after soaking the flank with water. In both instances the evaporation rate rose quickly, but using water the rate soon declined again, while in the experiment using mud the control value had still not been reached after more than 2 h, when the capsule was again allowed to sample room air (C).

The behaviour of the pig thus transforms the animal from one in which evaporative heat loss is small (Table 1) to one in which the evaporation rate is comparable with that of man. Moreover, since the evaporative rate remains high for a long period after the application of mud, the

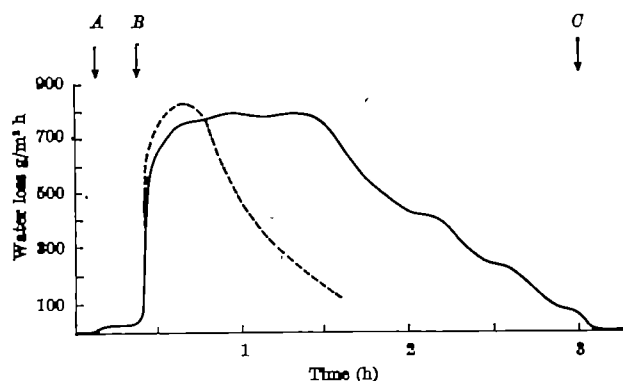


Fig. 1. Hypocutaneous water-loss from the skin of a pig measured by the ventilated capsule technique. At A the capsule was placed on the skin. At B mud (continuous line) or water (dotted line) was smeared over the skin. At C the capsule was removed from the skin surface.

Table 1. WATER-LOSS FROM THE SKIN OF VARIOUS SPECIES (g/m² h)

Species	Below 30°C dry bulb	Above 30°C dry bulb
Pig <sup>a</sup>	7-16	24-82
Sheep <sup>a</sup>	12	63
European cattle <sup>a</sup>	12-16	67-144
Man (at rest) <sup>10,11</sup>	6-10	150
Man (at work) <sup>12</sup>	—	1,200

animal is free to move some distance away from the mud wallow. Even sweating man must have access to water, but while man faces a problem of salt balance as a result of sodium chloride loss in the sweat, the pig avoids the problem. Its bristle coat probably helps to retain mud on the skin; moreover, in domestic pigs at least, the coat is too sparse to prevent contact with the skin, with the result that the evaporative cooling can be expected to cool the skin directly. If, on the other hand, the coat was thick and close, the mud might not be in contact with the skin and hence the efficiency of the cooling would be impaired.

Thus the pig, which at first appears to be unsuited to hot climates, has potentialities of high heat tolerance by virtue of its behaviour.

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### Chronic Thiouracil Feeding and Accumulation of <sup>125</sup>I in some Organs of C<sub>3</sub>H Mice

For many years radioactive iodine has been used as an indicator of iodine metabolism by the thyroid gland. Compounds such as thyroxine, labelled with <sup>125</sup>I, have served not only for that purpose, but also to study the peripheral metabolism of thyroid hormones. Gross and Schwartz<sup>1</sup> and Corey and Gross<sup>2</sup> have found that radioactive thyroxine accumulates in the liver and mammary gland tumours of C<sub>3</sub>H mice.

Thiourea compounds such as thiouracil (TU) and propylthiouracil (PTU) have been valuable tools in clarifying thyroid physiology. There is abundant experimental evidence to show that these goitrogens exert a thyroidal effect by blocking the synthesis of the gland's secretions and also an extra-thyroidal action on the peripheral

metabolism of thyroxine. Escobar del Rey<sup>3</sup> in rats, and Slingerland and Burrows<sup>4</sup> in humans, have presented enough data to indicate that TU and PTU interfere with peripheral de-iodination of thyroid hormone(s).

The experiments performed with rats by most of these researchers were done in animals given the goitrogens for periods not exceeding a few weeks. In our chronic experiments with C<sub>3</sub>H mice fed 0.3 per cent TU diet for periods of more than 11 months we investigated the iodine accumulation by gastrocnemius muscle, liver and intestine, in the hope of finding some more evidence that would indicate the extra-thyroidal effect of this goitrogen on iodine metabolism.

Three experimental and 3 stock-fed control animals were injected with <sup>125</sup>I after 11 months on the TU dietary regimen. Another 3 experimental mice and 3 stock-fed controls were similarly injected 16 months after feeding TU. Each mouse received subcutaneously 33.6 µc. of carrier-free <sup>125</sup>I as NaI. The radioactivity of the organs was determined with a Geiger-Müller counter 24 h after injection. The tissue samples were washed with isotonic saline and, after being weighed and minced, were placed in small, stoppered glass bottles for radioactivity readings. The amount of <sup>125</sup>I recovered from each of the 3 organs studied is shown by Fig. 1.

An analysis of variance of the results revealed a statistically significant difference ( $P < 0.001$ ) in the <sup>125</sup>I retained 24 h after injection between the different organs of mice given TU. There was a significant difference between muscle and intestine, and muscle and liver, but not between intestine and liver. The increasing iodine retention was especially noticeable in liver and intestine.

A statistically significant difference was also found between the amount of <sup>125</sup>I retained by all organs in the control animals and in those given TU for 11 months and for 16 months. Likewise, the difference in the amount of <sup>125</sup>I in the organs after 11 months and after 16 months of treatment was significant.

The foregoing results showed that 24 h after injection of <sup>125</sup>I, C<sub>3</sub>H mice, chronically fed a 0.3 per cent thiouracil (TU) diet for 11 or 16 months, have a significantly increased retention of the <sup>125</sup>I in gastrocnemius muscle, intestine and liver when compared with control animals fed no goitrogen. This may represent a reduced utilization

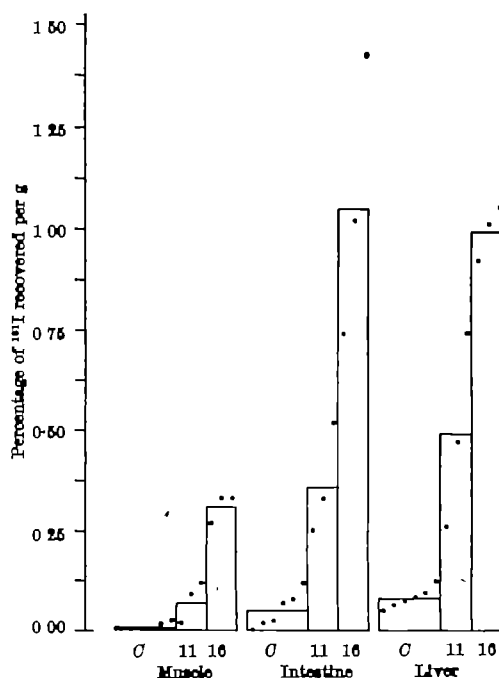


Fig. 1. Graph showing percentage recovery of <sup>125</sup>I from muscle, intestine and liver. C, controls, 11, 11 months on TU, 16, 16 months on TU.



of iodinated compounds and/or a decrease in the rate of  $^{125}\text{I}$  excretion in this strain of mice treated with TU. The iodinated compounds may be in the form of thyroid secretion or in the form of unknown  $^{125}\text{I}$ -labelled material.

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### Effect of Rifamycin on Protein Synthesis

In an earlier paper<sup>1</sup> we reported that rifamycins  $M_{14}$  (diethylamide of rifamycin B) and SV inhibited  $^{14}\text{C}$  L-isoleucine incorporation by cell-free extracts of *B. subtilis* strain ATCC 6633.

L-Isoleucine incorporation into protein was dependent on the addition of DNA to the reaction mixture, and the results of our experiments did not allow us to decide whether the inhibition of the incorporation of amino-acids, caused by rifamycin, was connected with an effect of the antibiotic on the synthesis of messenger RNA or with an inhibition of protein synthesis at the ribosome level; activation of amino-acids was not affected by rifamycins<sup>1</sup>.

We now report the effects of rifamycin  $M_{14}$  on  $^{14}\text{C}$  uracil incorporation into trichloroacetic acid (TCA)-insoluble material and on the *in vitro* incorporation of amino-acids into proteins in the presence of polyuridylic acid (poly U) and of high molecular weight RNA from *B. steierthermophilus*.

*B. subtilis* strain ATCC 6633 was cultivated and the extracts prepared as previously reported<sup>1</sup>. In all experiments of *in vitro* protein synthesis the supernatant of centrifugation at 80,000g (S-30) was prepared by the method reported by Matthaei and Nirenberg<sup>2</sup>.

Polyuridylic acid was a Mann product; stimulation of phenylalanine incorporation could be obtained only when

Table 1

Additions	C.p.m./mg protein	Percentage inhibition
Complete mixture	210	
Complete mixture + 0.03 $\mu\text{moles/ml}$ rifamycin $M_{14}$	210	
Complete mixture + 100 $\mu\text{g/ml}$ DNA	1,500	
Complete mixture + 100 $\mu\text{g/ml}$ DNA + 0.03 $\mu\text{moles/ml}$ rifamycin $M_{14}$	670	80
Complete mixture + 2 mg/ml RNA	1,850	
Complete mixture + 2 mg/ml RNA + 0.03 $\mu\text{moles/ml}$ rifamycin $M_{14}$	420	81

The reaction mixture contained the following, in  $\mu\text{moles/ml}$ : 100 *tris*-HCl, pH 7.4; 13 magnesium acetate; 50 ammonium acetate; 6 mercaptoethanol; 1 ATP; 5 phosphoenolpyruvate; 20  $\mu\text{g/ml}$  pyruvate kinase; 0.05 each of 20 L-amino-acids minus L-methionine; 0.03 GTP; 0.07  $^{14}\text{C}$  L-isoleucine (specific activity  $6.5 \times 10^4$  c.p.m./ $\mu\text{mole}$ ); 2 mg/ml S-30 protein. Total volume, 250  $\mu\text{l}$ . Samples were incubated at 37° C for 45 min, and the reaction was stopped with perchloric acid by the method of Ofengand *et al.*<sup>3</sup>. Radioactivity was determined by a windowless gas-flow counter.

Table 2

Additions	C.p.m./mg protein	Percentage inhibition
Complete mixture	450	
Complete mixture + 0.03 $\mu\text{moles/ml}$ rifamycin $M_{14}$	370	
Complete mixture + 80 $\mu\text{g/ml}$ poly U	4,150	
Complete mixture* + 0.03 $\mu\text{moles/ml}$ rifamycin $M_{14}$ + 80 $\mu\text{g/ml}$ poly U	1,050	84
Complete mixture† + 80 $\mu\text{g/ml}$ poly U + 0.03 $\mu\text{moles/ml}$ rifamycin $M_{14}$	2,670	40

The reaction mixture contained the following, in  $\mu\text{moles/ml}$ : 100 *tris*-HCl, pH 7.4; 13 magnesium acetate; 50 ammonium acetate; 6 mercaptoethanol; 1 ATP; 5 phosphoenolpyruvate; 20  $\mu\text{g/ml}$  pyruvate kinase; 0.05 each of 20 L-amino-acids minus L-phenylalanine; 0.03 GTP; 0.03  $^{14}\text{C}$  L-phenylalanine (specific activity  $74 \times 10^4$  c.p.m./ $\mu\text{mole}$ ); 2 mg/ml S-30 protein. Total volume was 250  $\mu\text{l}$ . Samples were incubated at 37° C for 45 min, and the reaction was stopped with perchloric acid by the method of Ofengand *et al.*<sup>3</sup>. Radioactivity was determined by a windowless gas-flow counter.

\* Rifamycin was incubated for 5 min at room temperature in the complete assay mixture before addition of poly U.

† Poly U was incubated for 5 min at room temperature in the complete assay mixture before addition of rifamycin.

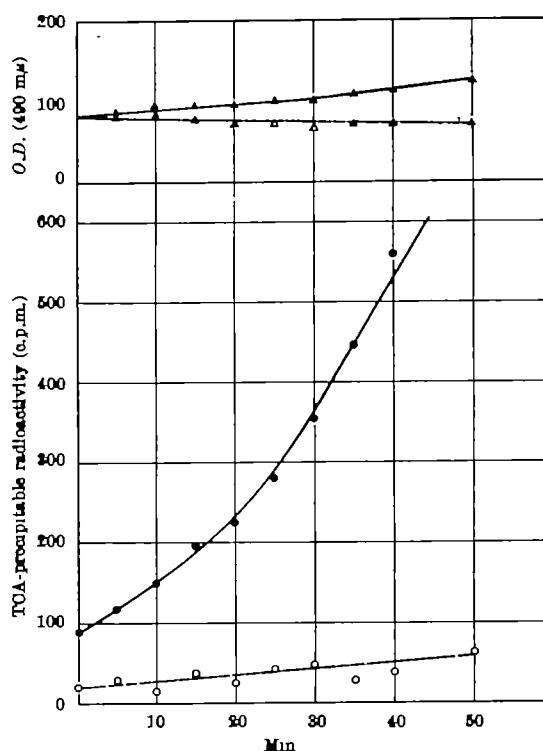


Fig. 1. Effect of rifamycin on  $^{14}\text{C}$  uracil incorporation into TCA-insoluble material. Cells were grown in medium  $A_{K-2}$  (ref. 6) supplemented with 20  $\mu\text{g/ml}$  guanine, 20  $\mu\text{g/ml}$  cytosine, 10  $\mu\text{g/ml}$  uracil. When the culture had reached an optical density at 490 m $\mu$  of 0.150 the culture was chilled, and the cells were centrifuged and resuspended in 0.1 M phosphate buffer, pH 7, with the same supplements and containing 0.2 per cent glucose and 2  $\mu\text{g/ml}$   $^{14}\text{C}$  uracil (specific activity  $2.24 \times 10^5$  c.p.m./ $\mu\text{mole}$ ). The suspension was divided in 2 tubes, one tube also contained rifamycin  $M_{14}$  at a final concentration of 5  $\mu\text{g/ml}$ . At 5-min intervals portions were taken and, after an optical density reading at 490 m $\mu$ , were treated with 2 volumes 7.5 per cent TCA. Precipitates were centrifuged and washed 3 times with 5 per cent cold TCA and dissolved in 1.7 N  $\text{NH}_4\text{OH}$ , the radioactivity was determined by a windowless gas-flow counter. (O---O) C.p.m./ml. of culture into TCA-insoluble material in the presence of 5  $\mu\text{g/ml}$  rifamycin  $M_{14}$ ; (●—●) without rifamycin  $M_{14}$ ; ( $\Delta$ --- $\Delta$ ) O.D. at 490 m $\mu$  of culture in the presence of 5  $\mu\text{g/ml}$  rifamycin  $M_{14}$ ; ( $\Delta$ — $\Delta$ ) without rifamycin  $M_{14}$ .

this product was separated from fragments of low molecular weight by precipitation from a solution in 0.5 M KCl with half volume ethanol.

$^{14}\text{C}$  L-phenylalanine and  $^{14}\text{C}$  L-isoleucine were obtained from S.O.R.I.N. (Vercelli, Italy) and  $^{14}\text{C}$  uracil from the Radiochemical Centre, Amersham, Buckinghamshire.

RNA from *B. steierthermophilus* was a gift of Dr. M. Arca. This RNA contained only two components of 17 and 23 S as shown by ultracentrifuge sedimentation patterns<sup>4</sup>.

DNA was prepared following the method reported by Marmur<sup>5</sup>.

In Tables 1 and 2 the effect of rifamycin  $M_{14}$  on the incorporation into protein of  $^{14}\text{C}$  L-isoleucine and  $^{14}\text{C}$  L-phenylalanine in the presence of high molecular weight RNA and of poly U is reported.

To test the effect of rifamycin on RNA synthesis, the incorporation of  $^{14}\text{C}$ -uracil into nucleic acids was followed by the method described by Dubin *et al.*<sup>6</sup>. Results are reported in Fig. 1 as TCA-precipitable counts per ml. of culture. The same figure shows the optical density of the cultures.

Fig. 1 demonstrates a strong inhibition of uracil incorporation into TCA-precipitable material in the presence of rifamycin. This inhibition, however, cannot be considered as direct evidence in favour of an action of rifamycin on RNA synthesis since rifamycin might cause a decrease of cell permeability (a decrease of cell permeability might be connected with the inhibition by rifamycin of the oxidation of exogenous substrates<sup>7</sup>). Alternatively, the decrease of RNA synthesis might be a secondary effect of

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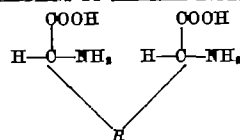
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For the intestinal transport of amino-acids, the carboxyl and amino-groups appear to be of key importance<sup>1</sup>. In the case of cystine, there has been suggestive evidence that the disulphide group might also play a part in the movement of the compound<sup>2,3</sup>. We present here simple but compelling data on the participation of the disulphide moiety in cystine transport. There is shown to be *in vitro* intestinal transport of cystine and homocystine against a concentration gradient, while 9 analogues which do not possess the —S—S— linkage are not transported.

L-Cystine-<sup>35</sup>S (Schwarz) had carrier added as necessary. Diaminopimelic acid in radiolabelled form was obtained from commercial suppliers (diaminopimelic acid-2,6-<sup>14</sup>C, Cal. Biochem., and the generally tritiated compound, Amersham Radiochemical Centre). L-Homocystine-<sup>35</sup>S

Results of the transport experiments are shown in Table 1. Cystine and homocystine were transported against a concentration gradient by the small intestine of the hamster. Within the limits of detection, none of the other analogues was transported. That is, replacing the —S—S— by other linkage groupings resulted in cessation of transport. Initial studies have also been performed utilizing everted intestinal sacs from the rat and mouse. Results in these species are so far identical with those in the hamster for the three compounds tested—transport of cystine but not of diaminopimelic acid or djenkolic acid. Results of the studies on possible inhibition of cystine transport by its analogues are shown in Table 2. None of the compounds, including the transported L-homocystine, inhibited L-cystine movement against a concentration gradient. Perhaps this is not too surprising; these compounds have limited solubility and, at the resulting low concentration, the transport system is probably not saturated.

Table 1. TRANSPORT OF L-CYSTEINE AND ITS ANALOGUES ( $1 \times 10^{-4}$  M) AGAINST A CONCENTRATION GRADIENT BY HAMSTER INVERTED INTIMAL SACCS



Composition of R	Names of compound	Transport*
$-\text{CH}_2-\text{S}-\text{S}-\text{CH}_2-$	L-cystine	20
$-\text{CH}_2-\text{CH}_2-\text{S}-\text{S}-\text{CH}_2-\text{CH}_2-$	L-homocystine	18
$-\text{CH}_2-\text{S}-\text{S}-\text{CH}_2-$	D,L-selenocystine	0
$-\text{CH}_2-\text{CH}_2-\text{S}-\text{S}-\text{CH}_2-\text{CH}_2-$	D,L-selenohomocystine	0
$-\text{CH}_2-\text{S}-\text{CH}_2-\text{S}-\text{CH}_2-$	L-cystekolic acid	0
$-\text{CH}_2-\text{S}-\text{CH}_2-\text{CH}_2-\text{S}-\text{CH}_2-$	L-homodiselenokolic acid	0
$-\text{CH}_2-\text{S}-\text{CH}_2-\text{CH}(\text{CH}_2\text{CH}_2-\text{S}-\text{CH}_2)-$	L-3,3'-(2-methylethylene-1,2-dithio)-dialanine	0
$-\text{CH}_2-\text{CH}_2-\text{S}-\text{CH}_2-$	D,L + allo-cystathionine	0
$-\text{CH}_2-\text{S}-\text{CH}_2-$	L + meso-lanthionine	0
$-\text{CH}_2-\text{CH}_2-\text{CH}_2-$	L + meso- $\alpha$ , $\beta$ -diammopimelic acid	0
(Nothing)	Diaminosuccinic acid	0

\* Amount gained in the serosal fluid ( $10^{-6}$  moles/400 mg intestinal sac).

Table 2. EFFECT OF ANALOGUES ON THE INTERNAL TRANSPORT OF L-CYSTEINE ( $1 \times 10^{-3}$  M) AGAINST A CONCENTRATION GRADIENT

Addend at maximum solubility	Per cent inhibition of L-cysteine transport
diaminosuccinic acid	0
L-diphenolic acid	0
L + meso- $\alpha,\epsilon$ -diaminopimelic acid	0
D,L-selenocysteine	0
L-homocysteine	0

was stated to represent interference, by the charge on the side-chain, with the transport system. The significance of this observation, and the relationship of the compound to cystine, were apparently not reported. A more recent paper, by Randall and Evers<sup>8</sup>, indicated that the small intestine of the rat did not transport diaminopimelic acid, at higher concentrations, against a concentration gradient.

Reasons for the transport of cystine and homocysteine, but not the analogues lacking the disulphide grouping, must centre about the presence of the —S—S— linkage in the first two materials. A conjectural possibility is that the transported moiety is cysteine, with its —SH— group. Small amounts of cysteine might be in equilibrium with cystine, or perhaps cysteine can be formed from the —S—S— compound by the action of the gut wall. Indeed, an abnormality of cystine-cysteine interrelations has been postulated in cystinuria<sup>9</sup>. The occurrence of the disulphide of cysteine and homocysteine in the urine of cystinuric individuals suggests an abnormal disulphide interchange<sup>9</sup>. One of the defects in cystinuria, or a result of the disease, may be an inability to cleave the —S—S— bond at the appropriate moment, leading to the eventual formation of inappropriate compounds. However, altered disulphide interchange or rotation of the groups at the —S—S— bond cannot be excluded at present.

We thank Barbara Lutters, Francis Vishno and Bunardy Evans for their assistance and Dr. K. McConnell and Dr. W. H. Gunther for their advice.

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## PHARMACOLOGY

### Inhibition of a Brain Protease by General Anaesthetics

We described previously a protease in rat brain cortex the activity of which increased after electrical stimulation of afferent nerves<sup>1</sup>. Further investigations have shown that this enzyme, besides being active on the synthetic substrates N-acetyl-L-tryptophan ethyl ester (ATrEe) and N-acetyl-L-tyrosine ethyl ester (ATyEe), is also capable of hydrolysing casein and that it is activated by the addition of cysteine. Its optimum pH is 7.1. A similar enzyme was extracted from rat brain by Guroff<sup>2</sup>.

The activity of this protease was found to be modified by various drugs administered *in vivo*<sup>3</sup>. It was noted, particularly, that ether and chloroform prevented the increase of enzyme activity induced by stimulation of the brain. More recently a systematic study of drug action

on the brain protease was undertaken *in vitro*. This report deals with the effects of the following anaesthetic agents: diethyl ether (DEe), chloroform (CHCl<sub>3</sub>), trichloroethylene (Cl<sub>3</sub>Et), halothane (Hal), methoxyflurane (MeOF), ethylvinyl ether (EVe) and divinyl ether (DVe).

The protease was prepared by adding 20 vol. of cold acetone ( $-20^{\circ}$  C) to freshly collected rat brains. After homogenization in a Waring blender and stirring for 2 h at low temperature, the precipitate was collected. After drying, the precipitate was ground up in 0.15 M sodium chloride and extracted at room temperature for 6 h. After centrifugation, the enzymatic activity of the supernatant was 3–4  $\mu$ moles of ATyEe hydrolysed/h/g wet brain. Addition of  $5 \times 10^{-3}$  M cysteine increased the activity to 7–8  $\mu$ moles/h/g. Tyrosine released by hydrolysis of casein was estimated by the fluorometric method<sup>4</sup> which allows measurement of a few nmoles of the amino-acid. The enzyme released 150–200 nmoles of tyrosine/h/g tissue without cysteine and 500–700 nmoles/h/g in the presence of cysteine.

The anaesthetic agents were used in saturated aqueous solutions. Varying amounts of these solutions were incubated with the quantity of enzyme corresponding to 100 mg of brain,  $5 \times 10^{-3}$  M cysteine and 0.8 ml. of a 1 per cent casein solution at pH 7.1 (0.025 M Tris buffer), in a total volume of 2 ml. After 1 h at room temperature, the proteins were precipitated with trichloroacetic acid (final concentration 6 per cent) and tyrosine was estimated in the filtrate. An identical sample was treated with trichloroacetic acid at zero time and its filtrate was used as blank. Control samples were incubated without anaesthetics.

Table 1. PROTEASE INHIBITION AND ANAESTHETIC POTENCY

	ml. of sat. sol./ml.	IC <sub>50</sub> mg/ml.	AD <sub>50</sub> mg/ml. (20°)
Diethyl ether	0.287	20.00	2.16
Divinyl ether	2.500*	7.75	—
Ethylvinyl ether	0.594*	4.75	—
Trichloroethylene	0.225	1.40	—
Chloroform	0.054	1.07	0.167
Halothane	0.150	0.56	0.0758
Methoxyflurane	0.183	0.43	0.0675

\* Extrapolated values.

Each agent was tested at several concentrations and the concentration necessary to inhibit 50 per cent of the enzyme activity was determined. Table 1 shows the results in terms of ml. of the saturated solution, and in mg/ml., calculated from the solubility of the agent in water at 20° C and from its specific gravity. For DVe and EVe, which have low solubility and low inhibitory action, the 50 per cent inhibition had to be extrapolated.

The significance of these findings depends largely on the correlation between enzyme inhibition and anaesthetic potency. The literature contains few reliable data on the potency of general anaesthetics. The most accurate values have been published recently by Cherkin and Catchpool on DEe, CHCl<sub>3</sub>, Hal and MeOF, determined in goldfish<sup>5</sup>. Fig. 1 shows the correlation for these agents between the concentration required for 50 per cent protease inhibition (IC<sub>50</sub>) and the concentration which caused anaesthesia in 50 per cent of the goldfish (AD<sub>50</sub>). No accurate values are available for the other three anaesthetics, but DVe and EVe are believed to have an activity of the same order as DEe and Cl<sub>3</sub>Et, similar to that of CHCl<sub>3</sub>.

This remarkable correlation ( $r = 0.991 \pm 0.010$ ) would add one more to the many physical and chemical properties of the anaesthetic agents associated with their pharmacological activity. Protease inhibition can probably be understood best in the context of the recent work of Pauling<sup>6</sup> and Miller<sup>7</sup>, which points to a combination of anaesthetics with the structured water surrounding protein molecules. This combination would probably include protein side-chains<sup>8</sup> and, by involving active sites of enzymes, could result in their inhibition. My efforts are

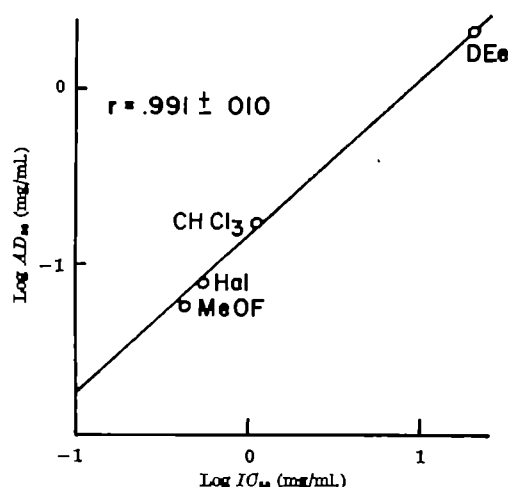


Fig. 1. Correlation between enzyme inhibition and anaesthetic potency. Abscissa: enzyme concentration inhibiting 50 per cent of enzyme activity ( $IC_{50}$ ); ordinate: anaesthetic potency according to Oberkin and Catapool (ref. 5). Both on log scale. DEe, diethyl ether; Hal, halothane; MeOF, methoxyflurane.

now being directed towards the purification of the enzyme and the analysis of other drug actions on it. These investigations may supply further information on the functional significance of the enzyme and its possible role in anaesthetic action.

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### Peri-renal Sarcoma Induced by Cellulose Wrapping

EXPERIMENTAL, perinephritic, renal hypertension may be produced by wrapping the kidneys in a variety of inert substances such as silk<sup>1,2</sup>, latex<sup>3</sup>, plastic solutions<sup>4</sup> and cellulose material<sup>5</sup>.

An attempt was made to produce hypertension in Wistar rats by wrapping the kidneys with 'Viaking' dialysis tubing (manufactured by Hudec Merchandising Corporation). This was abandoned in favour of unilateral renal artery clipping with contralateral nephrectomy; the majority of animals being killed. Four animals survived for 9 months, but were then killed because of abdominal swelling causing respiratory distress. Post-mortem examination revealed bilateral, firm, white tumour masses enclosing both kidneys in all animals and in one case (Fig. 1) invading the renal parenchyma. Two out of 4 rats showed blood-stained ascites with numerous small tumour nodules covering the peritoneum and the under surface of the liver. Microscopically the tumour was a fibro-sarcoma (Fig. 2), consisting of intertwining bundles of spindle-shaped cells, having round or oval nuclei. Mitoses, both normal and abnormal, were frequent and occasional bizarre, multinucleate giant cells were seen. Sections of the liver showed direct invasion of the parenchyma from the capsule.

Oppenheimer *et al.*<sup>6,7</sup> reported the development of tumours in 8 out of 23 animals similarly treated, and stated that the shortest time taken for the production of a tumour measuring 8 mm in diameter was 362 days from the time of kidney wrapping. The carcinogenic action of the



Fig. 1. Kidney with surrounding tumour showing direct invasion at lower pole ( $\times 1.5$ ).

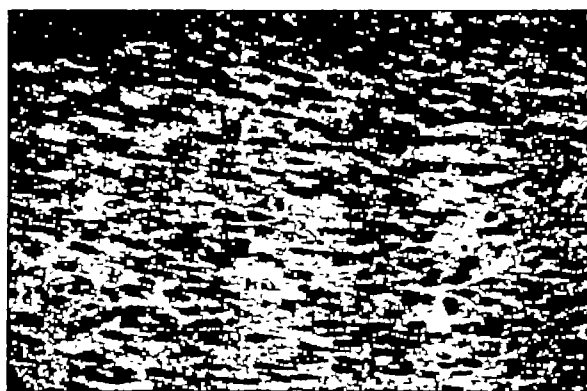


Fig. 2. Fibrosarcoma composed of bundles of spindle-shaped cells (haematoxylin and eosin  $\times 620$ ).

cellulose wrapping has been confirmed in this series, but the time of development of the tumours has been shown to be considerably shorter than in previous reports.

The observations preclude the method for the production of a colony of renal hypertensive rats for long-term observations, but may provide a useful method for the induction of experimental sarcoma in a cancer research programme.

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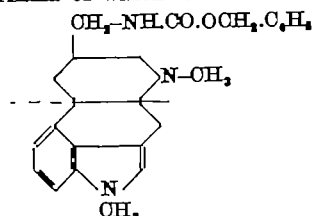
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# 1-Methyl-8 $\beta$ -carbobenzyloxy-aminomethyl-10 $\alpha$ -ergoline, a Potent and Long-lasting 5-Hydroxytryptamine Antagonist

It has been proved that derivatives of 8-methylergoline and 8-methylergoline specifically antagonize 5HT. In this group of substances the more active are *d*-lysergic acid diethylamide (LSD<sub>25</sub>) and 1-methyl-*d*-lysergic acid butanolamide (UML<sub>491</sub>). We now report a potent and exceptionally long-lasting 5HT antagonist, namely the 1-methyl-8  $\beta$ -carbobenzyloxy-aminomethyl-10  $\alpha$ -ergoline (MCE), a new compound synthesized in our laboratories<sup>1</sup>, the structural formula of which is:



Its *LD*<sub>50</sub> is 85 mg/kg intraperitoneally and 430 mg/kg *per os* in mice; more than 800 mg/kg *per os* in rats; and about 20 mg/kg intravenously in rabbits. This new compound has been tested for its pharmacological actions both *in vitro* and *in vivo*. The results obtained *in vitro* are summarized in Table 1. All the aqueous solutions were made with the aid of maleic or ascorbic acid in slight excess. When administered *per os* the compound was suspended in 5 per cent gum acacia.

Tests	Antagonists
Agonists ( $\mu$ g/ml.)	MCE <i>ED</i> <sub>50</sub> ( $\mu$ g/ml.) UML <i>ED</i> <sub>50</sub> ( $\mu$ g/ml.)
<i>Isolated rat uterus</i>	
5HT creatinine-sulphate (0.005–0.01 $\mu$ g/ml.)	0.00001 0.0005
Bradykinin (0.001 $\mu$ g/ml.)	5.0 50.0
Acetylcholine bromide (0.75 $\mu$ g/ml.)	5.0 20.0
<i>Isolated guinea-pig uterus</i>	
5HT creatinine-sulphate (0.5–5.0 $\mu$ g/ml.)	5.0 50.0
Histamine hydrochloride (0.05 $\mu$ g/ml.)	0.75–1.0 3.0
Acetylcholine bromide (0.01 $\mu$ g/ml.)	10.0 50.0
Edoquin (0.0025–0.008 $\mu$ g/ml.)	4.0 50.0
Bradykinin (0.15–0.05 $\mu$ g/ml.)	2.0–3.0 50.0
<i>Isolated guinea-pig seminal vesicles</i>	
Adrenaline hydrochloride (1.0 $\mu$ g/ml.)	0.1 50.0
Histamine hydrochloride (1.0 $\mu$ g/ml.)	5.0 10.0
Edoquin (0.1–1.0 $\mu$ g/ml.)	10.0 50.0

*ED*<sub>50</sub> = Concentration producing 50 per cent inhibition of the spasmodic effects of the agonists, estimated graphically.

In all the experiments antagonists have been applied only 3 min before agonists.

On the isolated guinea-pig ileum UML<sub>491</sub> is often spasmodic at concentrations from 1  $\mu$ g/ml upwards.

To study the anti-5HT action on isolated oestrous rat uterus we followed the procedure described by Stone *et al.*<sup>2</sup> in the work on 1-methyl-4-(5-dibenzo- $\alpha$ ,  $\epsilon$ -cycloheptatrienylidene)-piperidine hydrochloride (cyproheptadine), employing Tyrode's solution at 32° C.

On this test and with this procedure, after a single exposure to the extraordinarily low concentration of 0.00001  $\mu$ g/ml. of MCE the action of 5HT was reduced by about 50 per cent.

MCE behaves like cyproheptadine. In fact little or no inhibition occurs when the antagonist is present along with 5HT in the organ bath. After its removal by washing, the inhibitory activity gradually appears and becomes more and more evident at successive administrations of 5HT. UML<sub>491</sub> inhibition has a relatively rapid onset and reaches its maximal effect more rapidly than MCE. The comparison between the doses which produce about 50 per cent inhibition of the height of contractions shows that MCE is at least 500 times more potent than UML<sub>491</sub>.

At higher concentrations MCE irreversibly blocks the 5HT uterine receptors. MCE exerts a specific anti-5HT action on the uterine receptors. In fact, it reduces to half the spasmodic action of bradykinin and acetylcholine at concentrations at least a million times higher than those active against 5HT. Strangely enough, MCE as UML<sub>491</sub> has no specific anti-5HT activity on guinea-pig ileum. The inhibition of different spasmodic agents like acetylcholine, histamine, bradykinin and edoquin exerted on different smooth muscle preparations by MCE at high concentrations is probably due to a myolytic papaverine-like action of the compound.

*In vitro*, MCE has a weak adrenolytic activity on isolated guinea-pig seminal vesicles.

The results obtained *in vivo* are summarized in Table 2.

Table 2 shows that MCE *in vivo* is a potent 5HT antagonist, without any appreciable antiadrenaline, antihistamine, antiedoquin, antibradykinin and parasympatholytic action.

Tests	Antagonists
Agonists ( $\mu$ g/kg; administration route)	MCE Route of administration <i>ED</i> <sub>50</sub> $\mu$ g/kg Peak effect UML Route of administration <i>ED</i> <sub>50</sub> $\mu$ g/kg Peak effect
<i>Pithed rat (blood pressure)</i>	
5HT creatinine-sulphate (15–30 $\mu$ g/kg; i.v.)	i.v. 1.5 i.v. 1.0
Adrenaline hydrochloride (0.01–0.05 $\mu$ g/kg; i.v.)	i.v. >1000 —
<i>Local oedema (rat paw)*</i>	
5HT creatinine-sulphate (1 $\mu$ g injected into the paw)	s.c. 15 s.c. 16
<i>Guinea-pig bronchospasm†</i>	
5HT creatinine-sulphate (10–30 $\mu$ g/kg; i.v.)	i.v. 1.5 i.v. 1.0
Bradykinin (0.5 $\mu$ g/kg; i.v.)	i.v. >1000 —
Acetylcholine bromide (5–10 $\mu$ g/kg; i.v.)	i.v. >1000 —
Histamine hydrochloride (10–20 $\mu$ g/kg; i.v.)	i.v. >1000 —
Edoquin (0.2–0.8 $\mu$ g/kg; i.v.)	i.v. >1000 —
<i>Anaesthetized dog (blood pressure)</i>	
Adrenaline hydrochloride (1 $\mu$ g/kg; i.v.)	i.v. >1000 —
Acetylcholine bromide (3 $\mu$ g/kg; i.v.)	i.v. >1000 —
Histamine hydrochloride (8 $\mu$ g/kg; i.v.)	i.v. >1000 —
Edoquin (0.005 $\mu$ g/kg; i.v.)	i.v. >1000 —

\* Doepfner, W., and Cerletti, A. (ref. 3).

† Konetzki, H., and Röscher, R. (ref. 4).

*ED*<sub>50</sub> = Dose producing 50 per cent inhibition of the effects of the agonists.

MCE and UML<sub>491</sub> appear to be equipotent when their peak-effects are considered. When their durations of action are compared MCE is far more active than UML<sub>491</sub>. This is clearly shown in Fig. 1.

After subcutaneous administration the *ED*<sub>50</sub> of UML<sub>491</sub> and MCE is similar when their peak-effects (1 h and 3 h after dosing, respectively) are compared. 20 h after administration the *ED*<sub>50</sub> for UML<sub>491</sub> is 75 times higher than at 1 h, whereas that for MCE is only 5 times as high as that at 3 h. Therefore the 5HT antagonism of UML<sub>491</sub> has a rapid onset but it wears off quickly; the inhibitory effect of MCE develops slowly but lasts longer. The comparison of the *ED*<sub>50</sub> 20 h after subcutaneous administration shows that MCE is at least 15 times more potent than UML<sub>491</sub>. The guinea-pig bronchospasm is probably the best test to show the long-lasting effect of MCE. For instance, 15  $\mu$ g/kg of MCE, administered subcutaneously, reduce by 50 per cent the bronchospastic effects of 5HT for 24–48 h. To obtain the same block with UML<sub>491</sub> a dose more than 100 times as high is required.

Some of the 5HT effects *in vivo* are not antagonized even by relatively high doses of MCE: in dogs the effects of 5HT on respiration (hyperpnoea and tachypnoea followed by short-lasting apnoea) and in rabbits the 5HT effects on circulation and respiration (hypotension, bradycardia and apnoea). In this respect MCE behaves like UML<sub>491</sub>.

At a dose of 1 mg/kg, administered intravenously, MCE has no action on the blood pressure and respiration of dogs and cats and does not modify coronary and femoral blood flow and heart contractile force in dogs.

## HAEMATOLOGY

## Availability of Platelet Factor 3 and Activation of Factor XII in Thrombasthenia

RECENT reports by Hardisty, Dormandy and Hutton<sup>1</sup>, Hardisty and Hutton<sup>2</sup>, and Spaet and Cintron<sup>3</sup> have demonstrated that incubation of platelet-rich plasma with kaolin shortens the recalcification<sup>1,2</sup> and Russell's viper venom ('Stypven')<sup>3</sup> times of the mixture. These authors concluded that the effect resulted from availability of platelet coagulant activity (platelet factor 3 availability). Since the activity is confined to the sediment of the centrifuged mixture, is related to the platelet concentration and the anticoagulant used (citrate or ethylenediamine tetraacetic acid), it appears to be related to a surface influence of kaolin, accompanied by adhesion and aggregation of the platelets. In the recalcification system, Hardisty *et al.*<sup>1</sup> detected a marked abnormality with thrombasthenic platelets, which they suggested may be correlated with the failure of these platelets to react with adenosine diphosphate (ADP). We have examined nine thrombasthenic patients by the simultaneous use of the global recalcification test of Hardisty *et al.*<sup>1,2</sup> and the more specific Stypven time according to Spaet *et al.*<sup>3</sup>. In addition the activation of Factor XII was followed and the results indicate the existence of a further *in vitro* defect in thrombasthenia.

The kaolin clotting time was performed under the conditions described by Hardisty *et al.*<sup>1</sup>. The results confirm the findings of these authors in that the abnormality in thrombasthenic platelet-rich plasma was constant in all patients and over a range of platelet concentrations (Fig. 1). As already shown by Hardisty *et al.*<sup>1</sup>, we have confirmed that the abnormality is platelet-specific and not influenced by the presence of activated normal platelet-poor plasma.

Activation experiments were also performed as described by Spaet and Cintron<sup>3</sup> using Stypven. Fig. 2 illustrates the range of the results of clotting times of platelet-rich plasma of 9 control and 8 thrombasthenic subjects. There is clear differentiation into two groups, clotting time always being longer with patient platelet-rich plasma. Activated normal platelet-poor plasma gave results within the patient range.

In an identical system to that described by Spaet and Cintron<sup>3</sup> the activation of Factor XII was tested on a congenitally deficient stored substrate plasma. The results are illustrated in Fig. 3 and represent the range of 14 control experiments and 10 performed with thrombasthenic platelet-rich plasma. Initial activation is identical in platelet-rich plasma and platelet-poor plasma of patients

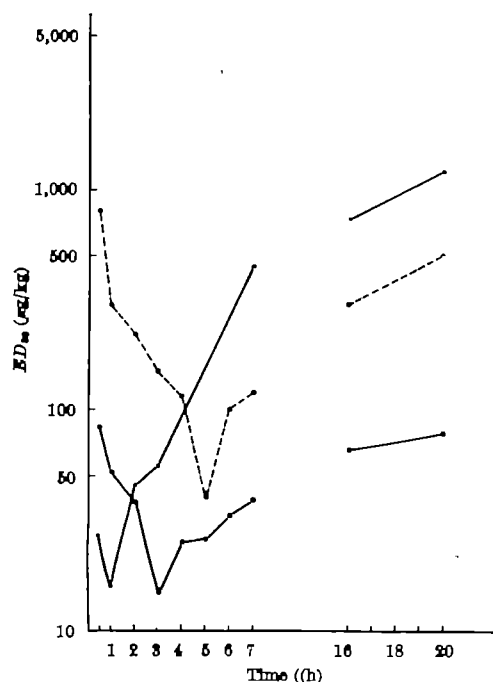


Fig. 1. Anti-5HT action of UML<sub>491</sub>, administered subcutaneously (●—●) and of MCE administered subcutaneously (○—○) or orally (○---○) at intervals before injection of 5HT to the rat's paw. Ordinate: ED<sub>50</sub> (doses producing 50 per cent inhibition of the oedema) of the antagonists (µg/kg), estimated graphically. Abscissa: time (h) after antagonist administration. The results for UML<sub>491</sub> are in accordance with those obtained by Berde *et al.*<sup>5</sup>

In mice MCE at doses higher than 2 mg/kg, administered intraperitoneally, increases barbiturate toxicity, decreases rectal temperature and reduces spontaneous motility. In rabbits MCE does not antagonize 5-hydroxytryptophan (5HTP) hyperthermia<sup>6</sup> at the dose of 1 mg/kg, administered subcutaneously.

According to Tedeschi *et al.*<sup>7</sup>, anti-5HT drugs, with an anti-5HT action on the central nervous system, antagonize the convulsant effects of tryptamine in rats. On this test we found that MCE behaves like 2-brom-*d*-lysergic acid diethylamide (BOL<sub>148</sub>), LSD<sub>25</sub>, and like chlorpromazine as described by these authors.

Its subcutaneous ED<sub>50</sub>, 3 h after administration, is about 75 µg/kg. Summing up, the new synthetic compound 1-methyl-8 β-carbobenzoyloxy-aminomethyl-10 α-ergoline (MCE) is a very active and specific antagonist of 5HT. Its acute and subacute toxicities are low<sup>8</sup> and its absorption from the gastro-intestinal tract is good (Fig. 1).

Owing to these favourable properties and to its extraordinarily long-lasting action MCE appears to be a suitable agent to study the still unknown part played by 5HT in human physiology and pathology.

We thank Sandoz Ltd. (Switzerland) for generous supplies of UML<sub>491</sub> and bradykinin, and Dr. C. D. Bianchi for his help in translating the test.

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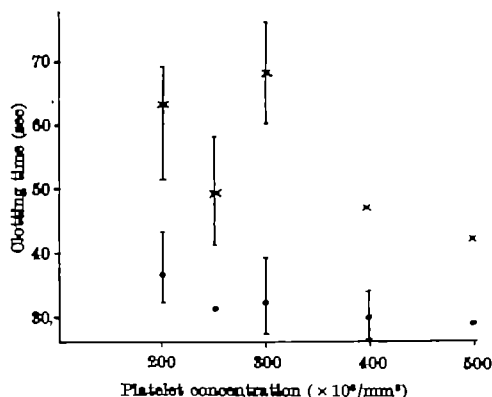


Fig. 1. The recalcification time of normal (●) and thrombasthenic (×) platelet-rich plasma after incubation with kaolin. The mixture containing 0.1 ml. platelet-poor plasma, 0.1 ml. platelet-rich plasma at the concentrations shown and 0.1 ml. kaolin (20 mg/ml. in buffered saline) was incubated at 37° C for 20 min and recalcified with 0.2 ml. calcium chloride 0.025 M. Each point represents the mean of 1-7 tests each performed in duplicate. The range of the mean of multiple tests is illustrated.

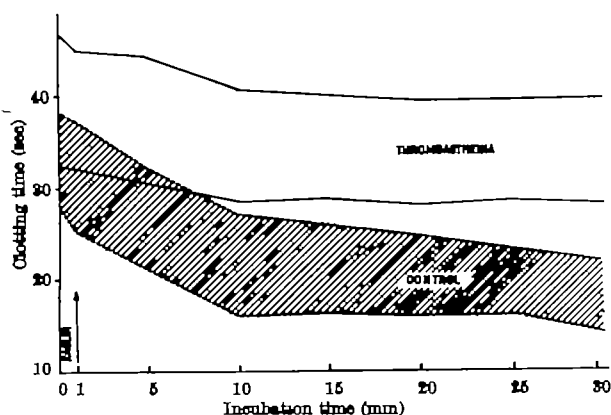


Fig. 2. Platelet Factor 3 availability measured by the Stypven time in controls and thrombasthenia. Incubation mixture was 0.5 ml. platelet-rich plasma and 0.1 ml. kaolin 50 mg/ml. at 37° C. 0.1 ml. of the mixture was transferred to a tube containing 0.1 ml. calcium chloride 0.025 M and 0.1 ml. Stypven 1:100,000 and the clotting time recorded.

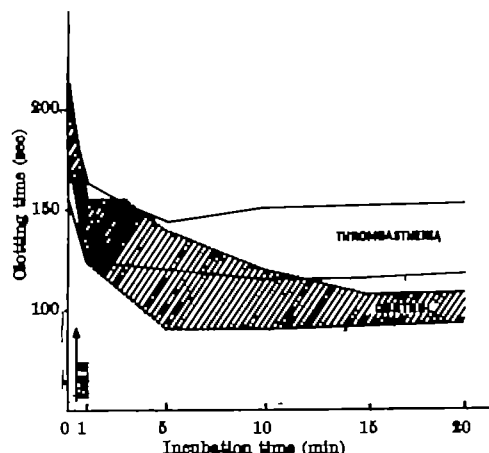


Fig. 3. Factor XII activation in platelet-rich plasma of controls and thrombasthenic patients. Activation mixture as before (Fig. 2). Sub-samples were diluted 1:20 in V.B.S. and 0.1 ml. of the diluted mixture transferred to a tube containing 0.1 ml. substrate plasma, 0.1 ml. 'Cephalin' (ref. 6) and 0.1 ml. kaolin (20 mg/ml. in V.B.S.) and recalcified. All additions to the substrate were made within 30 sec of testing.

and controls. However, activation continues in the control platelet-rich plasma and is maximal at 15 min, whereas normal platelet-poor plasma and the patients' platelet-rich plasma show no, or minimal, further activation.

These results indicate a parallel abnormality with thrombasthenic platelets measurable by recalcification, by the Stypven time and by a defect in the activation of Factor XII. It was therefore believed possible that platelet Factor 3 availability may depend on coincident optimal Factor XII activation or that Factor XII activation may influence the results of both tests. However, as already shown by Hardisty and Hutton<sup>3</sup>, congenitally deficient Factor XII platelet-rich plasma was found to give normal coagulation times in the presence of activated normal platelet-poor plasma, in the recalcification system. Furthermore, the Stypven times of kaolin activated Factor XII deficient platelet-rich plasma were normal even in the absence of normal plasma (Fig. 4). The latter test is therefore independent of Factor XII activation and seems to measure platelet activity more specifically. This is also confirmed by our observation that the addition of a partially purified contact product, prepared according to Wessler<sup>4</sup>, to a normal platelet-rich plasma or platelet-poor plasma does not influence the Stypven time.

If platelet aggregation is essential for the availability of platelet Factor 3, as is suggested by the defect in thrombasthenia, then it would be expected that the addition of a sufficient concentration of ADP would shorten the Stypven time of normal platelet-rich plasma in the

Spaet<sup>5</sup> assay. This was confirmed by the findings illustrated in Fig. 5, which demonstrates sub-optimal availability of platelet Factor 3, as the activity is less than that observed with kaolin. These results support the findings of Mustard, Hegardt, Rowsell and MacMillan<sup>1</sup> working with native platelet-rich plasma, who demonstrated, with the prothrombin consumption test, increased coagulant activity after ADP-induced aggregation. On the other hand, Hardisty and Hutton<sup>3</sup> were not able to demonstrate this effect of ADP, but the discrepancy is explicable on the basis of the ADP concentration utilized by these authors.

These results confirm the existence of a defect in the availability of platelet Factor 3 in thrombasthenia and indicate the presence of an unrelated abnormality of surface activation. It is probable that both defects result from the inaggregability of these platelets. The significance of the tests described by Hardisty *et al.*<sup>1,3</sup> and Spaet and Cintron<sup>5</sup>, in terms of platelet function, is the same, provided that plasma abnormalities are corrected in the first. The Stypven assay is more specific in the measure of platelet Factor 3 availability, in the sense that it is independent of the contact phase of plasma activity and the Factor XII activation induced by kaolin. The exact roles of platelet adhesion<sup>1,3</sup>, or of aggregation<sup>5</sup>, and of an eventual abnormality of the platelet membrane in thrombasthenia, remain to be established. It must also be considered that there may be little correlation between these *in vitro* abnormalities in thrombasthenia, hitherto unrecognized, and a real coagulation defect in this disease.

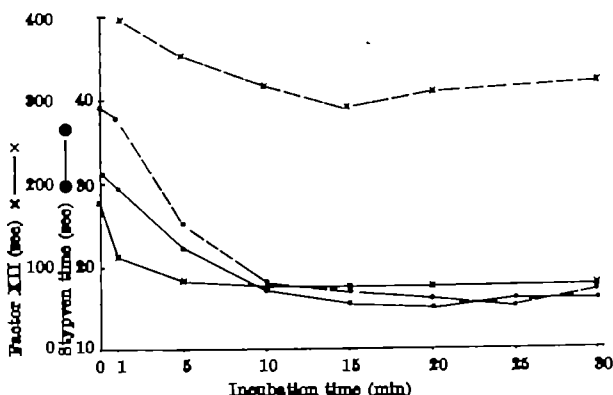


Fig. 4. The Stypven times and Factor XII activation in a control subject and one case of Hageman deficiency. The results of the control are represented by solid lines and those of the patient by broken lines.

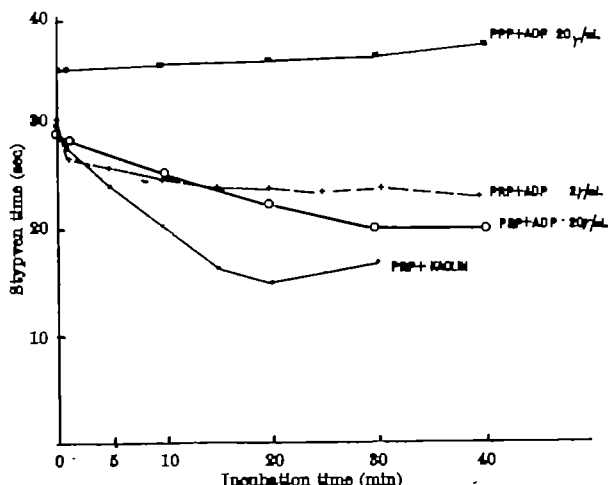


Fig. 5. Platelet Factor 3 availability measured by the Stypven time after ADP induced aggregation in relation with the final concentration of ADP, in normal platelet-rich plasma and platelet-poor plasma. Results with kaolin are illustrated below for comparison.



*Note added in proof.* Since this communication was submitted, Horowitz<sup>7</sup> has reported similar findings with 'Celite' incubated platelet-rich plasma and the Stypven time.

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### Haemoglobins of Adult and Foetal African Elephants

THE numerous special properties of human foetal haemoglobin (HbF) as compared with those of the human adult (HbA) have been rather thoroughly investigated<sup>1,2</sup>. Fewer data, however, are available on other mammalian foetal haemoglobins<sup>3,4</sup>.

In comparison with the adult blood pigment the characteristic alkali stability, slow electrophoretic mobility, and tryptophan band of human HbF were missing in the animal foetal haemoglobins previously examined, with the exception of the rhesus monkey<sup>5-7</sup> and the chimpanzee<sup>8</sup>. The isoleucine content is greater in all foetal haemoglobins compared with adult haemoglobin<sup>9</sup>, so far as is known.

This communication presents results on electrophoresis, alkali denaturation, ultra-violet spectrum, and potassium ferricyanide oxidation of adult and foetal haemoglobin from the African elephant (*Loxodonta africana*).

Pregnant elephants were shot during game control measures in the Masindi and Fort Portal areas of Uganda. It was possible to remove the specimens of blood within 0.5 h of the animals' death, and transport them in Dewar flasks to a mobile laboratory for investigations. Blood

specimens were examined from five adult elephants and two foetuses with ages of 5 months (foetus 1) and 12 months (foetus 2), respectively<sup>10</sup>.

In starch-block electrophoresis (veronal-veronal-sodium buffer, pH 8.8) and in starch-gel electrophoresis (tris-EDTA-borate, buffer, pH 9.0), only foetus 1 yielded two haemoglobin fractions. The haemoglobin from foetus 2 was identical with that of adult elephants. The slowly migrating fraction of foetus 1 (Fig. 1) represented 15 per cent of the entire pigment. The relative electrophoretic migrating velocities of adult human and African elephant haemoglobins agree substantially with those found by other investigators<sup>11,12</sup>.

No qualitative or quantitative differences in the kinetics of alkali denaturation<sup>13</sup> between the foetal haemoglobin and the adult haemoglobins were observed. The amount of alkali-resistant blood pigment from all investigated elephants was found to range between 95 and 97 per cent.

A 15 per cent labile fraction in the entire cyanmethaemoglobin haemolysate from foetus 1 was found during heat denaturation. This fraction corresponds quantitatively to the slowly migrating electrophoretic component. The haemoglobin from foetus 2 gave results identical with those of the adult elephants. Human HbF, in comparison with HbA, is less resistant<sup>14</sup>. The ultra-violet spectro-analysis (Fig. 2) of the slowly migrating fraction of foetus 1 showed, in contrast to the fast-migrating fraction and the adult haemoglobins, the typical tryptophan band which is known to be characteristic for human HbF<sup>15</sup>.

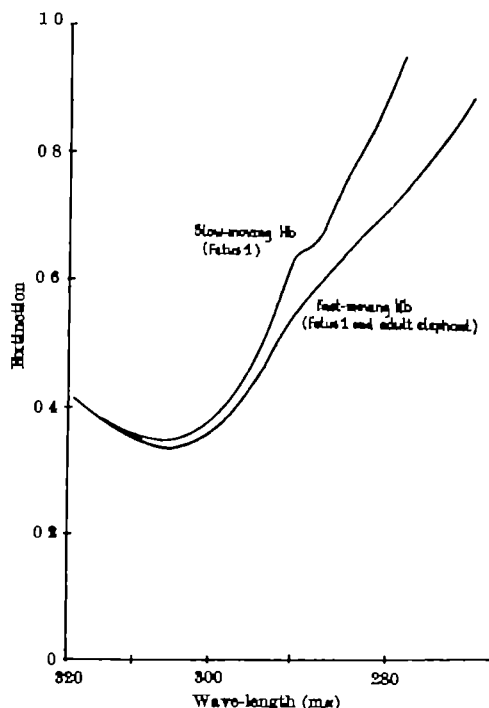


Fig. 2. Absorption spectra in the ultra-violet of the slow- and fast-moving haemoglobin of foetus 1 compared with the blood pigment of adult elephants

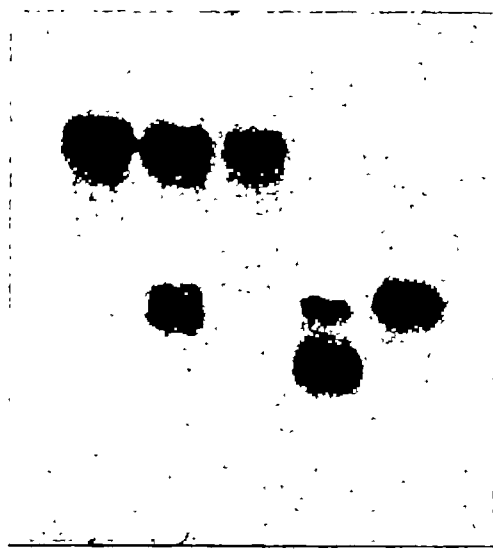


Fig. 1. Separation of haemoglobins by starch-gel electrophoresis at pH 9.0. From left to right, adult elephant, foetus 1, foetus 2, human cord blood, human adult blood. Horizontal line, that of application

The potassium ferricyanide oxidation proceeded three times faster in the slowly migrating fraction than in the fast-migrating fraction of foetus 1 and the adult elephant haemoglobin. As previously shown, the same differences exist in the oxidation speed between foetal and adult human blood pigment. It was concluded that the potassium ferricyanide oxidation is related to the dissociation of oxygen from haemoglobin<sup>16</sup>.

It can be stated, therefore, that a foetal haemoglobin was found only in the 5-month-old foetus and not in the 12-month-old foetus of the African elephant, the gestation time of which is 21 months. With the exception of alkali

denaturation, elephant foetal haemoglobin showed physico-chemical similarities to human HbF.

From a biological point of view it is interesting that blood from the older foetus has a higher oxygen affinity under standard conditions of temperature and pH than the blood of the younger<sup>17</sup>.

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## HISTOCHEMISTRY

### Use of Galactose Oxidase in the Histochemical Examination of Mucus- secreting Cells

GALACTOSE oxidase is an enzyme which oxidizes the primary alcohol group of galactose or galactosamine to an aldehyde<sup>1</sup>. The enzyme attacks not only free galactose but also terminal non-reducing galactose and galactosamine units when glycosidically linked. Detection of such units in mucopolysaccharides and mucoproteins produced by mucus-secreting cells can be accomplished by staining the aldehyde produced with Schiff's reagent.

Material collected from the slaughterhouse was fixed in 10 per cent formol-saline, and specimens were wax-embedded and cut at 4 $\mu$ . The sections were first treated with a solution of 0.4 per cent sodium borohydride containing 0.4 per cent sodium bicarbonate at room temperature for 1 h in order to reduce Schiff-staining groups already present in the tissues. After a wash in running water for 5 min the sections were treated with a solution containing 2-3 units per ml. of galactose oxidase (prepared according to Amaral *et al.*<sup>2</sup> but now obtainable from Worthington Biochemical Corporation, Freehold, New Jersey) and 1 per cent by volume of catalase (Boehringer and Soehne G.m.b.H., Mannheim, Germany) in 0.03 M-tris hydrochloride buffer, pH 7, at room temperature for 2 h. Following another wash in running water for 5 min, the sections were stained with Schiff's reagent<sup>3</sup> for 3 min, then rinsed in sulphurous acid for 4 min and in running water for 10 min. Aldehyde groups produced by the action of galactose oxidase were stained red by this reagent,

but the intensity of staining was less than that produced by periodic acid-Schiff staining because less aldehyde groups were produced by this method.

Sections of bovine cervix taken from a cow at pro-oestrus showed a great variation in intensity of staining from cell to cell, as is shown in Fig. 1. Some cells were not stained at all, whereas others were stained relatively strongly. This indicated that the mucus in the cells of the cervical epithelium was heterogeneous with regard to the number of terminal non-reducing galactose and galactosamine groups present. Another possibility is that the linkage of galactose and galactosamine to the penultimate sugar varied from cell to cell since that has been shown to cause variation in the rate of oxidation of model disaccharides by galactose oxidase (Roberts, G. P., unpublished results). However, since a treatment for 2 h with galactose oxidase is sufficient to oxidize all the available galactose and galactosamine groups, this later theory is considered unlikely.

Ovine submaxillary gland sections were not stained by this procedure, as is to be expected from the structure of the mucoprotein isolated from this source, most of the galactosamine units being substituted at the primary alcohol group by a sialic acid residue<sup>4</sup>. However, when the sections were subjected to an overnight incubation with neuraminidase, subsequent treatment with galactose oxidase produced aldehyde groups which could be stained with Schiff's reagent.

In order to obtain information about the linkage of sialic acid in sections of bovine cervix, all the terminal non-reducing galactose and galactosamine units already present were first oxidized with galactose oxidase for 2 h as already described. The aldehyde groups thus produced were then blocked by the aniline chloride method of Lillie<sup>5</sup> using a 2-h reaction time. Completeness of oxidation and blocking was checked at this stage by re-oxidizing with galactose oxidase and staining with Schiff's reagent. No staining was produced, thus confirming the effectiveness of this treatment. Sialic acid was then removed from the aniline chloride blocked sections by an overnight incubation at 37° C with a solution containing 0.004 per cent by weight of neuraminidase (Sigma Chemical Co., St. Louis, Missouri) and 10<sup>-4</sup> M calcium chloride in 0.1 M sodium acetate buffer, pH 5.1. Treatment of these sections with galactose oxidase produced aldehyde groups in some cells as shown by Schiff staining (Fig. 2). Here again there was a great variation in intensity of staining from cell to cell. This result indicated that at least some of the sialic acid in the mucus-secreting cells of the bovine cervix is linked to galactose and galactosamine. It is not possible at this stage to identify by histochemical means



Fig. 1. Bovine cervix treated sequentially with 0.4 per cent sodium borohydride containing 0.4 per cent sodium bicarbonate/galactose oxidase/Schiff's reagent.



Fig. 2. Bovine cervix treated sequentially with galactose oxidase/aniline chloride/neuraminidase/galactose oxidase/Schiff's reagent.

which of these two sugars is involved. This result suggests a heterogeneity in the mucus of bovine cervical epithelium with regard to sialic acid linkage.

We thank Dr. E. I. Garvie for advice and assistance in culturing *Dactylium dendroides* from which the galactose oxidase was prepared, Mr. N. G. J. Gruber for photographing the sections, and Dr. R. A. Gibbons for his advice.

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## HISTOLOGY

### Lesion of the Bone Matrix in Vitamin D-resistant Rickets

MICROBADIOGRAPHIC examination of compact bone from patients with vitamin D-resistant rickets has revealed that, in addition to the presence of wide osteoid borders, there is a lack of mineral around osteocyte lacunae and their canaliculi<sup>1,2</sup>. Histological examination of decalcified material hitherto has failed to demonstrate any abnormality in the organic matrix at these sites, suggesting the presence of mineral deficiency only<sup>3</sup>. We have obtained evidence for a lesion of the bone matrix, however, by applying the following modification of Schmorl's method<sup>4</sup>.

Compact bone from the fibulae of 2 patients (a 22-year-old male and a 17-year-old female) was removed in the course of orthopaedic procedures. It was fixed for 24 h in a mixture of formalin, mercuric chloride and picric acid in water, and afterwards for 2 days in 10 per cent formalin. Decalcification was carried out in 18 per cent ethylenediamine tetraacetic acid in water at pH 7.1 and 40° C for 3 days. Following this, the material was rinsed in water and cut in the frozen state at 20 μ. The sections were

stained in 0.01 per cent azure II in water (pH adjusted to 9.0 with ammonium hydroxide) for 30 min, rinsed and transferred to a solution of 20 ml. saturated picric acid in 200 ml. water. After a final rinse, the sections were mounted in laevulose.

In normal bone azure II heavily stains the walls of the lacunae and canaliculi. In bone from these patients, the stain was also concentrated at the sites where the mineral was lacking (Figs. 1 and 2). The localization of this abnormal pattern was not strictly perilacunar but rather extending from the lacunae toward the centre of the osteone, along the centripetal canaliculi. This pattern has been observed before in undecalcified bone sections on treatment with basic fuchsin<sup>4</sup>. The normal lamellar architecture of the matrix appeared to be interrupted at these sites. At high magnifications it was noted that neither lacunae nor canaliculi were enlarged and that the matrix around the canaliculi had taken up the stain in a distinct globular pattern. These globules measured up to 1 μ in diameter. Non-mineralized osteoid, for example at the inner circumference of growing osteones, did not take up the stain.

Attempts to determine the nature of the matrix-compound, which was responsible for the affinity for azure II, have so far yielded negative results. Prior digestion of the sections with pepsin and trypsin did not interfere with the pattern as revealed by azure II. Haematoxylin-, periodic acid-Schiff-, van Gieson-, Mallory- and sudan black-stains, as well as the acrolein-Schiff reaction for protein<sup>5</sup>, failed to bring out the lesion. Alcian blue was localized at these sites only very occasionally and in low concentration.

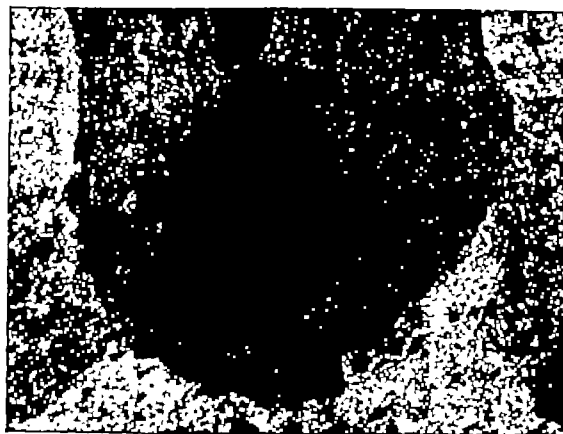


Fig. 1. Microradiograph of 100 μm undecalcified section from a 17-year-old patient with vitamin D-resistant rickets (× c. 200).



Fig. 2. 20 μm decalcified section from same patient, stained with azure II-picric acid (× c. 200).

The density of the perilacunar and pericanalicular regions to very soft (3 kV) X-rays was indistinguishable from the normal. In phase-contrast microscopy of 20 $\mu$  decalcified sections, the lesion was visible as a conglomeration of small dots, interrupting the lamellar pattern of the osteons.

These findings are consistent with the possibility that a physical characteristic rather than a chemically defined moiety of the matrix is responsible for the observed affinity for azure II. The lack of mineral around lacunae and canaliculi may be the result of impaired mineralization or of demineralization *in vivo*<sup>4</sup>. It is conceivable that a structural change in the matrix is involved in either of these processes and is revealed by the described deposition of azure II.

It is unlikely that the lesions resulted from long-continued treatment with high doses of vitamin D, as one of the patients had not received any vitamin D for 4 years.

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Lipid in the Matrix of Ageing Articular Cartilage

It is well known that with ageing, lipid material appears in the matrix of hyaline cartilage in the immediate vicinity of the cells, in the form of 'haloes' around the lacunae<sup>1</sup>. In the course of a histochemical and quantitative investigation of the lipid content of human cartilage, it has been observed that a more diffuse impregnation with fat occurs with ageing in the superficial zone of articular hyaline cartilage.

Post-mortem and amputation material has been used from 85 subjects ranging from 2 days to 80 years of age. Specimens from macroscopically normal areas have been taken from the medial femoral condyle of the knee joint (other joints being used as the source in two cases), fixed in formal calcium, and 10 $\mu$  frozen sections coloured with sudan black B in propylene glycol. Where a positive result has been obtained, the tissue had been subjected further to the Nile blue<sup>2</sup> and acid haematein techniques<sup>3</sup>, pyridine extraction being used as a control. In addition to the normal areas, a few osteoarthritic patches have been studied.

In cartilage from subjects under the age of 20 years, lipid cannot be detected in the matrix of the superficial zone. From the third decade onwards, however, a sudan-

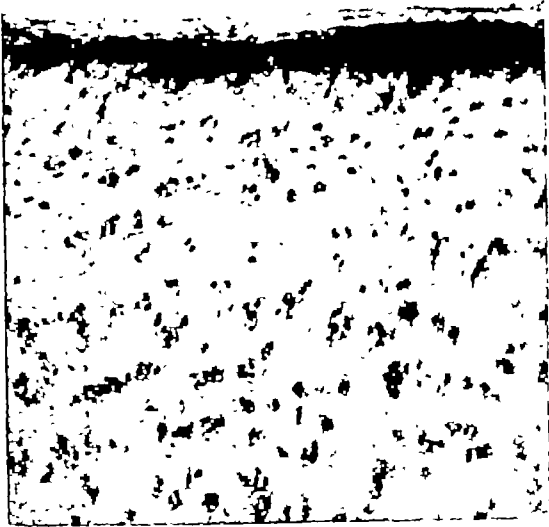


Fig. 1. Articular cartilage from medial femoral condyle of subject aged 80 years (sudan black  $\times 75$ )

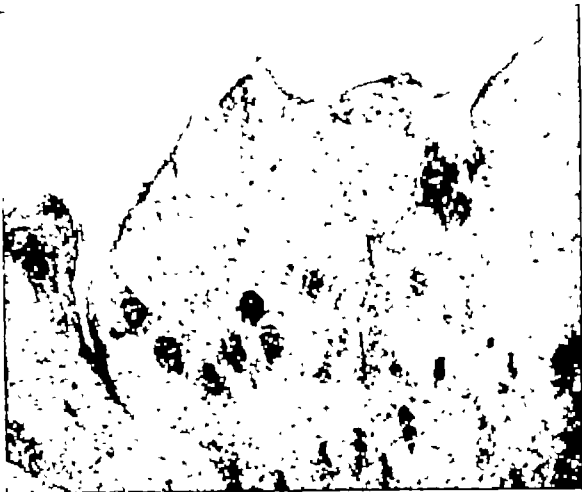


Fig. 2. Osteoarthritic cartilage from area adjacent to normal tissue shown in Fig. 1 (sudan black  $\times 75$ )

ophilic layer in this zone can be seen in an increasing proportion of the individuals studied (see Table 1 and Fig. 1). This layer varies both in depth and in staining intensity as seen in transverse section, both features tending to increase with age. The sudanophilic material is extractable with pyridine and the corresponding area is stained red and black by the Nile blue and acid haematein techniques respectively, thus denoting the presence of both neutral fat and phospholipid. In the osteoarthritic areas studied, no lipid layer could be seen (Fig. 2). No correlation was established between the presence of the lipid and the state of nutrition of the subject, although this may be due to the small number investigated.

The problem arises as to the origin of this lipid material. Fat is a normal and constant constituent of the chondrocyte<sup>4</sup> and presumably some extracellular fat may derive from the cell it surrounds, although the appearance of fat in the matrix thus can scarcely be considered physiological<sup>1</sup>. In an ultrastructural study of articular cartilage, Barnett *et al.*<sup>5</sup> describe the degeneration of superficial cells *in situ*, leaving a residuum of myelin figures and osmiophilic granules in the matrix. This mechanism could also contribute to the appearance described here.

Table 1

Age group	No. cases	Diffuse lipid layer in superficial zone	
		Present	Absent
0-20 years	6	0	6
21-40 years	7	4	3
41-60 years	11	10	1
61-80 years	10	8	2
81-	1	1	0

However, the uniform nature of the lipid distribution suggests that a diffusion process from the synovial fluid may take place; on this point it may be relevant that cartilage is readily permeable to lipid-soluble materials\*. Such diffusion could be a post-mortem phenomenon although, were this so, it seems probable that it would be manifest in young as well as old subjects, and in osteo-arthritis as well as normal areas.

The existence of this lipid-rich zone in life could influence or modify the nutrition of the cartilage cells, at least in so far as the free circulation of aqueous fluid through the matrix is concerned. Such an effect has been demonstrated in a 'Millipore' filter by Rendi and Reid<sup>7</sup>. However, until the work reported here has been extended, no interpretation as to significance can be made.

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## IMMUNOLOGY

### Passive Transfer of 'Allergic' Encephalomyelitis with Antibrain Serum Injected into the Lateral Ventricle of the Brain

It is generally believed that experimental allergic encephalomyelitis results from an interaction between nervous tissue antigens and cells which are engaged in a delayed hypersensitive response. Although experimental allergic encephalomyelitis can be followed by circulating antibodies that react *in vitro* with nervous tissue constituents, all experiments designed to transfer passively experimental allergic encephalomyelitis either with sera from diseased animals or with antibrain immune sera have failed<sup>1</sup>. This has been taken as evidence that antibody does not play any significant part in the development of experimental demyelinating disease<sup>2</sup>. A possible explanation for the failure of antibrain antibodies to induce encephalomyelitic lesions may be their inability to cross the blood-brain barrier in sufficient amount. In order to test the role of antibrain antibodies in the pathogenesis of experimental allergic encephalomyelitis, guinea-pigs with a cannula inserted permanently into the lateral ventricle of the brain were used in the present experiment. Administration of serum through the cannula by-passes the blood-brain barrier, and permits direct contact of injected antibodies and nervous tissue.

Experimental allergic encephalomyelitis was induced in guinea-pigs by a single intradermal injection over the sternum of 0.1 ml. of the following mixture: 0.5 g of fresh bovine spinal cord, or 200 mg of cord lipid, and 3 ml. of paraffin oil containing 9 mg of *Mycobacterium tuberculosis*. Sera from animals showing histological signs of disease were heated at 56° C, pooled, passed through a Seitz filter and stored at -20° C for three months. A complement-fixation assay revealed antibody to spinal cord lipid at a titre of 1/50.

A permanent cannula<sup>3</sup> designed especially for guinea-pigs was implanted into the left lateral ventricle. Operated animals were injected with sterile saline for three successive days, and thereafter with pooled sera from experimental allergic encephalomyelitis animals. Each animal received two 0.1 ml. injections of serum daily with a 12-h interval

between injections. Control cannulated guinea-pigs were treated with normal guinea-pig serum. Animals were observed carefully during the treatment, and those with weakness were killed. Paraffin sections of cerebrum, cerebellum and three levels of spinal cord were processed for haematoxylin-eosin staining. Some representative sections were stained for myelin using the Weigert-Pal technique. The intensity of disease was judged according to the extent of histological lesions.

Table 1 summarizes the incidence of experimental allergic encephalomyelitis in guinea-pigs injected intraventricularly with homologous antibrain or normal serum. Seven of eleven animals treated with serum from diseased guinea-pigs showed mild but definite lesions on histological examination. Two guinea-pigs of this group developed inflammatory lesions within three days of the beginning of treatment, that is following administration of only 0.6 ml. of antibrain serum. Histopathological changes in the nervous system were of minimal intensity, but characteristic of delayed hypersensitive reactions with demyelination. The lesions consisted of histiocytes and lymphocytes; no polymorphonuclear cells were noted. Most of the lesions were localized in the vicinity of the lateral ventricles, and some aggregates of mononuclear cells were situated directly beneath the ependyma. Small perivascular collections of inflammatory cells were observed in cerebrum, occasionally in cerebellum and spinal cord. Sections of control animals injected with normal serum were completely negative.

Table 1. EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (HAE) FOLLOWING PASSIVE TRANSFER OF ANTIBRAIN SERUM INTO THE LATERAL VENTRICLE OF THE BRAIN

Group	No. of guinea-pigs with HAE lesions	
	Negative	Positive
Treated with antibrain serum	4	7
Treated with normal serum	10	0

The present experiment demonstrates that experimental allergic encephalomyelitis can be passively transferred by administration of experimental allergic encephalomyelitis serum directly into the lateral ventricle of the brain. Although the antibody system involved in the demyelinating process is not characterized, it seems that antibrain antibody can elicit an immune response *in vivo* relevant to the pathogenesis of 'allergic' encephalomyelitis. The observations presented here offer *in vivo* evidence to support the *in vitro* findings of Bornstein and Appel<sup>4</sup> showing that a fraction of rabbit experimental allergic encephalomyelitis serum demyelinated myelinated cultures of rat or mouse cerebellum. Using the fluorescent antibody technique Sherwin *et al.*<sup>5</sup> demonstrated that antibodies with a specificity for myelin are present in the sera of rabbits immunized with spinal cord. The suppressive effect of complement-fixing antibrain antibody, described by Paterson and Harwin<sup>6</sup>, is not inconsistent with this report. It may be due to an antibody which has 'enhancing' properties such as certain factors demonstrable in tissue transplantation immunity. It should be mentioned that meningo-vascular lesions have been produced by means of intracisternal injection of heterologous antibrain sera<sup>7</sup>, but the character of the lesions was different from the injury seen in experimental allergic encephalomyelitis and in this investigation.

The mechanism which underlies the induction of experimental allergic encephalomyelitis by antibrain antibody is not clear, and several possibilities should be taken into account<sup>8,9</sup>. It appears that the pathogenesis of experimental allergic encephalomyelitis is more complex than was originally believed, and that in addition to sensitized cells of 'delayed' type and cell-bound antibody, circulating antibody can also take an active part in the mechanism responsible for demyelinating disease. Although this is possible, it could be argued that antibrain antibodies can attack brain *in vivo*, thus causing the lesions which are reminiscent of those of experimental allergic encephalomyelitis.

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### Effect of Trypsin Inhibitor Isolated from Sows' Colostrum on the Absorption of $\gamma$ -Globulin by Piglets

It is generally held that the absorption of whole proteins by piglets occurs only during the first two days of life. By this mechanism the baby pig acquires antibodies. For the first 5 days *post partum* the sow's colostrum contains a trypsin inhibitor<sup>1</sup> and it has been suggested<sup>2</sup> that the action of the trypsin inhibitor is to prevent the digestion of  $\gamma$ -globulins, so allowing the piglet to absorb them intact. Extending this theory further, it can be postulated that whole protein absorption ceases when there is insufficient inhibitor in the colostrum to inactivate all the trypsin entering the gut.

We prepared a solution of trypsin inhibitor from sow's colostrum according to the method of Leaskowski *et al.*<sup>3</sup>. The potency of the solution expressed in the units of Kunitz<sup>4</sup> was  $200 \times 10^4$ /ml. Each ml. of solution was capable of inactivating 30 mg of the sample of crystalline trypsin used in the assay. Sow's colostrum was chosen as starting material as it was thought that the inhibitor it contained could well be more effective *in vivo* than either the soya bean or bovine colostrum proteins used by earlier workers<sup>5,6</sup>. Leaskowski *et al.*<sup>3</sup> have demonstrated important differences in the properties of inhibitors isolated from these sources that show them to be different substances, so it seemed advisable to use the native protein.

Samples of  $\gamma$ -globulin were prepared by a combination of sodium sulphate precipitation and fractionation on a DEAE cellulose column from pig serum. The  $\gamma$ -globulin was labelled with iodine-131 by the method of McFarlane<sup>7</sup>.

Quantitative portions of labelled  $\gamma$ -globulin were given to two piglets in a litter of eight, 2 h after birth. Blood samples were taken 12 h later and the serum volumes of the piglets were estimated. Calculation showed that about 13 per cent of the activity administered was present in the serum protein.

The remaining six piglets were given a single dose of labelled  $\gamma$ -globulin at 60 h of age. Blood samples were taken 12 h later and serum volumes were calculated. With one exception, only negligible amounts (0.3–0.9 per cent) of activity were present in the serum proteins. One piglet still absorbed much  $\gamma$ -globulin (11 per cent in serum protein). This animal was discarded. The five remaining piglets were divided into an experimental group of three animals, each of which received 4 ml. trypsin inhibitor each hour from 74 h onwards, and a control group of two animals, given 4 ml. of distilled water each hour. At 80 h, all five animals received a second dose of labelled  $\gamma$ -globulin, accompanied by trypsin inhibitor or water. Doing with trypsin inhibitor or water continued for a further 12 h (92 h of age), when blood samples were taken and serum volumes estimated. During the 18-h period

(74–92 h of age) each piglet in the experimental group received inhibitor equivalent to at least 1 l. of colostrum<sup>1</sup>. There was no increase in either group in the quantity of labelled  $\gamma$ -globulin detectable in the precipitated serum protein fraction.

This result indicated that this inhibitor is incapable of inducing the absorption of  $\gamma$ -globulin in 3-day-old piglets. It would seem that the disappearance of trypsin inhibitor from colostrum does not account for the cessation of absorption of  $\gamma$ -globulin.

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### Spontaneous Occurrence of Autoantibodies Cytotoxic to Thymus Cells in the Sera of Mice of the 129 Strain

AUTOANTIBODIES of various types appear in the serum during autoimmune diseases; but it is not clear whether they are the cause or the result of the pathological process. Grabar<sup>1</sup> and Boyden<sup>2</sup> have postulated that autoantibodies are constantly present in low concentrations in the normal organism, their function being to transport normal catabolic products. This possibility that autoantibodies may have a physiological function was strengthened by the recent detection of autoantibodies in normal human sera<sup>3,4</sup> and in sera of healthy mice<sup>5</sup>. In the work recorded here, sera of normal adult mice of the 129 inbred strain were found to be cytotoxic for thymus cells of mice of strain 129 and of other strains in the presence of guinea-pig serum. Although the nature of the factor in 129 strain mouse serum has not yet been fully established, its behaviour in the cytotoxic test is similar to that of well-defined isoantibodies<sup>6,7</sup>. It will therefore be referred to here as an antibody.

Mice of an inbred 129/Rr colony maintained at New York University Medical School by Dr. E. A. Boyce were used as donors of sera in most experiments. In some experiments the tests were performed with the sera of 129 mice reared in other laboratories (129/J from the Jackson Memorial Laboratory, Bar Harbor, Maine, and 129/Rr from the Immunogenetics Research Unit, Duke University Medical Center, Durham, North Carolina, from which the New York University colony originated). The mice were bled either from the tail or from the retro-orbital sinus. The sera were separated within 5 h after bleeding and were stored at  $-10^{\circ}\text{C}$ . The cytotoxic test was a modification<sup>8</sup> of the method of Gorer and O'Gorman<sup>9</sup>. The guinea-pig serum used as a source of complement was absorbed with washed packed leukaemic cells until all cytotoxic activity against mouse thymus cells<sup>10,11</sup> had been removed. Cytotoxic titres were defined as the highest dilution of serum at which 50 per cent of the cells were killed.

The sera of all 129 mice 5 weeks of age or older contained an antibody which in the presence of guinea-pig com-

plement was cytotoxic for mouse thymus cells. Although the antibody was present in the serum of pregnant mice it was absent in suckling progeny, but appeared during the third week of life. Only thymus cells were sensitive to the cytotoxic effect of normal 129 serum; cells from spleen, bone-marrow, lymph nodes and peritoneal cavity were not affected. The thymus cells of all the strains of mice tested were susceptible, although there were differences in the sensitivities of different strains. Cytotoxic titres of 1:8 to 1:16 were obtained with thymus cells of the *C57BL/6*, *A*, *BALB/c*, and *C57L* strains. Lower titres were obtained with thymus cells of *C3H*, *C58* and *SJL/J* strains. The highest cytotoxic titres (1:16–1:32) were obtained with thymus cells of the 129 strain. Sera of individual 129 mice had the same cytotoxic effect *in vitro* on their own thymus cells as sera of other 129 mice. In no case were 129 thymus cells killed by guinea-pig serum alone, indicating that the cells were not coated with antibody *in vivo*. In the presence of guinea-pig serum, the sera of 129 mice were cytotoxic also for rat thymus cells, but not for rat spleen cells. In addition to its cytotoxic effect on thymus cells the serum of 129 mice was found to have a cytotoxic effect *in vitro* on the transplanted leukaemias *ERLD*, *H<sub>2</sub>RL9* (ref. 12) and a *BALB/c* radiation-induced leukaemia. There was no effect on many other tumour lines (including the sarcomas *Sal*, *BP8*, and the leukaemias *H<sub>2</sub>RL3*, *H<sub>2</sub>RL9*, *HL4* and *K36* (ref. 12)).

While cytotoxic tests thus indicated a specific cytotoxic effect of the 129 normal serum on thymus cells and some leukaemic cells, absorption experiments indicated that the antigen was of more widespread distribution. The cytotoxic activity of 129 serum was absorbed by all nucleated tissues tested, including thymus, spleen, and kidney of the mouse and rat. Cytotoxic activity was removed also by absorption with guinea-pig red cells but not with mouse, sheep and hamster red cells. The antigen from mouse thymus or spleen resisted boiling for 30 min. This treatment did not diminish the capacity of these tissues to absorb the antibody.

The cytotoxic antibody in the serum of 129 mice was destroyed completely by heating at 56° C for 20 min, and was inactivated by treatment with 0.01 M mercaptoethanol for 1 h. Preliminary fractionation by filtration through a 'Sephadex G-200' column, followed by analytical ultracentrifugation, indicated that the antibody is probably a 19S  $\gamma$ -globulin.

The antibody present in the sera of normal 129 mice differs from the autoantibodies previously described in mice<sup>12,14</sup> in its specificity and characteristics: it is cytotoxic in the presence of guinea-pig complement for thymus cells and for some leukaemic cell lines, but is absorbed by many different tissues; it appears earlier in the life of the animal than autoantibodies previously reported, is heat-labile and is probably a 19S  $\gamma$ -globulin.

Sera of individual 129 mice reacted *in vitro* with their own thymus cells. There was, however, no evidence for reaction of the cytotoxic factor with thymus cells *in vivo*, since the thymus cells of 129 mice were not killed by guinea-pig complement alone. The lack of access of the antibody to the thymus cells might be attributed to the separation of the thymocytes from the blood stream by a barrier to macromolecular materials<sup>15,16</sup>. Similarly, the placental barrier, which is relatively impermeable to  $\gamma$ -IM globulins<sup>17</sup>, probably accounts for the absence of the antibody from sera of neonatal mice. No pathological changes, such as are found in *NZB/BL* mice<sup>18</sup>, were found in the thymus of 129 mice. Indeed, the thymus of 129 has served in electron microscopic studies as a prototype of the structure of the normal mouse thymus<sup>19</sup>.

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## RADIOBIOLOGY

### Cysteamine-Induced Increase of Cellular Glutathione-level: A New Hypothesis of the Radioprotective Mechanism

It was reported recently<sup>1</sup> that incubation of *ELD* ascites cells or its sublines in a tissue culture medium supplemented with cysteamine causes an increase of the cellular content of the acid-soluble, non-protein-bound sulphhydryl groups (NPSH). The increase was found to be directly related to the cysteamine concentration in the medium. Expressed in SIF\*, the extent of NPSH increase was similar to the extent of the increased radioresistance expressed in DRF† of human kidney cells or mouse fibroblasts treated with cysteamine at comparable concentrations<sup>2</sup>. This similarity of SIF and DRF has been interpreted<sup>1</sup> as indicating that the mechanism of radioprotection by cysteamine is, in some way, dependent on the increased concentration of NPSH in the protected cell. The nature of this NPSH is not known at present.

Aliquots of freshly withdrawn *ELD* ascites tumour<sup>3</sup>, with a concentration between 150 and 250 × 10<sup>6</sup> cells/ml., were diluted with equal parts of Eagle's tissue culture medium supplemented with various amounts of cysteamine. To controls, the medium was added without cysteamine. 5 ml. volumes of the cell suspensions in closed, 10-ml. glass vials were incubated in a water bath at 37° C, and kept under gentle agitation for a period of 30 min. The cells were then centrifuged at 600g for 10 min and re-suspended in 20 volumes of tissue culture medium without cysteamine. The total NPSH content of the washed cells and the incubation medium were subsequently estimated in duplicate. Ellman's<sup>4</sup> method was used as previously described<sup>5</sup> except that NPSH extraction was made with 6 per cent perchloric acid instead of trichloroacetic acid in order to facilitate the subsequent determination of glutathione (GSH) (ref. 6). Cell counting was performed by a Coulter model B electronic particle counter. The standard error of a single determination of NPSH was 5.7 per cent of the mean as calculated from the differences between duplicate measurements. Of the total NPSH,

\* SIF, Sulphydryl increase factor denotes increase of NPSH relative to untreated controls.

† DRF, Dose reduction factor denotes the increase of radiation resistance relative to untreated controls.



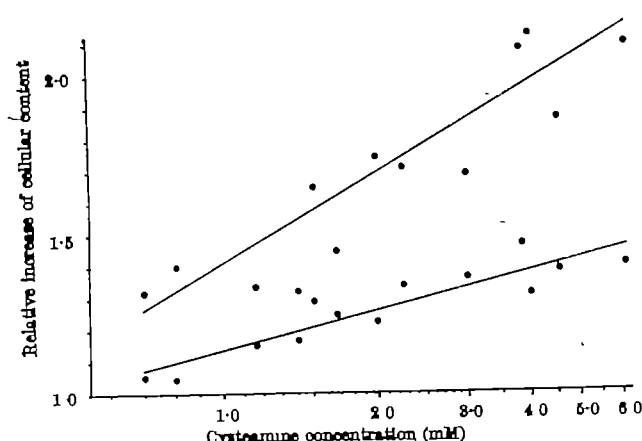


Fig. 1. Non-protein sulphhydryl (NPSH, ○) and glutathione (GSH, ●) level of *E. coli* cells treated with different concentrations of cysteamine, in relation to the level of the respective, untreated controls. The lines represent statistical regression lines with equation  $y = 0.981x \pm 1.409$  and  $y = 0.408x \pm 1.181$  for the increase of NPSH and GSH, respectively.

the amount of glutathione was determined specifically by the glyoxalase assay using the technique of Klotzsch and Bergmeyer<sup>4</sup>. In this case, the standard error of a single determination was 5.3 per cent of the mean.

In 13 separate tests, the concentration of cysteamine in the incubation medium was varied between 0.7 and 6.3 mM. The actual concentration of the compound was, in each case, established at the end of the incubation period. Fig. 1 illustrates the NPSH- and GSH-levels of the treated cells in relation to the respective controls. It can be seen that NPSH in the cells increased in a direct relationship to the amount of cysteamine in the medium, an almost ten-fold increase of the cysteamine concentration being associated with a nearly two-fold increase of cellular NPSH. The increase in NPSH was, in part, due to an increase of GSH which was also directly related to the cysteamine concentration. On the average, 37 per cent of the NPSH increase was due to a rise of the GSH-level. In the cells not exposed to cysteamine, practically the whole NPSH content,  $2.48 \pm 0.08 \times 10^{-8}$  μmoles/cell (mean  $\pm$  S.E. calculated from the values of the controls), consisted of GSH, which totalled  $2.42 \pm 0.11 \times 10^{-8}$  μmoles/cell.

It is reasonable to assume that incorporated cysteamine or some sulphhydryl-containing product of this compound of low molecular weight accounts for the non-GSH component of NPSH in the treated cells. No simple explanation can be offered for the increased GSH content. New formation of GSH in the cells during the incubation period in the amounts recorded would require an extraordinarily accelerated rate of synthesis, the feasibility of which is unknown.

Possible reduction of oxidized glutathione (GSSG), known to be affected by cysteamine<sup>5</sup>, could explain a part of but not the whole GSH increase. The conclusion is based on results obtained in assays performed by the method of Klotzsch and Bergmeyer<sup>4</sup> with eight different samples of our material. The results showed a GSSG content of  $0.200 \pm 0.060 \times 10^{-8}$  μmoles/cell. This amount equalled not more than 16 per cent of the total GSH content and was in agreement with measurements of the acid soluble disulphides in non-neoplastic material<sup>6</sup>. Thus, even if wholly reduced, it cannot account for more than an increase of the cellular GSH content by a factor of 1.16 on the average.

Mixed disulphides between glutathione and protein (GSSP) can be considered more likely as the possible source of the increased GSH content. The occurrence of such disulphides in our cell material is strongly suggested by the results of recent experiments designed to test this possibility. In these experiments, cells from 2 ml. ascitic fluid were treated with 2 vol. of 6 per

cent perchloric acid for 1 h, and the resulting precipitate was washed 3 times with 10 volumes of the acid. No measurable GSH or GSSG was detected in the last wash fluid. The disulphide bonds of the washed precipitate were then reduced by treatment with sodium borohydride essentially according to the technique of Moore *et al.*<sup>7</sup>. The reaction was carried out by dissolving the precipitate in 4 ml. of a 0.5 per cent solution of sodium lauryl sulphate and sodium borohydride at a temperature of 40° C. After treatment for 1 h, the excess sodium borohydride was destroyed by lowering the pH of the solution to 7 by means of 1 N hydrochloric acid. The proteins were then again precipitated with perchloric acid. After centrifuging, GSH, conceivably released from the proteins, could be demonstrated in the supernatant in amounts corresponding to about  $2 \times 10^{-8}$  μmoles/cell.

Mixed disulphides between cysteamine and protein (CSSP) have been demonstrated by Eldjarn and Pihl<sup>10</sup>. Possible release of GSH from GSSP, in association with the formation of CSSP, might explain the observed increase of intracellular GSH, as well as the occurrence of CSSP following cysteamine treatment of cells. The formation of CSSP resulting in a temporary blockage of vital sulphhydryl groups has been regarded as a possible mechanism for the radioprotective action of cysteamine. Release of intracellular GSH can also be considered as a significant factor in the radiation protection afforded by cysteamine treatment. This consideration is based on the direct correlation between cellular NPSH content and radioresistance found in experiments with different clones derived from a common cell population<sup>8,11</sup>, and with cells treated with varying doses of cysteamine<sup>1,2</sup>. GSH may protect by functioning as a scavenger of radiation induced free radicals, and as a specific agent for the elimination of radiation generated hydrogen peroxide via the glutathione peroxidase pathway<sup>12</sup>. If the idea that intracellular release of GSH affords radiation protection is accepted, it is reasonable to extend the search for potential radioprotectors to include compounds which can induce an increase of intracellular GSH.

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### Reactivation of Radiation-killed *E. coli* with Ribonuclease

RIBONUCLEASE has been reported to influence the action of X-rays on *E. coli*<sup>1</sup>. This communication shows that the presence of ribonuclease during irradiation did not influence the percentage of survivors, but presence of ribonuclease after irradiation and during incubation at 37° C increased the survivor ratio to a very high value.

*E. coli* B cells were routinely grown in Difco nutrient broth, supplemented with 0.2 per cent sodium chloride. Cells were grown with continuous aeration from an overnight culture to reach a logarithmic phase of growth in nutrient broth. Solid media were prepared by addition of 1.5 per cent Difco agar to the foregoing liquid media. Cells were gathered on nutrient agar plates at suitable dilutions and plates exposed to X-rays or ultra-violet rays, as the case may be, for the different doses of exposures. The plates either contained the graded amounts of ribonuclease before the exposure dose or graded amounts of ribonuclease were added after the exposures. Incubation of the plates after exposure was carried out at 37° C for the usual period of 18 h. Comparison of the colonies developed in plates containing ribonuclease with those in plates having no ribonuclease gave the difference in surviving ratio due to the influence of ribonuclease.

X-rays used were 80 kV, 17 m.amp at the dose rate at the place of irradiation of 340 rads/sec without filtration and ultra-violet dose rate was 30 ergs/mm<sup>2</sup> sec. The total doses of both X-ray and ultra-violet ray were so selected as to give about 0.1 per cent survival ratio for the bacterial cells.

Fig. 1 shows the variation in the percentage of X-ray survivors due to incubation in presence of various quantities of ribonuclease on plate. Curve *a* is the case when the ribonuclease was added to the plate before irradiation and remained on it during the incubation period. Curve *b* is for the case when the ribonuclease was added immediately after irradiation but was not present during the time of irradiation. Incubation of the plates continued in presence of the added amount of ribonuclease. The survivor percentage in the absence of ribonuclease was normalized to 100 and corresponding normalization with respect to this value for other cases of survivors in presence of various quantities of ribonuclease was done. Curve *a* shows that the saturation value of the increase in survivors reaches about twelve times in presence of 80 µg/ml. ribonuclease. Curve *b* shows that saturation value is as high as 45 times and is reached at 25 µg/ml. of ribonuclease concentration. Also the shapes of the curves are distinctly different; whereas in case of curve *a* the initial rise is slow, in case of curve *b* the rising part is very stiff.

When cells are exposed to X-rays in presence of ribonuclease, but freed from this immediately after irradiation and plated in absence of any ribonuclease, the number of survivors remains the same within the limits of experi-

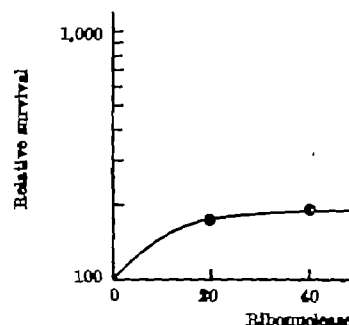


Fig. 2. Influence of ribonuclease on the survival of *E. coli* B cells after irradiation.

mental error for all the quantities (not shown in figure).

Fig. 2 shows the variation in violet survivors due to the in various quantities of ribonuclease curves for the case when ribonuclease was added before irradiation or after irradiation.

The results clearly indicate that ribonuclease during the incubation period is necessary for the recovery of survivors. Thus ribonuclease is necessary for the recovery of survivors rather than of protection. The difference between curves *a* and *b* of Fig. 1 is due to the fact that ribonuclease is inactivated during the incubation period. The available quantity of ribonuclease is less in case of curve *a* resulting in a decreased saturation value of the increasing initial part. However, the difference in the results of the reactivation of the survivors after X-rays and ultra-violet rays is not so large. Whereas the level for X-rays is about 45 times, for ultra-violet rays it is only about 12 times. This difference only highlights the difference in the action of X-rays and ultra-violet rays. Ultra-violet rays are known to produce thymine dimers<sup>1</sup>, which are repairable only by an excision-repair mechanism. For X-rays, there must be some other mechanism by which when affected can induce a repair mechanism. It has been observed that the effect of ribonuclease is to break down (ribosomal) to 4 S (soluble) RNA. It is conjectured only how this break down helps the cell to recover from the radiation damage. But this much is obvious from the results of the experiment for ultra-violet damage as that.

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## Cell Cycle of Mouse Embryo Fibroblasts in Continuous Culture

THE cell cycle in proliferating adult animals commonly takes the form of a continuous cycle. The synthetic period (*S*) is fairly constant in duration and the post-DNA synthetic period (*G<sub>2</sub>*) is also fairly constant in duration. Most of the variations in the duration of the pre-DNA synthetic period (*G<sub>1</sub>*) are known<sup>1</sup>. Among the variations yet reported are those found in

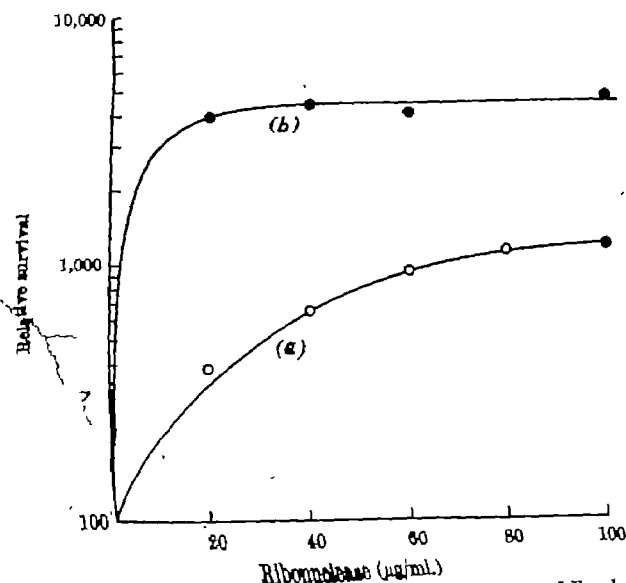


Fig. 1. Influence of ribonuclease on the X-ray inactivation of *E. coli* B cells. *a*, ribonuclease added before irradiation; *b*, ribonuclease added after irradiation.

rat gut (8.2-8.4 h) (ref. 2), in the chronically irradiated mouse gut (~8 h) (refs. 3 and 4) and in the neural tube of mouse embryos at 10 days gestation (probably ~8.5 h; ref. 5).

Embryonic tissue is one of the most rapidly proliferating cell systems known in mammals. In the present investigation the cell cycle was studied in the tip of the tail of the embryonic mouse at the 12th-13th day of gestation both in control animals and in animals that had been exposed to 5 days of continuous gamma irradiation at 50 rads/day. The tip of the tail of the embryonic mouse during the period is growing very rapidly. It behaves somewhat like a root tip. There is an area of high cell division at the tip and, as additional cells are produced, somites are formed behind the tip. Cell division, however, is not restricted to the tip as in root tips, and divisions continue in the newly differentiated somites.

Pregnant *Strong A* female mice at the 12th day of gestation as judged by vaginal plugs were injected with 50  $\mu$ Ci  $^3$ H-thymidine in normal saline (1.0 c./mM, Radiochemical Centre, Amersham) and killed at various intervals up to 20.5 h after injection. Embryos were fixed in 1:3 acetic-alcohol and stained *in toto* by the Feulgen method. Squash preparations of the tip of the embryonic tail were made in 45 per cent acetic acid. The cover glasses were removed by the dry-ice method and autoradiographs were made with Ilford 'K-5' liquid emulsion. Exposures were for 3 months. The irradiated mice were placed in the caesium-137 unit\* on the 7th day of gestation at a dose rate of 50 rads/day. Irradiation was continuous except for 0.5 h servicing intervals every other day. The irradiated mice were treated with  $^3$ H-thymidine and processed identically with the controls. Irradiation continued until they were killed. Autoradiographs were scored for labelled mitoses. From 3 to 12 tail tips were scored per pregnant female. At least 40 mitoses were observed in each tail tip and the results were summed for all the tail tips from one pregnant female.

Labelled mitoses for both the control and irradiated series are shown in Fig. 1. The data from the irradiated animals are very similar to those from the control group. The results indicate that this embryonic tissue has one of the shortest cycle times yet observed in mammals, about 8.5 h. An analysis of the cycle for both irradiated and control series indicates an *S* period of 6.5 h, a  $G_2 + M/2$  of 1 h and a  $G_1 + M/2$  of 1 h, where *M* represents the duration of mitosis.

Several reports show that continuous exposure of proliferating tissue to gamma-rays will shorten or lengthen the cell cycle. In plant root tips the cycle may be lengthened<sup>6,7</sup>, whereas in the intestinal crypt cells of the mouse the cycle is shortened<sup>8,9</sup>. The conditions and times of exposure were similar in this experiment to our previous work with intestinal crypt cells<sup>4</sup>. There, after a 5-day exposure of male *Strong A* mice to 50 rads/day, we found evidence of a shortened  $G_1$  period in the crypt cells of the

ileum. Here, on the other hand, in embryonic major change in the cell cycle can be detected because the cell cycle is already as short as possible given environmental conditions. Further studies of proliferating tissues are necessary before a statement can be made about the effect of ionizing radiation on the cell cycle in the mouse.

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## BIOLOGY

### Induction of Chromosome Aberrations by Aflatoxin

In 1960 an outbreak of 'Turkey X' disease in turkeys in Britain. This outbreak was traced to effects of groundnut meal which formed part of the food<sup>1,2</sup>.

The toxic factors (which are now called aflatoxins) were found to be metabolites of *Aspergillus flavus*<sup>3</sup>, which is a common contaminant of groundnuts, groundnut cake and meal, and maize<sup>4,5</sup> and other foodstuffs. Aflatoxin was found in the milk of cows fed on rations of groundnut meal<sup>6</sup>.

Recent work has shown that aflatoxin is the most active known liver carcinogen for man and also induces local tumours when injected in the livers of rats<sup>7</sup>.

Histological examinations have been made of induced tumours in rats, but, so far as is known, no previous attempts have been made to investigate the action of aflatoxin on chromosomes.

The present experiments were carried out on seedlings of *Vicia faba* (Sutton's prolific). The aflatoxin solution used consisted of 2  $\mu$ g aflatoxin B<sub>1</sub>, 56.4 per cent aflatoxin G<sub>1</sub>, and aflatoxin G<sub>2</sub> were present in small amounts. The aflatoxin was dissolved in alcohol. This solution was then added to water, and the alcohol was removed by evaporation.

Control experiments with roots treated with water which had been mixed with alcohol and then evaporated showed that there were no chromosome aberrations induced by any traces of alcohol which may have remained after evaporation.

Roots were treated under two experimental conditions. Half the roots were immersed for 3 h in 6.7  $\times 10^{-4}$  M aflatoxin at pH 5.8 and at 21°C. The other half were treated in water for 3 h. After this treatment all the roots in both experiments were returned to the bean culture jar and grew in water.

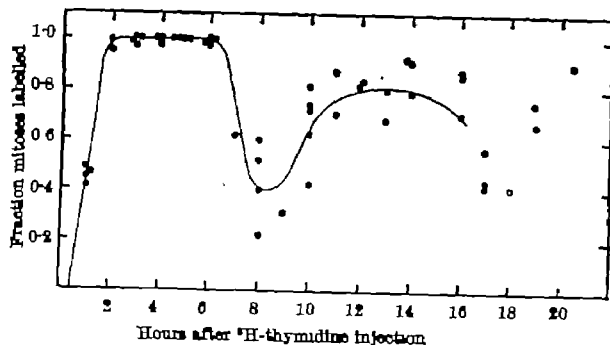


Fig. 1. Labelled mitoses in the tip of the tail of the embryonic mouse at 12-13 days gestation. O, Controls; ●, irradiated with 50 rads/day between days 7 and 12 of gestation. There is no major difference between the two series.

Table 1. ABNORMAL ANAPHASES IN ROOTS FIXED AT VARIOUS TIMES AFTER TREATMENT FOR 3 H IN AFLATOXIN

Fixation Water	Times (h)	3	6	12	24	30	48	72
	No. of anaphases examined	200	200	200	200	200	200	200
	% abnormal anaphases	0	2	0	1	0.5	1	1
Aflatoxin	No. of anaphases examined	195*	200	200	59*	59*	200	200
	% abnormal anaphases	41.5†	11.5†	2.5	16.9	30.7	25.5	2

\* The low mitotic index at these times reduced the available anaphases below the 100 per root normally examined.

† Roots at each of these times contained sticky chromosomes with bridges but few fragments.

At various intervals after treatment (timed from the beginning of the 3-h treatment) roots were fixed in 1:3 acetic alcohol. The fixation times were at 3, 6, 12, 24, 30, 48 and 72 h. The roots were then hardened in absolute alcohol and stored in 70 per cent alcohol.

Roots were hydrolysed at 20° C in 5 N hydrochloric acid for 40 min and stained in leucobasic fuchsin and squashed. Two roots were analysed from each treatment at each fixation time. Anaphases showing fragments and bridges were scored as abnormal. The results are shown in Table 1.

There is a highly significant ( $P < 0.001$ ) increase in the number of abnormal anaphases following treatment with aflatoxin. Most of the abnormalities consisted of chromosome fragments with occasional bridges, except at 3 h where both roots, and 6 h where one root, showed stickiness and many bridges. There was a considerable inhibition of mitosis (especially at 24 and 30 h) followed by a peak of divisions at 48 h.

Further experiments are in progress to compare the action of aflatoxin with that of well-known radiomimetic substances. It is also hoped to obtain samples of the separate aflatoxins for experiments.

Butler and Barnes<sup>1</sup> and other authors<sup>2-4</sup> have discussed the possible hazards of aflatoxin to man. These authors have directed attention to the possible part that fungal products may play in producing liver cancers in various parts of the world. So far there are no detailed investigations reported linking the occurrence of liver cancer with aflatoxin in humans. However, it is of interest that during an investigation into the induction of chromosome breaks in human blood cells in culture, being carried out in this laboratory, preliminary results have indicated that aflatoxin breaks human chromosomes<sup>11</sup>.

The aflatoxin used was a gift from the Tropical Products Institute, Department of Scientific and Industrial Research, to Prof. F. Dickens, of the Courtauld Institute, Middlesex Hospital, London, W.1.

*Note added in proof.* Recent experiments<sup>12</sup> have shown that aflatoxin treatment inhibits the synthesis of deoxyribonucleic acid from tritiated thymidine and inhibits mitosis, in cultures of human embryonic lung cell lines.

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## Individualized Whistle Contours in Bottle-nosed Dolphins (*Tursiops truncatus*)

In 1953, Essapian<sup>1</sup> suggested that individual bottle-nosed dolphins, *Tursiops truncatus* (Montagu), may have distinctive notes which each dolphin can recognize. From his context, in using the word 'notes' Essapian referred to the whistle component of *Tursiops* phonation.

In spite of the extensive work that has been done on the vocalizations of this and related dolphin species, this approach to the whistles as suggested by Essapian apparently has received no further consideration. Rather, the accent has been on an attempt to attach a particular whistle to a definite situation<sup>2</sup>. It has even been theorized that the various whistles could conceivably constitute a language<sup>3</sup>.

In the course of investigation relating to other aspects of cetacean behaviour, we recorded the vocalizations of five freshly captured *T. truncatus*, consisting of two adult females, one adult male and two sub-adult males. Vocalizations were audited for approximately three weeks following capture and in a great variety of circumstances. Our tapes show that with this group of animals, all taken from one wild school, each animal showed a clear tendency to emit the same whistle regardless of the situation. This becomes particularly clear when the tapes are slowed to 1/8 normal speed. The variety of situations to which they were exposed included isolation from the group (by which individual whistles were identified), feeding, entrance of divers into the tank, introduction of a strange dolphin into the tank, removal from a familiar tank to an unfamiliar one, and exposures to novel and sometimes disturbing stimuli including dead young from the same wild school. Although the whistle might vary slightly, the basic contour remained the same. The manner in which the whistle varied included number per minute, intensity, duration of the one whistle, and the abrupt breaking off of a whistle which had begun to be emitted. These variable units are potentially capable of conveying much information. For example, under mildly stressful situations, the whistles increased in number and intensity. In very alarming situations, the whistles ceased for a few minutes. The basic information transmitted, however, would be the identity of the animal doing the whistling.

The five predominant whistles for the five animals were clearly distinctive from each other at 1/8 speed. They consisted of the following whistles which can be compared with chart I of the whistle contours of *T. truncatus* as catalogued by Dreher and Evans<sup>4</sup>. Contour 1, corresponding to Dreher and Evans's contour 1, was a sharply rising single up-sweep, and was emitted in a series of one to nine whistles. Contour 2, a falling inflexion, corresponds with Dreher and Evans's contour 9. In our animal, this had a break toward the lower end and was never emitted more than twice in series, and usually only once. The third contour, a single unbroken up-and-down sweep, is the same as contour 3 of Dreher and Evans and was emitted almost constantly by one of the young males. It was made in series of 1-7 quick whistles and at 1/8 speed had a distinctive series of breaks in both rising and falling, somewhat resembling a scale as practised by a singer. Contour 4, corresponding to Dreher and Evans's contours 6 and 15, began with a falling inflexion, then rose and fell at least once. The rising and falling inflexions were sometimes continued without break for up to four more warbles. Our contour 5 somewhat resembles our contour 4, with the exception that it begins with a rising rather than a falling contour, and may warble from twice (Dreher and Evans's contour 5) up to five complete and unbroken warbles similar to contour 14 as presented by Dreher and Evans, which shows four complete warbles.

The most conclusive evidence which we can present regarding the individualization of these whistles is that, on being placed together, when one animal begins whist-

ling, others frequently follow suit resulting in a confused overlay. However, at slow speeds the individual whistles can be isolated. During these whistle sessions the individual contours were retained, and as they overlapped each other it was obvious that each animal was making its individual whistle. As our contours 4 and 5 varied only in the initial rise or fall of the warble, only the overlaid whistles of these two animals proved that they were distinctive and not variations of one contour.

On the basis of these observations we do not intend to state arbitrarily that a single animal has a vocabulary of a single somewhat variable whistle contour. However, this distinctive whistle occupied much more than 90 per cent of the total whistle vocabulary of any one animal during this three-week period following capture. Very rarely, however, an unfamiliar whistle was recorded. We do wish to point out, however, that our work to this point indicates a definite tendency toward an individualized whistle. Further investigation of this point is contemplated in the immediate future.

The whistle constitutes only one of the many phonations emitted by *T. truncatus*. Squeals, chirps, squeaks, squawks, barks, yelps, grating sounds, and echo-location bursts are common ones. Although some of these have been clearly shown to be used for echo-location<sup>4</sup>, others such as the squawk, even though containing very rapid pulses, may be indicative of the emotional state of the animal and not an echo-location device. Much work remains to be done in analysing these sounds in relation to behaviour.

Support for this work was received through grants from the U.S. National Institute of Mental Health (MH-07509-01) and the U.S. National Science Foundation (GB-1189). We thank William E. Evans of the University of California, Los Angeles, and William N. Sutherland of the Lockheed-California Company for assistance with the recording equipment, and J. B. Siebenaler of Florida's Gulfarium, Fort Walton Beach, for help with the capture and maintenance of the animals.

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## Correlations between the Architecture of Shoots and the Particular Fragments of them found as Fossils

OBSERVATIONS were made on plausibly-coniferous fossil shoots in silicified floras from arid southern central Australia. Such floras are found in an indurated siliceous matrix superficially resembling siltstone<sup>1</sup>, but occurring as eroded stones on various landsurfaces. The fossils, loosely oriented according to apparent bedding planes, are undistorted natural moulds of very high fidelity, often carrying details of surface morphology down to the microscopic dimensions of cell outline. They are not well expressed on fresh fracture faces, but only on weathered faces, by differential erosion. Of the plant debris fossilized within the matrix, one might expect the array presented at the surface of the eroded stony fragments to be dictated by chance, at the vagaries of erosion and weathering. However, the particular kind of fragment (branched or not, terminal or not, etc.) was found to be correlated with the architecture (and hence with the systematic status) of the shoot, despite fragmentation.

Correlations between characters of presentation and characters of shoot architecture were sought among 76 fossil shoots (of 21 different kinds) on characters including, on one hand, length of fossil, whether branched or not, and whether terminal or not, and on the other whether phyllotaxis was spiral, decussate, etc. Results of contingency comparisons between some of these features are given in Table 1.

Table 1

A	B	a	b	c	d	$\chi^2$ (Yates's correction)	Exact probability (Fisher's method)
Branches	Type	8	4	2	62	20.262	0.0000106
Branches	Spiral	5	60	6	5	13.112	0.00070659
Type	Spiral	5	60	6	3	23.663	0.00000917
Length	Branches	4	7	26	39	0.011	—
Length	Type	3	10	27	36	1.033	—
Length	Spiral	23	37	2	9	1.509	—

Association between characteristics A and B evaluated by  $\chi^2$  (Yates's correction) and by Fisher's exact method<sup>2</sup>. (Lengths greater than mean length were rated positive)

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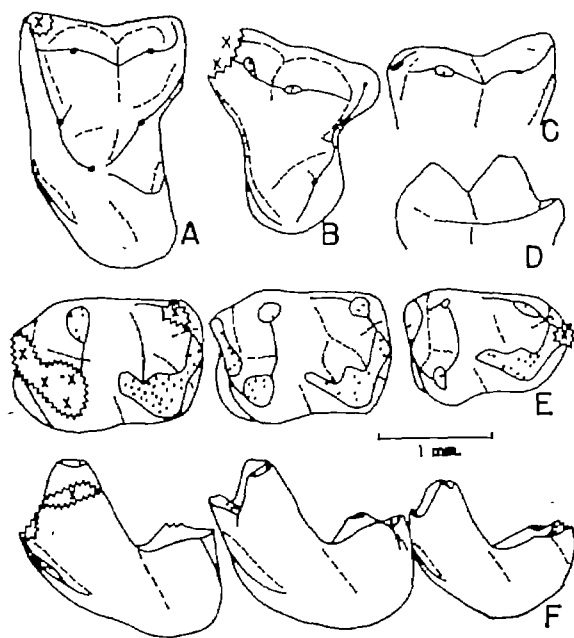


Fig. 1. A-D, *Palenochia minor*. A, left  $M_1$ , U.S.N.M. No. 9590. B, right  $P_4$ , A.M.N.H. No. 35443. C, labial part left  $M_2$  (not  $M_2$  as stated in ref. 5), U.S.N.M. No. 9595. D, labial view left  $M_2$ , U.S.N.M. No. 9595. E-F, *McKennaetherium laticum*, left  $M_1$ - $M_3$ , type specimen. E, occlusal view. F, labial and slightly anterior view. Areas with X are broken, dotted areas are wear facets, and dashed lines are concavities ( $\times 15$ )

mandible fragment with  $M_1$ - $M_3$  from Gidley Quarry<sup>5</sup>, Lebo Formation, Crazy Mountain Field, Montana.

Diagnosis: Most closely similar to *Palaschthon* and *Palenochia*. The lower molars differ from both these genera as follows: teeth relatively longer anteroposteriorly (except for the specialized  $M_2$  of *Palaschthon*); lateral walls of teeth more vertical; trigonid somewhat lower; posterior wall of trigonid somewhat less vertical; crista obliqua not extending up posterior wall of trigonid (also true in some specimens of *Palenochia*); talonid basin shallower; hypoconulid distinct on  $M_1$ - $M_3$ ; deepest point of talonid basin central;  $M_2$  shorter than  $M_1$ . Additionally differing from *Palaschthon* as follows: talonid narrower than trigonid; paraconid more distinct from parolophid and projecting more anteriorly; labial cingulum virtually absent; accessory protolophid absent; metaconid less bulbous; hypoconid relatively lower; no incipient third lobe on  $M_2$ ; hypoconulid single on  $M_2$ ; entoconid no more basin central;  $M_2$  shorter than  $M_1$ . Additionally differing from *Palenochia* as follows: parolophid less anterior; direct ridge from paraconid to protoconid absent; metaconid less posterior; base of enamel on labial side of trigonid rising much less steeply; hypoconid relatively lower; hypoconulid of  $M_1$ - $M_3$  somewhat lingual; hypoconulid lobe smaller but hypoconulid larger on  $M_2$ .

Table 1. MEASUREMENTS OF *McKennaetherium laticum*, TYPE SPECIMEN

	Length (mm)	Trigonid width (mm)	Talonid width (mm)
$M_1$	1.7	1.2	1.1
$M_2$	1.6	1.2	1.0
$M_3$	1.5	1.0	0.8

The genus is named for Malcolm C. McKenna; the specific name is a Latin word meaning pleasing.

Examination of specimens of *Palenochia* and *Palaschthon* has shown the presence in each genus of a previously unreported anterior tooth. In *Palenochia minor*, the alveoli of A.M.N.H. No. 35449 show that there are five antemolar teeth, the same number and presumably with the same homologies as those of *Paromomys*. In *Paromomys*  $P_4$  is two-rooted; in *Palenochia* it is one- or possibly two-rooted. The incisor of *Palenochia* is not particularly enlarged. In *Palaschthon alticuspis*, A.M.N.H. Nos. 35478 and 35489 have complete sets of alveoli, which indicate an

additional one-rooted tooth behind the moderately enlarged incisor. This additional tooth is probably  $I_2$ , although it could be  $P_1$ . The specimens used by Simpson<sup>6</sup> do not contradict the revised formulae. The lower dental formula of *Palenochia* and *Paromomys* is then, most probably,  $I1, O1, P3, M3$ , and of *Palaschthon*  $I2, O1, P3, M3$ . These genera are therefore not as aberrant as was previously thought.

I take this opportunity to present drawings of the upper dentition of *Palenochia minor* (Fig. 1 A-D). The figure given by Gidley<sup>5</sup> and copied by Simpson<sup>6</sup> is inaccurate.

*McKennaetherium*, *Palenochia*, *Palaschthon*, and probably *Plasiolestes* form a group of closely related genera that may provisionally be placed in the Paromomyidae pending a clarification of early prosimian interrelationships. The late Palaeocene genus *Navajovius* is generally similar to this group (its  $M_2$  is especially similar to that of *Palenochia*), but for the most part its similarities are confined to primitive characters. *McKennaetherium* and *Navajovius* are, in most of their known characters, the two most primitive primates as determined by comparison with possible near ancestors in the Insectivora<sup>7</sup>. Primitive erinaceoids, such as *Leptaecodon tener*, and leptictids seem the most likely near ancestors of the primates<sup>8,9</sup>, although this is uncertain. This conclusion implies that semimolariform fourth premolars may be primitive in primates, as further comparisons suggest even for the Eutheria as a whole<sup>10</sup>. The Microsypidae, placed possibly correctly in the Primates by McKenna<sup>3</sup> and Simons<sup>11</sup>, are unknown before the Eocene but at that time are not especially similar to the genera mentioned in this paragraph.

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\* Abbreviations. A.M.N.H., American Museum of Natural History; U.S.N.M., United States National Museum.

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## Loss of Suberin from Bark tissue rotted by *Armillaria mellea*

SUBERIZED tissue has for a long time been regarded as among the most resistant of plant tissues to fungal decay. Cork can be virtually immune from breakdown<sup>1,2</sup>, and the production of cork barriers is a common host reaction to fungal infection<sup>3</sup>. Nevertheless, there can be little doubt that suberin must eventually be degraded by microorganisms in the soil and litter; similarly, it is known that several fungi are capable of penetrating suberized barriers. Among these is *Armillaria mellea*, which was shown by Thomas<sup>4</sup> to be able to penetrate directly through the bark of host roots. Although this penetration was largely mechanical, Thomas reported some degradation of the cork layers in advance of the fungal hyphae. This suggestion of enzymatic breakdown of the cork layers has been largely discounted by later authors<sup>5,6</sup>.

Recent work here has, however, shown that the suberin content of bark tissue shows a marked decrease during rotting of the tissue by *A. mellea*. Ground bark tissue from the roots of *Brachystegia spiciformis* was extracted with ethanol, water and 5 per cent  $\text{Na}_2\text{SO}_3$  successively, and then the suberin, holocellulose and lignin contents

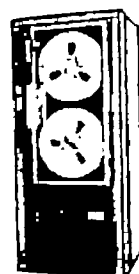
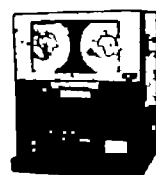
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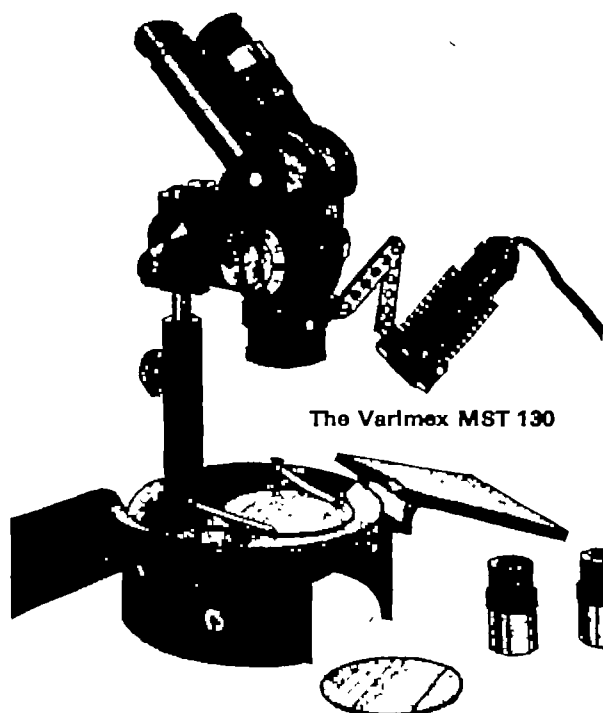
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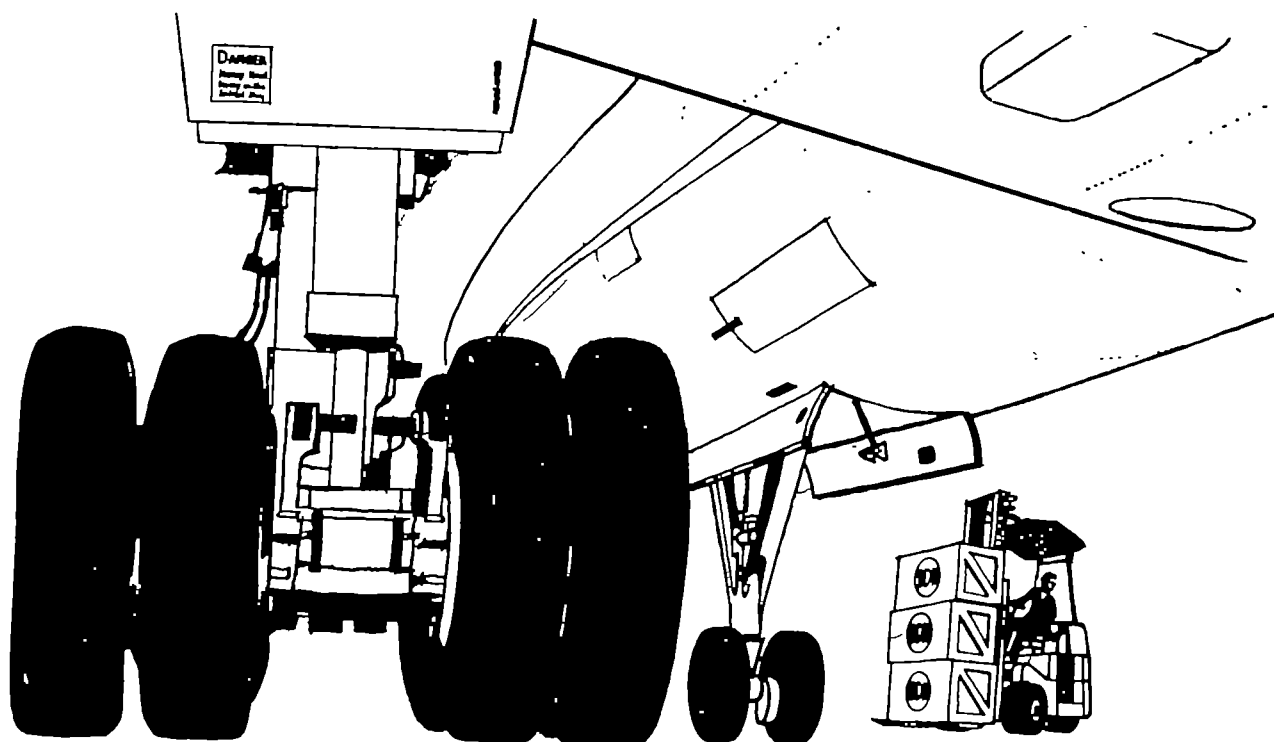
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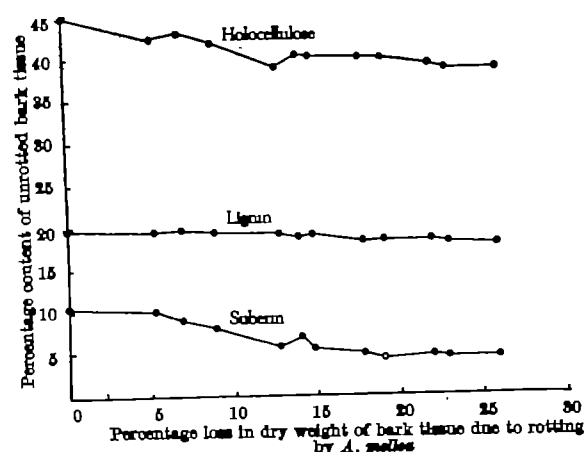


Fig. 1. Changes in composition of bark tissue due to rotting by *A. mellea*

were estimated, both before and after rotting by *A. mellea*. The suberin was estimated by the iodine-complexing method developed by Zetsche, Oholatnikov and Scherz<sup>7</sup> and the holocellulose and lignin separated by hydrolysis of the carbohydrate with 72 per cent  $H_2SO_4$ . Fig. 1 shows the changes in the content of these three fractions during rotting of the tissue by *A. mellea* over a period of ten months. During this period, the tissue lost more than 25 per cent of its dry weight; the holocellulose content had decreased by 14 per cent, the lignin by 9 per cent. The suberin content, on the other hand, had dropped from 10.8 per cent (of the original dry weight) to 4.7 per cent—a loss of 59 per cent of the original content.

These results indicate that suberin is lost from bark tissue during rotting by *A. mellea* and suggest the possibility of enzymatic breakdown of this substance by the fungus. It is hoped to be able to pursue this matter further, both with a larger range of fungi, and by investigation of the breakdown products of suberin degradation.

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## ENTOMOLOGY

### Influence of Radiation on Ovarian Maturation and Histolysis of Pupal Fat Body in Diptera

In *Calliphora* the medial neurosecretory cells of the brain are necessary for ovarian maturation<sup>1</sup>. They seem to act partly by inducing the release of a gonadotrophic hormone from the corpus allatum and to regulate the storage and mobilization of nutrients in the adult fat-body. Doane studied the interaction of ovary, corpus allatum and adult fat-body in mutant (female sterile (2) adipose) *Drosophila*<sup>2</sup> and concluded that the ovary itself, in developing under hormonal stimulus from the corpus allatum, mobilizes reserves in the adult fat-body by secreting a second hormone.

This system of developing organ (ovary), nutrient source (adult fat-body) and controlling mechanism (neurosecretory cells and corpus allatum) contains a second nutrient source variously called the 'larval fat-body'<sup>3</sup>,

'products of histolysis'<sup>3</sup> and 'pupal fat-body'<sup>4</sup>. It is a tissue left over from the pupal stage which rapidly disappears in normal flies as the ovaries begin to develop. Probably it is only a supplementary source of ovarian nutrients, because dietary deficiency in some adult Diptera both inhibits ovarian development and retards histolysis of the pupal fat-body<sup>4,5</sup>.

How is this histolysis controlled? Is it accomplished by direct action of the juvenile hormone and does maturation of the ovaries wait on this action? Or does the secretion of the corpus allatum stimulate the ovary, causing it to demand histolysis of the fat-body? Further questions are raised by the observation that, in Queensland fruit-fly *Dacus tryoni* Frogg (Diptera, Tryptetidae), doses of  $\gamma$ -radiation exceeding 4,000 rads also suppress ovarian maturation and delay histolysis of the pupal fat-body. Is this due to: (a) direct damage to the ovary and hence reduced 'demand' on the fat-body, or (b) damage to the brain or corpus allatum which prevents them from producing the gonadotrophic hormone, or (c) interference with a hypothetical mechanism by which the ovary controls the secretory activity of the corpus allatum, or (d) blockage of the system by damage at more than one point?

In the following experiments we tried to eliminate some of these alternatives. If (b) is correct, irradiation of the head should prevent ovarian maturation. We gave the following treatments to four randomly selected groups of pupae *Dacus tryoni*, using pupae 6–8 days old and, for convenience, a 120-kV source of filtered X-rays instead of  $\gamma$ -rays. The pupae were exposed on a 'Perspex' plate to a dose in air of 8,000 rads, as follows: Group A: control, not irradiated; Group B: whole body irradiated; Group C: anterior third of body irradiated; Group D: posterior third of body irradiated. The unirradiated portions of pupae in Groups C and D were shielded by lead blocks 1.2 cm thick. Scattering into the shielded parts seemed to be negligible when tested with X-ray plates. When adult flies emerged, they were provided with water, sucrose and enzymatic hydrolysate of yeast and kept at 25° C in a photoperiod of 15–16 h. After 5 weeks, the females were dissected. Ovarian development was scored on a subjective scale from I to IV. Stage I ovaries showed no apparent development over the ovaries of newly emerged flies and Stage IV was identified by the presence of mature eggs. The presence or absence of pupal fat-body was also recorded. The results are shown in Table 1.

Table 1

Group	Treatment	Nos. in each stage of ovarian development (I–IV) and Nos. with pupal fat-body (pfb)							
		I	II	III	IV	pfb	pfb	pfb	pfb
A	Control	0	0	0	0	0	54	0	0
B	Whole body irradiated	45	Not recorded	0	Not recorded	0	Not recorded	0	Not recorded
C	Anterior third irradiated	8	0	4	0	1	21	0	0
D	Posterior third irradiated	29	19	2	0	0	1	1	1

The difference in ovarian maturation between Groups A and B does not call for statistical analysis. If we pool the data from animals in Groups C and D under the headings Mature (Stage IV) and Immature (Stages I–III), they are significantly different ( $\chi^2 = 7.1$ ,  $P < 0.01$ ) and they also differ significantly ( $\chi^2 = 22.2$ ,  $P < 0.001$ ) in the proportion of animals which have histolysed their pupal fat-bodies. The ovaries of eight animals in Group C remained in Stage I and in seven animals of Group D they went on to Stage IV probably because of scattered radiation or variation in alignment under the lead shield.

Obviously irradiation does not directly prevent secretion by the corpus allatum and it apparently acts directly on some abdominal organ. Doane<sup>2</sup> suggested that the ovary itself may influence the secretory activity of the corpus allatum, and our results could mean that radiation blocks some stimulus from the abdomen, thus indirectly preventing ovarian development.

To test this hypothesis we applied farnesyl methyl ether, which mimics the gonadotrophic action of the juvenile hormones<sup>6</sup>, to the abdomens of newly emerged female flies which had been given a dose of 4,000 rads  $\gamma$ -radiation on the eighth day of pupal life. The farnesyl methyl ether was mixed with alumina dust to prevent it from spreading over the cuticle and perhaps to assist its entry into the haemocoel. The dose (12  $\mu$ g mixed with 15  $\mu$ g of alumina) was made large enough to induce marked muscular twitching in the legs of the recipients. Fifty-eight out of 132 treated females survived for 5 weeks but none showed ovarian development. This negative result suggests that the irradiated ovary may be incapable of responding to the gonadotrophic hormone from the corpus allatum, but tells us nothing about a possible stimulatory action on the corpus allatum by the ovary.

The histolysis of pupal fat-body seems to be partly dependent on ovarian maturation. But in these and more extended observations, irradiated flies lost their pupal fat-body slowly even though the ovaries did not develop. Presumably other demands cause a slow histolysis even in the absence of ovarian development, whether this is inhibited by radiation or by nutritional deficiencies in the adult diet<sup>4,5</sup>. These results suggest that radiation does not act hormonally and support the contention of La Chance and Bruns that it directly damages the nurse cells of the ovary<sup>7</sup>. This in turn probably reduces demand on the pupal fat-body and delays its histolysis.

We thank Prof. H. G. Andrewartha and Dr. E. M. Arnold for their advice. Samples of farnesyl methyl ether were given by Dr. J. Fellig of Hoffmann-La Roche Inc., and Mr. F. Hampshire of Union Carbide Australia, Ltd. Methods of applying farnesyl methyl ether were suggested by Prof. A. F. O'Farrell and Prof. A. Stock. The work was carried out in the Zoology Department, University of Sydney.

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## MICROBIOLOGY

### Growth Inhibition of *Escherichia coli* by some Basic Proteins prepared from the Same Strain

PONTECORVO<sup>1</sup> in his Leeuwenhoek lecture to the Royal Society said that "histones are characteristic intranuclear proteins in search of employment". Other workers have suggested that histones could be concerned with the regulation of RNA synthesis<sup>2,3</sup>. The findings of Gurley *et al.*<sup>4</sup> were more specific. They found that in a cell-free system DNA polymerase was inhibited by a lysine-rich histone fraction. Our own work points in the same direction. During a study of the cellular control of metabolism we isolated basic proteins of *Escherichia coli* and found that some of these preparations inhibited the organism from which the protein was isolated.

*E. coli* (NCTC 8196) was grown in 49 l. of Littauer and Kornberg's medium<sup>5</sup> for 4 h (end of logarithmic phase) with mechanical stirring and forced aeration at 37° C. The cells were collected in a Sharples centrifuge, and

stored deep-frozen until required. We isolated an acid-alcohol-soluble fraction by the method of Johns *et al.*<sup>6</sup>, recommended for the isolation of the arginine-rich fraction of histone from calf thymus. 50 g bacterial paste was homogenized with 350 ml. ethanol:water (70:30 v/v) in a Braun disintegrator (Shandon Ltd., London) with 50 g No. 12 Ballotini beads and continually cooled with CO<sub>2</sub> snow. The homogenate was centrifuged and the supernatant discarded. The deposit was washed once in the above solvent, and the supernatant again discarded. The deposit was then taken up in 100 ml. ethanol:N.HCl:water (70:25:5 v/v), transferred to a ball mill, and extracted at 1° for 20 h. After centrifuging, the deposit was re-extracted in 50 ml. acid ethanol for 4 h at 4° and centrifuged, and the deposit was re-extracted for a further 2 h with 50 ml. solvent. The precipitate was discarded. The supernatants were combined and dialysed three times for 4 h at 4° against 300 ml. ethanol. The precipitate which forms during dialysis was collected by centrifugation, washed with ethanol, then with acetone, and dried under vacuum.

We also isolated a perchloric-acid-soluble fraction by the method of Johns and Butler<sup>7</sup> recommended for the isolation of the lysine-rich fraction from calf thymus. 50 g of *E. coli* paste was homogenized with 400 ml. 5 per cent v/v of 0.76N HClO<sub>4</sub> in a Braun disintegrator, with 50 g No. 12 Ballotini beads, and continually cooled with CO<sub>2</sub> snow. After centrifuging at 1100 g for 10 min, the deposit was re-extracted in 100 ml. 5 per cent HClO<sub>4</sub>. The combined supernatants were clarified through a No. 4 sinter and trichloroacetic acid was added to the clear extract to bring the final concentration to 12 per cent (0.74 M). After standing overnight at 4° the precipitate was removed by centrifuging. The sediment was dissolved in 100 ml. ethanol and concentrated HCl (1 ml.) was added, followed by 50 ml. acetone. The white precipitate formed was washed three times with acetone and dried under vacuum.

The effect on the growth rate of *E. coli* was followed by diluting an overnight culture 1/1000 in Littauer and Kornberg's medium to which different concentrations of the preparations redissolved in distilled water were added. The growth of the organism was followed by measuring the increase in optical density at 580 m $\mu$  for 8 h.

The fraction soluble in perchloric acid did not increase or reduce the rate of growth of *E. coli* at final concentrations of 200  $\mu$ g/ml.

The acid-alcohol-soluble fraction gave variable results. This preparation was repeated 17 times; 3 preparations were 'active' because they were inhibitory to growth; 14 preparations were 'inactive'.

The effect of the 'active' preparation is shown in Fig. 1. Growth inhibition was proportional to the concentration used: 40  $\mu$ g/ml. was partially inhibitory and 80  $\mu$ g/ml. was completely inhibitory. Inactive preparations gave curves which were indistinguishable from the control.

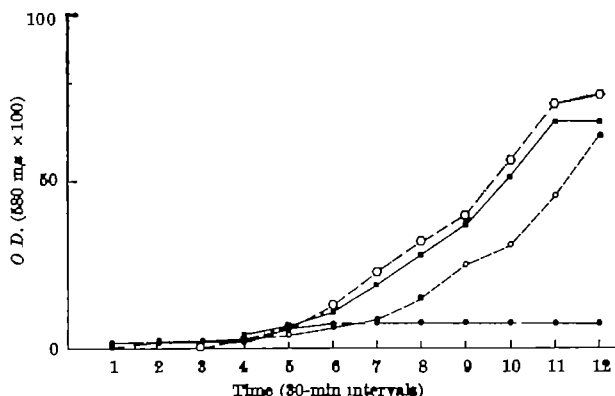


Fig. 1 Graded response assay of 'active' preparation: —●—, 80  $\mu$ g/ml.; —○—, 60  $\mu$ g/ml.; —□—, 40  $\mu$ g/ml.; - - - - - , control

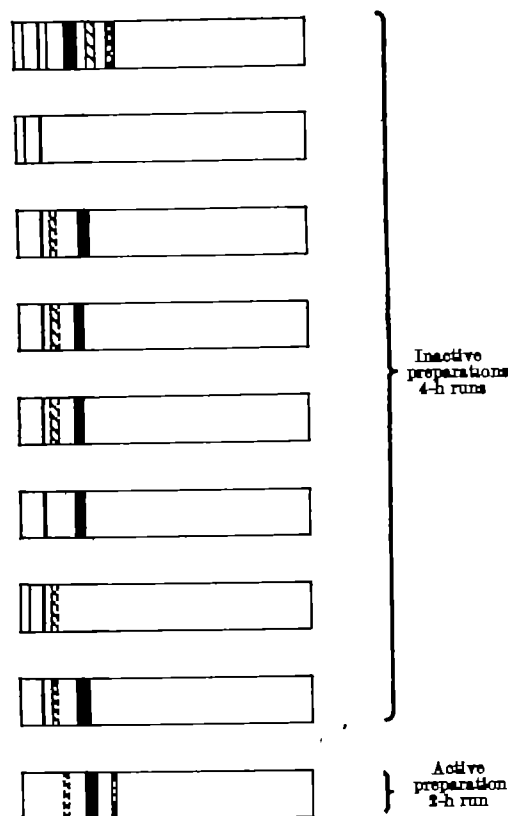


Fig. 2. Polyacrylamide-gel electrophoretic patterns of acid-alcohol-soluble fractions of *Escherichia coli*.

These active and inactive preparations were examined by electrophoresis in polyacrylamide gel after the method of Reisfeld *et al.*<sup>6</sup> The gels were made up in potassium hydroxide/glacial acetic acid (pH 4.3) and they were run for 2 or 4 h. Fig. 2 shows that the 'active' preparations have a thick band of fast-moving component which is missing in the 'inactive' preparations. The latter, however, contains bands of slow-moving material missing in the 'active' preparation. The exact nature of the bands is not yet known and we cannot explain why some preparations should be active and others inactive. Activity most probably resides in the fast-moving component missing from the 'inactive' preparations, but it is also possible that the slow-moving bands which are present only in the 'inactive' material antagonize the inhibitory activity. Although only 3 of the 17 preparations were 'active' the interest of this work is that we have obtained at least some preparations which were inhibitory to the growth of cells of the strain from which they were isolated. It could be, therefore, that in the growing cell also such proteins are concerned with regulation of growth.

We thank Dr. P. Akroyd for carrying out the gel-electrophoresis.

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## GENETICS

### Maleic Hydrazide, a Chemical Mutagen in *Drosophila melanogaster*

MALEIC hydrazide is one of the various reagents which induce breaks in the chromosomes of some plants at specific regions recognizable as heterochromatin<sup>1,2</sup>. It also inhibits growth in several species of plants, at least doubling the time taken to proceed through a complete mitotic cycle. Much of this delay in cell development has been explained by Evans and Scott<sup>3</sup> as due to severe depression in the rate of DNA synthesis. They also suggest that the aberrations are produced at the time of duplication, and that maleic hydrazide acts by interfering, in some way, with the normal process of duplication. It was also found by Compton<sup>4</sup> that it has a more pronounced effect on cell division in the roots than in the shoots. In view of its pronounced effects in plants, it was thought of interest to test this chemical for mutagenic action in *Drosophila*.

Males of a wild-type 'Oregon-K' (*Or-K*) strain, which has a spontaneous mutation rate of 0.3 per cent, were used. The standard Muller-5 test for sex-linked recessive lethals was used. The chemically treated medium was made up by stirring 0.4 per cent maleic hydrazide at 55° C into a standard medium. Seeding with live yeast was omitted. Flies reared on the standard medium started to emerge one day earlier than those reared on the chemically treated medium.

In a second experiment, one- to two-day-old males reared on the standard medium received abdominal injections of 0.7 per cent sodium chloride Ringer's solution containing 0.4 per cent maleic hydrazide.

The results are summarized in Table 1.

Table 1. FREQUENCY OF SEX-LINKED RECESSIVE LETHALS IN THE PROGENY OF MALES REARED ON FOOD CONTAINING 0.4 PER CENT MALEIC HYDRAZIDE

Brood	No. of sperm tested	No.	Lethals	%
1st	1,024	11		1.07
2nd	857	3		0.35
3rd	813	3		0.37

It is clear from Table 1 that the most advanced stage in the larval testis responded to maleic hydrazide. In the progeny of the injected males, it proved to be ineffective; only 12 mutations were produced in more than 3,000 chromosomes tested in four broods.

Since there is a possibility that maleic hydrazide, when given in the food, acts on auxocytes and since there are no auxocytes in the adult testis, this by itself could explain the lack of response for maleic hydrazide injected into adults.

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## SOIL SCIENCE

### Extractable Aluminium in Steam-sterilized Soils

CHANGES in the chemical properties of steam-sterilized soils have been frequently reported<sup>1-3</sup>. The temporary increases in ammonia-nitrogen and readily available manganese have attracted particular attention.

In recent studies of manganese toxicity a high content of aluminium was observed in lettuce plants grown in steam-sterilized soil. The effect of steam-sterilization

on the extractable soil aluminium was therefore examined; N-ammonium acetate at pH 4.8 was chosen as the extractant, as used in some of the more recent investigations<sup>4-7</sup>.

Soil samples were taken from five locations at this Institute in November 1963 and again in March 1964. Sub-samples (200 g moist soil) contained in 250-ml. conical flasks, loosely plugged with cotton wool, were treated as follows: *A*, control—no treatment; *B*, steam-sterilized in an autoclave at 10 lb./sq. in. pressure for 30 min; *C*, as *B* with 0.3 per cent of CaCO<sub>3</sub> by weight mixed in before steaming; *D*, as *B* with 0.3 per cent of CaCO<sub>3</sub> by weight mixed in after steaming when the soil was cool.

All the flasks were sealed with 'Parafilm' to prevent drying, and after storing for three weeks at room temperature (18°–23° C) aluminium was extracted from the moist soil and determined by the aluminon method<sup>8</sup>.

In November 1963 only a single soil sample per location was analysed, thus limiting the statistical examination of the results. There was an increase in extractable aluminium in steamed soil, the mean values for treatments *A* and *B* being 23.2 and 35.8 p.p.m. aluminium respectively (based on oven-dry soil). Liming reduced the levels of aluminium to just below that of the unsteamed soil (*C*, 21.4; *D*, 20.6).

The results for samples taken in March 1964 are given in Table 1. A highly significant ( $P < 0.01$ ) increase in extractable aluminium due to steaming without liming was recorded for all samples. Liming either before or after steaming (*C* and *D*) decreased the content of extractable aluminium as compared with treatment *B*. Averaged over the five sampling locations, the combination of liming with steaming (*C* and *D*) resulted in a content of extractable aluminium below that of the untreated soils ( $P < 0.01$ ). The mean pH values for the four treatments are given in Table 1.

Table 1. ALUMINIUM (P.P.M. Al IN OVEN-DRYED SOIL) EXTRACTED WITH N-AMMONIUM ACETATE (pH 4.8) FROM SOIL SAMPLES TAKEN IN MARCH 1964

Soil	Treatments				Mean
	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	
1	77	94	56	36	65.5
2	18	57	36	20	32.6
3	86	110	58	45	74.6
4	64	86	50	37	59.1
5	24	68	43	30	43.4
Mean	55.5	82.9	48.4	33.4	
Mean pH	6.1	6.1	7.6	7.7	

L.S.D.\* ( $P < 0.01$ ) for aluminium figures only.

Body of the table	4.18
Soil means	2.07
Treatment means	1.87

\* L.S.D.—least significant difference.

The marked differences in aluminium extracted from the corresponding samples at the two dates probably reflect a seasonal change, but no definite conclusion can be drawn until the investigation has been continued over a longer period. Comparable seasonal changes in extractable aluminium have been reported by Plucknett and Sherman<sup>4</sup>.

Kelley and McGeorge<sup>9</sup> noted some increase in aluminium solubility in heated soils, but Robinson<sup>10</sup> and Lutrick<sup>11</sup> failed to observe any effect of steam-sterilization in their studies.

The injury to young plants often reported in steam-sterilized soils has been variously attributed to the accompanying increases in ammonia and soluble manganese. Excessive uptake of manganese certainly occurs in plants growing in some steamed soils, and gives rise to symptoms of manganese toxicity which can readily be reproduced in sand culture at high levels of this element. Not all cases of sterilization injury can be explained satisfactorily in terms of excess manganese or ammonia-nitrogen, however. The present results show appreciable increases in the content of extractable aluminium in steamed soils, and it is suggested that aluminium may be an important factor in sterilization injury. In this connexion it is of interest that young plants have been reported to be particularly vulnerable to an excess of aluminium<sup>12</sup>. The results may also help to explain the response of plants

to additional phosphate in cases of sterilization injury, even where the level of soil phosphate would normally be regarded as fully adequate for plant growth.

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## Use of a Selective Cellulose Agar for Isolation of Soil Fungi

ADVANCES in soil microbiology have frequently been related to the development of selective isolation techniques. Such methods usually depend either on the provision of an energy source available only to a limited group of micro-organisms, or on the presence of an anti-microbial substance which allows a limited spectrum of micro-organisms to grow. Energy sources which have been studied by such methods include a range of monosaccharides, cellulose<sup>1,2</sup>, chitin<sup>3</sup>, xylan<sup>4</sup> and lignin<sup>5</sup>. The anti-microbial agents most widely used are the antibiotics, effective against various groups of soil organisms.

Concerning the isolation of fungi from the soil, it is clear that selective media have some advantages over 'non-selective' media, such as tap water agar and soil extract agar, but the exact nature of these advantages is often only imprecisely defined. Such definition requires detailed comparative studies, using the selective and non-selective agars in parallel.

During work on the rhizosphere of *Halimione portulacoides* (L.) Aell. in several salt marshes, such comparative information has been obtained concerning the value of cellulose agar prepared according to the method of Eggins and Pugh<sup>6</sup>. The soil plate technique of Warcup<sup>7</sup> was used on mud from two *Halimione* communities at Gibraltar Point, Lincolnshire, and one community at Parkgate, Cheshire, during the period October 1961 to July 1963. Fungi were recorded only as present or absent on the soil plates. In Table 1 the fungi listed were recorded as clearing the cellulose agar on more than 10 per cent of the soil plates in at least one of the three communities. These results are compared with those obtained for the same species using mud extract agar<sup>7</sup>. A modification of Warcup's technique, involving the subculturing of all fungi as they appeared, was practised with the mud extract agar plates, so that competition between species on the plates was greatly reduced.

Consideration of the fungi in Group 1 of Table 1 shows that where they were present in considerable numbers the individual frequencies of isolation on the two media were often remarkably similar. A detailed statistical analysis of each pair of totals showed that in no instance was there any significant difference between the two frequencies. Such tests were not applied where the combined total on the two media was below 20. Further analysis of Group 1 shows that, using fungal species as replicates, there was, for each community, no significant difference between the total isolation of Group 1 fungi on cellulose and on mud extract agar.

In two instances, placed together in Group 2, the results diverged from the pattern found for the previous group. Isolations of *Cephalosporium* spp. from the develop-

Table 1. COMPARISON OF FUNGI ISOLATED FROM RHIZOMORPHS OF *Habeoloma* BY THE SOIL PLATE METHOD, USING CELLULOSE AGAR AND MUD EXTRACT AGAR

	Gibraltar Point				Parkgate marsh	
	Developing marsh		Mature marsh		Cellulose	Mud-extract
	Cellulose	Mud-extract	Cellulose	Mud-extract		
Number of soil plates	112	112	112	112	112	112
Fungi—(percentage of plates colonized)						
Group 1						
<i>Ondocytium helveticum</i> (Ost.) Sacc.	1	12	2	7	14	4
<i>Dendryphiella sedula</i> (Buth.) Pugh + Moot	21	20	1	1	21	20
<i>Gliocladium roseum</i> (Link) Batnik	5	12	75	72	5	7
<i>Gliocladium stramonii</i> (Oorda) Hughes	12	12	8	4	10	11
<i>Gliocladium</i> sp. 68	0	0	12	5	0	1
<i>Nectria fraxinea</i> Peith. (conidial)	12	10	4	2	6	12
<i>Trichoderma viride</i> Pers. ex Fr.	12	14	2	5	22	24
<i>Verticillium nigrescens</i> Peith.	11	0	2	0	10	5
Group 2						
<i>Cephalosporium</i> spp.	49	67	22	26	49	63
<i>Fusarium culmorum</i> (W. G. Smith) Sacc.	11	18	12	14	10	23

ing marsh at Gibraltar Point were significantly different ( $P < 0.05$ ) on cellulose agar and mud extract agar, though no significance was found between comparative isolations in the mature marsh or at Parkgate. The figures from which this analysis was calculated were obtained from the primary data which were in the form of monthly totals based on equal numbers of Petri dishes of each medium. *Fusarium culmorum*, while being isolated with similar frequencies on the two media from the Gibraltar Point communities, showed some differences between the media at Parkgate ( $P \sim 0.1$ ).

These data suggest that where the abundant cellulose-decomposing species are concerned they may be isolated with similar frequency on mud extract agar and on cellulose agar. The two exceptions show a majority of isolations from mud extract agar, which suggests that there were components of these taxa which were unable to decompose the cellulose particles and were therefore not recorded from the cellulose agar plates. This is of interest as it agrees with the situation as observed during the work. *Cephalosporium* is a very difficult genus to treat in a strict taxonomic fashion on the accepted morphological criteria. It would seem probable that several species were involved in the totals given, especially those for the plates prepared using mud extract agar. *Fusarium culmorum* is also a taxon which is subject to some discussion, and it may be that the isolates assigned to this species on mud extract agar were not all valid *F. culmorum*, or that this species has a wider range of variation in biochemical potential than in morphology.

The detailed data include only those fungi which were relatively abundant, but an examination of the full lists and totals obtained in the study shows that few species were isolated on the cellulose plates, even in low numbers, which were not also found on the mud extract agar soil plates. It may be suggested that the use of cellulose agar allows no marked additional spectrum of cellulose-decomposing fungi to be isolated. This would be in accordance with the accepted view that, although fungi may have a potential for decomposing complex substances such as cellulose, they also grow well on less complex energy sources, as provided by the non-selective media. Pugh *et al.*<sup>6</sup> came to a similar conclusion regarding the ubiquitous cellulose-decomposing fungi present in all the sites they examined, though they did not consider the remainder of the fungi, isolated from a more limited number of sites, to be regularly isolated on non-selective media. This is at variance with the data presented here, and in this context it must be stressed that these present results were obtained by using the complete Warcup technique<sup>5</sup>. Although this is very laborious, it does considerably increase the number of isolates and of species which are obtained.

There are, however, some very considerable practical advantages to be gained by the use of cellulose agar for certain types of soil mycological investigations. As was suggested by the initiators of the technique<sup>6</sup>, it allows a

rapid assessment of the population of cellulose decomposing species to be made and, as many sporulate more readily and more copiously on this medium, their identification is made more certain. It does not fully inhibit the growth of mucoraceous fungi but their growth is usually sparse, so that they do not over-run the slower growing species as much as they do on non-selective media. Cellulose agar is at present being used to investigate the distribution of certain cellulose-decomposing species in a number of soils where no information is required concerning other fungi present.

We thank Dr. D. M. Loeel for her advice.

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## PSYCHOLOGY

### Correlation of Behaviour with Changes in Amplitude of Cortical Potentials evoked during Habituation by Auditory Stimuli

INCREASING attention has been directed to changes in the parameters of both evoked and spontaneous electrocortical activity in attempts to determine the electrophysiological correlates of behavioural awareness or attention, or the significance of sensory stimuli. In animal studies, habituation procedures have been used extensively and emphasis has been placed on the alterations which occur in the primary component of the sensory-evoked response. For example, it has been reported<sup>1,2</sup> that the iterative presentation of a brief acoustic stimulus induced a decrease in the amplitude of the primary response evoked in the specific auditory cortex. The behavioural criteria for habituation in these studies were not defined. Moreover, the sensory stimulus was presented over long periods of time and one would assume that any significance or meaning attached to it by the animal would have been lost long before a change occurred in the primary cortical response.

In order to study this question more fully, experiments have been carried out using five cat preparations with chronically implanted cortical recording electrodes. During the experiments the animals were housed in a sound-proofed cubicle and observations of behaviour were made by means of a closed-circuit television system. the camera

of which was placed in the roof of the cubicle. Tonal pips of 30 msec duration were used so that the frequency of the acoustic signal could be varied over a wide range. Frequencies between 800 and 10,000 c/s were employed and the tonal pips were presented from a loudspeaker also situated in the roof of the cubicle. The intensity of the stimulus at these different frequencies was equated for loudness to the cat<sup>3</sup>, being approximately 50 dB above the threshold for human hearing at 1,000 c/s. The acoustic signals were presented at intervals between 1 and 2 min.

Fig. 1A illustrates that within 30 presentations of a tonal pip of 3,000 c/s to an alert preparation, the secondary, surface-positive component of the potential evoked over the primary auditory cortex had decreased in amplitude by 50 per cent, while the primary, positive/negative complex still showed no significant change. This effect was specific for the 3,000-c/s stimulus, since potentials evoked by test stimuli of 10,000 c/s, presented before and after habituation to the 3,000 c/s tonal pip, still showed no significant change in either the primary or secondary components of the cortical response (Fig. 1B). Further repeated presentation of the 3,000-c/s stimulus was necessary before attenuation of the primary wave occurred.

It was difficult to correlate any precise behavioural observations with the attenuation of either the primary or secondary wave using stimuli of such short duration. This disadvantage was offset by increasing the duration of the tonal pip, a procedure which did not appear to produce any significant change in the latency, form or duration of the evoked responses. For example, the response evoked by a 30-msec tonal pip (Fig. 1A) was practically identical with the 'on' response initiated by a tonal stimulus of equal intensity and frequency, but lasting for five seconds (Fig. 2A). The only apparent difference between the two stimuli was in the intensity of the behavioural response which they evoked. The tonal pip produced little behavioural change other than an occasional ear movement, whereas the tone lasting 5 sec induced widespread cortical desynchronization and complete behavioural arousal. By using the latter criteria the behaviour of the animal could be correlated with the form of the evoked potential and the process of habituation followed more easily, for, as Sharpless and Jasper<sup>4</sup> have

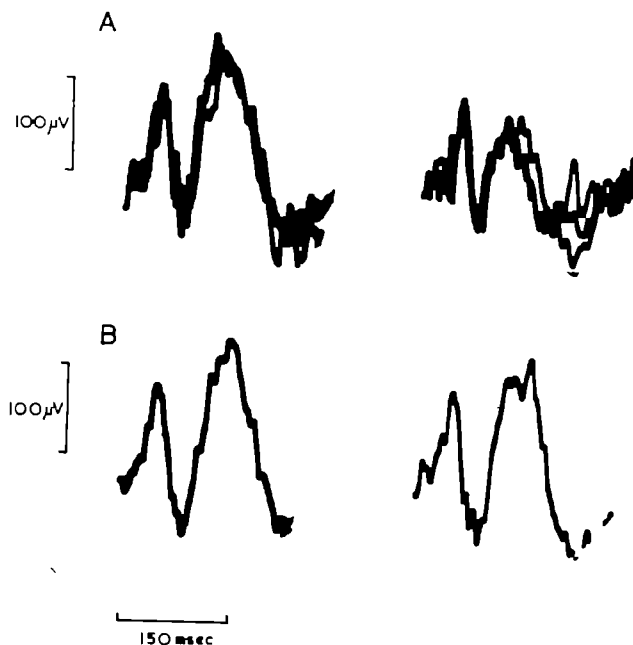


Fig. 1. A, Superimposed monopolar recordings of cortical potentials evoked in the primary auditory cortex by a tonal pip (3,000 c/s) before and after 30 presentations of the stimulus to the alert animal. B, Similar cortical potentials evoked by a test stimulus of 10,000 c/s before and after habituation to the 3,000-c/s stimulus. Polarity - positive deflection upwards

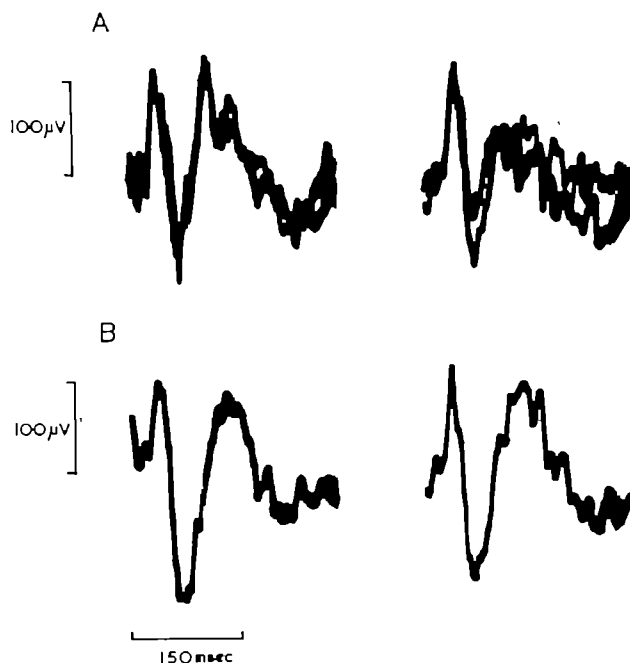


Fig. 2. A, 'On' responses evoked in the primary auditory cortex. Potentials initiated by a 5-sec, 3,000-c/s tonal stimulus before and after habituation to the behavioural arousal response. B, Similar potentials initiated by a test stimulus of 600 c/s, which still produced arousal before and after habituation to the 3,000-c/s stimulus. Polarity - positive deflection upwards

pointed out, the repeated presentation of a tonal stimulus results in the progressive reduction and final obliteration of the period of electrocortical desynchronization, accompanied by abolition of the behavioural arousal response.

Repeated presentation of a 5-sec tonal stimulus of 3,000 c/s at approximately 2-min intervals produced habituation of the arousal response, usually within 20 to 30 trials, and also the progressive reduction in the amplitude of the secondary component of the evoked 'on' response in the auditory cortex. Comparison of the 'on' potential to tones inducing behavioural arousal at the beginning of the experiment with potentials evoked by the same tone after habituation demonstrates this latter point, and shows that the amplitude of the secondary, surface-positive component of the auditory-evoked potential follows closely the behaviour of the animal (Fig. 2A). Indeed, in the case of Fig. 2 the amplitude of the primary wave exhibited little change during the initial stage of the experiment. It was only following further presentation of the 3,000-c/s stimulus and after abolition of the behavioural response that changes became apparent in the amplitude of the primary component. Moreover, the change in the secondary wave was specific for the potentials evoked by the 3,000-c/s tone. Test stimuli at a frequency of 600 c/s, which induced arousal before and after habituation to the 3,000-c/s stimulus, still induced cortical potentials without any significant attenuation in the amplitude of either the primary or secondary waves (Fig. 2B).

These results appear to substantiate the view that it is the amplitude of the secondary, surface-positive component of the responses evoked in the primary auditory areas, rather than that of the primary waves, which is more closely related to the level of significance of the stimulus, at least in terms of its arousal or attentive value.

B. J. KHY

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<sup>1</sup> Galambos, R., Sheatz, G., and Verner, V. G., *Science*, **123**, 376 (1956).

<sup>2</sup> Marsh, J. T., McCarthy, D. A., Sheatz, G., and Galambos, R., *Electroencephal. Clin. Neurophysiol.*, **12**, 244 (1961).

<sup>3</sup> Dworkin, S., Katzman, J., Hitchison, G. A., and McCabe, J. B., *J. Exp. Psychol.*, **28**, 381 (1940).

<sup>4</sup> Sharpless, S., and Jasper, H., *Brain*, **70**, 655 (1955).



## FORTHCOMING EVENTS

Wednesday, July 28

**SOCIETY FOR ANALYTICAL CHEMISTRY, SPECIAL TECHNIQUES GROUP** (Joint meeting with the Polarographic Society, at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Mr. K. G. Powell and Dr. G. F. Reynolds, "The Application of the 'Load-Line' Method to the Interpretation of the Effect of Resistance in Electrochemical Systems"; Mr. J. W. Howden: "Some Considerations in the Construction of a Cathode-Ray Polarograph".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

**POST-DOCTORAL FELLOW IN THE DEPARTMENT OF MATHEMATICS** to work on the application of quantum mechanics to conjugated molecules—The Registrar, The University, Nottingham (July 28).

**ASSISTANT LECTURER** (with a good honours degree (preferably also a higher degree), experience in research and/or industry, and preferably some teaching experience) in the DEPARTMENT OF APPLIED PHYSICS—The Secretary, Welsh College of Advanced Technology, Cathays Park, Cardiff, quoting Ref. No. DU878 (July 31).

**LECTURER OR ASSISTANT LECTURER** (preferably with interests in a branch of pure mathematics) in MATHEMATICS—The Registrar, University of East Anglia, Norwich Hall, Norwich, NOR 88Q (July 31).

**LECTURERS AND ASSISTANTS IN ZOOLOGY**—The Secretary of University Court, The University, Glasgow (July 31).

**RESEARCH ASSISTANT** in the DEPARTMENT OF GEOGRAPHY to work for one year on biogeographical aspects of the machair habitat in the Outer Hebrides—The Secretary of University Court, The University, Glasgow (July 31).

**RESEARCH ASSISTANT** (preferably with a higher degree and research experience in biochemical techniques) in the DEPARTMENT OF BOTANY, for a study, in association with Dr. G. G. Fritsch, of the effects of high pressures of oxygen on the enzyme systems of fungi and bacteria—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (July 31).

**SENIOR LECTURER (CLINICAL)** (with a higher qualification in surgery) in the DEPARTMENT OF SURGICAL NEUROLOGY—The Secretary, The University, Edinburgh (July 31).

**ASSISTANT LECTURER IN BOTANY**—The Secretary, Birkbeck College (University of London), Malet Street, London, W.1 (August 2).

**EXPERIMENTAL OFFICER** (with a degree or at least a Higher National Certificate in chemistry) in the DEPARTMENT OF CHEMISTRY to record infra-red and ultra-violet spectra required for research purposes and to organize the maintenance of spectrometers—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (August 3).

**ASSISTANT RESEARCH OFFICER OR RESEARCH OFFICER** (with training and experience in the use of a wide range of photogrammetric equipment) in PHOTOGRAMMETRY—The Registrar, University College of Wales, Aberystwyth (August 5).

**LECTURER** in the DEPARTMENT OF ELECTRICAL ENGINEERING—The Secretary, University College, Gower Street, London, W.1 (August 6).

**LECTURER OR SENIOR LECTURER** (with qualifications and research interests, preferably in some branch of mathematical statistics) in the DEPARTMENT OF PURE MATHEMATICS AND MATHEMATICAL STATISTICS—The Registrar, University College of South Wales and Monmouthshire, Cardiff (August 6).

**ASSISTANT LECTURER (veterinary graduate)** in ANIMAL HUSBANDRY—The Registrar, University of Bristol, Senate House, Bristol, 2 (August 7).

**PROFESSOR** (with teaching and research interests in the broad field of the sociology of industrial society, and an interest in the education of students of technology and science) of SOCIOLOGY—The Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol, 7, quoting Ref. OST 65/62 (August 9).

**LECTURER (2)** in PSYCHOLOGY—The Secretary, The Queen's University, Belfast, Northern Ireland (August 10).

**TUTOR** in the DEPARTMENT OF GEOLOGY—The Registrar, University College of Wales, Aberystwyth (August 10).

**LECTURER** (with an appropriate degree of a recognized university, and preferably experience in the food preservation industry) in FOOD TECHNOLOGY at Hawkesbury Agricultural College, Richmond, New South Wales (N.S.W. Department of Agriculture)—New South Wales Government Offices, 66 Strand, London, W.1 (August 12).

**CHAIR OF ENTOMOLOGY** at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, August 13).

**RESEARCH ASSISTANT** in the DEPARTMENT OF PSYCHOLOGY (candidates who have recently graduated will be considered)—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (August 13).

**CHAIR OF CONTROL ENGINEERING**—The Deputy Secretary, The University, Southampton (August 14).

**LECTURER IN AGRICULTURAL ZOOLOGY**—The Secretary, The University, Aberdeen (August 14).

**LECTURER** (with a special interest in radio chemistry) in the DEPARTMENT OF CHEMISTRY—The Secretary, The University, Aberdeen (August 14).

**SENIOR LECTURER, LECTURER, SENIOR TUTOR** and a TUTOR in the DEPARTMENT OF MATHEMATICS, University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 14).

**RESEARCH ASSOCIATES** and **POST-DOCTORAL FELLOWS** in the SCHOOL OF ENGINEERING SCIENCE, for research posts in the fields of noise and vibration, metal fatigue, applied super-conductivity, automatic control or electro-chemical machining—The Registrar, University of Warwick, Coventry, Warwickshire (August 16).

**SENIOR LECTURER IN CHEMICAL PATHOLOGY** in the DEPARTMENT OF PATHOLOGY, University of Lagos Medical School—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.1 (August 18).

**CHAIR OF CHEMISTRY** in the DEPARTMENT OF PURE AND APPLIED CHEMISTRY—The Registrar, University of Strathclyde, George Street, Glasgow, G.1 (August 20).

**SENIOR RESEARCH ASSOCIATE** (with a Ph.D. or equivalent research experience in any relevant discipline such as physics, chemical engineering or metallurgy) in the SCHOOL OF MATHEMATICS AND PHYSICS, to work on the properties of liquid metals at high temperature and pressures in collaboration with Prof. N. W. Cowell—The Dean, School of Mathematics and Physics, University of East Anglia, Wilberforce Road, Norwich 77H (August 20).

**LECTURER** (with postgraduate qualifications in psychological medicine) in PSYCHOLOGICAL MEDICINE at the University of Adelaide, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, August 21).

**RESEARCH ASSISTANT** (graduate) in the DEPARTMENT OF GEOLOGY AND MINERALOGY for X-ray work on minerals—The Reader in Mineralogy, Department of Geology and Mineralogy, University of Oxford, Parks Road, Oxford (August 21).

**LECTURER OR ASSISTANT LECTURER IN PHYSIOLOGY**—The Registrar, The University, Sheffield (August 23).

**LECTURER OR ASSISTANT LECTURER** (with interests in either high temperature inorganic chemistry or chemistry of non-transition elements) in INORGANIC CHEMISTRY—The Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol 7, quoting Ref. OST 65/72 (August 31).

**LECTURER** (preferably with a knowledge of plasma physics) in PHYSICS at the University of Natal, Durban—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, August 31).

**SENIOR LECTURERS OR LECTURERS** in the SCHOOL OF PHYSICS, University of Melbourne, to carry out teaching and research in theoretical, nuclear or solid state physics, or in electron and X-ray diffraction—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 31).

**ASSISTANT LECTURER IN BOTANY**—The Secretary, The Queen's University, Belfast, Northern Ireland (September 1).

**LECTURER** (with a good honours degree in engineering or physics and preferably research interests in the field of experimental reactor physics) in the NUCLEAR POWER GROUP of the MECHANICAL ENGINEERING DEPARTMENT—Dr. P. J. Grant, Mechanical Engineering Department, Imperial College of Science and Technology, London, S.W.7 (September 1).

**LECTURER/SENIOR LECTURER IN MATHEMATICAL STATISTICS** at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 3).

**SENIOR LECTURER IN PHYSIOLOGY**; and a **LECTURER OR ASSISTANT LECTURER** in PHYSIOLOGY (suitably qualified persons interested in any of the fields of animal physiology)—The Secretary, The Royal Veterinary College (University of London), Royal College Street, London, N.W.1 (September 14).

**VISITING PROFESSOR** (preferably with a research background in geo morphology and/or climatology) in GEOGRAPHY for one year at Aarhus University—Aarhus Universitet, Aarhus C, Denmark (November 1).

**ASSISTANT LECTURER** in the DEPARTMENT OF FOOD SCIENCE—The Registrar, University of Strathclyde, George Street, Glasgow, G.1.

**ASSISTANT LECTURER OR DEMONSTRATOR** in the DEPARTMENT OF PATHOLOGY—Prof. W. A. J. Crane, The University, Sheffield, 10.

**ASSISTANTS (2)** (graduates in geography or botany) in the DEPARTMENT OF GEOGRAPHY, to help with vegetation mapping and associated research projects—The Registrar, University College of Wales, Aberystwyth.

**HISTOCHMIST**, Basic Grade (preferably with an honours degree in chemistry or biochemistry or equivalent qualification) for the Sunderland Hospital Group Laboratory Service—The Hospital Secretary, Royal Infirmary, Sunderland.

**CHAIR IN COMMUNICATIONS THEORY**; and **CHAIR IN ACCOUNTING**—Dr. G. L. d'Ombra, Chairman, Electrical Engineering Department, McGill University, Montreal, Canada.

**FISHERIES OFFICER** (national of the United Kingdom or the Republic of Ireland, with a good science degree and experience in fisheries management, including marketing and processing) in Malawi, to collect statistics and develop and demonstrate to local fishermen improved methods of fishing appropriate to local conditions—The Appointments Officer, Room 301, Ministry of Overseas Development, Strand House, Stag Place, London, S.W.1, quoting Ref. RO 289/134/01.

**GRADUATE** (with an honours degree in agriculture or other appropriate science) to work on the bull register of the Breeding Research Division—The Personnel Officer, Milk Marketing Board, Thames Ditton, Surrey.

**JUNIOR RESEARCH ASSOCIATE** (preferably with a first or upper second-class honours degree in physics, chemistry or metallurgy) in the SCHOOL OF PHYSICS, to work on research projects in the fields of solid state physics and the physics of materials, under the direction of the Professor of Physics, and to register for a higher degree—The Registrar, University of Warwick, Coventry, Warwickshire.

**LECTURER (2)** in OPERATIONAL RESEARCH OR INDUSTRIAL ENGINEERING; **ASSISTANT LECTURERS** in OPERATIONAL RESEARCH; and an **ASSISTANT LECTURER** in PRODUCTION TECHNOLOGY—Prof. B. Hiron, Imperial College of Science and Technology, 14 Prince's Gardens, London, S.W.7.

**PHYSICAL CHEMISTS** to work in the field of thermodynamics, thermodynamics, high temperature chemistry, and on the deposition of metals by vapour phase reaction—The Director of Research, Fulmer Research Institute, Stoken Park, Bucks.

**RESEARCH ASSISTANT** (graduate or expecting to graduate this summer) in PLANT PHYSIOLOGY under the supervision of Prof. M. B. Wilkins, Professor of Biology—The Dean, School of Biological Sciences, University of East Anglia, Wilberforce Road, Norwich, NOR 77H.

**RESEARCH ASSISTANT** (with a Ph.D. or B.Sc. or equivalent in chemistry, mathematics or physics, and interested in the application of computer techniques to molecular theory) in THEORETICAL CHEMISTRY—The Secretary, Royal Holloway College (University of London), Eghfield Green, Surrey.

**RESEARCH ASSISTANT** (graduate in agriculture or agricultural botany) in CROP HUSBANDRY for work on the potato crop—The Secretary, The School of Agriculture, West Mains Road, Edinburgh, Scotland.

**RESEARCH FELLOW** (preferably graduate mathematician, physicist, aerodynamicist or physical chemist) in the DEPARTMENT OF AERODYNAMICS to carry out theoretical and/or experimental work on topics in the field of real-gas dynamics—The Registrar, The College of Aeronautics, Cranfield, Bedford.

**RESEARCH FELLOW** (with a higher degree or extensive postgraduate experience, and an interest in one of the following fields: particle mechanics, process dynamics, mass transfer or petroleum technology) in the DEPARTMENT OF CHEMICAL ENGINEERING—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 30/G.

**SENIOR LECTURER OR LECTURER** (preferably with a special interest in the geography of Africa) in GEOGRAPHY at the University College, Dar es Salaam (University of East Africa)—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.1.

**SENIOR OR CHIEF TECHNICIAN** in the SCHOOL OF MATHEMATICAL AND PHYSICAL SCIENCES, to work under the general guidance of the Professor of Experimental Physics on atomic beam research projects concerned with gas scattering, optical and electronic excitation and radiofrequency resonance—The Laboratory Superintendent, Physics Laboratory, University of Sussex, Fulmer, Brighton, Sussex.

**SENIOR PHARMACEUT** (full- or part-time)—The Physician Superintendent, Oakwood Hospital, Maidstone, Kent.  
**SENIOR RESEARCH ASSOCIATE IN THE SCHOOL OF CHEMICAL SCIENCES** for work in the preparation and spectroscopic study of dicyclic aromatic molecules or coordination compounds—Prof. S. F. Mason, School of Chemical Sciences, University of East Anglia, Wilberforce Road, Norwich, NOR 77H.  
**SILVICULTURIST** (national of the United Kingdom or the Republic of Ireland, with a good degree in forestry with a minimum of one year's relevant postgraduate training or experience) in Sabah, to supervise the silvicultural and geological research of the Forest Department, to study methods of natural regeneration of Dipterocarp forests, to establish species trial plots and afforestation techniques, and to analyse and record data from regeneration surveys—The Appointments Officer, Room 301, Ministry of Overseas Development, Bland House, Stag Place, London, S.W.1, quoting Ref. R0234/150/02.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

**Ministry of Technology. Forest Products Research 1964: Report of the Forest Products Research Laboratory Steering Committee with the Report of the Director of Forest Products Research.** Pp. vi+54+12 plates. (London: H.M. Stationery Office, 1965.) 6s. 6d. net. [16]  
**Ministry of Agriculture, Fisheries and Food. Bulletin No. 94: Potatoes.** Pp. iv+113+4 plates. (London: H.M. Stationery Office, 1965.) 8s. net. [16]  
**Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. No. 754, Vol. 249 (27 May 1965): The Central Nervous System of *Neostitius*.** By Prof. J. Z. Young. Pp. 1-25+plates 1-7. 21s.; \$1.15 dollars. No. 755, Vol. 249 (27 May 1965): The Buccal Nervous System of *Ocotopos*. By Prof. J. Z. Young. The Centres for Touch Discrimination in *Ocotopos*. By Prof. J. Z. Young. Pp. 27-67+plates 8-17. 18s.; 2.70 dollars. No. 756, Vol. 249 (27 May 1965). Functional Capacity of Molepine Pituitary Transplants in the Teleost *Poecilia formosa*, with a Comparative Discussion on the Transplanted Pituitary. By J. N. Ball, Madeleine Olverman, Anna M. Silber and K. D. Kallman. Pp. 69-99+plates 18-21. 18s.; 2.70 dollars. (London: The Royal Society, 1965.) [16]  
**Ministry of Technology. National Engineering Laboratory. Heat Bibliography 1964.** Pp. vii+507. (Edinburgh and London: H.M. Stationery Office, 1965.) 47s. 6d. net. [16]  
**Focus on Information and Communication. Papers by Rupert Crawshaw-Williams, James K. K. Fotherman, A. R. Meehan, Patrick Meredith, W. T. Williams and Barbara Wootton. Edited by Barbara Kyle.** Pp. vii+112. (London: Aslib, 1965.) 14s. [16]  
**Discovery Reports, Vol. 33, pp. 309-324: The Latitudinal Distribution of *Euphausia* Species in the Surface Waters of the Indian Ocean.** By A. de O. Baker. (London: Cambridge University Press, 1965.) 17s. 6d. net. [16]  
**Council for the Preservation of Rural England, Sheffield and Peak District Branch. Annual Report, April 1965.** Pp. 28. (Sheffield: Council for the Preservation of Rural England, Sheffield and Peak District Branch, 1965.) [16]  
**The Zoological Record. Vol. 98, Section 20, 1961: List of New Genera and Subgeneric Names Recorded in Volume 98.** Compiled by Leonard G. Mills. Pp. 19. 20s.; 2.25 dollars. Vol. 99, Section 3, 1962: *Porcifera*. By E. Ware. Pp. 17. 12s. 6d.; 1.80 dollars. Vol. 99, Section 11, 1962: *Tribolita*. Compiled by Dr. J. T. Temple. Pp. 46. 12s. 6d.; 1.80 dollars. Vol. 99, Section 16, 1962: *Pisces*. Compiled by G. Palmer and Dr. M. L. White. Pp. 85. 8s.; 4.25 dollars. Vol. 99, Section 16, 1962: *Amphibia*. Compiled by Alice G. O. Grandison, Pauline Curds and Sarah H. Bunney. Pp. 76. 8s.; 2.25 dollars. Vol. 100, Section 1, 1963: *Comprehensive Zoology*. Compiled by Dr. B. Markward. Pp. 27. 12s. 6d.; 1.80 dollars. (London: The Zoological Society of London, 1965.) [16]  
**The J. A. Radley Research Institute. Report for 1964.** Pp. 31. Technical Memorandum No. 1: The Sensitivity of Methods for Trace Analysis in Use at the J. A. Radley Research Institute. Pp. 4. (Reading: The J. A. Radley Research Institute, 1965.) [16]  
**The Stratigraphy and Structure of the Eastern Aden Protectorate.** By Dr. Z. R. Beydoun. (Bulletin Supplement No. 6 of "Overseas Geology and Mineral Resources.") Pp. vii+107+16 plates+3 coloured geological maps. (London: H.M. Stationery Office, 1964.) 27s. 6d. net. [16]  
**Genetics and Prokaryotes.** By Sir Gavin de Boer. (The Rede Lecture 1965.) Pp. 58. (London: Cambridge University Press, 1965.) 5s. net, 1 dollar. [46]  
**Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 2. Disinfection of Poultry Houses and Equipment.** Pp. 8. Leaflet No. 60: Part-Time Agricultural Classes. Pp. 4. Leaflet No. 143: The Cattle Crush. Pp. 4. (Belfast: Ministry of Agriculture, 1965.) [46]

### Other Countries

**Indian Forest Bulletin, New Series, No. 241 (Entomology): Prophylactic Efficacy of Various Insecticides in the Protection of Freshly Felled Timbers in Storage Against Insect Bore and Subterranean Termites, Part 2.** By B. N. Mathur, P. N. Chatterjee and R. S. Thapa. Pp. 23+3 plates. (Delhi: Manager of Publications, 1965.) [36]  
**Institut Royal Météorologique de Belgique. Bulletin Mensuel: Observations Météorologiques, Mars, 1965.** Pp. 26. Publications, Série A, No. 53: Données Rayonnement Solaire à Leopoldville, Période 1953-1962. Par L. Hostians et B. Ikwa. Présenté par M. Oerebant. Pp. 41. (Uccle-Brunelles. Institut Royal Météorologique de Belgique, 1965.) [36]  
**Comptes Rendus des Séances de la Douzième Conférence Générale des Poids et Mesures, Paris, 6-13 Octobre 1964.** Pp. 100. (Paris: Gauthier-Villars et Co., 1965.) [36]  
**Tate and Lyle. Annual Report of the Central Agricultural Research Station 1964.** Pp. 863. (Carrapichama, Trinidad. Tate and Lyle, 1965.) [36]  
**Chemistry, Oil and Gas in Rumania: News and Commentaries, No. 1, 1965.** Pp. 48. (Bucarest: Centrul de Documentare al Industriei Petrolului si Chimiei, 1965.) [36]  
**Unesco. Scientific Institutions and Scientists in Latin America. Venezuela (Ciencia). Fascículo 2.** Pp. vii+412. (Montevideo: Centro de Cooperación Científica para América Latina, en colaboración con la Organización de los Estados Americanos, 1965.) [36]  
**Metropolitan Life Insurance Company Statistical Bulletin, Vol. 46 (March 1965): Hospitalization for Mental Disorders. Longevity Has Changed Little in Past Decade. Large Accident Toll Among Infants. Lower Frequency of Disability in 1964.** Pp. 12. (New York: Metropolitan Life Insurance Company, 1965.) [36]

**United States Department of Agriculture. Farmers' Bulletin No. 2307: Controlling the Pink Bollworm on Cotton.** Pp. 12. (Washington, D.C.: Government Printing Office, 1965.) 20 cents. [36]  
**Annals of the New York Academy of Sciences. Vol. 122, Article 1: Research in Demyelinating Diseases.** By B. O. Alford and 87 other authors. Pp. 1-570. (New York: New York Academy of Sciences, 1965.) [36]  
**Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Bulletin 123: Latest Lower Triassic Ammonoidea from Hilemore Island and Northeastern British Columbia.** By H. T. Toset. Pp. 45+8 plates. Paper 64-2: Triassic Stratigraphy Near the Northern Boundary of Jasper National Park, Alberta. By D. W. Gibson. Pp. 11+144. 75 cents. Paper 64-20: The Dubawnt Group, District of Keewatin and Mackenzie. By J. A. Donaldson. Pp. iv+11. 75 cents. Paper 64-24: Structural Analysis of Part of a North Coal Mine, Michel, British Columbia. By D. K. Morris. Pp. iv+13 (4 plates). 75 cents. Paper 64-28: Leaf River Map-Area, Quebec and District of Keewatin. By L. M. Stevenson. Pp. 11+10. 75 cents. Paper 64-22: Big Bend Map-Area, British Columbia. By J. O. Wheeler. Pp. v+27. 75 cents. Paper 65-5: Lower Cretaceous Floras of Western Canada. By W. A. Bell. Pp. 86 (16 plates). (Ottawa: Queen's Printer, 1965.) [36]  
**History Under the Sea: a Handbook for Underwater Exploration.** By Mondel Peterson. Pp. xiii+108+56 plates. (Publication 4533.) (Washington, D.C.: Smithsonian Institution, 1965.) [36]  
**Proceedings of the United States National Museum, Smithsonian Institution. No. 8508, Vol. 116: Contributions to the Knowledge of the Hemerobidae of Western North America (Hemiptera).** By W. Makahara. Pp. 205-222+plate 1. No. 8504, Vol. 116: A Contribution to the Study of the Genus *Sphaerocera* Latreille in Central and South America (Diptera: Sphaeroceridae). By O. W. Richards. Pp. 223-243. No. 8506, Vol. 116: Herpetology of the Zuni Mountains Region, Northwestern New Mexico. By Frederick R. Gehlbach. Pp. 245-252. (Washington, D.C.: Government Printing Office, 1965.) [36]  
**United States Department of the Interior: Geological Survey. Geophysical Abstracts, No. 220, May 1965.** By James W. Clarke, Dorothy B. Vitaliano, Virginia B. Neesebel, and others. Pp. ii+345+34L. (Washington, D.C.: Government Printing Office, 1965.) 25 cents. [36]  
**National Science Foundation. Weather Modification: Sixth Annual Report for Fiscal Year ended June 30, 1964.** Pp. v+68. (Washington, D.C.: National Science Foundation, 1965. Available from U.S. Government Printing Office.) 80 cents. [36]  
**Republic of South Africa. Science Bulletin No. 369: A Conspectus of South African Spiders.** By R. F. Lawrence. (Plant Protection Series.) Pp. iv+64. (Pretoria: Government Printer, 1964.) R. 1.25. [36]  
**Japanese Contribution to the International Geophysical Year and the International Geophysical Co-operation, Vol. 6.** Pp. 167. (Tokyo: National Committee for the International Geophysical Co-ordination, Science Council of Japan, 1964.) [36]  
**Republic of South Africa: Department of Commerce and Industries. Thirty-Second Annual Report of the Division of Sea Fisheries for the period 1st April, 1960, to 31st March, 1961.** Pp. 267. (Reprint from *Commerce and Industry*, May, 1964.) (Pretoria: Government Printer, 1964.) [36]  
**United States Department of Commerce: Weather Bureau. Technical Paper 51: Two- to Ten-Day Rainfall for Return Periods of 2 to 100 Years in the Hawaiian Islands.** Prepared by John F. Miller. Pp. vi+34. 90 cents. Technical Paper No. 52: Two- to Ten-Day Precipitation for Return Periods of 2 to 100 Years in Alaska. Prepared by John F. Miller. Pp. ii+30. 60 cents. (Washington, D.C.: Government Printing Office, 1965.) [36]  
**Smithsonian Contributions to Astrophysics, Vol. 81, No. 6: Second Catalog of Hourly Meteor Rates.** By Charles P. Olivier. Pp. 171-180. (Washington, D.C.: Government Printing Office, 1965.) 20 cents. [36]  
**Consed Permanent International pour l'Exploration de la Mer, Charlottenlund-Slot, Danemark. Bulletin Statistique des Pêches Maritimes, Volume XLVII pour l'Année 1962. Rédigé par Arni Fridriksson.** Pp. xii+54. (Copenhagen. Andr. Erod Hest et Pils, 1965.) 25 Kr. [36]  
**Smithsonian Miscellaneous Collections, Vol. 149, No. 3. The Relationships of *Quercus grisea* (Rodentia: ?Hesperodontidae).** By Clayton H. Ray. Pp. 12+1 plate. (Publication 4606.) (Washington, D.C.: Smithsonian Institution, 1965.) [36]  
**Commissariat à l'Énergie Atomique. Étude des Valeurs et des Prix du Plutonium à Long Terme: un Modèle Paramétrique Simplifié.** Par Jacques Gammens et Henri Pallot. (Études Économiques. Rapport ORA-R 2795.) Volume 1: Exposé Général de l'Étude. Volume 2: Développement Mathématique du Modèle Paramétrique Simplifié et Application—Annexe. Partly translated into English. Pp. 189. (Paris: Documentation Française, Secrétariat Général du Gouvernement, Direction de la Documentation, 16 rue Lord Byron, 1965.) [36]  
**Smithsonian Annals of Flight, Vol. 1, No. 2. The First Airplane Diesel Engine: Packard Model DR-980 of 1923.** By Robert B. Meyer. Pp. vii+48. (Washington, D.C.: Government Printing Office, 1964.) 60 cents. [36]  
**Federal Radiation Council, Washington, D.C. Implications to Man of Irradiation by Internally Deposited Strontium-90, Strontium-90, and Cesium-137. A Report of an Advisory Committee from the Division of Medical Sciences: National Academy of Sciences—National Research Council.** Pp. v+34. (Washington, D.C.: Federal Radiation Council, 1964.) [36]  
**Background Material for the Development of Radiation Protection Standards: Protective Action Guides for Strontium-90, Strontium-90 and Cesium-137. (Staff Report No. 7 of the Federal Radiation Council.)** Pp. iv+44. (Washington, D.C.: Government Printing Office, 1965.) 80 cents. [36]

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## HIGHER EDUCATION IN BRITAIN

THREE of the papers presented at a discussion on universities and social fabric, arranged jointly by Section J (Psychology) and Section N (Sociology) at the meeting of the British Association for the Advancement of Science at Southampton in September 1964, have recently been published (edited by Prof. J. Drever) in *The Advancement of Science* (22, 29; May 1965). In the interval they have become even more pertinent to developments in higher education and to some current discussions about university places, and particular shortages, such as those of engineers and physicists. This was apparent at the Home Universities Conference in December 1964, for example, although ostensibly that Conference was concerned with university teaching methods and with the finance of universities.

In the first of these three papers, Prof. J. Drever himself discusses need, choice and supply in higher education. His experience in the United States as well as in Britain leads him to suggest that more ought to be known about the academic motives of our young people before we can plan ahead with confidence. It is not just that the mounting scale of investment in higher education leads to questions concerning the rate of failure or to the relation between supply and demand in particular occupations being viewed with greater concern. There is, Prof. Drever suggests, a possibility that the demand for places in higher education may be self-limiting in some way and that this limitation may not be an adaptive one. The delicate balance between ambition and impatience, between conformity and impatience, may easily be upset, with consequences that may not be predictable.

Again, as regards choice, he points out that the Robbins Report has scarcely faced the real issues. It is unrealistic to suppose that the needs of the community are invariably met by the decisions of its adolescent members or to assume that the changing demands of research and industry are necessarily reflected in an educational system which tries to keep central planning and direction to a minimum. There are indeed no easy answers to such questions, and the whole tenor of the replies recently given in Parliament to questions about the work of the Committee on Manpower Resources for Science and Technology suggests that a much more realistic view is now being taken both by the Minister of Technology and the Secretary of State for Education and Science. Britain cannot, Prof. Drever observes, afford the luxury of complete freedom of the American way, but it is equally questionable whether some measure to eliminate or minimize the element of chance which often determines the choice of curriculum necessarily means a serious infringement of liberty.

So far as engineering and technology are concerned, attention is already being directed to the correction of some bias which acts against the choice of these careers. Further action, however, may well be expected when the examination by the Tavistock Institute of Human Relations on factors affecting the status of engineers in society is completed by mid-September. Even so, as Prof. Drever emphasizes, there will remain the question of standards, and here also we know too little to take them for granted. In the face of the present uncharted drift of academic standards, we need objective standardized tests at various crucial stages in the educational process. These would have to be kept flexible and

sensitive to new developments, and this Prof. Drever maintains is possible.

The second paper, by Dr. W. Taylor, discusses university culture and social change, and once again directs attention to the lack of knowledge about important aspects of university life. Few would dissent from the view that the work of universities is central to the processes of social and technological change in an industrial society, but there is little hard evidence of the effect of residence in hall on the student on which to base proposals for expansion of such institutes. Obviously to judge soundly the extent of such influence on students, something should be known of the values and attitudes of student sub-cultures. Dr. Taylor urges that we need many more systematic analytical investigations of the interaction of the various groups that make up the university, the social processes that are involved in providing and receiving a higher education and of the way in which universities relate to other institutions within society and respond to the demands made on them. He calls for clarification of some of the observations of the Robbins Committee, asking how university life is to compensate for differences in home background, and exactly what type of social habitat the Committee had in mind for a university.

It is salutary to be reminded that there is little firm evidence to support the wide claims that are made for the effects of the residential life, and Dr. Taylor recognizes that it is much easier to be critical than constructive about the socializing function of the university: reconciliation of ideological diversity, individual liberty and a sufficient measure of consensus to make social life possible cannot be easy. Even more to the point, he insists that it is above all with the intellectual hazards of university life that we have to be concerned. It is these that are most threatened and also most commonly overlooked, and it is also the values and types of judgment represented by scholarship and the scientific temper which, as Dr. Taylor justly remarks, must be fought for anew with each group of students entering the university. Halls of residence, college units and moral tutors are no substitute for curricular reform, well-equipped libraries and laboratories and improved teaching and examining.

Even more specifically than Prof. Drever, Dr. Taylor puts first things first, nor does Prof. A. Rodger fall behind him in the third of these papers on capacity and inelination for university courses. Further, he underwrites Prof. Drever's insistence on the importance of inelination, which does not always run with capacity. Refusal to recognize this may involve both social waste and individual misery. The warning of Lilje's *The Abuse of Learning* has implications far outside Germany. Moreover, excessive preoccupation with capacity can lead to too much stress being placed on attainment and too little on intelligence.

That is a criticism that has been authoritatively voiced since the Southampton meeting of the British Association, but Prof. Rodger goes on to add that there is a further danger that we may fail to appreciate properly some of the pointers provided by recent work on the relation between capacity and social class. Here he is thinking of social class as a factor facilitating or hindering the inelination to study, and more especially of the task of assessing fairly the candidate whose capacity and inelination for university work have, in a sense, become

atrophied through unfavourable home circumstances. Since we already know that both capacity and inclination are influenced by various environmental factors, this may well mean in practice that available information on the relations between attainment intelligence and social class is likely to lead to underestimates of our reserves.

It could also be asked whether sufficient attention has yet been given in the universities, either in planning courses or curricula, or in the provision of facilities and methods of teaching, to the effect of the influence of an increasing number of students from homes with no tradition of learning or facilities for study. Both considerations become the more important when concern with university finance raises the question of benefit to the community from the investment involved. Prof. Rodger raises a further related point. There is also the danger that addiction to specialization may make it impossible for us to investigate systematically the limits of educational and occupational versatility. Quite rightly, he urges that in these days of rapid and extensive technological change, and consequent occupational change, versatility of both capacity and inclination deserves far more study than it receives. For all the talk about general or broadly based education at any level, this question of versatility or adaptability is scarcely mentioned. Yet if rigidity is to be checked, and if automation, for example, is not to lead to unemployability as well as unemployment, this aspect is of paramount importance.

Prof. Rodgers is inclined to put some considerable measure of responsibility for the present situation of specialization on academic circles, though he recognizes that the University Grants Committee has for some years been fostering the notion of the joint honours degree. If these became common, he thinks that our present ignorance would be reduced a little and at the same time our students could be given a chance to find out methodically what they are best at and enjoy most. Systematic studies of planned procrastination in the choice of school and university courses, he suggests, are much more likely to advance our knowledge of capacities and inclination than any amount of early specialization.

Finally, he refers to the danger that concern for the younger age-groups may lead us to neglect the special problems of the mature university student. For various reasons, these are increasing in numbers and many students for higher degrees are men and women anxious to make progress in their chosen line. Often these will, of necessity, seek part-time courses; some may desire to change their discipline; and Prof. Rodgers reminds us that sometimes the older, experienced part-time students are better fitted than others to pursue particular researches. Studies of reserves of capacity and inclination for university courses should accordingly take account of the potential among the mature, whose needs are not invariably met by extra-mural courses and are frustrated by the customary Government parsimony in that field.

These suggestions are not likely to be particularly welcomed by Ministers and their administrators, especially when they involve or imply freedom for rigorous experiment. Prof. Rodgers admits that projects for research in social science must be technically sound, administratively convenient and politically defensible, but the last two of these three criteria may tend to exclude experiment, and he adds that we must be prepared to use survey methods extensively. Nevertheless, unless due attention is paid to the points made in these three papers, Britain is

unlikely to avoid serious errors in the expansion of higher education and a waste of talent and of public money that can ill be afforded.

The discussion at the Home Universities Conference\* on the report of the Hale Committee on University Teaching Methods, which Sir Edward Hale himself opened, brings out the importance of these views. Sir Edward was content to emphasize his own experience of the value of the tutorial in teaching him how to read up a subject, to think clearly for himself, and to use language to communicate his thoughts. He added that it was necessary for every student to stretch his ability to the utmost: the purpose of the tutorial was not to make things easy for him. If university expansion necessitated reduction in any form of teaching, the last thing to go should be teaching by discussion with students of their written work.

Prof. R. J. Blin-Stoyle also stressed the vital importance of effective contact between staff and students, including a continuous and firm relation between a student and at least one member of the staff, in which the student became increasingly independent. He added that, at least in science, effective and stimulating teaching stemmed from a background of research and enquiry, and the teaching system must leave time for the university staff to put in a substantial research effort. Plans for university expansion which endanger this balance are unlikely to be effective. He also referred to the desirability of flexibility and transfer from one department to another, and his remarks fully substantiated much that Prof. Rodgers, Prof. Drever and Dr. Taylor had said at Southampton.

Throughout the discussion at the Conference these points were reiterated in different ways. Prof. E. Wright referred, for example, to the importance of the library in giving the student a sense of belonging to a community of scholars. Moreover, while Mr. H. J. Perkin referred to the desirability of excellence as a teacher being rewarded, and being seen to be properly rewarded, and while this was in support of what the Robbins Committee had said, it should not be at the expense of research. Prof. Wright again referred to the real danger of universities being regarded more and more as service institutions, professional training schools, or technological institutions. However, both Prof. A. G. N. Flew and Dr. F. G. Healey, in remarks that linked this discussion with the subsequent one on the financing of universities, referred to the importance of using university institutions more economically and using the teaching machine to do precisely those things that the teacher does not do, for taking all those service courses which are meant to equip the student to do another course better.

The discussion on the financing of universities started from the problem of striking a balance between the freedom of a university to develop its academic programme and the legitimate demand of Government for some control over the large sums of public money provided, at least to the extent of being satisfied that a reasonable return was forthcoming. Like the discussion at Southampton, however, it began by emphasizing the absence of reliable information on this latter aspect. As Mr. S. F. Burman, the Pro-Chancellor of the University of Birmingham, observed in opening the discussion, there is an

\* Report of the 1964 Home Universities Conference, University of London Senate House, 11th and 12th December, 1964. (The Report of the Committee on University Teaching Methods. The Financing of Universities.) Pp. 76. (London: The Association of Commonwealth Universities, 1965.) 5s.

almost complete lack of meaningful statistics on which valid judgements can be made as to the worthwhileness of any particular form of expenditure; the formulae by which we justify our capital expenditure are crude; and there is no efficiency audit on what we do.

First, Mr. Burman suggests, we should work out our actual costs for tuition per student, department by department, taking into account maintenance, capital and all the other factors involved, and thus avoiding the considerable distortions in the present method. The work already done on specific research grants could well be extended to grants for the research which arises naturally out of tuition or the individual purposes of the academic staff. Enquiry into the wider aspects of capital expenditure again might well reveal a correlation between teaching costs, research costs and capital costs. He also thought that it might be useful if Britain's provision of finance to universities were devised so as to provide each university with a positive incentive to the optimum utilization of that finance, with freedom to vary the cost/quality balance. Moreover, Mr. Burman said frankly that the mounting cost of university education forced consideration of the question of whether we were justified in accepting any student who presented himself or herself, with appropriate entrance qualifications, to be educated at the public expense in the discipline of his or her choice, irrespective of whether there was an ultimate demand for those with that particular type of training.

In pointing out that, if university education is regarded as training for a vocation, we must make some careful attempts to measure the demand, Mr. Burman was not merely endorsing Prof. Drever's argument. He was emphasizing the truth which it is so fashionable to ignore—that university education is not a right, but a privilege, and as such carries responsibilities. These responsibilities, it might be added, are commonly ignored by those who are most forward in urging that the universities are primarily places of vocational or professional training. The realism which marks this whole discussion has some implications that may well prove awkward for the Minister of Technology and the Secretary of State for Education and Science unless their declared outlook has changed. It is not merely that the discussion provides welcome and unmistakable evidence of a readiness on the part of the universities to look more closely into their financial control, efficiency, organization and methods of government. Dr. H. M. Taylor, Vice-Chancellor of the University of Keele, seized the opportunity to refer to the importance of a firm assurance of sufficient resources to enable general developments to be planned five years ahead without erosion by rising costs. There was sanity also on the question of students' fees, and Sir Edward Hale strongly supported Dr. Taylor's argument for raising these. This was, however, questioned by Dr. M. B. Sutherland and Prof. A. G. N. Flew, who preferred the introduction of a loan element in student finance, and were supported by Mr. A. W. Jones.

These details are of secondary importance, however, compared with the extent of the evidence that the universities themselves are prepared to think realistically on matters of finance, not only in terms of control of expenditure and their own internal administration but also in terms of teaching methods and the benefit to their students and to the community. Obviously this involves some fresh thought about the purpose of a university, for no sound judgment can be formed as to the return on

university expenditure unless we are clear in our minds as to what that purpose should be. Here it would seem that the universities are ahead of the Government; it is surely significant that both at Southampton and at the Home Universities Conference attention should be directed as emphatically to the gaps in our knowledge as to the effectiveness and appropriateness of many existing practices. Primarily, the need is for fact-finding rather than research, between which Lord Heyworth's Committee has since drawn a sound distinction. However, the necessary enquiries call for studies in the social field, and the recommendation of the Heyworth Committee for a considerable extension of social research is emphatically endorsed. It may not be too much to add that if the Government is really concerned to ensure the maximum public return from expenditure on higher education, it would be wise to ease the pressure on universities to increase the number of places. Certainly, this should be done while answers are being sought to questions such as were raised by Prof. Drever and other participants in the discussion at Southampton, as well as at the Home Universities Conference. If any choice has to be made, more finance should be provided for the social research required before that for further expansion of the universities.

## THE WORLD OF ARIEL

### The Golden Age of Wireless

By Asa Briggs. (The History of Broadcasting in the United Kingdom, Vol. 2.) Pp. xvi + 688 + 54 illustrations. (London: Oxford University Press, 1965.) 75s. net.

LIKE broadcasting itself, Prof. Asa Briggs has made a significant contribution to the history of our times. His first book in a projected four-volume history of broadcasting in the United Kingdom described the birth of wireless. This, the second, is concerned with the period from 1927, when the British Broadcasting Corporation ceased to be a private company and became a public corporation, up to the outbreak of war in 1939. The period has been chosen with care for it represents more than the passing of a limited experiment of interest mainly to the participating few. It stands for the establishment of a national service with implications and repercussions not only for British citizens but also, because of the way it was shaped, for humanity. The shaping, as Briggs makes crystal clear, was due to the leadership of a man who, above all, stood inflexibly for the dissemination of truth and the upraising of moral and cultural standards. Not for Reith to project a Government or cheap commercial interests. Thus, for all that, Briggs has continued his story for a period of one year after the departure of the B.B.C.'s first Director-General, this book is almost entirely an account of Reith's successful attempt to build a large-scale public corporation.

To obtain his information Briggs has had to dig deep and wide. Diaries, memos, newspapers, journals, conversation, Government and many other sources of information have been used and brought together with great scholarship and skill to produce a book which everywhere pays tribute to the genius of Reith even when recording his imperfections. It should be a source of delight and interest especially to those who lived through the period under review. Many nostalgic memories will be revived and half-forgotten incidents recalled. Older readers will, for example, remember Stuart Hibbard's memorable "The King's life is moving peacefully towards its close", John Hilton, Howard Marshall and John Snagge, the bulletins about the *Thetis* disaster in 1937, the first broadcast Promenade Concerts, football match recordings by George

Allison, Band Waggon, Saturday Night Music Hall, and the Sunday Night Services from St. Martin-in-the-Field's, Stephen King-Hall on Children's Hour, outstanding broadcasts to schools, and many, many others.

Although television was well established by 1939, its range was still limited to the London area and viewers were comparatively few. For those, this book will bring memories of studio plays and pantomimes, the Boat Race and the Coronation, Jasmine Bligh and Elizabeth Cowell and other well-known personalities. Among the half-forgotten facts which will occasion surprise is that, up to the outbreak of the Second World War, no broadcasting of any kind took place before the daily service at 10.15 a.m. and no regional variations were permitted before the start of Children's Hour.

The value of *The Golden Age of Wireless*, however, lies less in programme remembrances and more in the revelations behind them. The scientific and technological developments required for the transmission of radio waves over long distances are accurately portrayed and are only excelled by Briggs's fascinating account of how Baird's intrepid struggle to establish a successful means of televising ended in tragic failure. The political warfare with successive Governments about ultimate control of broadcasting, the cheese-paring attitude of Ministers and senior Civil Servants, and the ineptitude of a prominent Postmaster-General, help to explain at least some of the reasons which led to apparently slow progress in the development of regional broadcasting and programmes designed especially for overseas listeners. Students of management, too, will find much that is familiar in the way in which Reith built up a large organization on functional lines with constant adaptation to meet changing need, and where the span of control and the matching up of authority and responsibility were well conceived and executed.

The vastness of the book and the detail of its record, however, may account for its greatest imperfection. Many students of social history and sociology would wish to have read more of Briggs's views on the impact of wireless on public taste and opinion. Has the broadcasting of 'good' music led to a real improvement in standards? Among the more intelligent only? What of the effect of 'poor' music and variety on the less intelligent? Were the B.B.C. right to establish such a marked middle-class image? What effect has there been on politics, and on the practice of religion? Has individuality suffered and the creative spark been dimmed? Should not much more have been done to use the radio as a deliberate means of adult education? Has the B.B.C. played its part in the evolution of an industrial society the continuance of which depends on the success of its manufacturing industries? Could knowledge of developments in science and technology have been more widespread? What Briggs has successfully done is to provide the necessary information which others could use to dig even deeper in unearthing the answers to some of the questions posed. What he has done even better is to pay public tribute to the man who so built the B.B.C. that its reputation for integrity stands unrivalled in broadcasting systems throughout the world.

T. H. HAWKINS

## TECHNOLOGY AND APPLICATION OF RADIOACTIVITY

Die Technischen Anwendungen der Radioaktivität  
Band 1. Von Dr. Engelbert Broda und Dr. Thomas Schönfeld. 3. verbesserte und stark erweiterte Auflage. Pp. 372. (Leipzig: Veb Deutscher Verlag für Grundstoffindustrie, 1962.) 32.50 D.M.

**D**RS. Broda and Schönfeld's well-known book on the technological applications of radioactivity has now reached its third edition. There has been so much

progress in the subject that it has become necessary to redistribute the material into two volumes, the first of which has now been published. The second volume is promised for the near future.

The basic plan of the previous editions has remained unaltered; but the chapters in the second edition on "Absorption and Scatter of Radioactive Radiation in the Technique of Measurements" and "The Technological Applications of the Effects of Radioactive Radiation on Matter" will appear in Volume 2. The chapter on protection against radiation remains at the end of Volume 1. Examples of stable isotopes used as markers, mainly in cases where radioactive isotopes of elements are too short-lived, have been given in this volume, though without any description of working methods and measurements, since these are not the main topic.

The initial chapter contains a concise summary of the basic facts of radioactivity necessary for a full understanding of the technological applications, followed by a survey of methods of measurement. There is also a review of the various applications of radioactivity to the wide field of chemical analysis and a discussion of the methods of analysis by activation, by indicators, by isotope dilution, etc. The remainder of this volume deals with more specific technological applications in the mining and oil industries, in general and specific problems of the chemical industry, and finally in agriculture and forestry. The treatment of all these items is not exhaustive with regard to detail; the reader who requires detail can look it up in the bibliography attached to each separate section.

The book is very clear and readable, and the text benefits from the avoidance of detail and from the occasional judicious use of examples. For anyone who requires information about the existing technological applications of radioactivity this book will prove a suitable and reliable source.

W. M. DALE

## III-V COMPOUND SEMICONDUCTORS

### Physics of III-V Compounds

(Wiley Series on the Science and Technology of Materials.)  
By Prof. O. Madelung translated by Dr. D. Meyerhofer.  
Pp. xiv + 409. (London, New York and Sydney: John Wiley and Sons, Inc., 1964.) 98s.

**T**HE discovery by Welker of the III-V compound semiconductors had the effect of enormously widening the field of semiconductor research not only by the inclusion of the III-V compounds themselves but also by suggesting that there might exist other classes of binary, ternary and even more complex semiconducting systems. The resulting comparative investigations of different semiconductors have been extremely valuable in elucidating the underlying principles governing their behaviour. This applies particularly to the problem of band structure, as is made clear in the second and final chapters of Prof. Madelung's book, *Physics of III-V Compounds*.

One question that must be answered before discussing the book in detail is whether its appearance is timely. There is a contradiction to overcome here since if one waits for a topic to become quiescent before writing a book on it, one may find that readers are no longer interested. The author, I think, justifies himself in the preface where he correctly states that the main features of the phenomena observed in III-V compounds were correctly understood by about 1962 and that present-day research is largely directed towards the solving of technological problems. To this one might add the investigation of phenomena, in themselves interesting, for which some III-V compound provides a particularly suitable medium. It certainly seems unlikely that the picture of the III-V compounds presented in this book will undergo any considerable modification in the near future, though a comparison with Hilsenrath's slightly later review at the



semiconductor conference in Paris does show some quantitative changes.

After an introductory chapter in which the history and crystallography of the III-V compounds were discussed, the author discusses band theory as it applies to these substances. This is supplemented by some brief remarks concerning the chemical binding which emphasize the persistent failure of the 'band' and 'bond' pictures to coalesce. The review given in this chapter is of considerable value though in the case of the k.p. theory it would have been better if either less detail or more explanation had been given.

The chapter on optical properties is probably the best in the book. It consists of a review of optical methods of studying band structure, with illustrations drawn from the III-V compounds, and is to be recommended to anyone interested in understanding how these techniques are used. The degree of sophistication and subtlety in experimentation and interpretation is very well brought out; by comparison the picture derived from the transport properties lacks precision. It is only where a detailed numerical calculation has been carried out that one feels confident in the analysis of the experimental data. This situation probably explains why the chapter on transport properties has much more of the character of a compilation than that which precedes it. The same is almost inevitably true of that dealing with mixed crystals and ternary compounds.

The subject of impurities and defects must have been difficult to deal with, since it is in this area that many of the unsolved problems crop up. Consequently there are probably more controversial assertions here than elsewhere, though it seems to me that fair treatment has been given to conflicting points of view.

The survey of p-n junction phenomena is probably the place at which the timing of the book was rather unfortunate owing to the discovery of the diode laser. Only the briefest account of this subject is given, and it will certainly bulk larger in any subsequent edition Prof. Madelung may bring out.

There is a very complete bibliography up to the beginning of 1964 and the division into subject and substance indexes is useful. The translation only rarely reveals that the book was originally written in German and is only positively infelicitous on the dust jacket. This book should prove of great value to all those interested in the physics of III-V semiconductors.

J. E. PARROTT

## ASSESSMENT OF OPTICAL SYSTEMS

Fourier Methods in Optical Image Evaluation

By Dr. E. H. Linfoot. (The Focal Library.) Pp. 90. (London and New York: The Focal Press, 1964.) 50s.

THE assessment of optical systems has, traditionally, been predominantly by spot diagrams derived from ray-traces. That of photographic emulsions has been in terms of subjective 'graininess' and 'limiting resolution'. These means are no longer adequate for the assessment of modern high-performance optical systems.

Even if the spot-diagram fully represented the optical performance, its interpretation would remain ambiguous; it is not evident whether a concentrated core surrounded by a relatively weak but extended halo is better or worse than a comparatively uniform spot-distribution of moderate size. In fact, of course, the spot-diagram distribution does not represent the image structure even qualitatively when the aberration-spreads and diffraction-spreads become comparable, since the diffraction-spread corresponding to an aberrant zone (Babinet's principle) may then exceed the whole ray-theoretic image spread.

The fact that almost all images of interest are eventually presented to the human retina provides some justification for the subjective assessments traditionally

used in photography. It has become increasingly clear, however, that 'graininess' and 'limiting resolution' are not well defined either conceptually or operationally, and they do not obey any practicable calculus.

Finally, the assessments traditionally applied to optical systems and to photographic emulsions have been mutually isolated. There has been no means of combining spot-diagrams, diffraction-spreads, and photographic characteristics of noise and resolution into an overall assessment of a photographic optical system.

In recent years, these difficulties have been overcome by the application of the methods of communication theory, and their further development for optical applications. Because of the wave-nature of light, an optical image is effectively band-limited, and because of the particle-nature of light the image has a finite signal-to-noise ratio. It follows that the image has a finite information content. Roughly speaking, this means that it is capable in principle of distinguishing one out of a finite set of object intensity-distributions. Useful assessments can be formulated in terms more or less closely related to the extent to which a practical optical system approaches the theoretically possible discriminating-power corresponding to its size and the amount of available light.

The basic analytical tool for these assessments is the Fourier theory. The object- and image-distributions are analysed into their orthogonal sinusoidal components. These components are propagated through an optical system with (in a useful approximation) change only in amplitude and phase; this is a much simpler situation than that for point-components of the object distribution, which may change shape in a complicated manner. The performance of the whole system can be expressed completely (in this approximation) by specifying, as a function of frequency of the sinusoids, the change in signal-to-noise ratio and contrast between object and image distributions. The treatment is implicitly wave-theoretic, although ray-approximations can be derived in some circumstances; photographic effects are included. From these full descriptions it is possible to abstract figures of merit, each of which may be expected to be especially useful in particular circumstances; in particular, it is possible to specify the performance in relation to a prescribed object-set. It turns out that most of the intuitively satisfactory abstractions are closely related, so that it is seldom necessary to make a decision between them.

Dr. Linfoot has played a leading part in these developments, and his book provides a welcome exposition of the basic theory, and of some illustrative results, within the pages of a single compact volume. A careful balance has been maintained between the mathematical and physical content; the basic Fourier theory is collected in an appendix, and the whole treatment requires for its understanding no mathematical technique beyond elementary analysis. After a preliminary chapter exposing the problems and intentions, the processes occurring in optical and photographic imaging are treated by Fourier methods. This leads up to quality assessments of optical and photographic images, and numerical results are given illustrating the interaction between wave aberrations and photographic spread in some selected special cases. The general theory is equally applicable to receiving surfaces other than photographic, and in particular to photoelectric image devices.

The standard of production of the book is high. The paper and layout appear excellent, and the type-face untiring to read. The price works out at about sixpence-halfpenny per numbered page. Since this is more than the cost of duplication on the best present-day office copiers, it raises the question of what part printing ought to play in the dissemination of scientific knowledge to-day. This is not, of course, a reflexion on the merit of the book itself, which can be confidently recommended.

P. FELLGETT



## MAGNETIC RECORDING TECHNIQUES

Proceedings of the Conference on Signal Recording on Moving Magnetic Media

Berichte der Konferenz über die Signalspeicherung auf Bewegten Magnetischen Medien, Budapest, 15. 18. Oktober 1962. Pp. 470. (Budapest: Akadémiai Kiadó, Publishing House of the Hungarian Academy of Sciences, 1964.) 12 dollars.

THIS is a well-produced book of some 470 pages which gives the full text of all the papers that were read at a conference on "Signal Recording on Moving Magnetic Media" held in Budapest during October 15-18, 1962. There are 37 contributions, about two-thirds of them from East European countries, principally East Germany and Hungary. The remaining one-third of the contributions are from West Germany, Britain, the United States, Belgium and France.

It is stated that the conference was originally planned as a Hungarian rather than an international one and this, no doubt, has influenced to some extent the geographical distribution of the contributors as well as, in some cases, the presentation of the material submitted.

The papers are all published in their original languages, about two-thirds of them in German but with contributions also in English, French and Russian. As is to be expected with the record of a conference of this kind, the papers vary both in merit and in length, while the diversity of subject-matter is such that it is difficult to indicate the range covered by quoting a few general categories into which the papers may be divided. Indeed, the Editor, faced with this difficulty of presenting the papers under a series of headings, has wisely taken refuge in printing them in the alphabetical order of the authors' surnames. As some indication of range let it be said here that, taking six papers more or less at random from the index, the subjects dealt with include a 1½-in./sec magnetic recording system for stereophonic music, the magnetic recording of seismographic data, a proposed new double 8-mm magnetically striped film, the recording of single television pictures on magnetic disks, the measurement of fluctuations of speed in magnetic tape equipment and the application of information theory to recording systems.

Because of the width of the field covered, it is inevitable that there will be few private purchasers for this book who will have a close interest in all of the papers, the more so since four languages are used. Some private purchasers might therefore feel that the equivalent of 12 dollars is a high price to pay for the fraction of the book that they want.

The volume remains, nevertheless, an excellent permanent record for library use, but some caution is necessary if only because progress in this field is still active and what was an up-to-date account in 1962 may be less satisfactory in 1965. For example, there is an excellent paper by Gondeesen surveying the techniques of synchronizing magnetic tape sound recordings with picture film for television use. The survey is confined to practice in West Germany and, except for the frequency modulated track used in connexion with the Electronic-Cam system, the only type of tape synchronizing track described is that recorded transversely over the whole width of the tape. However, an arrangement using two narrow tracks down the centre of the tape and carrying the synchronizing information recorded in push-pull is now increasingly used in West Germany; it is included in the present Deutsche Industrie Norm, or D.I.N., standard, and information about it was published in Britain, by the same author, more than a year ago. The 1962 paper in this volume, excellent though it is, therefore now tends to give a misleading impression of the state of these techniques even in West Germany alone. Survey papers are perhaps particularly open to the risk of being overtaken by events, but

some of the theoretical papers in this collection also appear to be somewhat dated.

It must, therefore, be borne in mind that this volume is a record of material that was presented in 1962, some of it not written specifically for international publication, some of it not, perhaps, entirely new at the time. With this proviso the book can be recommended as an interesting and valuable record of the situation in 1962 over a wide range of magnetic recording activities. The publication is of particular interest because it makes available papers from a number of technical authors in the East European countries whose work is not too well known in Britain or the United States.

H. DAVIES

## GLASS-CERAMICS: A NEW TECHNOLOGY?

Glass-ceramics

(Non-Metallic Solids: a series of Monographs, Vol. 1.) By P. W. McMillan. Pp. viii+229. (London and New York: Academic Press, 1964.) 47s. 6d.

GLASS has been very neatly defined as "an inorganic product of fusion which has cooled to a rigid condition without crystallizing". No such succinct definition of a ceramic has ever been formulated and we fall back on a rather unwieldy description of a heterogeneous, polyphase material, based essentially on oxides, consolidated by processes of vitrification and sintering to produce a complex of crystals and pores involved in a glassy matrix. It is of course true that modern technology has moved to the production of relatively pure single-phase polycrystalline aggregates of certain oxides, but this is really the tidy end-point of a progressive development rather than a basically new departure. Now, with the advent of the glass-ceramic, the one technology seems to absorb the other, and from the ordinary processes of glass-making emerges a new class of material, conforming exactly to the crystal-glass description of a ceramic, but with many improved characteristics.

Historically, this has its origin in an odd experiment by Réaumur, who in 1739 subjected glass bottles to a prolonged heat treatment in a mixture of gypsum and sand and converted them into opaque, porcelain-like objects, apparently completely crystallized into a radial network of acicular crystals. It was not until the work of Stookey and his colleagues at the Corning Glass Works was disclosed in 1957 that the technological possibilities of this early experiment were revealed. Normally, in the manufacture of transparent glass, every care is taken to avoid crystallization, but for special effects—opal glasses and glasses coloured by colloidal metals—limited crystallization has played a useful part, although perhaps only very small quantities of crystalline materials may be involved. The new technology of glass-ceramics involves the main body and main constituents of the glass, and the resulting product, which is white and opaque, is almost fully crystallized to a grain size usually less than one micron (which is 20-50 times smaller than that in a normal ceramic), and with a complete absence of pores.

The main developments have been made in America at the Corning Glass Works (although it is known that some development has been done in the U.S.S.R.) and most of the work still remains in the journals and in the patent files. Some years ago the Nelson Research Laboratories of the English Electric Co., Ltd., started work in this field and independently produced a range of devitrified glasses of controlled thermal expansion characteristics which could be made to 'fit' various metals. P. W. McMillan, author of *Glass-ceramics*, has been largely responsible for this development, and he has now produced the first book on devitrified glass to be published in Britain. This is a most welcome pioneering effort, and presents, within a modest compass, a very

readable account of crystallization and devitrification and a survey of glass types and nucleating agents for controlled crystallization. The basic requirement is the introduction of submicroscopic catalyst particles into the glass at a temperature below that at which major crystalline phases could grow quickly, and to disperse the catalyst through the glass so that there are a great number of nucleating centres. One of the important catalysts used by Stookey was titanias: McMillan has made a particular study of the use of metallic phosphates.

The glass-ceramic process itself begins with conventional melting and shaping of the glass. It must then be brought to, and held at, a temperature plateau, some 50° C above the annealing temperature, to nucleate, and then brought slowly to a crystal growth plateau which will be some 50° C below the temperature at which the predominant crystal phase is likely to redissolve. Apart from the obvious change in appearance produced by this heat treatment, there is a small (~1 per cent linear) dimensional change, a marked increase in strength and a change in thermal expansion which can be manoeuvred to meet particular requirements.

The mechanical and electrical properties are discussed at some length: strengths up to 50,000 lb./in.<sup>2</sup> are reported, with a notional breaking strain of  $2-3 \times 10^{-3}$ —at least twice that of ordinary glasses and ceramics. Electrically, especially from the point of view of dielectric loss, there is not much to choose between glasses, ceramics and glass-ceramics, but the last can show higher electric strength. Among possible applications, one, in the field of cooking ware, is probably well known to the public, but more importantly there are a number of possibilities in the electrical field, perhaps, very interestingly, in micro-miniaturization.

Little or nothing is said in the book about the economics of the new technology: how far glass-ceramics will invade the chosen field of other materials will depend very largely on this aspect. The book, which is modestly priced by present standards, is the first in a series of monographs on non-metallic solids, a series which is manifestly away to a good start and which, one hopes, will sustain this early promise. N. F. ASTBURY

## NEW RESOLUTIONS FOR BIOCHEMISTS

### New Biochemical Separations

Edited by Dr. A. T. James and Dr. L. J. Morris. Pp. ix+424. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1964.) 83s.

IN recent years there have been a number of significant developments in the methods which are used for the separation and isolation of naturally occurring compounds. The aim of *New Biochemical Separations* is "to provide up-to-date explanations of the most modern refinements in separation techniques and to include experimental data of their application to biochemical problems". The various methods are described by different authors who are experts in their particular fields and who have, in most cases, been responsible for the developments in the technique they describe. Gas-liquid chromatography, thin-layer chromatography and gel-filtration receive particular attention since they have developed most rapidly recently. This volume is not therefore a complete manual of separation techniques, but it is an important contemporary complement to more catholic text-books such as *Chromatography* by Lederer and Lederer and *Separation Methods in Biochemistry* by Morris and Morris.

The first five chapters deal with the application of gas-liquid chromatography to the separation of radioactive

compounds, steroids, alkaloids, carbohydrates, coenzyme A esters, bile acids and amino-acids. In some cases it is helpful or even necessary to convert the sample into more volatile derivatives by esterification, trifluoroacetylation, or silylation before chromatography. Adequate details or literature sources are given for these preliminary steps. It is emphasized that the chromatographic procedure and the nature of the derivative should be varied where possible in order to achieve the maximum degree of separation and certainty of identification.

The use of cross-linked dextran gels for the fractionation of amino-acids, peptides, proteins and polysaccharides is described in two chapters. Thin-layer chromatography has been used for the separation of amino-acids and their *N*-2,4-dinitrophenyl derivatives; full descriptions of the methods are given. The separation of 1-dimethylamino-naphthalene-5-sulphonyl derivatives of amino-acids by thin-layer chromatography (*Experientia*, 20, 559; 1964) may well prove more popular eventually in view of its high sensitivity. The separation of alkaloids by conventional paper chromatography as well as thin-layer chromatography is described in detail and illustrated by numerous tables of *R<sub>F</sub>* values. Although reversed-phase paper chromatography and chromatography on alumina- or silica-impregnated paper have been useful for the separation of hydrophobic compounds, it seems that thin-layer chromatography is proving to be a more powerful tool. This trend is reflected by the inclusion of chapters on the separation of steroids, triterpenoids and bile acids by this method.

The remainder of the book is devoted to the use of various techniques for the separation of lipids especially those containing polar groups. Progress in the lipid field has been inhibited by the paucity of analytical methods. The present accounts of the use of thin-layer chromatography, chromatography on paper impregnated with silicic acid, and counter-current distribution are important, therefore, in view of the increasing interest in the lipid field.

This book is well produced and remarkably free from errors. Contributions from German authors have been translated into English. Literature references up to 1963 are to be found in most chapters. It is to be hoped that further editions will appear from time to time in order to focus attention on present-day trends in an important aspect of biochemistry. D. T. ELMORE

## POISONS IN INDUSTRY AND RESEARCH

### The Halogenated Hydrocarbons of Industrial and Toxicological Importance

By Dr. W. F. Von Oettingen. (Elsevier Monographs on Toxic Agents.) Pp. x+300. (Amsterdam, London and New York: Elsevier Publishing Company, 1964.) 60s.

THE study of toxic compounds is now in an exciting phase. New techniques, biochemical and electron microscopic, are bringing a mass of new information and new ideas are springing up. Because carbon tetrachloride has been studied for such a long time, the application of the new techniques to the lesion following this poison yields results which can be laid against existing knowledge more profitably than for any other toxin. Inhibition of protein synthesis which occurs *in vivo* can be reproduced *in vitro* and shown to be due to a failure of ribosomal function; this can be seen by the electron microscopist as detachment of granules from membranes of the endoplasmic reticulum.

Furthermore, it is becoming clear that there are general reactions to injury at the cellular level which may occur whatever the toxic agent (degranulation of the endoplasmic reticulum is an example). An advancing under-

standing of these reactions sheds light on the organization of the normal cell. The work of Magee in showing that dimethyl nitrosamine is demethylated by enzymes in the liver to form a highly toxic alkylating agent has extended the field of 'lethal synthesis' from a rare event to being perhaps the key to hepatotoxicity.

None of these developments is mentioned in Von Oettingen's *The Halogenated Hydrocarbons of Industrial and Toxicological Importance*, despite the fact that there is an extensive discussion of experimental work on carbon tetrachloride.

Von Oettingen discusses eighteen halogen derivatives of methane, ethane, ethylene and butadiene. Each compound is dealt with in two sections, one dealing with clinical and industrial data, and one with experimental work. The sections dealing with chloroform and carbon tetrachloride occupy one-third of the book. There are more than 800 references for these compounds alone. The juxtaposition of clinical reports with a collection of the experimental work by a worker of great experience, the author of the well-known U.S. Public Health Service Bulletin No. 414, is very helpful. However, in our opinion, this admirable attempt to present both sides of the field carries with it responsibilities to those readers familiar with one side only which the author does not fulfil. There is no attempt at synthesis or critical discussion in either section. This must make the experimental sections heavy going to anyone not familiar with the field. Among a very wide range of references, dealing with older work, as well as with some of the most recent, a number of key papers are missing which will limit the value of the bibliography to the beginner. In the chapter on carbon tetrachloride, there is no mention of the work of Christie and Judah in the section on hepatic cell injury, none of Seneviratne in the section on circulatory changes. There is no mention at all of Oberling and Rouiller's classic electron microscopic studies, or of any other electron microscope work.

However, for the research worker who is acquainted with the work of the past twenty years, the many references to the older work, though unselective, are very useful.

ELIZABETH and A. E. M. MOLMAN

## A LANDMARK IN ARTERIAL PATHOLOGY

### Arterial Disease

By Dr. J. R. A. Mitchell and C. J. Schwartz. Pp. xv+411. (Oxford: Blackwell Scientific Publications, 1965.) 95s. net.

IN his foreword to *Arterial Disease*, Sir George Pickering correctly states: "... this is careful, methodical work on an important subject which comes to a relatively novel, and I think probably correct, view of the nature of the disease. It is beautifully illustrated and presents new knowledge against a background of the old. It should make a substantial contribution to the advancement of knowledge in an important and hitherto poorly understood field".

The contents of this volume are fascinating and presented in a lucid, direct and sound, if, perhaps, slightly authoritarian manner.

A brief introductory outline of the problem and a description of the material analysed are followed by a text presented in three distinct, albeit closely related sections, namely: Part 1, "The Oxford Necropsy Survey" (ten chapters); Part 2, "Arterial Diseases—A Review" (five chapters); Part 3, "Methods" (one chapter).

In Part 1 the authors present, in relation to historical background and to more recent work, their findings in the hearts and/or vascular trees of several hundred patients—both 'selected' and 'unselected' (criteria detailed)—subjected to autopsy at their uniquely situated 'outpatient' hospital which they state has a 90 per cent autopsy rate

on patients who die therein. Their subjects were all more than thirty-five years old. It would have been illuminating to learn something of the state of the arteries and hearts of infants, children, adolescents and young adults in the same population—perhaps the topic for a future report by these authors? The actual years during which their material was collected is not stated. This omission could be of considerable importance to future workers.

The morphology and localization of 'arterial plaques' (precisely defined), the nature and extent of aortic disease, the relationship of myocardial lesions to coronary artery disease, as well as the morphology, incidence and implications of 'large cardiac lesions', distribution and prevalence of coronary stenosis, pathology encountered in carotid and vertebral arteries and structure of thrombi, are described and critically assessed.

Part 2 is devoted to a critical analysis of present knowledge and prevailing theories of the aetiology and pathology of the diseases studied, an assessment of the possible import of individual peculiarities and of those environmental factors at present considered by many investigators to play aetiological parts of greater or lesser significance. An absorbing analysis has been made of the pathogenesis of arterial plaques in man and of the relevance, or irrelevance (for understanding lesions in man), of experimental contributions to the study of arterial disease and particularly thrombus formation. This section is nicely rounded off with a refreshing, stimulating and critical concluding chapter on the implications of all the preceding for those therapeutic régimes which, at present, are widely and often slavishly instituted for myocardial infarction.

Part 3 should prove useful to all those studying arterial supplies to any organ, although, of course, the blood supply to the heart is here dealt with primarily. It provides full details of the techniques used by the authors. Many other writers in this field might have enhanced the value of their own work by providing similar technical details, so necessary if others are to acquire comparable data. In writing this section, then, the authors are to be congratulated.

It should be apparent, at once, that this is an unusual addition to the literature in this field, for it is an absorbing mixture of clinical, pathological and experimental evidence on the nature and possible (perhaps even probable) pathogenesis of that important group of human arterial diseases—namely, the thrombo-occlusive, degenerative lesions—affecting the main arteries of the human body, with particular reference to the coronary arteries and the myocardial lesions.

As for the latter, the authors tacitly assume they are invariably secondary to coronary occlusion(s). This view on the pathogenesis of myocardial necrosis and/or fibrosis has, for many years, been accepted unquestioned by most pathologists and clinicians. My own deliberate hesitancy in blindly adhering to this conception derives from experiences in other fields where massive parenchymal necrosis (of non-toxic origin) is known to occur in the absence of vascular occlusion, for example, nutritional hepatic necrosis. Moreover, there is now some evidence that myocardial infarction may occur—and not rarely—without preceding coronary infarct; the latter may, indeed, be secondary to the myocardial lesion(s) (see *Arch. Path.*, 78, 432 (1964), on "Low Incidence of Coronary Thrombosis in Myocardial Infarction"). Furthermore, since uterine artery narrowing, and even occlusion, may apparently succeed uterine involution after pregnancy, it seems not impossible that involutionary changes in coronary arteries may perhaps also be consequent on 'disuse atrophy' of the actively used 'hypertrophic' myocardium they once supplied. And what better place to question prevailing dogma than in a review of this book which does this so often and so well.

Indeed, this volume should constitute a landmark in the development of our knowledge of these diseases which

must, in a progressively ageing world population, become of even greater importance than they are at present. It provides a long-overdue critical examination of accepted dogma—a 'spring-cleaning' of our thoughts and of the literature on arterial diseases. The facts and arguments, so lucidly presented, invalidate many of the shibboleths used by workers in this field. Thus, for example, dogmatically held views on the relations of fatty streaks to fibrous plaques in arteries are discredited.

The authors are to be congratulated on the directness with which they state their case and views. Whether or not their views are accepted entirely, it is refreshing and helpful, in a field so befogged with hazy thinking, and encumbered by canonical and often obscure statements, to read: "In our view, the thrombogenic concept is the most satisfactory hypothesis [for the genesis of arterial diseases] so far formulated . . ." "There is little doubt that today the emphasis is changing, with thrombosis emerging as the factor of paramount importance in the development of occlusive arterial disease." They suggest that ". . . our knowledge [may be advanced] more rapidly by focusing attention on the circulating blood, rather than on the vessel wall lesions, which may be the result and not the cause of thrombosis"; and finally—"As we have seen, this is a thrombotic disease and, if any relationship does exist between environmental factors and cardiac infarction, we should not try to explain it in terms of 'atherogenesis' but of 'thrombogenesis'."

Full appreciation of this fact-packed, thoughtful and thought-provoking book might, I think, have been facilitated by a five or ten page summary of the main facts and conclusions presented. Also, this analysis would, perhaps, have been enhanced by greater attention to the development of arteries in relation to their diseases and hence by a fuller consideration of the possible roles of arterial remodelling, regeneration and repair in the pathogenesis of occlusive vascular diseases.

Nevertheless, this volume should certainly be of considerable interest to the innumerable pathologists, experimentalists and clinicians who are, to-day, forced to address themselves to this group of diseases, so frequently the cause of premature deaths of mature adults as to constitute the most important man-killer of our time.

T. GULLMAN

## RECENT ADVANCES IN LIPID RESEARCH

**Metabolism and Physiological Significance of Lipids**  
Proceedings of the Advanced Study Course held at Cambridge, September, 1963. Edited by R. M. O. Dawson and D. N. Rhodes. Pp. ix+657. (London, New York and Sydney: John Wiley and Sons, Ltd., 1964.) 147s.

**M**ETABOLISM and Physiological Significance of Lipids comprises the collected papers presented at an advanced study course on "Lipids" held in Cambridge in September, 1963. The contributors include many of the world's leading workers in this field of biochemistry. The range of topics discussed and the wealth of detailed information which the book contains indicate clearly both the enormous increase in interest in the biochemistry and physiology of lipids, which has taken place especially in the past two decades or so, and also the challenging problems at present confronting research workers. It is evident that the advances in our knowledge of lipids with which this volume is concerned owe much to the development of modern techniques, particularly to chromatographic procedures and to the use of isotopic tracer methods for the elucidation of metabolic pathways in tissues.

In addition to the two major sections on metabolism and on the physiological significance of lipids, shorter specialized sections deal with lipid absorption, lipids of

the nervous system and the association of lipids and proteins in biological systems. Brief reports of the contributions to two symposia, one on the fatty acid composition of lipids and another on techniques, are also included.

In the section on metabolism of lipids, the biosynthesis and inter-conversion of fatty acids in animal and plant tissues are discussed by several contributors. In animal tissues *de novo* synthesis from acetyl CoA and malonyl CoA, elongation and desaturation reactions all seem to be involved; in plant tissues there is no direct evidence at present of elongation of palmitate to stearate or of conversion of the latter acid to oleate.

The synthesis of characteristic branched-chain fatty acids by mycobacteria is also discussed. Other papers in this section deal with recent work on the biosynthesis of sterols, triglycerides and phospholipids. The metabolism of phosphoinositides is described and a hypothetical scheme for their possible role in the transport of cations across membranes is presented. Lipases and phospholipases are the subjects of several contributions, and work on the biochemical characterization of these enzymes is reviewed; the recognition of phospholipase activity in tissues and of the enzymic re-acylation of lysophosphatides has raised the question of the physiological significance of these enzymes in the selective turnover of fatty acids in tissue phospholipids.

In papers dealing with lipid absorption, recent advances in the physicochemical aspects of fat digestion and the mechanisms of transport of lipid material across the intestinal mucosa are reviewed; differences are reported in the mode of transport between different species and between different strains of rats.

Papers on the physiological significance of lipids cover a wide variety of problems. The role of plasma triglycerides in fat transport, the metabolism of short- and long-chain free fatty acids of plasma by various species and the difficult question of the part played by lipids in blood coagulation are reviewed. In other papers in this section lipid metabolism in ruminants and the biosynthesis of milk lipids are considered. Fatty infiltration of the liver as a result of nutritional deficiency or following exposure to toxic agents is discussed in two articles and the hypothesis is proposed that inhibition of lipoprotein formation plays a part in the development of fatty liver. Several contributions are orientated towards the more physicochemical aspects of lipids as membrane components, and others deal with the effects of drugs and hormones on lipid metabolism. In a paper on the lipids of bacterial membranes, the isolation of amino-acid esters of phosphatidylglycerol from a number of micro-organisms is described and their physiological function in bacterial membranes is considered.

Lipids of the nervous system are the subject of four contributions which cover biosynthetic mechanisms, the presence of lipolytic enzymes in nervous tissue and their possible significance in demyelination, a review of lipid metabolism in myelin and the lipidoses.

The section on lipid-protein associations includes an extensive and stimulating discussion of the part played by lipids in mitochondrial function.

Those responsible for the organization of this conference on lipids and the editing of its proceedings deserve great praise for their part in producing this excellent collection of authoritative articles. The book is well produced and will be invaluable, not only to biochemists but also to scientists in other disciplines whose interests involve lipids. Each paper includes a useful bibliography together with a summary of the audience discussion which followed its presentation. In view of the great amount of information the book contains and because some aspects of lipid biochemistry are discussed from different points of view by several of the contributing authors, a rather more detailed index would have been an advantage.

G. R. WEBSTER

## CAMBRIDGE MEETING OF THE BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

By R. DEMBITZER

Administrative Assistant to the Local Executive Committee

THE 127th meeting of the British Association is to be held this year in Cambridge during September 1-8.

Many members of the Association are familiar with Cambridge and many others have taught or been taught or undertaken research here, but as this article tends to cover a wide field it may not succeed in covering all aspects of the meeting or describe those parts of Cambridge to the satisfaction of every reader. For this I apologize.

Members will be coming to Cambridge at a period of turmoil in the University, in the educational world and in man's ideas of the role of science in the future of the country. Cambridge, like most other universities, has been facing the prospects of the post-Robbins legislation with some apprehension, but it appears now that a number of its fears will not be realized. However, the 'brain drain' has undoubtedly alarmed the authorities and the governmental bodies concerned, and the general hope is that as a result there will be better research facilities and more money for departments and their projects. But changes are slow, and some displeasure is being voiced around. On the other hand, the fact that more emphasis is being placed on the need for scientists and technologists to open the door of the future should give the British Association a new lease of life and an urgent *raison d'être*. It might even be possible to hope that the public at large will realize that the right climate for the growth of those strange human beings, 'scientists' and 'technologists', will come about only through the work of organizations such as the British Association.

Cambridge has been an exciting place to live in for the past few years. An unprecedented period of building has changed the face of University Departments and of Colleges, and much has been altered. Many Colleges are struggling for expansion, some more than others, and visitors who knew Cambridge ten years ago would not recognize many of the newer buildings. During the

meeting of the British Association, building will be in progress at Corpus Christi, St. Catharine's, King's, Magdalene, Newnham, which will prevent these Colleges from taking a major part in the reception of members; there is work taking place on an extension for St. John's College and on the kitchens and buildings of Trinity College.

The most notable change, however, has been the growing importance of the research worker. The Bridges Report, an internal enquiry, pointed out to anyone left in doubt the lack of facilities which were available for members of the University who were not also Fellows of Colleges, and some of the Colleges have made great efforts to overcome this and to offer new fellowships. A new graduate College, to be known as University College, will come into being soon to accommodate University officers who do not cover subjects suitable for undergraduate teaching or are on the administrative roll of the University. The University Centre, also a post-Bridges innovation, will offer additional facilities for other members of the University who fall in this group. With the introduction of Clare Hall, Leckhampton House and Darwin College, we witness the growing awareness of the Colleges of the problem of their research students, a class of people who have so far been given a less than justifiable position in the lives of the College. The three institutions named are all private efforts of Colleges acting either independently or in co-operation. This is certainly not the end of this movement.

Besides Churchill College, which was founded in 1959 to cater mainly for the scientist and the technologist, members of the British Association will be able to see the unfinished buildings of New Hall and Fitzwilliam House. There is one argument, however, which is militating against the geographical growth of the University. As the area covered by the University Departments and the Colleges is extended, the students and the staff will have to travel greater distances to reach them, and this

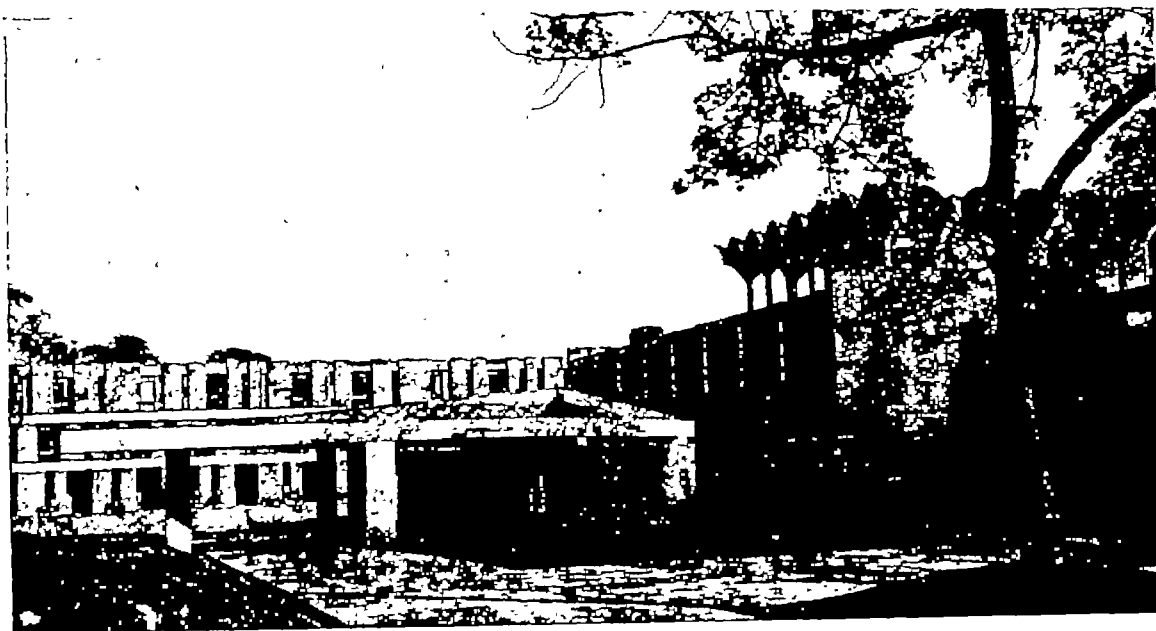


Fig. 1. Fitzwilliam House, University of Cambridge. Courtesy of City Architect's Department, Guildhall, Cambridge



Fig. 2. Part of Sidgwick Avenue site economics and politics building. Courtesy of City Architect's Department, Guildhall, Cambridge

eventually may lead to a 9 a.m.-5 p.m. University, with students cycling to one end of town to reach the University Department for the lectures, returning to their College late to complete their work in the College rooms or library. Though this is an inevitable process, there is hope that it can be contained.

### The British Association and Cambridge

The third meeting of the British Association (it had been founded in York in 1831) was held in Cambridge in 1833 under the presidency of the Reverend Alan Sedgwick, professor of geology. Meetings were held regularly in Cambridge every twenty-five or so years: 1845, 1862, 1904, 1938, and now in 1965. Of the past meetings, possibly the most significant was that held in 1904, because Mr. A. J. Balfour was president during the period of his premiership. Scientists took advantage of the eminence of their president to tackle him on the question of State-aid to the Universities—the beginning of university grants as we know them to-day. The last meeting here was held in 1938 under the presidency of Lord Rayleigh. It was at this meeting that the idea of a quarterly report was instituted; this resulted in the Association's journal *The Advancement of Science* which from May of 1965 is now published monthly.

The organization of the scientific programme of the meeting has changed very little over the years, although the number of sections, which were six in 1833, has grown to fifteen, and prominence is now given to the social sciences equally with the physical and biological sciences. Sociology and education are among the new sections of the Association since it last met in Cambridge.

### Science in Cambridge

The 127th meeting is being held at a time when the country at large is becoming increasingly aware of the need for scientists and technologists. Cambridge scientists have grown in relative terms; there are many more scientists (in the widest sense) now than ever before and they account for almost half the total undergraduate population, but are well over the fifty per cent mark in the graduate and teaching population of the University.

On occasions like this, figures speak clearest. It is difficult to give groupings which will be generally acceptable, as each University and indeed every University person has different ideas of what discipline fits into which grouping, but even so it is interesting to compare the rates of growth:

	TOTAL STUDENTS (ALL SUBJECTS)		
	Undergraduates	Postgraduates	Total
1928/29	5,226	705	5,931
1954/55	6,749	1,185	7,934
1959/60	7,575	1,422	8,997
1964/65	7,701	1,847	9,548

Of the foregoing figures, the scientists represent the following proportions (figures for postgraduates in parentheses):

	I	II	III	IV	Total	Total as % of all
1928/29	202 (41)	1,110 (239)	544 (15)	181 (58)	2,067 (348)	30.5 (48.7)
1954/55	507 (90)	1,620 (407)	714 (60)	198 (59)	1,939 (616)	43.5 (52.0)
1959/60	465 (95)	1,786 (607)	894 (118)	150 (52)	3,295 (866)	43.5 (60.9)
1964/65	2,659 (744)	773 (190)	65 (87)	8,527 (901)	45.8 (53.7)	

Notes: Group I, mathematics; II, natural sciences (including metallurgy, medicine, veterinary medicine); III, engineering and chemical engineering; IV, agriculture (including estate management and land economy).

Science has always been of fundamental importance in Cambridge, and there is little need, one feels, for it to be 'advanced' here, but its extent may still be a surprise to members of the Association. Those coming to Cambridge after an absence of some years, or who have not had the opportunity of acquainting themselves with some of the latest changes in the scientific field, should go round and make enquiries. On the occasion of the visit to Cambridge of such large numbers of scientists, many Departments are taking the opportunity of holding 'At Homes', and members will be welcome everywhere.

New buildings have emerged all over the city. The main development of the arts faculties has taken place on the Sidgwick Avenue site, designed by Hugh Casson and Partners at a cost so far of about one million sterling, and here in the next few years will be accommodated the bulk of the lecture rooms, staff rooms, research facilities and libraries of the arts faculties. On the science side, there has been no such wholesale concentration, but a gradual re-development of the sites is going on and is likely to go on well into the 1970's.

The two main areas of the scientific departments are the New Museum's site (Bene't Street, Free School Lane, Corn Exchange Street, Pembroke Street) and the Downing Site (Downing Street, Tennis Court Road, Downing College, Downing Place). Both sites have considerably changed over the years, but still accommodate more than twenty world-famous laboratories, all cramped for space. On the New Museum's site, during 1959-60 the Shell Co. paid for a four-storey block for the Chemical Engineering Department. The University has lost its only large multi-purpose hall, The Examination School, which has been taken over by the Department of Physics and which is being adapted as further laboratories and staff rooms.



On the Downing site there have been four major additions since the Second World War. The Public Health Laboratory was built in 1950. Close to it is the Veterinary Anatomy building, a pleasant five-storey extension to the older buildings of the Anatomy Faculty. In 1961 work started on the Psychology building, which was completed in 1963, the same year in which appeared the third stage of the Biochemistry building, built with the aid of the Wellcome Foundation.

In the early 1970's Addenbrooke's Hospital will have completely vacated its building in Trumpington Road and moved to the new buildings which are being built in Hills Road. Part of the new site, away from the town centre, has now been open for four years; but the final transition will take another six or seven. Once they have left, there will be a space of about six acres which, it is expected, will become available for re-development for the use of some of the scientific departments. Eventually, it is hoped, all the scientific departments will have buildings as modern and well equipped as the Chemical Laboratories in Lensfield Road and the Engineering Laboratories in Trumpington Street. The Chemical Laboratories were completed in 1960, at a cost of £2.25 million, and offer accommodation for over 200 research students and teaching staff, and class rooms and laboratories for 500 undergraduates. The Engineering Laboratories are still undergoing a rebuilding period and will not be completed for a few years. However, the Department, the largest in the University with a teaching staff of 80, will eventually be able to accommodate 1,000 undergraduates and 200 research students.

Probably the most interesting development in the field of research in Cambridge has been the growth of Government-sponsored bodies which have found a home here, and whose personnel have been involved and are involved only in a tenuous way with the University. The Medical Research Council have several units, of which three will be visited during the meeting, the Applied Psychology Unit, the Microbiology Research Department, which is attached to Addenbrooke's Hospital, and the Dunn Nutritional Laboratory. The Agricultural Research Council have a large unit at Babraham, concerned with animal physiology, and the National Institute of Agricultural Botany own extensive grounds for the testing of new seeds on the Huntingdon Road.

#### Programme of the Meeting

After the presidential address by Sir Cyril Hinshelwood on "Science and Scientists" on September 1, the scientific programme will start on the next day. This consists mainly of the reading of formal papers followed by the discussion on a theme or themes decided on by the various Section Committees. The keynote for the section work is given by the President in his presidential address. About 300 papers are being presented for discussion, and these will receive the attention of a total membership which will approach 3,500.

A random selection of the papers which will be read at the meeting does show that there will be a very great weight on the side of technology, and some of the lectures have such titles as "Technology in a Liberal Education", "The Interaction of Technologies", "Science Pure and Applied", "Automatic Safe Landing of Aircraft in All Weathers", "Technology and the Good Life", "The Cultural Technologist". While it would be irrelevant to sort out the scientific value of one paper from another one, it is likely that one of the most visually exciting talks will be one given by Prof. B. J. Mason, the president of Section A (Mathematics and Physics), who will speak about physics of raindrops and hailstones, accompanied by demonstrations.

In 1961, at its meeting in Cardiff, the Association for the first time held a one-day symposium on the subject of "Food and Population". This was very successful, as it dealt with a theme which had attracted the imagination of

the country at large on the work of the Freedom from Hunger Campaign which had just started. A speaker on that occasion referred to fuel and power being second in importance only to food and population, and this is one of the reasons for a choice of a one-day symposium, which will be held on September 6. Among the speakers will be Lord Hinton, Lord Robens, Sir Harold Hartley, and Prof. H. J. Kruisinga, and the theme which they will be discussing will be taken up by the various sections in their own activities. The main nationalized undertakings and oil companies are coupling this with an exhibition on the resources and need for fuel and power. It will be held in the Union Society and will last for two weeks after the meeting itself.

#### Excursions

An integral part of the meeting of the British Association is to pay visits to many places of interest, both of a scientific nature and of pleasure in the host region. Cambridge will be no exception, and this year more than 138 excursions have been organized.

Cambridge is probably poorer than many parts of the country in establishments of an industrial nature, and East Anglia has traditionally been the granary of England. But the City and the University make up for it very easily by housing a high concentration of research establishments. Among those which will be seen by the members of the Association are the giant industrial complex of Pye's, makers of all kinds of electronic apparatus, some for use in academic research, others for military uses, others for domestic television and radio. Pye's were founded by a Cambridge man, formerly of the Cavendish Laboratory at the end of the nineteenth century, and have now become a household name. The same link with the University is at the origin of the Cambridge Instrument Co., and of Cathodeon, who make electronic instruments, and others. The Fisons Group of Companies have some of their research departments in the area, and members will see the Pest Control Research Laboratories at Harston and the Laboratories at Chesterford Park. The Welding Research Association at Linton, near Cambridge, undertakes research on behalf of more than 300 member bodies and has close connections with the University's own Department of Metallurgy.

#### Young People's Programme

The lecturers who will take part in the programme for the Young People will be Dr. J. E. Gabb (research medical officer of the Institute of Aviation Medicine), who will talk on "Medical Aspects of Space Flights", Dr. F. A. Vick (member for research, United Kingdom Atomic Energy Authority) on "Exploration in Atomic Energy", Prof. Herman Bondi (professor of applied mathematics, King's College, London) on "Gravitation" and Prof. H. J. Eysenck on the "Measurement of Intelligence". The Science Fair, which has become a regular feature of the programme for young people, will be held in Homerton College, one of the best known of the Teachers Training Colleges. Many schools in the Cambridge area will take part and have been working hard at projects which they will display during the week of the meeting.

The two evening discourses, which will be held in the University Senate House, will be delivered by Prof. F. Hoyle, who will talk about recent work in the field of cosmology and astronomy, and Dr. E. B. Worthington on the "International Biological Programme".

Following last year's successful broadcast by Southern Television of some of the talks of the British Association, this year the B.B.C. will broadcast live lectures on four mornings during the meeting. These will be transmitted from the Lady Mitchell Hall, situated in the new complex of buildings on the Sidgwick Avenue site.

Statistics for this article have been obtained from the *University Reporter* and *Cambridge New Architecture* (second edition, April 1965).



## VIRUSES AND TUMOUR CAUSATION

## AN APPRAISAL OF PRESENT KNOWLEDGE

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A HOST of viruses have now been found which induce tumours in one way or another, and their number has increased so rapidly of late that the facts concerning them appear well-nigh chaotic. The present time seems right to try to bring order into what is known and then to ask what it may mean for the neoplastic diseases as such. An obvious first step is to try to group those viruses of vertebrates that belong together, as made plain by their production of typical tumours within the living organism. The phenomena witnessed when they act on cells freed from the restraint of the body by cultivation *in vitro* or in other ways will be noticed only where directly pertinent\*.

During half a century of search many viruses have been come upon which start and maintain tumours in chickens, but few have been discovered that do the like in other creatures. They all have this in common: each changes normal cells into neoplastic cells having features which it determines, multiplies within these cells, causing what appears to be their autonomous proliferation, and can be recovered from them in a condition to produce new growths of identical sort in other, similar hosts. They do everything in short that a real cause can do, and hence they can be termed 'comprehensive' tumour viruses. For most purposes, though, 'do-all' seems better, a word sanctioned by usage for more than three centuries, *vide The Oxford English Dictionary*.

"Do-all: One who manages the whole business; a factotum. 1633 D. Rogers Treat. Sacraments II. 7. 'It is conscience which is the do-all in the soule.'"

"1701 Pepys Diary, ed. by Jackson, 1879, VI. 233. 'The Cardinal is the do-all.'"

## The Do-All Tumour Viruses

The first comprehensive virus was procured in 1908 by Ellerman and Bang from the cells of a chicken leukaemia. Their discovery, though soon enlarged through their finding of other viruses causing chicken leukaemias of different sorts, was still-born into the world of knowledge because the leukaemias were not then deemed neoplastic diseases. A little later numerous viruses were obtained from the solid tumours occurring in chickens, typical neoplasms according to the criteria already established through the scrutiny of transplantable rat and mouse tumours. Each of these viruses produced growths of only the sort from which it had been obtained, yet they differed so widely in effect that the yield of neoplasms was various. Not until 1933 was a mammalian tumour virus found—in rabbits by Shope. It causes the benign, epidermal papillomas often present on the common wild American rabbits known as cottontails, trapped in the south-western United States. In 1934 Lucké reported renal carcinomas to be well-nigh epidemic in the leopard frogs of New England, and later he and others described the virus responsible for these notably malignant growths. In 1958, Shope procured another mammalian virus, one causing the subcutaneous fibromas occasionally present on wild Virginia deer.

To most investigators these meagre findings in creatures remote from man seemed to throw little light on the tumour problem; but in 1951 Gross obtained a do-all virus from the cells of a mouse leukaemia. This caused a stir because the tumours of mice had long been the common currency of cancer research, having yielded numerous facts which

proved applicable to the neoplasms of man. Forthwith a host of workers strove to get viruses from 'spontaneous' mouse leukaemias, and now not a few have been obtained, and some from rats as well.

The do-all virus longest and most closely investigated, now known as the RSV, was procured in 1911 from an anaplastic chicken sarcoma (the RS). It acted only on fibroblasts, and throughout more than forty years caused growths of identical sort, and these only. The viruses next procured from fowls also acted only on fibroblasts, yet each had its own determining effect on the character of the resulting tumours, a rifted, intracanalicular sarcoma, for example, in one instance and an osteochondrosarcoma in another. As time went on many other chicken viruses were come upon which affected cells of other types in ways characteristic of them individually, which results in a widely diverse yield of neoplasms.

The frog virus acts only on the convoluted tubules of the kidney, producing carcinomas of a distinctive sort. Often the malignant cells contain 'inclusion bodies', consisting of aggregates of the virus, but nearly as often these are lacking and the growths then wholly resemble classical cancers. The fibromas due to the action of the deer virus on fibroblasts have a morphology so unvarying as to bespeak strict morphological control by the virus. Some of the mouse viruses inducing leukaemia were wholly specific in their effects when first procured, for example, that of Friend which caused only reticulo-endotheliomas, but cells rendered leukaemic by one virus can become superinfected with another<sup>1</sup>, and for this and further reasons the problem of what is referable to each is too complex for present analysis. Very recently epidemics of leukaemia have resulted in great losses of cattle in Denmark and northern Germany, and much goes to show that they are caused by a virus.

The fact that the RSV actuates the RS tumours it initiates was strikingly demonstrated after they had been examined for fifteen years<sup>2</sup>. There then occurred a period of six months during which the growths were remarkably benign and no virus could be obtained from their tissue despite persistent efforts. But after many serial transfers during this period the growths again became actively malignant, recovered the ability to metastasize, and yielded virus in abundance. Titration tests have since shown that changes such as these are determined by the amount of virus the neoplastic cells individually contain, this determining both their aspect and their behaviour<sup>3</sup>. The same general principle holds true of the growths caused by the Shope rabbit virus, though their morphology is not affected. It elicits papillomas more slowly after partial inactivation by heating, and these lol along even when becoming big, and they frequently regress.

Needless to say, the role of the do-alls must be cyclic under natural conditions, else they could not continue to exist. Spontaneous sarcomas resembling the RS occur sporadically, and they yield viruses related immunologically to the RSV; but how this latter can maintain itself is still a problem, so sensitive is it to inactivation. The same holds true of the virus causing the peculiar chicken sarcoma rifted with blood sinuses, this growth reappearing spontaneously on a single occasion a few years after it was first observed. In contrast the Shope virus causing papillomas is so hardy and so readily transferred from rabbit to rabbit that growths due to it are abundant in several regions where cottontails are

\* No references will be cited to familiar papers, but only to those reporting overlooked facts or such as deserve special stress in the present connexion.

numerous. The renal cancers caused by the frog virus have remained almost epidemic in the leopard frogs of New England ever since they were first noted thirty-one years ago, but no one has yet ascertained how the virus is transmitted. That of the deer fibromas passes from one animal to another by way of the trees against which they rub. A few viruses of the leukaemias of rodents reach the young through the placenta or the milk, but how the generality is transferred has still to be found out.

Do-all viral agents capable of causing tumours of several distinct sorts of chickens have occasionally been come upon, but in most if not all instances they owe their ability to the association of several viruses of differing neoplastic potentialities. In 1917 a 'lymphoma complex' made its presence plain in White Leghorn chickens raised under crowded conditions in the Middle West of the United States, and it gradually caused such huge losses that a government-supported Regional Poultry Research Laboratory was set up in 1937 to combat it. The complex is indigenous to the White Leghorns and it consists of mingled viruses which individually cause leukaemias, lymphomas, several differing sarcomas and two diseases which are not neoplastic. Each of these agents elicits antibodies; yet still the complex cannot be controlled, and it is now prevalent in White Leghorns and chickens of some other kinds from Maine to California.

Each do-all virus has a single species as its natural habitat, and though it may cause growths on inoculation into closely related animals it fails to maintain itself permanently in them. The RSV, for example, is capable on test of producing tumours in pheasants, turkeys and ducks, but it never does this under natural conditions. The Shope papilloma virus, so readily transferred in cottontails, very occasionally causes a growth in one of the many jack rabbits living among them, but it gets no further in this species. Nor is it found in swamp hares, snow shoe and domestic rabbits, though it causes vigorous papillomas in them all on inoculation. The frog virus is indigenous to leopard frogs only. The deer virus produces growths in no other herbivora, and the rat and mouse leukaemia viruses cause growths spontaneously only in these creatures.

#### Do-All Viruses causing Successive Neoplastic Changes

The two do-all viruses which cause benign tumours are capable of more besides this. They can bring about secondary carcinomatous changes in the cells of these growths.

*The papilloma virus.* Very occasionally a Shope papilloma procured from a trapped cottontail has in its midst an epidermal carcinoma which has taken off directly from its virus-infected cells. This happens much more often in the exuberant papillomas induced by inoculating the virus into domestic rabbits; and in these it occurs relatively soon, and the cancers are often multiple. They, like the papillomas, can be serially transplanted\*.

The papillomas are strikingly similar in morphology whereas the derivative carcinomas are widely various. Occasionally one of them seems to differ from the benign growths only in being disorderly, aggressive and metastasizing, but thence their diversity ranges all the way to complete anaplasia, and even those appearing papillomatous on first arising tend to become anaplastic eventually by consecutive, step-like alterations such as are not infrequently observed to occur in human cancers. Owing to their derivation they are now called the Vx carcinomas. Seven of them have been transplanted serially and great efforts have been made to procure a 'cancer virus' from them, but none has been obtained. This failure is the more impressive because six of the seven have yielded the papilloma virus. The growth from which it has never been got (the Vx2, now in its 27th year of serial transplantation) contained the powerful antigen of the papilloma virus throughout its first 3.5 years of maintenance, its rabbit hosts proving completely resistant on inoculation

therewith, but after 4.5 years more not even this sign of it was left\*. Since then the animals carrying the tumours have regularly proved, on inoculation, as susceptible to the virus as normal animals. In contrast the Vx7 (now in its 18th year of maintenance) has continued to harbour recoverable papilloma virus, and this has recently been obtained from its tissue in such quantity as to have been chemically purified by a method that has consistently failed with extracts of the Vx2 (ref. 6). Like this latter it has been completely anaplastic from the first.

These findings make plain that the papilloma virus acts only to initiate the secondary carcinomas it induces, although on occasion it persists in them long. The Vx2 is now generally regarded as a cancer of the classic sort because no causative agent can be demonstrated in it, and as such it is widely utilized for experiment. Not so with the Vx7. Requests for it are few because it still harbours the papilloma virus.

*The milk virus of mice.* A virus first recognized in 1936 as existing in the milk of certain strains of mice is only now mentioned because of features that have been deemed to set it apart. Actually it is the most successful of the do-all viruses because transferred from mother mice to their offspring in the first milk they receive and in this way passed on to generation after generation. Indeed, it can only be avoided if the young are wet-nursed from birth by mice of a strain from which it is absent. It does not manifest itself in the young receiving it until they are adults, and in them only if hormonal and nutritive conditions are right. It then causes tiny growths in the mammary glands of females, doing so even if the surrounding tissue of the same kind happens to be undergoing involution. The little growths—equivocally termed 'hyperplastic nodules'—are actually true neoplastic entities\*, not only in morphology but also in their ability to proliferate serially if freed from the glandular tissue hedging them about, and transplanted to favourable sites in other mice\*\*\*. They are 'conditional adenomas', dependent for success on influences promoting their growth, as are not a few other neoplasms, notably certain cancers of the human prostate. Whether they are left *in situ* or transplanted, carcinomas take off from them often. In this way they become responsible for the frequency of mammary cancers in mice carrying the milk virus, and the more effective this latter is, in causing adenomas, the more frequently do malignant growths arise from them\*. The adenomas are all alike in mice of the same strain, whereas the cancers are even more various than those deriving from rabbit papillomas. In both instances uniformity is succeeded by diversity.

The milk virus elicits a distinctive antibody when introduced into adult mice of strains previously lacking it, yet it never calls forth any in mice receiving it when new-born, a fact understandable in terms of immunological tolerance\*\*\*\*, and of immunological paralysis later because of its continued presence. This being so, one might suppose that the conditions would be right for the recovery from the carcinomas of some related, derivative 'cancer virus', yet these have never yielded anything except the milk virus, which may ride along in their tissue throughout years of serial transplantation, only to be lost eventually. Its scope of action is exceedingly narrow. In no other species have the adenomas or a causative virus with similar attributes been found\*.

In sum, the milk virus is like the papilloma virus in causing benign tumours from which cancers arise and resembles it further in serving as no more than an initiator of the malignant growths.

\* The milk virus, when first discovered, seemed to provide a lead to the causation of mammary cancers in women, but no preliminary 'hyperplastic nodules' have been present in their breasts or in those of rats or rabbits, animals that also have mammary carcinomas. Furthermore, the incidence of these growths in women has given no indication of any noteworthy familial tendency to them, and the most ardent search, with the electron microscope, of normal and malignant human mammary tissue has failed to disclose any virus particles although those of the milk agent of mice can be readily seen by means of this instrument.

### The Disclosures provided by the Do-All Viruses

The number of do-alls is small, their limitations are great, and they are a motley lot, yet they have revealed some fundamental facts. They first enabled investigators to perceive how secretly a virus can act to cause tumours, producing growths which, both in appearance and behaviour, seem intrinsically cellular. A reciprocating partnership between cell and virus has been found to exist, as has also an exquisite, host-within-a-host relationship of virus, cell and organism. Two types of immune reaction by the body have been brought to light, one directed against the tumour cells as such, the other against the virus, both of them frequently present together when a tumour fares badly in a new host. The do-alls have also provided examples, resembling those in man, of the two differing ways whereby normal cells attain to the cancerous state, namely, by a direct, one-step change, or by two steps, the normal cell first becoming a benign tumour cell and later a malignant one by further discontinuous alteration.

In sum, what is known about the do-alls would enable one to understand how every tumour could be caused by a virus if all were produced by agents having their capabilities; but much speaks against any such assumption, as will be made plain farther on.

### The Synergistic Effect of Associated Do-Alls

Certain changes that have recently taken place in the virus causing the RS chicken tumour have large interest as disclosing what can happen to a do-all in the course of time.

Throughout almost half a century the RSV, though often tested, produced only sarcomas and these in fowls. Then in 1957 Zilber reported that it would cause haemorrhagic cysts as well on inoculation into newborn rats, and fibromas in new-born rabbits<sup>11</sup>. Soon afterwards he and some Scandinavian investigators showed that it would give rise to lymphomas also in young rats and mice, and that in new-born Syrian hamsters, cotton rats, guinea-pigs, ferrets and monkeys of several sorts it would cause sarcomas resembling the typical RS<sup>12-14</sup>. Now every strain of the RSV maintained in America and Europe has been found to produce such tumours in rodents.

What is the reason for the new capabilities of the RSV as observed in these widely separate lands? One can scarcely attribute them to a sudden burst of widely effective virus variants after the lapse of nearly half a century during which only a few had been procured, and these of limited effect in fowls<sup>15</sup>. Recent collateral discoveries tell much. Throughout decades no sign was found of contagion when chickens carrying the RS were purposely kept crowded in coops together with normal fowls. Even when the tumour tissue was repeatedly fed to these latter in quantity, growths failed to arise. Yet a few years ago Burmester, of the Regional Poultry Research Laboratory for study of the lymphoma complex, reported that pullets cooped with adult chickens carrying RS sarcomas not only developed these in their subcutaneous tissue but visceral lymphomas as well<sup>14</sup>. He had previously found that fowls carrying viruses of the complex often appear healthy, and this though the agent causing visceral lymphomatosis is transmitted to them both by contact and through the egg, as he had already shown<sup>14</sup>. Now his further experimentation made plain that the 'contagiousness' of the RSV is due to association with it of this other virus<sup>16</sup>. Yet here was not merely an instance in which each of the two agents produced growths characteristic of it in the same chicken. The lymphoma virus had so empowered the RSV that it was really contagious, causing typical RS sarcomas in normal chickens. More recently some California workers, purposely utilizing Leghorns known to harbour the lymphoma complex, have shown that virtually all the agents in this category can help or hinder the oncogenic action of the

RSV. Incidentally they expanded a finding already mentioned here, showing that chick embryos are very often infected with one virus or another of the complex, a fact of importance in relation to the utilization of such embryos to prepare vaccines.

The history of the introduction of the lymphoma complex into Europe provides additional support to the supposition that the new ability of the RSV to cause tumours in mammals is due to a synergistic association with it of the agents of this complex. Andrewes, working intensively with the RS and other chicken tumours from 1926 until the Second World War, sought leukaemias in English fowls, and lymphomas such as the complex causes, but found none<sup>17</sup>. American poultry records show that White Leghorns of a stock known to carry viruses of the complex were imported to Scandinavia a few years prior to the discovery by workers in these countries that the RSV is oncogenic for rodents, and a little later the complex was introduced into England—the last European country from which this new ability was reported. A Swedish worker has recently commented on "the almost omnipresent lymphomatosis virus" in the fibroblastic tissue of chickens<sup>18</sup>. It is now known to be present in the U.S.S.R.

All things considered, one might do well to reserve the term RSV for the uncontaminated virus—which may be isolated again—and to designate as 'RSV' any strain producing growths that indicate another virus is acting with it.

On coming on a new virus we are inclined to take for granted that it has always been what it is when we find it, and not reckon with its past. The RSV has been 'forced', to use a gardener's term, by frequent passage in chickens of many kinds and countries throughout its long propagation. Hence abundant opportunities have existed for other viruses besides those of the 'complex' to join it. Perhaps they have.

### The Viruses Initiating Neoplastic Change

**Polyoma virus.** The ordinary chemical and physical agents known to be capable of inducing tumours in animals do no more than convert normal cells into neoplastic cells which then multiply, forming tumours for reasons still unknown. Several viruses also possess this initiating power. The most enlightening is an exceedingly infectious virus found by Gross in 1951. This agent maintains itself by producing widespread, cellular injury in healthy-looking mice, and if the conditions are right, a few scattered cells amidst the multitude of the injured undergo a change which is its antithesis: they are rendered neoplastic and proliferate as a result, forming tumours which grow progressively and can be maintained indefinitely in suitable hosts. The virus does not act, like a do-all, on cells of only a single kind but on many, which results in widely various tumours—hence its present name, the 'polyoma' virus. Furthermore, its neoplastic capabilities are not limited, as are those of the do-alls, to species nearly related to that in which it is indigenous, but are wide in scope, growths resulting after its inoculation into rats, rabbits, guinea-pigs, hamsters and ferrets. In both these fundamental respects the polyoma resembles the chemical and physical initiators. Like these agents also, and in striking contrast to the do-alls, the virus fails to have any determining influence on the morphology of the tumours it induces. That depends on the kind of cell exposed to it, as in the case of the chemical and physical oncogens. In another, even more significant, respect it often resembles these agents: the virus is left behind as the neoplastic cells proliferate. Its disappearance is usually very gradual, though. Not only do most of the growths that are serially transplanted fail eventually to yield it any longer but sometimes not even an antigenic trace of it remains, although earlier it had elicited antibodies<sup>19</sup>. Now, like the rabbit papilloma virus that

initiated the Vx2, it has failed to persist even as a passenger virus.

The primary effect of the ordinary chemical oncogens is injurious, with neoplastic change as an incidental occurrence. So too with the polyoma virus. When the most powerful of chemical oncogens, 9,10-dimethyl-1,2-benzanthracene (DMBA), is applied to a broad expanse of mouse skin nearly all the myriad epidermal cells exposed to it are damaged, but here and there one of them is converted into a neoplastic cell, which results in scattered growths. The oncogenic action of the polyoma virus is of the same sporadic sort: it never causes any wholesale conversion of cells to the neoplastic state. The initiating abilities of the chemical agents can often be superimposed, which results in a larger yield of tumours than if either had been used alone. Precisely this happens when the polyoma virus is brought to bear on cells previously X-rayed: a much larger proportion of them become neoplastic<sup>10</sup>.

With all these likenesses to the generality of chemical oncogens, there yet remain two basic differences. The polyoma is contagious, infecting whole colonies of mice, and it persists for a considerable time in the proliferating cells it has rendered neoplastic. But these are differences readily understandable in terms of the intimate relationship existing between viruses and cells. Obviously on most occasions the polyoma virus acts as no more than an ordinary chemical initiator of neoplastic change, and chemical indeed it is, though of a distinctive sort as shown by its recent analysis<sup>8</sup>.

Very occasionally the polyoma fares well in the cells it has rendered neoplastic. This was the case in the exceedingly rare tumour from which the virus was first obtained, a carcinoma of the parotid gland. The virus had so flourished in the cells of this neoplasm as to be procured from it in an oncogenic state, and in some other parotid cancers resulting from its inoculation it has fared as well. Evidently the cells of these special growths are peculiar in providing it with a wholly favourable milieu during its sojourn in them, yet it has not undergone conversion to a real do-all virus. When again inoculated into mice it proves oncogenic in the same indirect fashion as before, causing widespread, smouldering, cellular injury with neoplastic changes as a by-product.

Here a fact that is seldom taken into account deserves stressing. The initial neoplastic findings with the polyoma were obtained in animals in which it is foreign, namely, inbred mice of laboratory strains. Its native hosts are house-mice and it exists widespread in these, often causing epidemics in slums, barns and granaries, as shown by blood tests<sup>11</sup>. Tumours never result from its action in them but only the typical, injurious cell disease; and though on access to laboratory mice, animals of distinctively different strains, it produces similar epidemics, with neoplasms as an occasional result, these are so infrequent as not to increase perceptibly the statistical incidence of tumours as compared with the number occurring in the uninfected members of the colony. Though the virus was primarily obtained from a parotid carcinoma occurring in a laboratory mouse, it caused growths only in new-born animals of this kind and only months after inoculation. In new-born hamsters, widely alien hosts, it produces them within a few weeks, and hence its neoplastic effects have been examined mostly in these.

The polyoma provides an extreme instance of Theobald Smith's law according to which a damaging parasite tends to become a symbiont when the relationship between it and its native host has existed long.

**Human initiating viruses.** The fact that the tumours due to the polyoma virus occur incidentally to a chronic, non-neoplastic disease has brought up the question whether similar happenings may not occur in man. So widely various are the many hundred, ordinary chemical oncogens, and so multifarious are the viruses in their capabilities—and uniquely intimate in their relationships

to cells—that it would be strange indeed if no agents of this sort initiated tumours in man.

Actually cancers have now and then appeared at sites where one or another of several well-known human viruses had produced skin injury many years before, leaving behind disordered tissue. These instances of long-delayed oncogenesis, for example the malignant melanomas which occasionally arise amid old small-pox vaccination scars, remind one of the cancers sometimes occurring in aged persons where their skin was burned in youth, as also of those carcinomas occurring in men uncircumcised when young. Not so with the cancers occurring where herpes simplex and herpes zoster viruses have previously produced their characteristic injuries of the skin. Here, epidermal carcinomas have occasionally arisen much sooner than those induced in man by the generality of chemical oncogens—after 6 weeks to 5 years in the case of herpes simplex<sup>12</sup> and 3 months to 3.5 years in that of herpes zoster<sup>13</sup>. The cancers traceable to the effects of herpes simplex have been situated where 'fever blisters' had previously occurred on the lips of ageing people who were unusually sensitive to sunlight. Since sunlight is itself an oncogen it seems likely that the tumours were actually the outcome of co-carcinogenesis.

A great number of other viruses productive of human diseases and residing long in the body have recently been injected into animals to learn their oncogenic possibilities. Because of experience with the polyoma virus, new-born hamsters have generally been used. Out of more than a hundred viruses tested, only three have yielded tumours. All are adenoviruses<sup>14,15</sup>, agents often causing inflammation of the lymph glands and tonsils of young persons, and persisting in their tissues for a longer or shorter period. The growths induced have been mostly "primitive, undifferentiated sarcomas with epithelioid traits", and some have been obtained in new-born rats and mice. They can be serially transplanted in adults, but none has yielded any oncogenic virus, though viral antigens persist within them, as serological tests have shown. Occasionally, remnants of virus particles can be perceived in the tumour cells with the electron microscope<sup>16</sup>.

A virus of rhesus monkeys, termed SV40, which does no more than produce vacuoles in their renal cells when cultivated *in vitro*, also induces tumours in new-born hamsters, but not in adults, though the tumours can be propagated serially in these. At times they yield a little oncogenic virus, and again none at all, though still containing viral antigens<sup>17,18</sup>.

Obviously the conditions under which the adenoviruses and SV40 give rise to tumours are exceedingly unnatural. Not only are the viruses introduced into sucklings of a remote species, but also the injection is done at the time when immunological tolerance is readily evoked, as witness the findings with the milk virus, and under such circumstances they soon become safely ensconced. For viruses very swiftly become fixed on living cells of the kinds susceptible to them, and these protect them from such circulating antibodies as they may subsequently call forth from their hosts<sup>19</sup>.

At this writing more than fifty viruses have been procured from rhesus monkeys, but none native to these creatures has caused genuine tumours in them, nor have any been obtained from the growths arising spontaneously in hamsters.

#### The Combined Effects of Tumour Viruses and Ordinary Chemical and Physical Oncogens

The increase in neoplastic changes when the polyoma virus is brought to bear on cells previously X-rayed is not the sole instance of the joint effect of tumour viruses and ordinary chemical, oncogenic agents. When areas of scarified rabbit skin were exposed to the oncogenic hydrocarbon DMBA, during the few days of healing after inoculation with the Shope virus, papillomas completely resembling those ordinarily caused by this agent arose,

but they underwent carcinomatous changes much sooner and oftener than did control growths on the same rabbits; yet the DMBA had, as usual, been injurious to the epidermal cells exposed to it while they were proliferating to cover the scarified expanses of skin. As a result the papillomatous layer formed very slowly and remained much more shallow than that of the controls. Nevertheless, carcinogenesis was hastened and rendered extraordinarily frequent<sup>24</sup>.

Recently, several human viruses ordinarily non-neoplastic, notably that of *herpes simplex*, have been found to induce tumours in mice previously painted with one chemical carcinogen or another in an amount too small to be oncogenic in itself<sup>25,26</sup>. The carcinomas thus induced have been of various sorts, like those due to the generality of chemical initiators.

### Effect of Do-All Viruses on Neoplasms actuated In Ways Unknown

The Shope papilloma virus has remarkable effects on the benign papillomas induced by tarring the skin of domestic rabbits. The growths differ distinctively in morphology from those caused by this agent, yet they have the typical character of neoplasms. After intravenous injection the virus often localizes in the benign tar tumours, resulting in very rapidly enlarging, mongrel growths, composed of cells that exhibit individually the merged morphological traits of the papillomas due to these differing oncogens. The virus has similar effects on many of the carcinomas which result from tarring cottontail rabbits. When small pieces of the cancerous tissue are returned to the donor animals, after exposure *in vitro* to a virus suspension, not only do their cells proliferate with prodigious rapidity, as compared with those of control grafts, but also they are now much more aggressive, and the morphology of the resulting tumours undergoes marked alteration. The virus has again acted not only as a synergist but as a transforming agent<sup>21</sup>.

The findings have been very different when the RSV was injected into the bloodstream of chickens carrying anaplastic sarcomas induced with tar or with the polycyclic hydrocarbon, 3:4-dibenzanthracene (DBA)<sup>22</sup>. The morphology of these tumours—which differ slightly yet distinctively from the RS—underwent no alteration, nor did their growth-rate change. Yet the RSV had become associated with them as shown by its recovery from the tumours of a second serial transfer. Here it may be recalled that the anaplastic Vx7 has never shown any sign of the formative influence of the papilloma virus, its constant companion; nor indeed have such tar carcinomas of cottontails as were anaplastic when experimentally infected with the virus. It may well be that such simplified growths no longer possess the power to assume the papillomatous form.

### Recovery of Extraneous Do-All Viruses from Tumours of Unknown Cause

Recently several investigators have procured leukaemia viruses by extracting the tissue of solid mouse and rat tumours long maintained for experimental uses and of unknown cause, notably the Ehrlich carcinoma, now sixty years old. This finding was the more surprising because the animals carrying the growths did not themselves have leukaemia. Yet a reasonable explanation lies at hand. Many non-neoplastic viruses are known to become associated with tumour cells, which thereafter protect them, as already stated<sup>27</sup>, from circulating antibodies, with the result that they persist as passengers. That the barrier interposed by the cells can act in both directions was clearly proved by a continuance of the tests just described which showed that the RSV was present in DBA tumours without giving any sign of itself. Some of the growths resulting from a third transfer of these growths regressed and wholly disappeared, and at the sites where this had happened typical RS tumours arose, obviously because

the RSV was no longer held in check but set free to act on the adjacent fibroblasts, elements which are especially susceptible to it during reactive proliferation<sup>28</sup>. It may well be that stray particles of leukaemia virus had become associated with the cells of the tumours of unknown cause and were restrained by them from producing disease, even after multiplying to a potentially pathogenic amount.

Persistent efforts have been made in the United States to find viruses which would lodge in human tumours and destroy their cells. Some have been procured: but they have killed normal tissue cells as well. No sign has been obtained that any tumour of man has been abruptly invigorated or transformed during its course, as happened when the Shope virus infected the tar tumour of rabbits. The paradoxical fact should here be mentioned that the Vx7 carcinoma, though carrying the papilloma virus in a potentially active state, has grown only about half as fast throughout the years as the Vx2 and almost never metastasizes as this latter usually does.

### A Grouping of the Neoplastic Viruses

When the tumour viruses are viewed together it can be seen that nearly all belong to one or the other of two basically different categories, with a few bridging instances between. The viruses of the do-all group exist in a perfect partnership with the tumour cells they maintain as 'autonomous'; whereas the initiating viruses have no such actuating role but resemble the ordinary chemical and physical oncogens in their effects.

The polyoma virus has the role of an initiator when producing tumours from which it entirely disappears, but it also provides enlightening intermediate instances. As already stated, it causes no tumours in the wild house mice to which it is indigenous but only the widespread, injurious, cellular disease characteristic of it, whereas in mice of laboratory strains it induces sporadic neoplastic changes in addition. Only rarely is it obtained in an oncogenic state in the tumours it thus starts off, and then it has not become a do-all but brings on the same zigzag course of events as previously, producing cellular injury, with neoplastic changes as a by-product. If the resulting growths are long maintained by serial transfer, the polyoma ultimately disappears from them, not even an antigenic trace remaining<sup>19</sup>.

The tumours initiated by the adeno- and SV40 viruses in new-born hamsters provide intermediate instances of a simpler sort. Again as has been stated, the growths induced by adenoviruses fail on serial propagation to yield these agents in an oncogenic condition, though antigenic traces of them persist and sometimes remnants of virus particles<sup>29</sup>. The SV40 virus also tends to disappear during serial transfer of its tumours, but it leaves behind an antigen, and occasionally a little oncogenic virus<sup>30</sup>.

Now for a telling fact. The tumours due to all three of the bridging viruses continue to grow as actively as ever while these agents are undergoing drastic reductions in pathogenic ability and are disappearing more or less completely. Sometimes those due to the polyoma virus grow even more vigorously<sup>31</sup>. This being so, it is difficult to suppose that their influence has exceeded the initiatory. Indeed, one may question whether the polyoma virus has really an actuating effect on the cells of the few tumours in which it flourishes or merely multiplies within their cells because these provide it with a notably favourable medium.

The chief difference, needless to say, between the bridging initiators and the chemical and physical oncogens in their oncogenic relationship, is that the latter have no ability to increase in quantity and ride along in the cells rendered neoplastic by them.

Two of the do-alls, the rabbit papilloma virus and the milk virus, serve as initiators on occasion, but their role in this respect differs markedly from that of the generality of such agents. Instead of acting on normal cells they

bring about carcinomatous changes in the cells of the benign tumours they have already engendered. Though they sometimes persist throughout long periods in the resulting cancers, as happens in the case of the Vx7 and many of the mammary cancers that take off from adenomas, they may ultimately be lost, as in the notable instance of the Vx2. Their role is solely that of initiators; and so too, in all probability, is that of the herpes viruses in bringing about carcinomatous changes in the injured cells of the epidermal lesions they produce in man.

### What can be the Meaning of the Neoplastic Viruses?

The many facts assembled here bring home a striking truth. Except in chickens, neoplastic viruses are exceedingly rare. Very few mammalian do-alls have been found since 1911, and almost as few that act as initiators. This is the more remarkable because, as already pointed out, viruses are so intimately associated with cells and so numerous and diverse in their effects on them.

Any attempt to consider the generality of mammalian tumours in terms of present virus knowledge can be no more than tentative because what viruses are and do has consistently transcended expectation. They were long regarded as tiny bacteria differing from the usual only in that they were most exacting in their nutritive requirements. Not until the 1930's was their obligate association with cells perceived, and only later was it realized that these are their chemical workshops. What a hubbub of surprise and of surmises about the nature of life followed on crystallization of the tobacco mosaic virus! When a study by highly qualified geneticists of what seemed an inherited tendency to breast cancer in mice led on to the discovery of the milk virus, this astounding possibility had not even entered man's thought! Perhaps to-morrow some cleaving discovery on the causation of tumours by viruses will demolish the inferences of to-day; yet what we now know would be worth little if none was made.

On marshalling the neoplastic phenomena which must be understood, whatever their cause, one is confronted with the vast, multifarious array of mammalian tumours initiated by ordinary chemical and physical oncogens. Can it be that most of these growths are actuated by hidden do-all viruses? None of the innumerable efforts to procure such agents from them has succeeded save in dubious instances. Perhaps the techniques of search have been inadequate, a view fostered by the outcome of recent experiments in which mice with their resistance weakened by irradiation developed viral leukaemias. But in this connexion the fact should be recalled that solid rat and mouse tumours, long maintained by serial transfer and of unknown aetiology, sometimes yield a lurking leukaemia virus on extraction, though their hosts had been free from this disease.

Efforts to get actuating viruses from mammalian neoplasms arising 'spontaneously' have also drawn a blank save in the case of the rabbit papilloma, deer fibroma and some mouse and rat leukaemias. Not so with the spontaneous chicken tumours. A do-all virus has been procured from every one of these that could be maintained long enough for adequate test. Since this is the case, one might suppose that chicken tumours induced by chemical oncogens would also yield viral agents. But not so! Though they closely resemble spontaneous growths in all essential particulars, a virus has almost never been procured from them. This paradoxical state of affairs is peculiar to fowls, as is also the prevalence and wide distribution of a lymphoma complex.

There is a more substantial obstacle than failure to obtain viruses to the assumption that such agents may be the actuators of the generality of mammalian growths, namely, the need for a perfect partnership between cells and virus if the latter is to function enduringly in this

way. The gradual disappearance of the polyoma virus from all except a few of the tumours it has initiated well illustrates this need, as does also the dwindling and loss of the SV40 and adenoviruses from the tumours they have started off in hamsters, animals to which they are strange. The cells are the stronger party in these imbalances, remaining vigorously autonomous like those initiated by ordinary chemical oncogens. The do-alls are the only viruses known to maintain the neoplasms they induce, and since each of them causes tumours of only a single sort—and in a single species under natural conditions—one would have to invoke a microcosm of these agents to account for the macrocosm of tumours which arise spontaneously in a single mammalian species, and different microcosms for such species as are not closely related.

This does not rule out the possibility that a few human tumours may be caused by do-alls. The electron microscope has recently disclosed in the blood of certain leukaemic patients particles which have a peculiar form much like that of the viruses causing some mouse leukaemias. Yet even if viruses turn out to be a cause of this disease in human beings, and perhaps of a lymphomatous neoplasm (Burkitt's disease)—now killing children in Africa—this limited finding cannot be regarded as an Open Sesame to the cause of the generality of human growths. It certainly has not proved to be such in mouse, rat and rabbit.

There are other possibilities. The 'RSV' of chickens, when acting together with a lymphoma virus, produces tumours of several sorts in rodents. Similar synergistic associations of mammalian viruses, which result in a widening of their effective oncogenic range, may conceivably occur, though no sign of any such happening has been found in man.

The fact that certain viruses, some of them oncogenic, disappear from view with the electron microscope for a brief period soon after entering cells—'go into eclipse', that is to say—has made it tempting to suppose that the generality of tumours, however induced, may contain causative viruses that remain undisclosed by any present means of demonstration. The rabbit papilloma virus provides a suggestive instance in point, being seldom recovered from the vigorous growths it causes in domestic rabbits. Indeed, it cannot be seen even with the electron microscope in cottontail papillomas which are actively proliferating, although they yield it in abundance. Only after the cells stop dividing and begin to differentiate into squamous elements does it become perceptible. Can one suppose that viruses actuate most, if not all, of the tumours of unknown cause yet remain permanently invisible? To imagine this is to come up against the jolting fact that nearly all of the oncogenic viruses now known elicit readily recognized, specific antibodies which make their existence known. Even when no virus can be seen or got from the rabbit papilloma it calls forth a strong antibody bespeaking its presence.

Needless to say, initiating viruses may exist in man which have not been discerned as yet. Serological tests have shown that one of the adenoviruses causing tumours in new-born hamsters exists widespread in Britain, and on isolation it has again produced hamster growths. In several human tumours excised in the United States adenoviruses have also been found; but their finders concluded that they were probably mere passengers in these growths. The ordinary maladies which have given the adenoviruses their name occur mostly in young persons; yet statistics show the incidence of cancer to be at its lowest during adolescence, and it undergoes no substantial increase until after the mid-thirties. Nevertheless, the possibility remains that the adenoe, like the herpes viruses, may occasionally engender a tumour, though one arising after a much longer time. In appraising the importance for cancer causation of such presumptive happenings, one should keep in mind the heavy loss of human life resulting from the misdeeds



of glands secreting hormones in pathological amounts. In comparison with their effect, what viruses may do seems negligible. Certain hormones not only initiate tumours but also drive them on. Some years ago Huggins discovered that not a few prostatic carcinomas are dependent for their entire existence on the male sex hormone, and that if its influence is excluded or nullified by means of a female hormone, the growths may dwindle and vanish even though they had metastasized<sup>17,18</sup>. Here is extraneous actuation, in which a virus can have played no part, in contrast to the intracellular effect of the do-alls.

The fact that the rabbit, deer and frog do-alls are directly transmitted from one individual to another under natural conditions, resulting in tumours, leads one to ask whether the recorded incidence of human growths yields any data suggesting a similar transfer of neoplastic viruses. Save in the debatable case of Burkitt's disease, statistics speak convincingly against any such happening—a most fortunate, bed-rock fact because maladies due to viruses so occupy public thought to-day. With cancer destructive to almost one person in six, and arising in numerous others who are saved in one way or another, it inevitably happens that now and again it will manifest itself in several individuals who have lived in the same locality or house. Chance accounts entirely for this, yet the fear of contagion persists in not a few persons. Seldom is the fact considered that throughout the long history of civilized man, huge, comprehensive tests which bear on this very possibility have unwittingly been made through the crowding together of hordes of human beings in army camps, ghettos, almshouses, prisons and the like. The dread of communicable diseases was far greater in old times than now, keeping the acumen of medical men whetted and quarantines rigorous; yet no reports of any unusual prevalence of tumours in people living cheek by jowl have come down to us from out of the past. Many epidemiologists in recent years have made a world-wide search for places or societies in which cancer has seemed exceptionally frequent, and not a few have been found; but always they have been traced to the action on the human body of chemical or physical initiators—exerted sometimes through living agents, for example, the tubercle bacillus and the *Bilharzia* parasite—or else to abnormal, genetic susceptibility to oncogens, as in the instance of the unfortunate 'moon children' frequent in an inbred tribe of Central American Indians, albinos who mostly die of epidermal cancers if they live long enough for tropical sunlight to have its carcinogenic effect on their unpigmented, naked skin<sup>19</sup>.

The investigation of viruses has revealed nothing whatever so far about the actual character of the changes cells undergo when rendered neoplastic, and though the do-alls are responsible for the independent behaviour of the cells in which they multiply, how they affect these remains unknown. The reason why cells act in an autonomous way after conversion to the neoplastic state by oncogens which merely initiate, including certain viruses, is also still conjectural. So, too, is the reason for the discontinuous, irreversible steps whereby neoplasms sometimes go from bad to worse. Our ignorance in all these matters stares us in the face.

In 1961, 326 American scientists<sup>20</sup>, as also many elsewhere, were known to be working on the tumour viruses. Their number is greater to-day. Why should there be this huge concentration of effort on so meagre a material? It is because numerous men of keen ability and specialized knowledge of widely different kinds have lately come to realize that even a single tumour virus offers unique opportunities for discovery. Study of some of the destructive viruses, those producing precise cell injuries, can be rewarding because one can learn through them, by way of the lesions they cause, the function of this or that item of cell equipment. But the tumour viruses offer vastly more in conferring an independent, aggressive life on

cells. No matter that this life is pathological: to learn about the normal by inducing the pathological has long been a prime stratagem of investigators. The tumour viruses provide instances of this sort ready-made. Hence most of the new workers give little thought to the problem of tumour causation in relation to what it now means to man. They are bent on learning how normal cells become neoplastic and proliferate independently. It may well be that through what they ultimately discover tumours can be fought with an understanding of them. The minority of virus workers, those who view tumour causation in its practical aspects, have a more immediate task. Confident, through knowledge already gained, that neoplasms seldom if ever arise 'spontaneously', that nearly all are occupational diseases of the body acquired while running the gauntlet of life, they are eager to learn more about why tumours occur, and among other questions they keep asking themselves what roles viruses have in causing and maintaining these. The intermingling of the facts newly won by the two sets of workers, the 'hows' and the 'whys' as one might call them (and fortunately some are of both sorts), is not really chaotic but powerfully evocative of thought and venture.

No one can tell what change or experiment may next reveal as concerns tumour causation. As William Blake once wrote: "To the man of imagination Nature is imagination itself"<sup>21</sup>.

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## OBITUARIES

## Sir Edward Bailey, M.C., F.R.S.

SIR EDWARD BAILEY, a former director of the Geological Survey of Great Britain and the Museum of Practical Geology, also for seven years previously professor of geology in the University of Glasgow, died in London on March 19, at the age of eighty-three.

The range of his international reputation may be gauged from his election to foreign membership of the national scientific academies of Belgium, Norway, Switzerland and the United States. This reputation mainly stemmed from his investigations over a long period in the south-western Grampians and Inner Hebrides, where he played a prominent part not only in explaining the complicated sequences and the repetition of rock types in schists, but also in unravelling the succession of events in ancient volcanic episodes. Much of his work in other fields, though less well known, proved equally stimulating. His strongly developed sense of the heroic, however, brought praise from some but impassioned criticism from others.

Edward Battersby Bailey was born at Marden in Kent, where his father was a medical practitioner. When the family moved to Kendal, Bailey attended Kendal Grammar School, whence, in 1899, he won an open scholarship to Clare College, Cambridge. There he showed outstanding ability by achieving first-class honours in both geology and physics in Part II of the Natural Sciences Tripos in one year (1902); also in that year he joined the Scottish branch of the Geological Survey of Great Britain. This service with the Geological Survey had two interruptions: the first between 1915 and 1919, when Bailey served with the Royal Garrison Artillery and was severely wounded on the Somme in 1916, and again near Ypres in 1918; he was awarded the Military Cross and the French Croix de Guerre with palms, and he was made a Chevalier of the Legion of Honour. At the close of 1929 he left the Geological Survey for the geology chair at Glasgow, which he relinquished in March 1937 to return to the Geological Survey as its director, an appointment which he held for eight years, culminating in his knighthood.

In his book *The Geological Survey of Great Britain* (1952) Bailey recorded his enjoyment on joining the Geological Survey at such an invigorating centre as Edinburgh, for he realized that he was to research "among a jostling crowd of problems awaiting solution". Having acquired at school and at his university extraordinary physical strength and mental courage, he became dedicated to geological problems and his alert mind brought insight to them. His flair for original thought was aided by a skill in reassessing and building on the observations made by others. He soon succeeded in clarifying the Carboniferous volcanic history of the Campsie Fells in Stirlingshire. In Argyllshire, with O. T. Clough and H. B. Maufe, he demonstrated in 1909 that Devonian lavas and tuffs flanking Glen Coe—which occupied an elliptical area bounded by a curved fault and measuring nearly 30 square miles—had been let down more than a thousand feet into the midst of Highland schists. This 'cauldron subsidence' was inferred to be sub-aerial and to have been formed, in part at least, at the time of the volcanic activity; it was proved to be partly contemporaneous with the broken ring of intrusive igneous rocks associated with the curved fault. Shortly after the announcement of this discovery came that of Bailey's interpretation of the Highland schists in the neighbourhood of Fort William and Ballachulish. He claimed them to have been folded in a recumbent manner and accompanied by fold faults or slides which in places had cut out great thicknesses of rock. But R. G. Carruthers (who died on the day of

Bailey's cremation) in 1913 disputed the correlation made by Bailey of some of the units in the succession. Bailey was unconvinced of his errors until 1930 when others had pointed out that current bedding provided incontrovertible evidence of the original order of stratal deposition; he then corrected various details in his interpretation of the tectonic pattern, but the concept of recumbent folding has survived. Bailey's final reassessment of this work appeared in his second edition of the *Geology of Ben Nevis and Glen Coe* (1960).

Further distinguished work was undertaken by Bailey and his colleagues in the Inner Hebrides, where they elucidated the complicated history of the Tertiary volcanic centre in Mull and the various stages of the igneous activity there. Sir John Flett in 1937 stated this to be one of the most wonderful chapters of the Geology of Britain. Here in Mull, Bailey described gravitational differentiation, ring bosses, pillow lavas, crater lakes and ring dykes. The last name took its currency from Bailey's first usage in 1915. After his return from the First World War in 1919, Bailey was placed in charge of the Survey's West Highland field unit. He took over supervision of the team-work in other parts of Mull, Ardnamurchan and Coll, and he brought to publication the one-inch geological map of Mull and the two classical memoirs thereon (1924, 1925). During this period he re-examined the Cretaceous sands on the west side of Loch Aline and from their very high silica content he deduced them to be the desert shore sands of the Chalk sea. The  $\text{Fe}_2\text{O}_3$  impurity being only 0.02 per cent, the deposit was later developed as a source of optical glass sand, and between 1940 and the autumn of 1948 a quarter of a million tons were shipped from a mine excavated in it.

While at Glasgow, Bailey continued his investigation of the manifestations of Earth movement and, inspired by his studies with R. M. Field and L. W. Collet on the effects of Palaeozoic submarine land-slippling near Quebec, he wrote with Dr. John Weir on faulting in Kimmeridge times which had produced spectacular effects off the coast of East Sutherland. He also wrote his book, *Tectonic Essays: mainly Alpine* (1937).

His plans for directing the Geological Survey were thwarted by the needs of the War years. The activities of the staff were then diverted to appraising, for concentrated utilization in the War effort, the country's natural mineral resources. These included coal, iron-ore, barytes, fluor spar, tungsten-ore, potash-feldspar, water and oil. The Survey's headquarters were partly occupied by another Government department, and as a spare-time activity Bailey was appointed in 1940 lieutenant commanding the Geological Survey and London Civil Defence Region section of the Home Guard, which duties he undertook with enthusiasm until 1942. In 1943 he had a short spell in Malta to advise on water resources there. He retired in 1945. He continued geological work after retirement, at first in Iran, and then in Turkey, the latter with Prof. W. J. McCallien. In recent years with the last-named he studied a problem in Apennine tectonic geology.

His keen walking and mountaineering activities led him to support the Scottish Youth Hostels' Association, of which he was successively Glasgow District chairman, vice-president and honorary president. He much enjoyed writing biographies of geologists. His book on Charles Lyell was published in 1962, and before he died he had prepared a study on James Hutton.

Bailey was elected to the fellowship of the Royal Society in 1930 and he received a Royal Medal in 1943. From the Geological Society of London he received the Bigsby, Murchison and Wollaston Medals. Honorary

doctorates were awarded to him by the Universities of Belfast, Birmingham, Cambridge, Edinburgh, Glasgow and Harvard.

His married life with Alice Meason started in 1914 and was ended by her death in 1956; they were devoted to each other and had a son and a daughter. In December 1962 he married Miss Mary Young, who survives him.

C. J. STUBBLEFIELD

### Dr. C. M. Merrihue

DR. CRAIG M. MERRIHUE, physicist of the Smithsonian Astrophysical Observatory and associate of Harvard University, Cambridge, Massachusetts, died in a mountain-climbing accident on March 14, 1965.

Born in Schenectady, New York, on July 8, 1933, he was awarded the B.A. degree from Harvard University in 1956, and the Ph.D. from the University of California at Berkeley in 1964. He lived in Cambridge, Massachusetts, with his wife Sandra and their son Jeffrey.

Dr. Merrihue's professional accomplishments were, of course, mainly ahead of him. However, in his short career he demonstrated an unusual combination of theoretical skills and experimental aptitude, and made important contributions to science. His first published work placed upper limits on a  $\beta$ -branch in  $^{124}\text{I}$  by examining with a mass spectrometer the xenon with deutron-irradiated tellurium. Other investigations helped determine the isotopic composition of the rare gases in several meteorites, with particular emphasis on xenon anomalies, which were being intensively studied in the laboratory of Prof. J. H. Reynolds at Berkeley, California.

One of Dr. Merrihue's most important discoveries was the finding of unusually large anomalies in xenon isotope composition in chondrules of the Bruderheim meteorite. These chondrules displayed the highest  $^{136}\text{Xe}/^{138}\text{Xe}$  of any meteoritic material yet examined, and supported the suggestion of a very primitive origin for chondrules. For this work he received the first Nünninger Meteorite Award.

His analysis showed large differences in the amounts of both anomalous  $^{136}\text{Xe}$  and total xenon in different minerals of the matrix. These matrix xenon variations were non-

linearly correlated, and distinctly different from the xenon observed in all other chondrules investigated. He used these observations as further evidence for the *in situ* origin of excess  $^{136}\text{Xe}$ . As a result of these investigations, Bruderheim is at present the only meteorite for which we have such complete xenon and krypton data on separated minerals.

In conjunction with the Bruderheim measurements, Merrihue embarked on a series of computer calculations. These included Monte Carlo calculations of the production of xenon isotopes by possible early solar-system irradiations of meteoritic target elements, and investigations of rare-gas diffusion. He also noted the great power of combining neutron activation of meteorites with mass spectrometry of the rare gases as a tool for determining the chemical abundance, and in favourable cases the isotopic abundance, of a list of elements. This technique also provides an elegant method of potassium-argon dating of small samples. He also made rare-gas measurements of a magnetic separate from a Pacific red-clay deep-sea sediment, and found isotopic anomalies suggesting the possible presence of extraterrestrial dust in this material. These applications of mass spectrometry to problems of meteorites and cosmic dust and of the history of the solar system were imaginative and fruitful and provided many important new data.

Merrihue was a quiet, modest, unassuming man with intense enthusiasm for life. He was deeply involved in the public problems of his time, taking an active interest in conservation, civil liberties, and political issues. He was concerned alike for the individual and for society.

A skilful mountain climber, he had led an expedition to the Andes, had climbed in British Columbia and in the Karakoram in Pakistan, and was planning an expedition to the Hindu Kush in Afghanistan. While he and Daniel Doody, a member of the American Everest Expedition, were climbing on the side of Mt. Washington, they both fell and were killed.

Because of his theoretical interests and practical skills as a scientist, and because of his experience as a mountain climber, Dr. Merrihue was a conspicuous candidate for the position of scientist on America's first manned expedition to the Moon.

## NEWS and VIEWS

Chief of the C.S.I.R.O. Division of Biochemistry and General Nutrition: Dr. H. R. Marston, F.R.S.

IN August 1965, Dr. Hedley R. Marston relinquishes the position of chief of the C.S.I.R.O. Division of Biochemistry and General Nutrition, Adelaide, having reached the age of retirement. When the Division was established in 1944 he was appointed as its first chief. He had been associated with the division of animal nutrition since its formation in 1927. It has always been Marston's firmly expressed belief that the only safe road to practical advance in applied science is through the understanding of the underlying natural phenomena, and his own research fully confirms this. His earlier work included investigations of the thermo-dynamics of ruminant nutrition, and fundamental studies of the protein requirements for wool production. His name, however, is most widely known for his work on the nutritional importance of trace elements, in particular of cobalt, copper and zinc. He and his colleagues were responsible not only for showing that soil deficiencies of copper and zinc prevented the growth of many food crops and that deficiencies of either cobalt or copper may prove fatal to sheep, but also for tracing an ultimate role of cobalt to its incorporation, after conversion to vitamin B<sub>12</sub>, by micro-organisms of the rumen, into methyl-

malonyl co-isomerase. It must be a lasting satisfaction to him that his work has brought prosperity to many parts of the world, and not least to the region in which he was born and lived, for his birth-place was Bordertown, on the edge of what was then known as the 'ninety mile desert'. Marston, like other men of high mental stature, is never slow to acknowledge his debt to others. In conversation, he mentions many names with affection, perhaps most frequently those of C. J. Martin, his former chief, Sir David Rivett, who was the architect of the Australian C.S.I.R., and Sir Frederick Gowland Hopkins, in whose laboratory he worked in 1936-37.

Zoology at the University of Cambridge:

Prof. T. Wels-Fogh

DR. TORBEN WELS-FOGH, who has been elected to succeed Prof. C. F. A. Pantin (*Nature*, 183, 506; 1959) as professor of zoology in the University of Cambridge from October 1966, was born in Aarhus, Denmark, on March 25, 1922. He studied zoology in the University of Copenhagen and already as a student of twenty-two he showed his extraordinary scientific talent by winning a gold medal for a zoological prize essay on soil ecology. In 1947 he took his degree as *Magister scientiarum* in zoology and became assistant in research to the retired Prof. August

Krogh, who in his private laboratory had taken up an investigation of the flight of insects. The participation in these studies led Weis-Fogh into a number of interesting problems ranging from the mechanical problems of flight, muscle- and nerve-physiology to metabolism and chemical investigations on the properties of mechanical components in the structure of the flying mechanisms. After Krogh's death in 1949, Weis-Fogh acted as head of Krogh's private laboratory until 1953 (supported by the Anti-Locust Research Centre, London). During 1953-54 he joined Prof. F. Buchthal at the Neurophysiological Institute as associate lecturer in the University of Copenhagen. In 1954 his connexion with the University of Cambridge began with a visit to the Department of Zoology as Fellow of the Rockefeller Foundation. From 1956 he was Balfour Student of the University of Cambridge and member of Trinity College, but in 1958 he was asked to return to Copenhagen to be professor in zoophysiology and head of the Zoophysiological Laboratory B of the University of Copenhagen. In 1961 he acted as Prather lecturer in biology in Harvard University. He is a Fellow of the Royal Danish Academy of Sciences and Letters and of the Danish Academy of Technical Sciences.

The professorship in Copenhagen was a research professorship with no formal teaching duties, and the work of Weis-Fogh and his collaborators in the years since 1959 has resulted in a series of important papers on many subjects, among which may be mentioned the co-ordination of motor activity in flight, diffusion problems, tracheal respiration and a number of papers on a peculiar rubber-like protein, resilin, found in the locust cuticle. Though Weis-Fogh considers research his main activity, his general interest in the development of Danish science forced him to spend time and energy on organization problems. He has been in the forefront of the fight for re-organization of the University of Copenhagen and of the structure of Danish science. He will be missed in Denmark not only as an inspiring scientist but also as an organizer and teacher of advanced students. While regretting his departure from Denmark, his colleagues wish him success in his new position and hope that it will lead to a strengthening of the ties between British and Danish science.

#### Mechanical Engineering in the Massachusetts Institute of Technology : Prof. A. H. Shapiro

PROF. ASCHER H. SHAPIRO, well known for his work on fluid dynamics and a leader in revising and improving engineering education in the United States, has been appointed head of the Department of Mechanical Engineering at the Massachusetts Institute of Technology. He has been on the teaching staff of the Institute for twenty-five years. He succeeds Dr. H. Guyford Stever, who has been appointed president of the Carnegie Institute of Technology in Pittsburgh. Emeritus Prof. O. Richard Soderberg has been acting head of the Department pending the appointment of a permanent successor to Prof. Stever. Prof. Stever also was head of the Institute's Department of Naval Architecture and Marine Engineering. Prof. Soderberg has also been acting head of this Department on an interim basis and he will continue in this capacity until a successor to Dr. Stever is appointed. Prof. Shapiro is at present chairman of the Faculty of the Massachusetts Institute of Technology. Prof. Shapiro has received international recognition in recent years for his development of motion-picture films as a significant component of engineering education. He was founder and first chairman of the National Committee for Fluid Mechanics Films, which, under sponsorship of the U.S. National Science Foundation, is producing films which have already been so influential as to have inspired similar efforts in other areas of engineering education. He is at present chairman of the Committee on Educational Films of the Commission on Engineering Education.

His research and professional activities for industry and Government have been principally related to the engineering problems of power production and propulsion engines. During the Second World War, he directed for the Navy Bureau of Ordnance a laboratory charged with the development of torpedo engines. He was a member of the Lexington Project which in 1948 evaluated the technical feasibility of nuclear-powered aircraft, in the course of which he invented a new form of nuclear-aircraft-propulsion system. In 1953, he directed "Project Dynamo", which evaluated for the Atomic Energy Commission the technology and economics of nuclear power for civilian use and which predicted the competitive economic position which nuclear power is assuming to-day. In the area of Government service, he has been a member of advisory groups to the National Advisory Committee for Aeronautics, and to the Office of the Secretary of Defence, and he is at present a member of the Scientific Advisory Board of the U.S. Air Force.

#### Physics in the University of Saskatchewan :

Prof. L. Katz

PROF. LEON KATZ, professor of physics and director of the Linear Electron Accelerator Laboratory at the University of Saskatchewan, Saskatoon, has been appointed head of the Physics Department at that University. He succeeds Dr. R. N. H. Haslam, who was appointed dean of arts and science at the University in 1964, and who is resigning the headship in physics to devote more time to his duties as dean. Dr. Katz has been with the University of Saskatchewan since 1946. He is best known for the part he played in establishing on the Saskatoon campus the recently opened linear accelerator laboratory, which is Canada's newest and largest laboratory for nuclear research. Besides extensive research in the field of nuclear physics, Dr. Katz has also conducted investigations in thermodynamics, micro-wave spectroscopy, and the ratio of specific heats of gases. Before joining the University of Saskatchewan, Dr. Katz was an engineer in the Electronics and Electro-Mechanical Division of the Westinghouse Electric Manufacturing Co., Pittsburgh. Dr. Katz was born in Poland, but when he was quite young his family emigrated to Canada and he attended public and high school in Toronto. He gained B.S. and M.S. degrees from Queen's University, Kingston, and was awarded a Ph.D. by the California Institute of Technology. Dr. Katz is a Fellow of the Royal Society of Canada, a Fellow of the American Physical Society, a member and past president of the Canadian Association of Physicists, and a member of the Physical Society (Great Britain). He was chairman of the Canadian Association of Physicists Committee set up to acquire a high-energy machine for Canada.

#### Marine Nuclear Propulsion

In a written answer in the House of Commons on July 7, Mr. F. Cousins, the Minister of Technology, stated that at June 30 about 95 qualified scientists and engineers were engaged on research and development on marine nuclear propulsion within his Department, including the Atomic Energy Authority and the Ship Research Association. This figure compared with 140 in 1964, 180 in 1963, 95 in 1962, 55 in 1961 and 30 in 1960. The cost of research and development in this field had been as follows: £0.2 million in 1961-62; £1.4 million in 1962-63; £2.2 million in 1963-64; £2.0 million in 1964-65; £0.3 million in the first quarter of 1965-66.

#### Grants to University Students

In a written answer in the House of Commons on July 2, Mr. A. Croxall, the Secretary of State for Education and Science, stated that total expenditure on grants to university students in England and Wales, by the Scottish Education Department and under the

Ministry of Education State Scholarship scheme in the academic year 1961-62, was £23.1 million, or £29.3 million gross before deductions were made for assessed contributions by students and their friends. For 1962-63 the corresponding figures were £28.6 million and £35.3 million, and for 1963-64, £31.5 million and £39.3 million. The estimated expenditure by local education authorities on maintenance allowances for school pupils over compulsory school age in 1964-65 was £1.3 million.

### Medical Education

IN a statement in the House of Commons on June 29, the Prime Minister said that the Queen had approved the recommendation that a Royal Commission on Medical Education should be appointed with the following terms of reference: To review medical education, undergraduate and postgraduate, in Great Britain and in the light of national needs and resources, including technical assistance overseas, to advise the Government on what principles, future development, including its planning and co-ordination, should be based. In particular, in the light of these principles, and having regard to the statutory functions of the General Medical Council and the current review by that Council of recent changes in the undergraduate curriculum, to consider what changes might be needed in the pattern, number, nature or location of the institutions providing medical education or in its general content and to report.

The Prime Minister said that the Queen had approved the appointment of Lord Todd as chairman, and the names of the other members would be announced later. The appointment of this Commission marked the importance which the Government attached to a fundamental review of the whole structure of medical education, its organization, content, and claims on resources. Meanwhile, the Government was reviewing the immediate measures which could be taken in the field of medical manpower, and the appointment of this Commission would not delay any action which needed to be taken as a result of this review. In reply to questions, Mr. Wilson added that while no decision as to new medical schools could be taken before the Royal Commission had reported, action could, of course, be taken on the immediate measures required without waiting for the Commission's report.

Following a similar Statement in the House of Lords on the same day, Lord Taylor added that Nottingham had been approved by the previous Government as the site of a new medical school, and an academic plan for that medical school had just been published; discussions would now proceed between the University there and the University Grants Commission. He also confirmed that although further medical schools could not immediately be created, it was not clear whether the best way of meeting the immediate need was by establishing more medical schools or by increasing the intake of existing ones. He emphasized that the Government was carrying out a review now, without waiting for the Commission to report, on the immediate measures that could be taken in this field to increase the supply of medical students and doctors.

### Overseas Development and Service Bill

IN moving the second reading of the Overseas Development and Service Bill in the House of Lords on July 1, Lord Taylor, the Parliamentary Under-Secretary of State for Commonwealth Relations and the Colonies, said that the Bill extended and developed the provisions of the Colonial Development and Welfare Acts of 1959 and 1963 and the Overseas Service Act of 1961, being concerned both with capital development and technical assistance. Under Clause 1, the Bill raised the ceiling on the amount which could be spent on Colonial development and welfare, and advanced the date by which the money could be spent for a further four years. Since its provisions overlap

with the existing Act of 1963, it provided for five years in all, from April 1, 1965, to March 31, 1970. As much of the money provided under the previous Act remained unspent, the total amount available in the new period would be £95 million in grants and £40 million in loans for dependent territories only, compared with £68.5 million for grants and £32 million for loans in three years only, under the previous Acts. The population of the dependent territories, however, had fallen from 18.75 million to 5.5 million as territories became independent. Under the previous Act the ratio of grants to loans was slightly more than 2:1, and this has now been increased so that 70 per cent of the aid available in the Bill would be by grants. These were considered to be more important than loans, as they do not put a further burden on the developing territories. Such sums would only be available for individual schemes within Colonial Development Plans, and under the main Act such Plans had to be approved by the Colonial Secretary, who consulted fully with the Minister for Overseas Development. Grants were available only towards the execution of plans which had first been approved; all the advisory services of the Ministry of Overseas Development could be brought to bear on the development problems of these territories.

Under Clause 2 the Overseas Service Act, 1961, was repealed and replaced, and the present Overseas Service Air Scheme was extended to cover people who were performing public or social services in overseas territories outside the Civil Services. Forty-one agreements have been signed under this Scheme, and at its peak more than 15,000 officers had been designated. More than 10,000 officers were still in posts under the Scheme in both independent and remaining dependent countries, of whom about 5,000 were members of the Overseas Civil Service, the rest being designated contract officers. Emphasis had been placed on maintaining, through a large number of short-term contracts, a considerable body of officers in the field to help overseas Governments to attain independence and to develop that independence as effectively as possible: the need for this help had not been reduced by independence. Although about 1,800 appointments were made under the Scheme in 1964, the vacancies outstanding at the end of the year exceeded the appointments made. With one major exception, the Government had not yet chosen the bodies to whom help (under the Scheme) should be offered, but it had begun a process of consultation so as to list a large number of bodies who might be eligible and then to assign some order of priority in the offers of help which could be made. It was estimated that the cost of this extension would rise to a maximum of about £1.5 million a year; the whole Scheme is at present costing the Government between £15 and £16 million a year.

### Mount John University Observatory, New Zealand

THE University of Pennsylvania, United States, and the University of Canterbury, New Zealand, formally opened the Mount John University Observatory, New Zealand, on July 10. The Observatory is the result of a joint project which began in 1961, and is situated at 3,500 ft. on the top of Mount John near Lake Tekapo in South Canterbury. It was, in fact, in limited operation before the official opening, and a 16-in. reflector telescope and an 8-in. refractor telescope lent by F. M. Bateson (a New Zealand research associate of the University of Pennsylvania) were operative. In use also was a 10.5-m. astrophotographic camera, which is utilized for taking pictures of large areas of the sky, and which was originally installed in the University of Pennsylvania's old Cook Observatory at Wynnwood. Pennsylvania has sent a renovated and rebuilt 18-in. refractor telescope which was originally installed in the University's old Flower Observatory, Upper Darby. This telescope is being stored until a structure can be built to house it. The University also

has plans to send a 39-in. reflector telescope to the New Zealand site. The University of Pennsylvania has entered into an informal working relationship with the General Electric Company. The General Electric Space Sciences Laboratory plans to collaborate with Pennsylvania and Canterbury at the Mount John Observatory on research into the atmosphere and surface features of other planets. Besides helping with the reconditioning of the 18-in. refractor telescope, the Company has provided a digitized photometer.

The original agreement between Pennsylvania and Canterbury has been renewed for a 10-year period dating from April 20, 1965, and will be renewable for subsequent 10-year periods. Pennsylvania will support a full-time astronomer at the facility to make observations and train students. He will be the astronomer in charge. Canterbury will provide two people for technical support. Students from both universities will train at the Mount John Observatory. At least one assistantship will be made available annually for a Canterbury graduate enrolled in Pennsylvania's Graduate School of Arts and Sciences. Pennsylvania has made arrangements to send two graduate students to the observatory during the 1965-66 academic year, one in September and one in January. A technician and a weather observer are already on duty at the site.

#### Benjamin Gompertz, F.R.S. (1779-1865)

JULY 14, 1965, marked the centenary of the death of the distinguished mathematician Benjamin Gompertz, who became one of the leading actuaries of his day, being the first actuary of the Alliance Assurance Company from its foundation in 1824 on the initiative of Nathan M. Rothschild and Sir Moses Montefiore, until his retirement in 1847. Gompertz married the sister of Sir Moses in 1810. Although Gompertz retired a year before the formation of the Institute of Actuaries, he was made an honorary member in recognition of his distinguished work in the actuarial field, and particularly his papers on life contingencies which were submitted to the Royal Society in 1820 and 1825. The Council of the Institute of Actuaries, to commemorate the centenary of his death, is to publish a commemorative article in its *Journal* (91, Part II, available after September 1965) and has hung a photographic reproduction of a portrait of Gompertz at Staple Inn Hall.

#### The American Philosophical Society

*Year Book 1964* of the American Philosophical Society as usual includes lists of officers and committees, members, lectures, meetings, biographical memoirs and, in the report of the Committee on Publications, a list of publications during the year (Pp. 780. Philadelphia: American Philosophical Society, 1965). Besides the Charter and Laws of the Society, the *Year Book* includes Dr. E. G. Conklin's brief history of the Society, and reports from the Committees on the library and on research. From the Pension Fund the latter Committee approved 292 grants totalling 273,600 dollars during the year; from the Johnson Fund, 56 grants amounting to 53,900 dollars; and from the Daland Fund for Research in Clinical Medicine, four fellowships, three of 8,000 dollars and one of 7,000 dollars. Two grants, one of 1,000 dollars and one of 900 dollars, were made from the Michaux Fund. Lists of recipients of grants and notes on the investigations in progress are included in the Committee's report.

#### Museums Journal

THE *Museums Journal* for June (65, No. 1; 1965) includes an informative article on the Public Libraries and Museums Act, 1964, so far as it concerns local authority museums. All previous Acts on museums are repealed, and Mr. John Edwards summarizes the Act in non-legal language. Although local authority museums

now have the power to charge for admission, it is doubtful whether many will avail themselves of this clause in the Act. Mr. E. L. Seyd gives a full and illustrated description of the Cannon Aquarium and Vivarium at the Manchester Museum. This has been provided in a typical exhibition gallery, and apart from its educational function has been much appreciated by the general public. Mr. Kenneth Hudson, in an article on "The Taming of Industrial Archaeology", feels that we must recognize that many individuals are "historical defectives", in the same way as we recognize colour-blindness. It is the present task of those interested to sell the ideas of the preservation of early industrial monuments and activities to the public. The issue also includes the annual report of the Council of the Museums Association for the year ended March 31, 1965.

#### The National Lending Library

THE National Lending Library for Science and Technology, which is trying to make its collection of recent conference proceedings as comprehensive as possible, has issued an *Index of Conference Proceedings Received* during 1964, together with a few received earlier (Pp. lv+60. Walton, Boston Spa: National Lending Library for Science and Technology, 1965). The *Index* is in two parts: the first part lists the title of the conference proceedings, giving place and date of conference, as well as conference number; the second lists subjects alphabetically by key words taken from the titles of the conference proceedings. Some 1,400 conferences are listed, and it is proposed to publish further indexes quarterly.

#### Fire Research Literature

THE Joint Fire Research Organization of the Department of Scientific and Industrial Research and Fire Offices' Committee has issued a list of recent reports, etc., published during 1959-August 1964 (Pp. 16. London: Department of Scientific and Industrial Research and Fire Offices' Committee Joint Fire Research Organization, 1964). The list covers, first, Stationary Office Publications, obtainable direct from H.M.S.O., and, secondly, other publications, obtainable free of charge from the Director, Joint Fire Research Organization, Boreham Wood, Hertfordshire. The latter are classified under: occurrence of fire; fire hazards; initiation and development of combustion; fire resistance; fire-fighting; and general, including works of reference.

#### Biological Characterization of Human Tumours

DURING May 27-29 a symposium was held on "The Biological Characterization of Human Tumours" under the auspices of the European Committee for Human Tumour Investigations, which was formed in 1961 in London during a meeting at the Chester Beatty Research Institute (see *Brit. Med. J.*, i, 1752; 1961; and *Nature*, 189, 627; 1961). Most of the contributions will be published in the new *European Journal of Cancer*, including those dealing with the main problems of "Long Term Cultures of Organized Human Tumours" (Prof. Etienne Wolff), "Enzymology of Human Tumours" (Dr. G. E. Boxer), "Histochemistry and Tumour Characterization" (Dr. R. J. G. Willighagen), "Evidence of Immunologic Reaction to Autochthonous Cancer in Man" (Dr. C. M. Southam), and "Viruses and Human Tumours" (Dr. R. J. C. Harris). In addition, a number of short papers on various aspects were presented, and the common difficulties arising out of work with human samples were thoroughly debated. A subsequent meeting of the European Committee discussed the formation and extension of study groups and working plans for the future.

#### C.S.I.R.O. Publications

THE Commonwealth Scientific and Industrial Research Organization, Australia, has issued a list of publications

of the Organization and of its predecessors to December 31, 1964 (*List of Publications*. Pp. 58. East Melbourne: Commonwealth Scientific and Industrial Research Organization, 1965). The *List* includes details of handbooks and monographs, journals, bulletins, pamphlets, circulars, annual reports, divisional publications and publications in the *Land Research Series* and soil publications. A subject index is provided.

#### Federation of European Biochemical Societies

THE Federation of European Biochemical Societies has created the post of secretary-general and has appointed Prof. W. J. Whelan to this office. The chairman of the Federation for 1965-66 is Prof. K. Zakrzewski. The Federation, founded in 1964, now unites the membership of twenty-one biochemical societies in the European area, and its activities include the arrangement of an annual international meeting, summer schools in advanced research techniques, charter travel to international congresses and, through a twice-yearly bulletin, information on biochemical meetings in Europe. The third Federation meeting will be held in Warsaw during April 4-7, 1966, and will include a symposium on "Properties and Structure of Genetic Elements" and colloquia on "Biochemistry of Blood Platelets" and "Mitochondrial Metabolism". Information on this meeting may be obtained from the Secretariat, Third Federation Meeting, Polskie Towarzystwo Biochemiczne, 16 Freta Street, Warsaw, 40, and on other Federation activities from Prof. W. J. Whelan, Royal Free Hospital School of Medicine, 8 Hunter Street, London, W.C.1.

#### University News:

##### Aberdeen

THE following appointments have been made: *Professorships*, Dr. H. F. W. Taylor (newly created third chair of chemistry); Dr. K. Walton (newly created second chair of geography); Dr. J. R. Symons (newly created second chair of psychology); Dr. J. R. Mallard (newly created chair of medical physics); *Senior Lectureships*, Dr. J. D. Lambert and Dr. D. R. Wallace (mathematics); *Lectureships*, Dr. R. Shilton, Dr. L. S. D. Glaeser and Dr. J. A. Duffy (chemistry); M. S. Philip (forestry); *Research Fellowship*, A. L. Fallas (chemistry).

##### Belfast

DR. J. C. BROWN has been appointed to the chair of computer science. The following lecturers have been appointed: Dr. J. J. Rooney (physical chemistry); Dr. O. M. White (physics); R. Jennings, J. D. Williams, A. S. Bahrani and W. J. Skelton (mechanical engineering); A. E. Hadden, D. M. Luke, H. R. Marten and K. H. Edwards (electrical engineering).

##### Bristol

THE following appointments have been made: *Readerships*, Dr. A. Kellar (physics); Dr. J. F. Nye (experimental physics); Dr. A. F. W. Hughes (zoology); Dr. J. B. Chappell (biochemistry); Dr. R. T. Severn (civil engineering); *Lectureships*, Dr. N. W. Ashcroft (theoretical physics); Dr. M. Green (organic chemistry); Dr. R. Parikh (pure mathematics); R. G. Morgan (civil engineering).

##### Cambridge

MRS. A. GREGORY has been elected to the Perrott-Warrick studentship in psychical research at Trinity College.

##### Edinburgh

THE following lecturers have been appointed: Dr. F. W. Winton and Dr. J. F. Peutherer (bacteriology); Dr. S. S. Brown (clinical chemistry); Dr. D. H. Mills (forestry and natural resources); T. G. I. Hamnett (social anthropology); J. S. D. Ritchie (veterinary pathology); Dr. A. H. Maddy and Dr. A. W. Ewing (zoology).

#### London

THE following titles have been conferred: *Professor*, Dr. R. Miledi (biophysics, in respect of his post at University College); Dr. R. W. Tiffen (applied mathematics, in respect of his post at Birkbeck College); Dr. T. Estermann (mathematics, in respect of his post at University College); Dr. D. R. Wilkie (experimental physiology, in respect of his post at University College); *Reader*, Dr. T. O. Griffith and Dr. S. Zienau (physics, in respect of their posts at University College); Dr. G. Stephenson (mathematics, in respect of his post at the Imperial College of Science and Technology); Dr. M. Whitear (zoology, in respect of her post at University College); Dr. N. C. Freeman (aerodynamics, in respect of his post at the Imperial College of Science and Technology); Dr. G. G. Meynall (microbiology, in respect of his post at the Lister Institute of Preventive Medicine); Dr. J. H. Calloman, Dr. H. J. Milledge, Dr. J. H. Ridd and Dr. M. L. Tobe (chemistry, in respect of their posts at University College).

#### Sussex

DR. W. LEDERMAN, at present reader in mathematics, has been appointed professor of mathematics.

#### Announcements

DR. B. R. NIJHAWAN, director of the National Metallurgical Laboratory, Jamshedpur, India, has been elected president of the Indian Institute of Metals for the year 1965-66.

THE tenth Congrès Européen d'Hématologie will be held in Strasbourg during August 23-28. Further information can be obtained from Dr. J. Lewin, Centre de Transfusion Sanguine, 10 rue Spielmann, Strasbourg.

THE ninth international conference on the "Biochemistry of Lipids" will be held at Noordwijk during September 5-9. Further information can be obtained from Dr. J. Boldingh, Unilever Research Laboratorium, Mercatorweg 2, Vlaardingen, Holland.

THE fifth Western National Meeting of the American Geophysical Union will be held in Dallas, Texas, during September 1-3. Further information can be obtained from Dr. J. C. Harrison, Hughes Research Laboratories, 3011 Malibu Canyon Road, Malibu, California.

MEETINGS of Commission 3 and Sub-Commissions 6b and 6c of the International Institute of Refrigeration will be held in Prague during August 31-September 9. Further information can be obtained from the Secretary, International Institute of Refrigeration, 177 Boulevard Malesherbes, Paris, 17.

THE twentieth annual meeting of the Society of General Physiologists will be held at the Marine Biological Laboratory, Woods Hole, Massachusetts, during September 1-4. The programme will include a symposium on "The Specificity of Cell Surfaces". Further information can be obtained from Roger Milkman, Department of Zoology, Syracuse University, Syracuse, New York 13210.

THE seventh congress of the International Union of Game Biologists will be held in Belgrade and Ljubljana during September 5-11. The programme will include two symposia on "Chemical Products and Techniques used by Man and their Effects on Wild Life" and "Damage to Agricultural and Forest Crops caused by Game". Further information can be obtained from Prof. S. Valentinoic, Gerbiceva 60, Ljubljana.

**CORRIGENDUM.** In the article entitled "The Auckland Islands" (*Nature*, 203, 26; 1964) it was stated that the expedition referred to in the article was from the Australian Museum. It was, in fact, dispatched by the New Zealand Department of Scientific and Industrial Research and the Dominion Museum, Wellington, New Zealand.



## THE SMITHSONIAN ASTROPHYSICAL OBSERVATORY

MORE than 350 astronomers representing approximately twenty countries will participate in two space-science conferences to be held at Cambridge, Massachusetts, this summer as a part of a celebration marking the seventy-fifth anniversary of the Smithsonian Astrophysical Observatory.

The Smithsonian Observatory, in co-operation with the Harvard College Observatory, will sponsor two symposia, the first concerning meteor orbits and dust (August 9-13), and the second concerning aeronomy, or the investigation of the Earth's upper atmosphere by means of artificial satellites (August 16-20). The Smithsonian Observatory has long been a centre for research in both aeronomy and meteoritics, and specialists in these fields from around the world are expected to attend, including scientists from Iron Curtain countries.

Two popular lectures open to the public will also be delivered: the first on August 10, entitled "The Falling Stars", given by Dr. Peter Millman of the National Research Council of Canada, and the second on August 17, entitled "The Aurora Polaris in the Magnetosphere", given by Dr. Sydney Chapman of the Geophysical Institute, University of Alaska.

The Smithsonian Astrophysical Observatory, a bureau of the Smithsonian Institution, Washington, D.C., was founded in 1890 by Samuel Pierpont Langley, third secretary of the Smithsonian and a pioneer in aeronautics and early manned flight. The Observatory's early research was concerned with the relationship between solar and geophysical phenomena.

In 1955, the Observatory headquarters and research facilities were moved to the grounds of the Harvard College Observatory in Cambridge, Massachusetts. Dr. Fred L. Whipple, the well-known Harvard astronomer, was appointed director of the Observatory at that time. The emphasis of Smithsonian research was shifted to the

'space sciences' in response to growing international space programmes.

The Observatory gained widespread recognition with the launching of Russia's *Sputnik I*. The Observatory was developing a world-wide network of satellite-tracking cameras at the time, and it already had an international organization of volunteer visual observers in operation. This group of amateur observers, called "Moonwatch", provided many of the first data leading to the determination of *Sputnik's* orbit.

The Smithsonian Observatory has continued its tracking operations under a grant from the National Aeronautics and Space Administration. Optical data from its network of Baker-Nunn cameras are used to-day both in support of the federal space programme and for the research of its own scientists who use the information in investigations of the upper atmosphere, celestial mechanics, the Earth's gravitational potential, and geodesy.

Besides satellite tracking and its related research projects, more than fifty Observatory scientists are engaged in varied researches ranging from theoretical astrophysics and meteoritics to exobiology and cometary studies. As part of the National Aeronautics and Space Administration's Orbiting Observatory programme, Smithsonian scientists are also preparing instrumentation for future satellite and balloon flights.

The Smithsonian Observatory's data-gathering facilities include the world-wide network of twelve Baker-Nunn satellite-tracking cameras, an unmanned network of sixteen camera stations in the mid-western United States for photographing bright meteors, a system of radar and optical tracking stations on the Virginia coast for observing simulated or 'man-made meteors', and, with the Harvard College Observatory, a radar system at Havana, Illinois, for the detection of ionized trails from micro-meteorites.

## LACK OF HOMOLOGY IN THE OSCILLATIONS OF NEUTRON STARS

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THE examination of highly condensed matter is important in itself as a fundamental problem in physics. Its importance has recently been increased because of a possibility that the presence of dense stellar matter in the form of a neutron star may be responsible for some of the celestial X-ray sources now known to exist. Oppenheimer and Volkoff<sup>1</sup> discussed the dynamical stability of neutron stars by calculating free energies. A variational principle was used by Misner and Zapolaky<sup>2</sup> for such investigations. Both approaches were used by Wheeler *et al.*<sup>3-5</sup>, who extended the problem to more general cases. This article presents some results of recent calculations which show how various possible forms of the nuclear potential are related to the general stability of high-density objects.

For stars to be stable they must first of all be in hydrostatic equilibrium. Models were, therefore, constructed by solving the general relativistic equations of hydrostatic equilibrium as derived by Oppenheimer and Volkoff<sup>1</sup>. In

order to estimate the dynamical stability of these models, Chandrasekhar's variational principle<sup>6-8</sup> (in its corrected form) was used. According to this principle, a sufficient condition for the occurrence of dynamical instability is that the square of the circular frequency of a radial oscillation,  $\omega^2$ , be negative. Chandrasekhar's trial function, which assumes homologous oscillations, was used.

Both the expressions for the hydrostatic equilibrium and for the dynamical stability contain the equation of state, the relation between the pressure and energy density,  $P = P(\epsilon)$ . It may be noted that the effect of nuclear forces enters our problem through this relation. When the stellar density approaches the normal nuclear density ( $3.7 \times 10^{14}$  g/cm<sup>3</sup>), the constituent particles generally experience attractive forces; but as the density increases further, repulsive forces eventually predominate. To see the effect of these forces, three different types of nuclear potential were chosen. The first, originally developed by Skyrme<sup>9</sup>, is a simple three-body effective



nuclear potential which represents fairly well many features of the more complicated many-body approach. In the present investigation, the relativistic Skyrme equation of state as given by Saakyan<sup>10</sup> was used. We shall refer to models of this kind as 'Skyrme models'. The other two potentials, denoted by  $V_\beta$  and  $V_\gamma$ , are neutron-neutron potentials derived by Levinger and Simmons<sup>11</sup>, which also are based on experimental data in the region of normal nuclear density. The case of non-interacting fermions is also considered for comparison. These are designated 'ideal' gas models. The principal differences among these potentials may be summarized as follows: For densities in the range of  $10^{12}$ – $10^{14}$  g/cm<sup>3</sup>, the Skyrme potential has the largest attractive term, and the  $V_\beta$  and  $V_\gamma$  potentials have a less attractive term, but the  $V_\gamma$  potential is somewhat more attractive than the  $V_\beta$  potential. For densities higher than about  $10^{14}$  g/cm<sup>3</sup>, the  $V_\gamma$  potential is the most repulsive, the  $V_\beta$  potential is the least repulsive, and the Skyrme potential is intermediate in its repulsive effect.

In a physically realistic equation of state, the pressure is not allowed to become indefinitely large, for two reasons. The first is the relativistic restriction that the pressure should not exceed one-third of the total energy density<sup>12</sup>. Zeldovich, however, argued that for some strong interactions this condition can be violated and that the absolute limit on pressure should be  $P \leq \epsilon$ , which implies that the velocity of sound cannot exceed the velocity of light<sup>13</sup>. (Here,  $P$  is the pressure and  $\epsilon$  is the energy density.) All nuclear equations of state considered here violate these limitations as the density is increased. Therefore, when one of these pressure saturation conditions is reached, the asymptotic equation of state  $P = \epsilon$  or  $P = \epsilon/3$  is used. The composition of pure neutrons was used throughout the work recorded here.

Generally, the mass of a degenerate star is uniquely determined for a given central density through the condition of hydrostatic equilibrium. The following general feature is common to all kinds of equations of state, independent of the nature of nuclear forces: With increasing density, the mass rises toward a principal peak, beyond which it falls and oscillates. Infinite series of such damped oscillations were predicted as the central density approaches infinity<sup>4</sup>. For each equation of state examined, about three such peaks were identified above the principal peak. When nuclear forces are not taken into account, the binding energy (the proper mass minus the gravitational mass) generally becomes negative for sufficiently high densities<sup>4–6,14</sup>. However, this negative binding in the region of high density does not occur when either the  $V_\beta$  or  $V_\gamma$  potential is applied to baryonic mixture<sup>11</sup>. The properties of the  $V_\beta$  and  $V_\gamma$  neutron star models (pure neutron configuration) are similar to the corresponding types of the baryon stars. The Skyrme models lie about halfway between  $V_\beta$  and  $V_\gamma$  models, with the principal peak at about 1.7 solar masses. The binding energy never becomes negative for the Skyrme models. The 'ideal' gas models are less massive than the  $V_\beta$  models, with the principal peak at about 0.7 solar masses. More detailed information on these models can be found in a thesis<sup>14</sup>.

In Fig. 1 the square of the circular frequency,  $\omega^2$ , is plotted as a function of the central energy density.  $\omega^2$  is expressed in relativistic units where  $c = G = 1$ , and the unit of length is  $1.86 \times 10^6$  cm. The solid curves represent the models with the pressure saturation condition  $P \leq \epsilon$ , and the dashed curves the models with the condition  $P \leq \epsilon/3$ .

The calculations exhibited in Fig. 1 of  $\omega^2$  are based on Chandrasekhar's formula, which in turn was based on a trial function in which the mode is constrained to pulsate homologously. The implicit assumption is made that such a pulsation is the fundamental model of lowest energy. If this were so, then the region of the diagram corre-

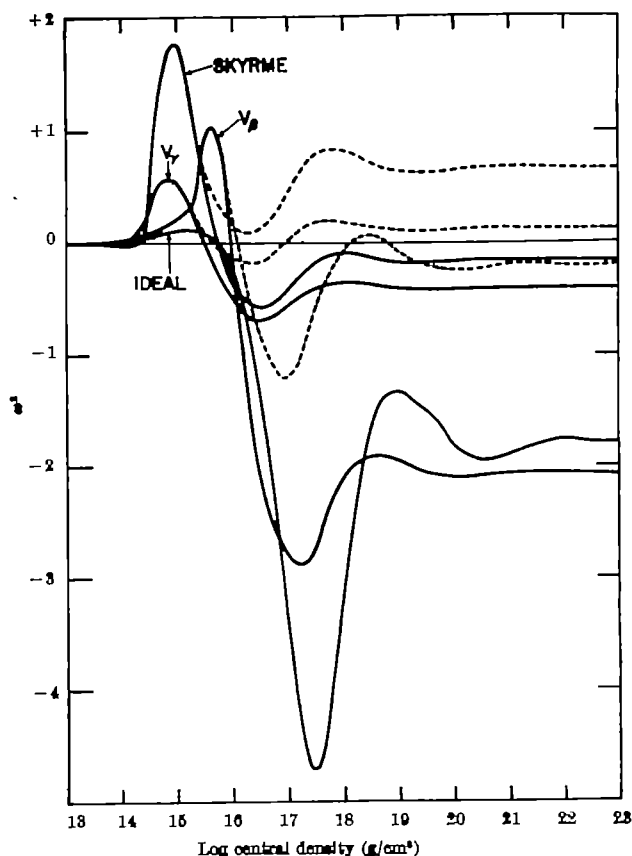


Fig. 1. Square of the circular frequency of the oscillations of a neutron star configuration based on the assumption that the fundamental mode consists of homologous radial pulsations. ---,  $P \leq \epsilon/3$ ; —,  $P \leq \epsilon$ .

sponding to  $\omega^2 > 0$  should be the stable region, and the region corresponding to  $\omega^2 < 0$  should be the region of instability.

The basic result found in these calculations is that  $\omega^2$  oscillates as a function of central density, and approaches a finite value after a number of damped oscillations as the central density goes to infinity. For some cases three such oscillations are recognizable in Fig. 1. This is a feature common to other stellar parameters, such as mass and radius. This property is found to be independent of the type of equation of state used.

All 'ideal' gas models were found to be stable along the lower branch of the principal mass peak in the region of neutron stars. The  $\omega^2$  changes sign from positive to negative at the principal mass peak, which is also the point of greatest binding energy, as expected. The two smaller  $\omega^2$  peaks in higher density regions roughly correspond to the lower branch of the second and the third mass peaks. (These are actually the third and the fourth peaks if the white dwarf peak is included.)

Nuclear forces generally increase the value of  $\omega^2$ . The greatest contributions to the integrals which must be evaluated to obtain  $\omega^2$  occur in the outer parts of the models, where the density is relatively low. Hence it is the attractive character of nuclear forces near ordinary nuclear density which must be responsible for the increased values of  $\omega^2$ . Furthermore, it may be seen that  $\omega^2$  is more positive when the pressure saturation condition  $P \leq \epsilon/3$  is applied than when the less restrictive condition  $P \leq \epsilon$  is used.

It is evident that the failure of all values of  $\omega^2$  to become negative past the top of the principal neutron star peak violates a fundamental theorem concerning instability which is based on energy principles (theorem 17, p. 61, ref. 4). Hence it is evident that the homologous

vibration of these neutron star models cannot represent accurately the fundamental mode. The departures from homologous displacements must be much greater for models based on equations of state with nuclear force terms included. Hence more complicated trial functions must be used to test for instability of such models.

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## FORMATION OF PRECIOUS OPAL

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**P**RECIOUS opal is distinguishable from other opaline silicas by being essentially amorphous, as shown by J. B. Jones, J. V. Sanders and E. R. Segnit<sup>1</sup>. The relation between the remarkable particulate structure and the reflected colours of precious opal has been described by J. V. Sanders<sup>2</sup>, who found that this mineral consists of regularly packed uniform spheres of amorphous silica a few tenths of a micron in diameter. From electron micrographs, Sanders concluded that the spheres must have grown in suspension by deposition of silica on to precipitated nuclei and then the spheres must have been later packed together, possibly during a filtration process.

The purpose of this article is to describe the spontaneous formation of brilliantly coloured, opal-like masses of 0.1- $\mu$  spheres of colloidal amorphous silica by aggregation from a sol and to point out the physical similarity between this and other types of similarly coloured, regular aggregates formed from inorganic and organic colloidal particles in this range of particle size.

The preparation of sols of uniform spherical particles of amorphous silica by depositing soluble silica on pre-formed nuclei has been described by M. F. Bechtold and O. E. Snyder<sup>3</sup> and the characterization of such silica has been summarized by me<sup>4</sup>.

In a 30 per cent silica sol of particles about 100 m $\mu$  in diameter, prepared by George W. Sears of this laboratory, he and I observed that after two years in quiescent storage in a 1-gallon bottle an intermediate layer showing brilliant colours in reflected light had been formed at the boundary between a dense, white, concentrated layer of colloid at the bottom and a more dilute opalescent sol remaining above.

To investigate the effect of pH, a number of 4-oz. samples of colloidal silica were withdrawn from the intermediate coloured layer, diluted with equal volumes of distilled water, adjusted with hydrochloric acid and dilute ammonia to pH values ranging from 3 to 10, and then allowed to stand in sealed glass or polyethylene bottles for another two years. In samples at pH 5 and 7, coloured layers formed within a period of 3 months; in all cases, coloured layers appeared after two years. Above pH 7, the coloured region consisted of a thin, dense intermediate layer of uniform thickness. Between pH 4 and 7 at the boundary between the concentrated and dilute regions, brilliant platelets were formed, growing upward into the supernatant liquid. These have the appearance of leaf-like or blade-like crystals, and will be referred to as 'pseudo-crystals'. These objects have been found repeatedly in silica sols of relatively uniform particle size averaging about 100 m $\mu$  in diameter; in most cases the initial silica

concentration was 5-10 per cent by weight of silica and the pH between 5 and 7. In these circumstances, the blade-shaped, coloured pseudocrystals grow to a length of 2 mm, with relatively straight sides but with the upper edges less well defined.

The colour of the pseudocrystals depends on the angle from which the light is reflected. As the bottle containing the specimen is carefully turned, new pseudocrystals of different colours come into view. It should be noted that a given pseudocrystal can be seen only by one eye at a time, since the other eye is usually not at the correct angle to see the reflected light. The colours range from brilliant yellow through yellow-green or chartreuse, orange, deep red, blue and violet. These pseudocrystals are extremely fragile and disappear if the container is jarred or slightly shaken, and are formed again only after the mixture has stood again for several months.

Unsuccessful attempts were made to produce pseudocrystals more rapidly by centrifuging sols to bring about settling in several hours, but the silica particles were pecked into white, opaque layers, leaving a translucent, almost transparent supernatant liquid. It is apparent that time is necessary for the particles to attain the required perfection of packing.

The coloured material has been isolated in dry form as chalky white masses of silica gel, still showing weak interference colours, by permitting the sol containing the coloured layer to evaporate very slowly over a period of two more years, so that the silica in the supernatant liquid is drawn down slowly on the coloured masses without disturbing them. More rapid evaporation, which obviously must involve movement of water from the bottom to the top of the mass, disturbs the structure. The slowly dried, fragile, opaque material was heated to 900° C over a period of 12 h and then slowly cooled. When the mass was impregnated with water or preferably with benzene or alcohol, the interference colours were again observable. The refractive index of the impregnating liquid had a marked effect on the reflected colours. Water with a refractive index of 1.33, as compared with 1.46 for the amorphous silica, gave a white opaque mass in which green flecks of colour could be seen. Normal butyl alcohol, refractive index 1.39, gave an almost transparent mass, showing green-blue flecks of colour in reflected light. Carbon tetrachloride, refractive index 1.46, gave a perfectly transparent mass which, however, showed slight green reflexions. An oil with a refractive index of 1.6 gave deep red reflexions, while liquids of still higher refractive index resulted in a white opaque mass with a brownish tinge.

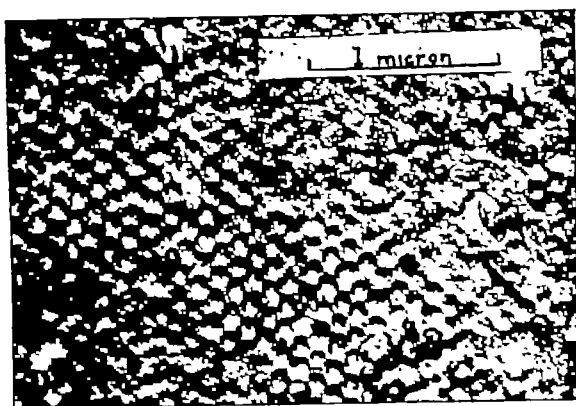


Fig. 1. Electron micrograph of replica of polished section of close-packed spheres of colloidal silica showing reflected interference colours ( $\times c. 25,000$ )

The bulk density of solid pieces of the slowly dried silica, still showing interference colours, dried and fired to  $1,000^{\circ}\text{C}$ , was  $1.53\text{ g./c.c.}$ , corresponding to 69.6 per cent by volume of amorphous silica. This approaches the value of 72 per cent by volume, corresponding to close-packed, uniform spheres. An electron micrograph of a replica of a polished section is shown in Fig. 1.

**Formation of natural opal.** The brilliantly coloured but very fragile layers containing the pseudocrystals, viewed through the glass walls of the container, look exactly like natural precious opal. There is little doubt that the colours originate from uniform arrays of silica spheres as Sanders<sup>1</sup> has described in the case of natural opal. However, in natural precious opal the spheres are larger, and the space between them is at least partially filled with hydrated amorphous silica which serves to harden the mass. Such a cementing process probably occurred after the spheres were packed in regular array, by long-continued impregnation with soluble silica. Cementing or reinforcing of suspended aggregates of silica particles has been carried out in the laboratory to produce mechanically strong silica gels<sup>2</sup>. Impregnation of dense-packed masses of opal may have occurred by a similar but far slower process in Nature, since the solution would have to pass through the mass in order to achieve such a uniform deposition of silica.

It is probable that a critical step in the formation of natural precious opal was the creation of spheres of amorphous silica in dilute suspension and subsequent slow settling in quiescent underground pools. Since geyser waters are often supersaturated with silica, it is conceivable that in some circumstances the rate of cooling might be such that relatively large uniform particles of colloidal silica were formed. In undisturbed subterranean pools such particles might have become concentrated, and uniformly packed aggregates may have been formed just as observed in the laboratory. Once such uniform aggregates had been formed, further hardening of the structure by deposition of silica within the interstices over thousands of years could result in the type of structures described by Sanders *et al.*

There remains the question as to why uniform spherical particles of amorphous silica should become packed together in regular arrays as pseudocrystals. In the silica-water system, the changes which occur spontaneously are those which lower the area of the silica-water interface, since there is an interfacial surface energy of about  $80\text{ ergs/cm}^2$  (ref. 6). Minimum interfacial area is achieved when the surfaces of such particles are brought together, excluding the water from the areas of contact. The formation of these points of contact represents the first stages in reducing the overall silica-water interfacial surface area. Greatest reduction in interfacial area is obtained with the closest packing of spheres in which each sphere makes the greatest number of contacts with sur-

rounding spheres. In a sol containing charged particles of different sizes, particles which are either smaller or much larger than the average will not fit as perfectly into the growing uniform array of the pseudocrystals, and their inclusion is thus thermodynamically less favoured. Unless the particle colliding with the surface is held with several points of attachment, it will be repelled by the surface of the pseudocrystal which is of like charge. This is analogous to the fact that a potassium ion does not fit into the lattice of a growing crystal of sodium chloride, and is thus excluded. Thus spheres of like size tend to fit into a given growing pseudocrystal.

**Analogous systems.** Analogous aggregation of spherical particles of colloidal size to form highly coloured masses has been observed with organic materials. N. Xeros<sup>3</sup> reported that a virus in an insect caused the formation of iridescent nodules which showed brilliant colours in reflected light; when the virus is purified it is obtained as a mass showing iridescent colours. The properties of this lattice, consisting of very uniform spherical particles,  $130\text{ m}\mu$  in diameter, is further described by Williams and Smith<sup>4</sup>.

The close packing of uniform polyvinyl toluene latex particles in the size-range of  $100\text{--}1,000\text{ m}\mu$  has been described by Alfrey, Bradford, Vanderhoff and Oster<sup>5</sup>. The spontaneously formed, close-packed, crystalline array acted as a diffraction grating and particle sizes in the range of  $302\text{--}481\text{ m}\mu$  were determined by the diffraction method.

Another instance of uniformly arrayed colloidal particles showing brilliant interference colours is the formation of the so-called 'schiller layers' from colloidal hydrated iron oxide. The structure and arrangement of regularly packed masses of uniform colloidal particles of this type have been described by Watson, Cardell and Heller<sup>10</sup>.

In instances in which the spontaneous regular and uniform aggregation of charged colloidal particles occurs, it appears that at least one of the dimensions, whether the diameter of a sphere, or the width of a rod-like particle, is of the order of  $100\text{ m}\mu$  or more. Possibly this is because uniform arrays of colloidal particles are most easily recognized when the particles are in this size range and thus reflect monochromatic light of visible wave-length. However, this is also the size-range in which slow sedimentation occurs. Particles larger than about  $100\text{ m}\mu$  in diameter or thickness tend to settle so that there is a gradual increase in concentration at the bottom of the container; conditions are thus favourable for the slow segregation of particles into regular aggregates or pseudocrystals, each consisting of particles of a particular uniform size.

On the other hand, in the case of smaller particles, the Brownian motion is so strong that the rate of sedimentation is negligible and the particles do not become concentrated by settling. If the concentration is increased rapidly by centrifugation, the particles become packed randomly since there is no time for segregation and for ordered arrangements to be developed. However, if the size is extremely uniform, as with virus or certain latex particles, a relatively rapid increase in concentration can still result in the formation of a highly ordered array.

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## COMPENSATING INFRA-RED SPECTROSCOPY

## THE POTASSIUM BROMIDE WEDGE TECHNIQUE

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THE compensating method of infra-red spectroscopy (sometimes called the differential or difference method) is a technique by which the spectrum of one component of a mixture may be completely eliminated from the recorded spectrum by introducing the component into the reference beam of a double-beam spectrometer. The method is also useful when, as frequently happens, information is required about a minor constituent of a mixture, where, because of overlapping of infra-red absorption bands, it is difficult or even impossible to obtain such information.

The technique is commonly used with liquid samples by compensating with known compositions in fixed path-length cells<sup>1</sup> or by using a variable path-length cell containing one constituent in the reference beam of the spectrometer and adjusting the width of the cell until this constituent is fully compensated in the sample beam<sup>2</sup>.

With insoluble or solid samples the control and variation of the solid layer or film required for compensation becomes difficult as the maximum thickness required is rarely more than 0.005 in. In an application of compensating infra-red spectroscopy to polymeric materials Willis and Miller<sup>3</sup> determined the free monomer content of

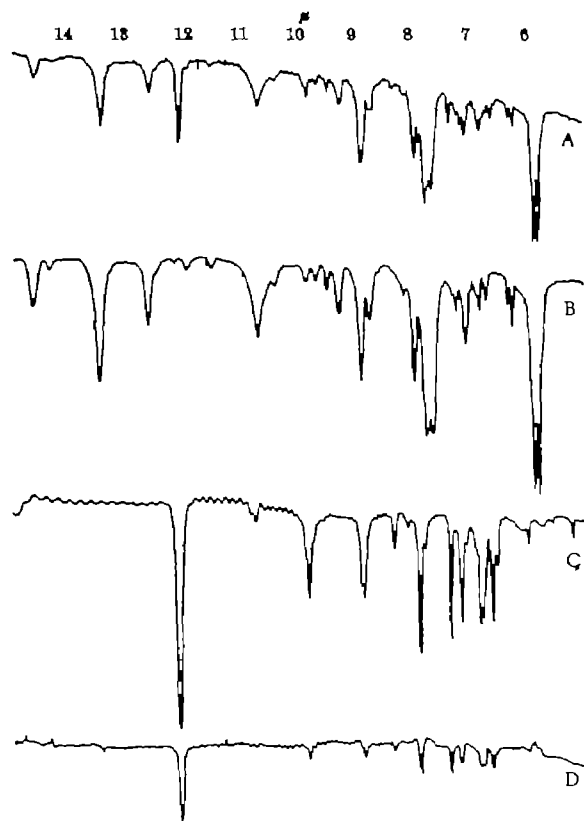


Fig. 1. A, mixture of *n*-butyl hydrogen phthalate and *p*-di-*tert.* butyl benzene; B, *n*-butyl hydrogen phthalate; C, *p*-di-*tert.* butyl benzene; D, mixture A compensated with B in the reference beam

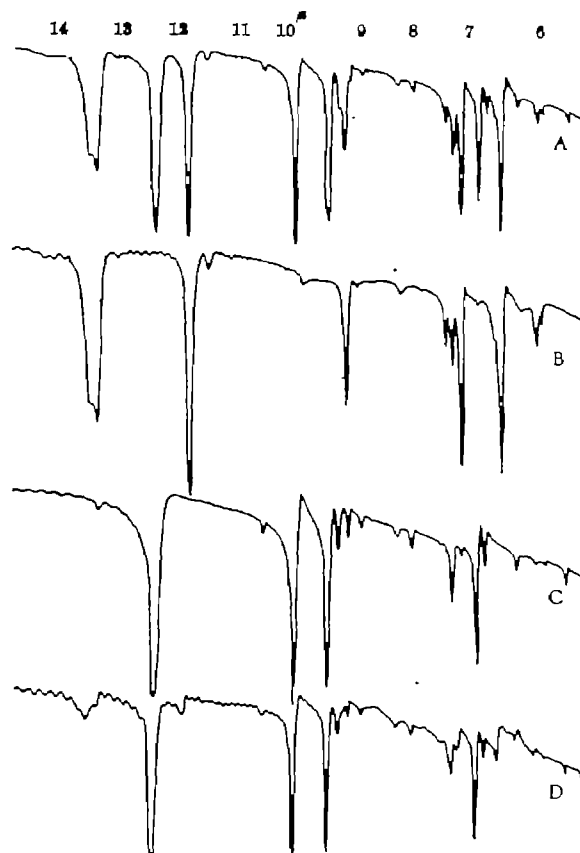


Fig. 2. A, mixture of *p*-dibromobenzene and 1,3,5-tribromobenzene; B, 1,3,5-tribromobenzene; C, *p*-dibromobenzene; D, mixture A compensated with B in the reference beam

poly(methyl methacrylate) by using compensating wedges of monomer-free polymer in the near infra-red (1–3 $\mu$ ) where greater thicknesses may be used. Similarly, by using a thin polymer wedge Willbourn<sup>4</sup> determined the degree of branching of polyethylene and Harvey and Peters<sup>5</sup> the methyl groups in the same material.

The field of application of compensating techniques may be greatly widened, however, by using potassium bromide wedges containing one of the constituents of a mixture. By this technique compensated spectra of mixtures may easily be obtained in the 'finger-print' region of the spectrum.

The extension of the usual technique of producing a parallel-faced disk of potassium bromide by pressure *in vacuo* to producing a rectangular wedge proved difficult but was solved satisfactorily by one of us (J. T.), and diagrams of a suitable die (patent applied for) in plan and sections are shown in Fig. 5. This die under a pressure of 25–26 tons makes wedges of potassium bromide 24 mm  $\times$  13 mm in size with a slope of one in twelve. (A wedge 13 mm  $\times$  13 mm  $\times$  2 mm taper requires 12 tons pressure.) No doubt it could also be used with other suitable infra-red transparent materials.

The method of preparing a wedge is to grind together by hand in an agate mortar approximately 1.5 g of spectroscopic grade potassium bromide with a suitable weight of the compensating constituent of a mixture (0.2–2 mg) using, if necessary, a small amount of a volatile solvent. The mixture is then ground again in a vibrating

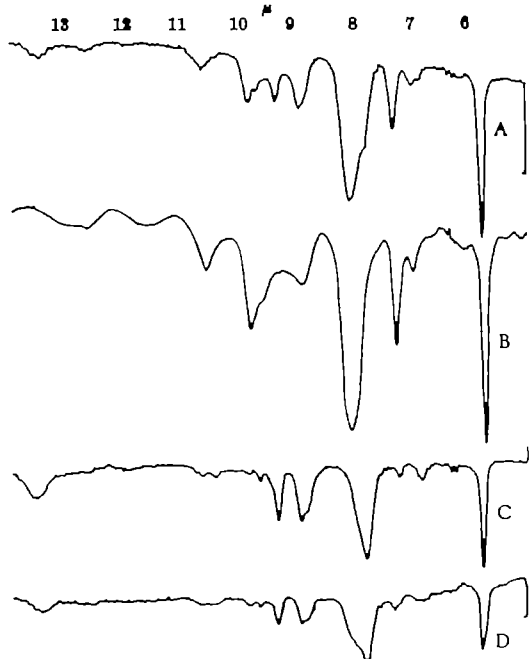


Fig. 3. A, polyvinyl acetate plasticized with dibutyl phthalate; B, polyvinyl acetate; C, dibutyl phthalate; D, composition A, compensated with B in the reference beam.

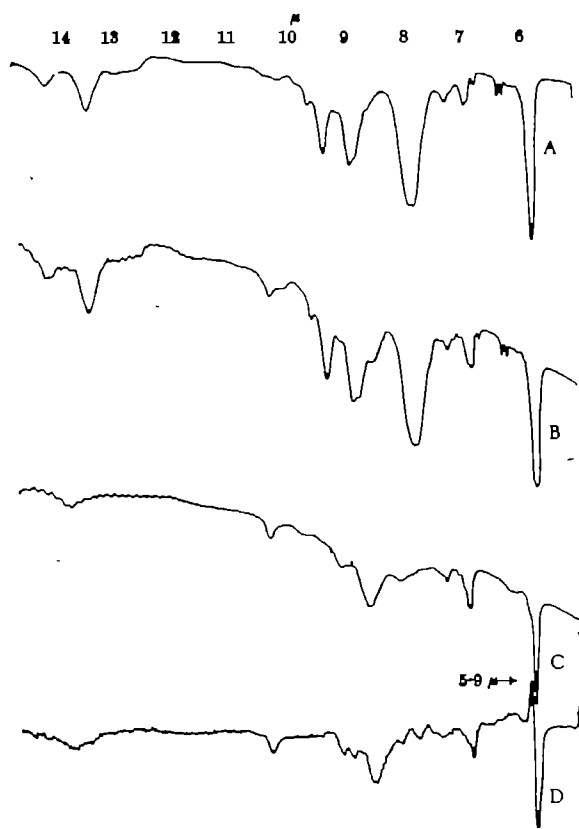


Fig. 4. A, 46 per cent linseed oil-modified glycerol alkyl resin; B, 63 per cent linseed oil-modified glycerol alkyl resin; C, bodied linseed oil; D, composition B compensated with A in the reference beam. This compensated spectrum shows the excess of bodied linseed oil C in B. The negative absorption at 5.9 μ is due to the higher acid value of A.

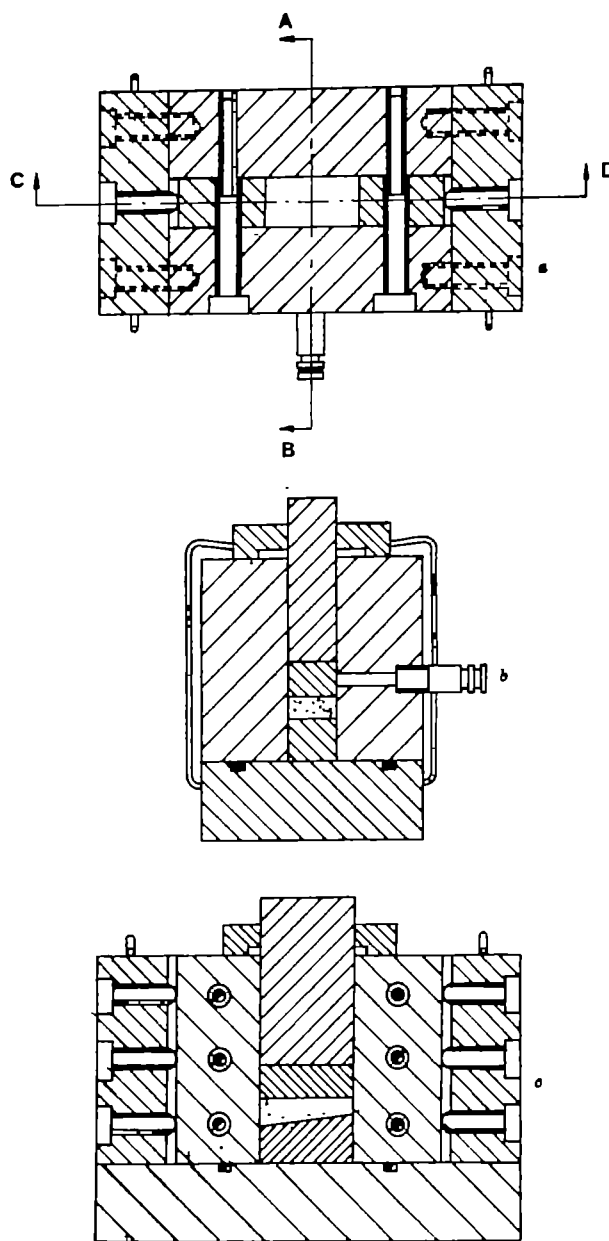


Fig. 5. Diagram of infra-red wedge die: a, plan (ram removed); b, section through AB; c, section through CD.

mill, thoroughly dried and pressed *in vacuo*. The resulting wedge will last indefinitely if stored in a desiccator.

In order to compensate for the optical displacement of the image of the reference beam in the spectrometer, a second wedge of the same size made from pure potassium bromide is clipped to the face of the constituent-containing wedge so that a rectangular block is formed. The combined wedges are then placed in a holder provided with a micrometer adjustment which allows the thickness of the constituent-containing wedge in the reference beam to be varied. With this holder placed in the reference beam of the spectrometer, the transmission is adjusted with a comb to approximately 80 per cent at a frequency at which the major constituent is transparent. The sample is prepared in the form of a potassium bromide disk and placed in the sample beam; the instrument is run and the major absorptions of the known constituent balanced out by adjusting the micrometer screw. With the instrument thus adjusted the complete spectrum of the mixture less that of the known constituent may be obtained.

Over-compensation may result in a spectrum containing negative bands which would be difficult to interpret. It may be advisable, therefore, to undercompensate and leave weak, identifiable bands of the major constituent.

If the known constituent preponderates in the mixture, the concentration of the sample in the disk must be increased. A point is reached, however, beyond which the available energy decreases very rapidly and the instrument response becomes sluggish, since speed of response is a function of the transmitted energy. In such cases it is advisable to reduce the scanning speed or to reach a compromise between the instrument response and the intensity of the absorptions of the minor constituent. The optimum operating conditions are when the absorption of the mixture at a common wave-length is 60–70 per cent.

The preparation of disks of different sample concentrations is time-consuming. A wedge containing the sample can advantageously be used in the sample beam in a holder similar to that in the reference beam, thus providing a wide choice of path-lengths.

Such a wedge, indeed, can be used in non-compensating techniques in order to arrive quickly at a suitable concentration of sample.

Infra-red spectra obtained by this technique are shown in Figs. 1–4. They were obtained with a Grubb Parsons G.S.2A instrument run at a speed of  $1\mu/2$  min, which is half the normal speed.

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## EFFECT OF COLLOIDAL PARTICULATES ON FOAM FRACTIONATION

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CHEMISTS, biologists and engineers have used foam fractionation to remove from aqueous solution soluble organic compounds and also inorganic cations and anions by complex formation with surface-active agents<sup>1,2</sup>. The process utilizes the surface-active properties of soluble organics to provide accumulation at the air-aqueous solution interfaces associated with bubbles. Froth flotation, which in contrast involves a three-phase system including a solid phase, has been applied to numerous commercial processes including ore purification and industrial waste treatment<sup>3,4</sup>. This process relies on the flotation of particles by aeration and bubble attachment which have been made selectively oleophilic by the addition of a surface-active collector. Most froth flotation processes involve coarse suspensions, and little attention has been paid to colloidal-size particulates.

For the removal of soluble organics by foam fractionation, limitations may arise owing to low surface activities of the organics and to the low concentrations which may be involved. For such solutions, the addition of colloidal particulates may provide more efficient separations through the replacement of the foam fractionation mechanism by a froth flotation mechanism. The aeration of a combined process stream containing both soluble organics and particulates may produce the effective removal of both constituents. Investigations have been made of the foam fractionation-flotation of systems containing stannic oxide<sup>5</sup> and silver iodide<sup>6</sup> together with cationic surface-active agents. The objective of this investigation is the quantitative determination of the effect of positively charged ferric oxide particulates (or polynuclear species) on the separation of an anionic surfactant, dodecyl sodium sulphate (DSS), from aqueous solution. The influences of pH, feed surfactant concentration, and feed particulate concentration are established. A brief discussion of the stannic oxide-CTAB system is included.

The hydrolysis of ferric chloride in vigorously boiling distilled water provided a positively charged ferric oxide sol<sup>7</sup>. The sol contained 292 mg/l. trivalent iron (Fe<sup>3+</sup>) as measured by the standard phenanthroline method<sup>8</sup>. Dilutions were made for the foaming experiments, and the pH was adjusted from the range 2.0–3.1 to the required values with sodium carbonate solution. The experimental apparatus was similar to that used for the ion flotation of dichromate<sup>9</sup>. The foam column was 130 cm high and 1.0 cm in diameter; nitrogen gas was saturated with water, passed through a calibrated rotameter, and diffused through a 36 $\mu$ , porous metal dispersion disk.

In each experiment 2,000 ml. of feed sol, including varying concentrations of dodecyl sodium sulphate (DSS; 95 per cent active), were added to the column. The sol was foamed until all foaming had ceased, which required 35–50 min, with foam removal from a port located 48 cm above the feed sol-level. After the completion of each experiment the residual sol volume was measured volumetrically, the residual concentration of trivalent iron was determined<sup>8</sup> (accurate to  $\pm 0.05$  mg/l.), and the residual concentration of DSS was measured by a two-phase titration technique<sup>10</sup>. Temperature was maintained constant at 25°C.

**Foamability.** The extreme influence of the presence of colloidal particulates on the foamability and separation of solutions containing surfactants of opposite charge to the particulates is shown in Table 1. The negatively charged stannic oxide sol was prepared by reacting sodium stannate with sulphuric acid in boiling water and by peptization with ammonium hydroxide; the pH of the sol was 7.4 (ref. 5). The feed sol, of volume 800 ml., contained 2,000 mg/l. of stannic oxide and 50 mg/l. of cetyltrimethylammonium bromide (CTAB), a cationic surface-active agent. Foam was collected from a port located 12 cm above the feed sol level. The solutions of CTAB were in distilled water. For the ferric oxide-DSS system, equivalent solutions of pure DSS were prepared with the pH lowered to approximately 2.5 with hydrochloric acid and elevated to 5.0 with sodium carbonate. In Table 1,

Table 1. INFLUENCE OF COLLOIDAL PARTICULATES ON FOAMABILITY

Stannic oxide-CTAB system		
Foaming time: 10 min		
Air rate: 2,520 ml./min		
pH, 7.4		
50 mg CTAB/l. solution	Stannic oxide (2,000 mg/l.) - CTAB (50 mg/l.) sol	
$V_f/V_i$	0.0	0.009
$\sigma_r$ , mg/l.	50.0	5.0
Air rate: 10,140 ml./min		
$V_f/V_i$	0.034	0.333
$\sigma_r$	40.0	1.0
Ferric oxide-DSS system		
Foaming time: 40 min		
Nitrogen rate: 4,500 ml./min		
pH, 5.0		
25 mg DSS/l. solution	Ferric oxide (117 mg/l.) - DSS (25 mg/l.) sol	
$V_f/V_i$	0.0	0.543
$\sigma_r$ , mg/l.	25.0	0.7
Nitrogen rate: 8,000 ml./min		
$V_f/V_i$	0.0	0.403
$\sigma_r$	25.0	0.9

$V_f$  is the volume of collapsed foam (ml.),  $V_i$  is the volume of feed sol or solution (ml.), and  $x_i$  is the concentration of surfactant in the residual sol or solution after foaming (mg/l.).

**Influence of pH.** A series of experiments was conducted to establish the effect of hydrogen ion concentration on the foam fractionation-flotation of ferric oxide-DSS sols. In order to clarify the notation used herein, for each experiment the following material balance equations may be written:

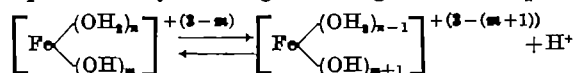
$$V_i = V_f + V_r \quad (1)$$

$$x_i V_i = x_f V_f + x_r V_r \quad (2)$$

$$z_i V_i = z_f V_f + z_r V_r \quad (3)$$

$x_i$  and  $z_i$  are the concentrations of DSS and trivalent iron in the feed sols, respectively,  $V_r$  is the volume of residual sol after foaming (ml.),  $x_r$  is the trivalent iron concentration in the residual sol, and  $z_r$  are the concentrations of DSS and trivalent iron in the collapsed foam, respectively. In these experiments, the nitrogen rate was 4,500 ml./min (at 25° C and 1 atm.), the feed concentrations of DSS were 15 and 40 mg/l., the feed concentrations of ferric oxide were 58.5 and 17.5 mg/l., and the pH was varied from 3.1 to 6.0.

Fig. 1 indicates the variation in the collapsed foam volume and in the DSS enrichment ratio ( $x_f/z_f$ ) with pH. For both sets of feed concentrations, at pH = 3.0 no foam was obtained. Elevation of pH to about 4.5 brought about extreme increases in  $V_f$  and decreases in the enrichment ratios, both of which tended to become constant at pH = 5.0. It appears that as the pH was elevated from 3.0, the ferric oxide complex was transformed by the removal of hydrogen ions from the waters of hydration, with each hydrogen ion removed yielding a hydroxide ion which remained associated with the complex, thereby reducing the charge of the complex:



where  $n$  is an integer ranging from 6 to 4, and  $m$  is an integer ranging from 0 to 2. Sebba<sup>11</sup> has discussed such

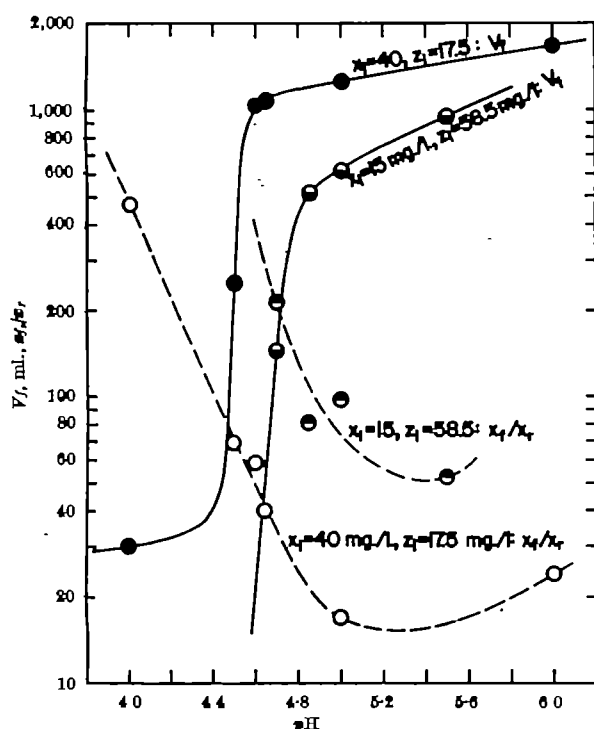


Fig. 1. Relations between  $V_f$ ,  $x_f/z_f$ , and pH

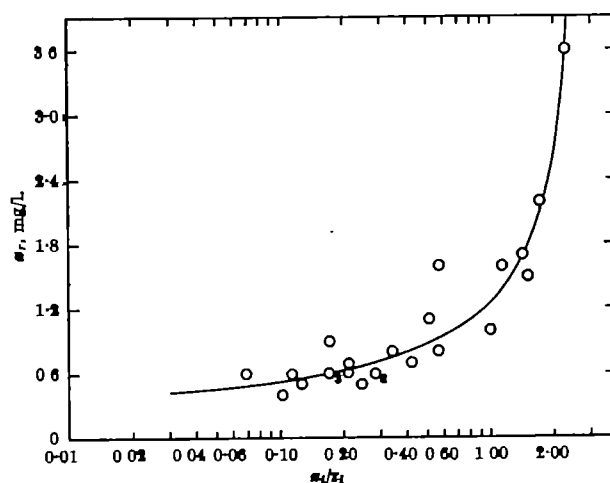


Fig. 2. Relation between  $x_r$  and  $x_i/z_i$

an interconversion of species for aluminium oxide. As the pH was raised, the charge on the hydrated complex was diminished and the formation of polynuclear species (containing oxygen bridges) was further enhanced. Such species would be more readily floated and would tend to promote foam stability. The formation of polynuclear species could be detected by optical density measurements on the feed sols, and it was found that the optical density, measured with a spectrophotometer at 500 mμ, increased with pH. The increase was more pronounced at higher trivalent iron concentrations.

For both sets of feed concentrations, the residual DSS concentrations decreased as the pH was increased, owing to the larger volumes of foam which were produced. For  $z_i = 17.5$  mg/l., the trivalent iron concentration in the residual sol decreased with increasing pH, while for  $z_i = 58.5$  mg/l.,  $z_r$  increased with increasing pH. These results are presented in Table 2.

Table 2. EFFECT OF pH ON RESIDUAL CONCENTRATIONS  
 $x_i = 40.0$  mg/l.,  $z_i = 17.5$  mg/l.

pH	$x_r$ , mg/l.	$z_r$ , mg/l.
3.1	8.0	0.35
4.0	5.0	0.30
4.5	4.2	~0.05
4.50	1.3	~0.05
4.55	1.6	~0.05
5.0	2.6	~0.05
6.0	2.0	~0.05

$x_i = 15.0$  mg/l.,  $z_i = 58.5$  mg/l.

pH	$x_r$ , mg/l.	$z_r$ , mg/l.
4.70	0.9	1.48
4.65	0.9	2.10
5.0	0.6	2.10
5.5	0.6	5.00

**Influence of concentration variations.** A second series of experiments was conducted to investigate the effect of feed concentrations of trivalent iron and DSS. In these experiments  $x_i$  was varied from 10 to 40 mg/l.,  $z_i$  was varied from 10 to 146 mg/l., the nitrogen rate was 4,500 ml./min, and the pH of each feed sol was maintained at 5.0. The residual DSS concentrations are related in Fig. 2 to the ratio of DSS to trivalent iron in the feed. As  $x_i/z_i$  was increased,  $x_r$  remained relatively constant and then rose sharply above a feed ratio of approximately 1.0. The total range of variation of  $x_r$  was 0.4–3.6 mg/l. For these same experiments, the residual trivalent iron concentrations could be related to  $x_i$  and  $z_i$  by a single equation:

$$z_r = 7.24 (10^{+5}) x_i - 9.60 z_i (3.20 \log_{10} x_i - 0.60) \quad (4)$$

For a total of 24 experiments the average deviation of values of  $z_r$  calculated with equation (4) from experimental values was 25 per cent. (Average deviation =

$$\frac{\text{calculated value} - \text{experimental value}}{\text{experimental value}} \times 100.)$$

An extensive discussion of the separation of ferric oxide (trivalent iron) has been reported<sup>7</sup>.



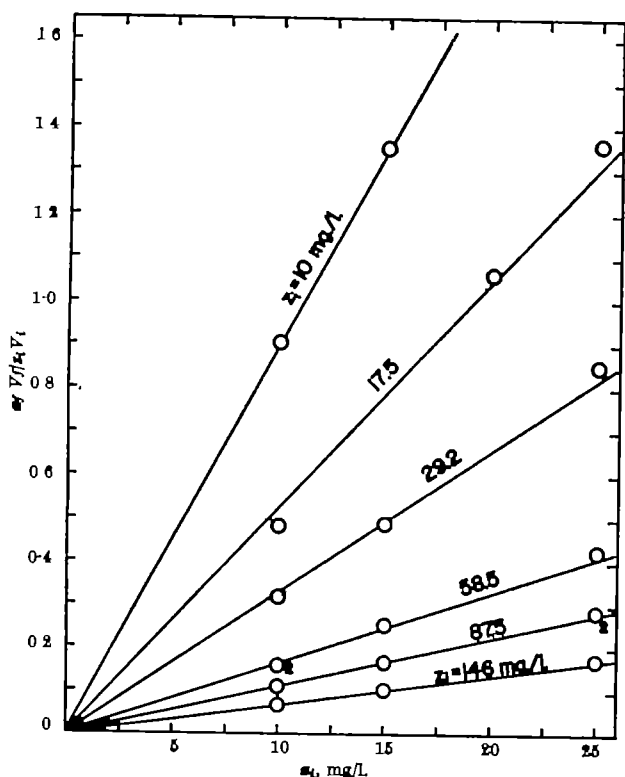


Fig. 3. Relations between  $x_f V_f / x_i V_i$  and  $x_i$ , with parameters of  $x_i$ .

Considering the utilization of colloidal particulates to enhance the separation of surfactants, a useful parameter is weight of surfactant removed in the foam per unit weight of trivalent iron in the feed,  $x_f V_f / x_i V_i$ . In Fig. 3 this parameter is related to  $x_i$  and  $x_i$ , and for each value of  $x_i$  it increases linearly with  $x_i$ . The slopes of the lines in Fig. 3 were found to be inverse power function of  $x_i$ , and since  $V_i$  was held constant in all the experiments the following equation was obtained:

$$x_f V_f = 1.692 x_i^{-0.032} x_i \quad (5)$$

Equation (5) shows that the weight of surfactant carried

into the foam was directly proportional to the feed concentration of surfactant and was a rather weak function of the feed trivalent iron concentration. The average deviation for equation (5) was 2.1 per cent for the 24 experiments. For constant  $x_i$ , as  $x_i$  was increased,  $V_f$  increased and  $x_f$  decreased, with the rates being virtually equal.

**Conclusions.** (1) The addition of colloidal particulates to solutions containing oppositely charged surfactants produces large increases in foamability, thereby promoting the separation of the surfactant into the foam. Through the addition of such particulates, the foam fractionation mechanism is replaced by a froth flotation mechanism, and the particulates together with the adsorbed surfactant are readily carried into the foam owing to preferential adsorption at the gas-solution interfaces associated with the bubbles.

(2) For the DSS-ferrie oxide system, pH has a pronounced effect on the quantity of foam produced and on the DSS enrichment ratio. This behaviour is magnified over a rather narrow pH range of 4.4-5.0, and is apparently caused by the interconversion of ferrie oxide complexes.

(3) For the DSS-ferrie oxide system at a constant pH of 5.0, the residual concentration of DSS is related to the feed ratio of DSS to trivalent iron, increasing sharply above a feed ratio of 1.0. The weight of DSS carried into the foam is directly proportional to the feed concentration of DSS and is a weak function of the feed concentration of trivalent iron.

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## INVERSE RELATIONSHIP OF GLUCOSE-6-PHOSPHATE LEVELS AND GLUCOSE UTILIZATION IN 6C3HED LYMPHOMA

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EVIDENCE has accumulated that glucose-6-phosphate inhibition of the hexokinase reaction may be a factor in the utilization of carbohydrate in cells. Product inhibition has been observed with hexokinase isolated from several mammalian tissues, both neoplastic and non-neoplastic<sup>1,2</sup>. The possible regulation of glucose utilization in hexokinase activity is further suggested by the finding of Hees and Chance<sup>3</sup> that addition of glucose to Ehrlich ascites tumour cells increases the level of glucose-6-phosphate to 1  $\mu$ mole/g of cells, that is, a concentration greater than 1 mM if the space occupied by cell matter is taken into consideration. In addition, the concentration of glucose-6-phosphate was highest at the time of maximal inhibition of glucose utilization, and the initially rapid rate was associated with the lower levels of hexose monophosphate; furthermore, the activity of hexokinase as well as phosphofructokinase of Ehrlich ascites tumour is relatively low in comparison with the other enzymes of

glycolysis, as reported by Racker and Wu<sup>4</sup>, and is thus a likely candidate for a controlling step.

This concept that product inhibition of hexokinase is an important regulatory factor in glucose utilization within the cell has recently been given added support by the work of Rose *et al.*<sup>5</sup> in an investigation of erythrocytes in which a linear relationship was observed when the reciprocal of the glucose-6-phosphate level was plotted against the rate of glucose utilization. These workers used an artificial electron acceptor, methylene blue, to stimulate glucose utilization by means of the phosphogluconate pathway, and inosine, which is utilized preferentially, to inhibit glucose uptake.

It was, therefore, of interest to examine the relationship of glucose-6-phosphate levels and rates of glucose utilization in cells capable of respiration in order to provide further understanding of the mechanism of the Pasteur effect in ascites tumour cells. The 6C3HED mouse

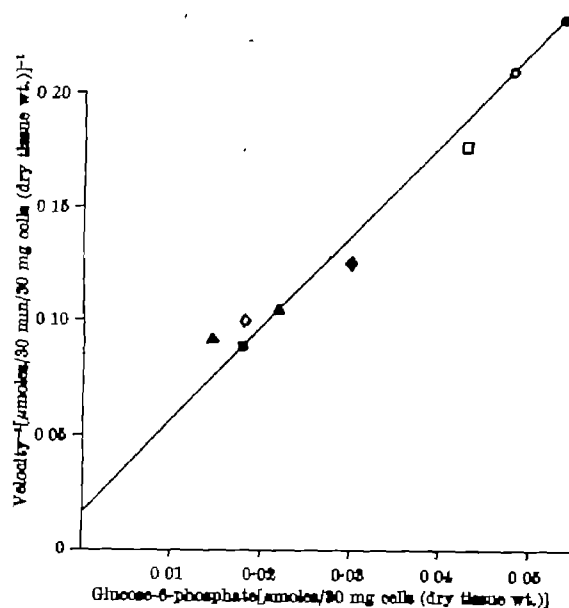


Fig. 1. Conditions are the same as described in Table 1. The symbols represent the following conditions: ○, control; ●,  $\beta$ -OH butyrate,  $1 \times 10^{-3}$  M; □, antimycin A,  $1.7 \times 10^{-4}$  M; ◆, ethanol,  $6.5 \times 10^{-4}$  M; △, rotenone,  $7 \times 10^{-5}$  M; ◇, nitrogen as the gas phase; ▲, antimycin A,  $8.4 \times 10^{-4}$  M; ■, rotenone,  $1.4 \times 10^{-5}$  M.

lymphoma is useful for this purpose since these cells have a low rate of glucose utilization and thus may be more susceptible to product inhibition. In addition, the changes in glucose utilization, induced by a wide variety of compounds, are more marked than in the Ehrlich ascites tumour cells.

For example, it was noted that  $\beta$ -OH butyrate decreases glucose utilization in this neoplasm without having observable effects on respiration, in contrast to tumours such as the Ehrlich and hyperdiploid ELD ascites form where  $\beta$ -OH butyrate had no effect on glycolysis. Also, ethanol has been observed to inhibit the oxygen uptake of rat liver mitochondria<sup>8</sup>, and preliminary investigations indicated that this lipid-soluble compound inhibits respiration and stimulates glucose utilization in the 6C3HED lymphoma. In addition, two well-known inhibitors of respiration, antimycin A and rotenone, produce the characteristic stimulation of glucose uptake and lactate accumulation in these cells, resembling the effect of anaerobiosis, and the effect of these inhibitors can be investigated at different concentrations to give a wide range of rates of glucose utilization.

6C3HED lymphoma cells were maintained in female C3H/ST mice and were collected seven days after implantation. The ascites tumour cells were isolated by low-speed centrifugation, were washed twice with  $\text{Ca}^{++}$ -free Krebs-Ringer phosphate and were resuspended in this medium.

The procedures of incubation and methods of analyses were essentially the same as described in a previous paper<sup>7</sup> and glucose-6-phosphate was determined by the method of Hohorst<sup>9</sup>.

Initially the effect of time on glucose utilization was examined, and like Ehrlich Lettré ascites tumour cells which show an initial burst of glucose utilization in the first 30 sec, and then a marked decrease in the steady state, the rate of glucose utilization in the lymphoma cells, and lactate production as well, was lower after this initial transient and linear for at least 20 min. The steady-state level of glucose-6-phosphate was reached within 80 sec, and did not change from this initial maximum.

The distribution of glucose-6-phosphate in the cells and the medium in which they were suspended was next investigated since the relationship between glucose-6-

phosphate level and rate of glucose utilization based on end-product inhibition would depend on the intracellular glucose-6-phosphate concentration. On centrifugation at 5° C of cells incubated with glucose, it was found that glucose-6-phosphate was associated entirely with the cell fraction, indicating that glucose-6-phosphate was completely intracellular.

An experiment, depicted in Fig. 1, was then carried out in which the rate of glucose utilization was modified by a wide variety of agents. The values for the levels of glucose-6-phosphate were plotted against the measurements of glucose utilized. In this typical experiment there is a linear inverse relationship between these two variables, and the relationship holds regardless of whether ethanol or the conventional inhibitors of respiration were used to stimulate glucose utilization or whether  $\beta$ -OH butyrate was used to inhibit glucose utilization.

The effects of these substances on the related processes are reported in Table 1. In addition to the measurements of respiration the values for the end-products of glycolysis are also reported in Table 1, and it was observed that the ratio of lactate/glucose increased as the degree of respiratory inhibition increased.

It is apparent that glycolysis was not stimulated to the same extent by respiratory inhibitors and anaerobiosis. Furthermore, the degree of stimulation of glycolysis was not only dependent on the inhibitor concentration at low concentrations but also at high concentrations. In the case of rotenone, glycolysis reached an optimum, and then a slight decline was observed. The decline was significant, since it could be reproduced in experiments where higher concentrations of rotenone were used. This decline is considered to arise from the breakdown of ATP required for glucose phosphorylation since the inhibition of glucose utilization and lactate production was also observed with progesterone, amytal or antimycin at concentrations which stimulate ATPase.

The finding that ethanol inhibits respiration and stimulates glycolysis in a manner similar to the other respiratory inhibitors suggests that lipid solubility is important in influencing these relationships.

In contrast, the inhibition by  $\beta$ -OH butyrate of glucose utilization cannot be attributed to the Pasteur effect since in none of the experiments carried out with  $\beta$ -OH butyrate did it produce a significant change in the rate of respiration.

The possibility that  $\beta$ -OH butyrate might influence glucose utilization by interfering with the reactions of glycolysis *per se* was ruled out by the observation that  $\beta$ -OH butyrate under similar conditions was not observed to have any effect on anaerobic glycolysis.

In order to determine whether the effect of  $\beta$ -OH butyrate is mediated by mitochondrial interactions, the effect of other diphosphopyridine nucleotide (DPN)-dependent substrates as malate, fumarate, citrate, glutamate and  $\alpha$ -ketoglutarate was examined. The

Table 1. EFFECT OF SUBSTANCES WHICH MODIFY GLUCOSE UTILIZATION ON RELATED PROCESSES

Additions	Final concentration (Molar)	Oxygen uptake (μmoles)	Glucose utilized (μmoles)	Lactate accumulated (μmoles)	Lactate/Glucose
Control		6.4	5.2	5.1	0.99
Antimycin	$1.7 \times 10^{-4}$	3.5	5.9	6.5	1.11
	$3.4 \times 10^{-4}$	1.9	10.8	16.1	1.49
	$6.8 \times 10^{-4}$	0.5	12.0	15.6	1.31
Rotenone	$7 \times 10^{-5}$	1.9	9.8	13.6	1.39
	$1.4 \times 10^{-4}$	0.9	11.8	16.0	1.43
	$1.4 \times 10^{-4}$	0.5	11.2	16.2	1.45
Nitrogen		—	10.3	14.8	1.44
Ethanol	$6.5 \times 10^{-4}$	3.9	8.4	10.1	1.23
$\beta$ -OH butyrate	$1 \times 10^{-3}$	6.6	4.5	4.3	0.96

6C3HED lymphoma ascites tumour cells equivalent to 30 mg dry tissue weight were incubated in Warburg flasks in  $\text{Ca}^{++}$ -free Krebs-Ringer buffer containing 40 μmoles of 6-(hydroxy methyl) aminocaproic acid and 10 μmoles of phosphate buffer, pH 7.4. Glucose and  $\beta$ -OH butyrate were tipped in from the side arm after 10 min of temperature equilibration; all other compounds were present in the centre compartment during this period. Incubation was carried out for 30 min at 38° C. The values reported represent those obtained for the 30-min incubation period. Total volume—3.0 ml.

Table 2. EFFECT OF DPN-DEPENDENT OXIDIZABLE SUBSTRATES ON GLUCOSE UTILIZATION AND GLUCOSE-6-PHOSPHATE LEVELS

Additions	Oxygen utilized (μmoles)	Glucose utilized (μmoles)	Lactate accumulated (μmoles)	Glucose-6-phosphate levels (μmoles)
None	5.1	4.9	2.7†	0.031*
Fumarate	5.0	5.1	4.3	0.019
Malate	4.9	4.6	6.3	0.035
β-OH butyrate	5.2	3.7	1.8	0.031
α-Ketoglutarate	5.2	3.7	2.4	0.013
Citrate	5.1	3.1	1.5	0.034
Glutamate	5.0	1.5	1.3	0.048

\* At zero time this value was 0.005 μmoles per flask.

† At zero time, this value was nil.

COSHMD ascites tumour cells equivalent to 25 mg dry tissue weight were incubated as described in Table 1 for 30 min at 38° C. All substrates including glucose, which was present in all flasks, were in a final concentration of 0.01 M.

experiment reported in Table 2 indicates that with the exception of α-ketoglutarate these substrates produced decreases in glucose utilization with a corresponding linear increase in the levels of glucose-6-phosphate. Furthermore, there were no significant changes in the rate of respiration. It would therefore appear that the effect of β-OH butyrate is mediated by the electron transport system and/or its associated phosphorylation.

It is also to be noted that glutamate, citrate and α-ketoglutarate inhibited lactate production, as did β-OH butyrate. It is also possible that malate and fumarate cause an inhibition of lactate production from glucose, but the inhibition may be masked by the production of lactate from these metabolites.

It is difficult to correlate hexokinase activity and glucose-6-phosphate level on a quantitative basis in the intact cell in view of the many factors which influence hexokinase activity. Since glucose-6-phosphate and ADP compete with ATP for the enzyme<sup>1,2</sup>, the  $K_i$  of product inhibition is also dependent on the concentration of these nucleotides. It is nevertheless of interest to calculate the approximate intracellular level of glucose-6-phosphate. The amount present in the steady state was observed to be 0.05 μmoles/30 mg dry tissue weight or 0.2 g wet weight of cells, that is, c. 0.25 μmoles/ml. If the concentration is calculated on the assumption that 80 per cent of the packed volume is water<sup>3</sup>, the value obtained is  $3 \times 10^{-4}$  M, which approximates that of the  $K_i$  observed for several mammalian tissue hexokinases<sup>1,4</sup>.

The present experiments indicate that the hexokinase reaction is completely responsive to a wide range of glucose-6-phosphate levels as had been observed in the erythrocyte by Rose<sup>5</sup>. The present work, where several respiratory inhibitors were used to alter glucose utilization, indicates that product inhibition of the hexokinase reaction is an important factor in glucose utilization in this neoplasm under aerobic and anaerobic conditions, and that the Pasteur effect in this neoplasm is possibly mediated by glucose-6-phosphate inhibition.

However, it is not yet possible to determine whether hexokinase is the major pacemaker in the overall reactions of glycolysis in these cells having a low rate of glucose utilization. The possibility that the relationship between glucose-6-phosphate levels and glucose utilization observed in these experiments is secondary to a change in some intermediate or effect which varies directly with glucose-6-phosphate, as fructose-6-phosphate or phosphofructokinase activity, cannot as yet be ruled out. Regen *et al.*<sup>1,6</sup> have observed that the activity of hexokinase in heart muscle was found to be inversely related to the glucose-6-phosphate levels, which they considered were governed to a large extent by the activity of phosphofructokinase on the basis that, with anoxia, phosphofructokinase activity was increased, as apparent from a lowering of the substrate concentration and an increase in the product concentration associated with a faster glycolytic flow.

In this regard, Hees and Chance<sup>7</sup> observed relatively high concentrations of fructose-1,6-diphosphate (1–3 mM) in ascites tumour cells having a high rate of glucose utilization, a finding which suggests that hexokinase activity does not control the overall reactions of glycolysis

in these cells under the conditions of their experiments. Whatever the means by which the primary control occurs, it is nevertheless necessary that there must also be control over the initial irreversible step of glycolysis since, as pointed out by Lonberg-Holm<sup>13</sup>, failure to exhibit control would lead to endless increases in intracellular phosphates.

It is not anticipated that the relationship between glucose utilization and hexokinase product concentrations will hold under those conditions which greatly alter phosphate concentrations, since it has been observed that inorganic phosphate partially releases the glucose-6-phosphate inhibitions<sup>8,14</sup>. In our earlier investigations, it had also been observed that glucose utilization by way of the hexose monophosphate oxidative shunt pathway in these cells was enhanced by the artificial electron acceptor, phenazine methosulphate, and the addition of higher levels of inorganic phosphate produced a further stimulation of this pathway.

The observation that β-OH butyrate and other DPN-dependent oxidizable substrates inhibit glucose utilization might be explained on the basis that these substrates compete with pyruvate for the respiratory chain. However, the finding that lactate is also inhibited by these substrates suggests that this explanation does not suffice for the decreased glucose utilization. Furthermore, the possibility that all these substrates compete with glucose for entry into the cell also appears unlikely.

The finding that glucose utilization can be regulated by several DPN-dependent oxidizable substrates is of especial interest since it occurs under conditions where respiration is unchanged. It is possible that the changes which occur under these conditions are analogous to those which occur in the operation of the Pasteur effect, and since glucose utilization can be readily manipulated by varying the concentration of the DPN-dependent substrate, an investigation conducted with this convenient system may provide considerable insight into this incompletely understood control mechanism. In particular, it is of interest to investigate the influence of these substrates on changes occurring at the mitochondrial pyridine nucleotide level.

It should also be pointed out that the Crabtree effect is much less pronounced in this neoplasm than that observed in the Ehrlich ascites tumour cells<sup>15</sup>, and that the intracellular concentration of glucose-6-phosphate of the lymphoma (c.  $3 \times 10^{-4}$  M) is considerably lower than that calculated on a similar basis from the data reported by Hees and Chance<sup>7</sup> on the Ehrlich carcinoma, that is,  $1.3 \times 10^{-4}$  M. In view of this correlation and the recent demonstration that respiration of mitochondria isolated from ascites tumour cells is inhibited by glucose-6-phosphate accumulation<sup>16</sup>, it is suggested that the level of glucose-6-phosphate plays a part in the inhibition of respiration produced on glucose addition.

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## NUCLEATING SUBSTANCE(S) IN HUMAN SALIVA

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**F**UNDAMENTAL step in the process of mineralization is the formation of nuclei of crystal growth. In this connexion, the isolation of substances that can induce nucleation is basic to the understanding of calcification in living organisms. As a result of the interest directed toward the calcification of bone, collagen has been identified as a substance capable of inducing the formation of nuclei of hydroxyapatite crystal growth from a metastable solution<sup>1-3</sup>. Mineralization occurs, however, in many areas other than bone, and in some cases minerals other than hydroxyapatite are formed (for example, oral calculi, kidney stones). It is of interest to study these systems, as new calcifying catalysts may be discovered as a result<sup>4</sup>.

We wish to report evidence indicating the presence of a substance(s) in human saliva capable of inducing the formation of nuclei of hydroxyapatite crystal growth.

Saliva preparations which induce calcification have been reported previously<sup>5,11</sup>. This calcification was interpreted as due to nucleation. These investigators, however, did not employ sensitive methods for detecting pre-existing nuclei (seed crystals)<sup>6,12</sup>. One billion nuclei of hydroxyapatite (seed crystals)  $25 \text{ \AA} \times 50 \text{ \AA} \times 100 \text{ \AA}$  weigh only  $4 \times 10^{-4} \text{ \mu g}$  and thus will escape detection by the usual chemical methods, but can be detected by other means recently developed<sup>6,12</sup>. With these new methods of testing for pre-existing nuclei<sup>6,12,13</sup> it appears from the present investigations that the preparations of the foregoing workers<sup>5,11</sup> were already 'nucleated'. In the presence of pre-existing nuclei, calcification represents crystal growth and does not provide rigorous evidence for the existence of an organic compound(s) capable of inducing nucleation. Wasserman *et al.*<sup>14</sup> examined calcification of plaque material *in vitro*. Von Der Fehr and Brudevold<sup>11</sup> criticized this work, suggesting that pre-existing nuclei may be present. In the same paper Von Der Fehr and Brudevold described the preparation of a calcifiable material by alternate dipping and drying of paper disks into saliva. According to the authors, the material contained "minute mineral deposition". Material prepared in our laboratories in a manner similar to that of Von Der Fehr and Brudevold was already 'nucleated' (Tables 1 and 2). In the hope of obtaining nuclei-free material, these investigators<sup>11</sup> have demineralized formaldehyde-treated calcified saliva matrix with EDTA at a pH of 7.0 for 24 h. The organic residue remaining did re-mineralize. However, nuclei for hydroxyapatite crystal growth still remain after decalcification for 24 h. in EDTA at a pH of 7.0 as shown in our laboratories<sup>14</sup>. Moreover, the formaldehyde-treated saliva matrix represents a derivative.

The saliva used in our work was obtained from human donors who chewed paraffin and it was used within 2 h. The saliva with or without treatment was centrifuged at 8,720*g* at low temperature in the Spinco preparative ultracentrifuge. The supernatant fluid was practically bacteria free.

The extracts used in this work were prepared as follows: 20 ml. of centrifuged saliva were each placed in hemispherical troughs and two glass slides were mechanically rotated through each one of these troughs for 3 h at 0.5 r.p.m.<sup>15</sup>. Air was blown past the slides so that, as the slides emerged from the troughs, drying took place. In this manner, a film was deposited at one end of the slide. Such film was practically bacteria free, although it contained considerable bacteria without the centrifuging step. The

film represents a continuous concentration of the solid in air and a partial dissolution of the dried material, the more soluble material being redissolved leaving the more insoluble material on the slide.

The films were placed for 20 h in solutions similar to those which were utilized in our earlier studies for calcification *in vitro* of hypertrophic epiphyseal cartilage and collagen<sup>1,3,6,8</sup>. Thymol was added at a level of 10 mg/l. The calcifying solution contained 70 mM NaCl, 22 mM NaHCO<sub>3</sub>, and 5 mM KCl per litre. Calcium was added as CaCl<sub>2</sub>. Phosphate phosphorus was added as the NaH<sub>2</sub>PO<sub>4</sub>: Na<sub>2</sub>HPO<sub>4</sub> mixture (4:1). The pH was 7.3 and the temperature was 37° C. In some instances, radioactive calcium-45 or phosphorus-32 labelled phosphate or both were added to the solution. The calcified substances were then counted, using a gas flow counter with a high-speed scaler or an ambient type liquid scintillation counter, and the amount of incorporated calcium and phosphorus calculated.

As seen in Table 1, films prepared from centrifuged saliva contained considerable calcium and phosphate, suggesting the presence of nuclei (seed crystals). Evidence that the calcium and phosphate are present in the form of pre-existing nuclei (seed crystals) is given in Table 2. There is considerable calcification after treatment with CuCl<sub>2</sub>, which would inactivate nuclei-free matrix as seen in Table 3. Such films therefore are not suitable for establishing the existence of nucleating substance(s) in saliva.

To prevent the formation of calcium phosphate nuclei (seed crystals), removal of calcium was undertaken. For this purpose, an aliquot of saliva was titrated with disodium EDTA<sup>16</sup> and the calculated amount of EDTA necessary to bind all the calcium was added to another portion of saliva. However, saliva treated in this manner gave no film. The EDTA changed the solubility of the material. The air-dried solid re-dissolved each time the slide was rotated into the saliva. To overcome this difficulty, an amount of oxalic acid equivalent quantitatively to the amount of calcium present was added to saliva. This was followed by centrifugation, which

Table 1. Ca AND P\* CONTENT OF FILMS PREPARED FROM CENTRIFUGED SALIVA

Sample No	Ca and P Incorporated (μg)
1	Ca 69.1
	P 26.4
2	Ca 86.3
	P 38.0
3	Ca 120.0
	P 54.5
4	Ca 124.0
	P 43.6

The total Ca and inorganic phosphate P were determined in the initial saliva. This was followed by the addition of radioactive <sup>45</sup>Ca and <sup>32</sup>P. The Ca and P contents of film were then calculated from the counts in the liquid scintillation counter.

\* Phosphate P.

Table 2. INFLUENCE OF CuCl<sub>2</sub> ON THE CALCIFICATION OF FILMS PREPARED FROM CENTRIFUGED SALIVA

Treatment	Ca and P Incorporated (μg)
Control	Ca 908
	P 734
Control	Ca 556
	P 416
Control	Ca 1,020
	P 722
CuCl <sub>2</sub> (1 mM)	Ca 621
	P 540
CuCl <sub>2</sub> (1 mM)	Ca 357
	P 288
CuCl <sub>2</sub> (1 mM)	Ca 404
	P 357

Films prepared from the supernatant of saliva centrifuged at 8,720*g* (ave.) were placed in 1 mM/l. CuCl<sub>2</sub> solution for 10 min. Controls were placed in H<sub>2</sub>O. After 10 min all slides were rinsed in water, then placed in calcifying solution (15 mg per cent Ca · 5 mg per cent P) to which <sup>45</sup>Ca and <sup>32</sup>P were added. After 20 h the films were removed from the calcifying solution, rinsed and counted, using an ambient type liquid scintillation counter.

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Table 3. THE INFLUENCE OF  $\text{CuCl}_2$  ON THE CALCIFICATION OF 'NUCLEATED' AND 'NON-NUCLEATED' SALIVA FILMS

Treatment	Ca and P incorporated ( $\mu\text{g}$ )
Control	Ca 75.1 P 89.4
Control	Ca 73.8 P 40.8
$\text{CuCl}_2$ (1 mM/l)	Ca 1.66 P 0.739
$\text{CuCl}_2$ (1 mM/l)	Ca 1.50 P 0.691
Nucleated then $\text{CuCl}_2$ (1 mM/l)	Ca 147.0 P 83.1
Nucleated then $\text{CuCl}_2$ (1 mM/l)	Ca 44.2 P 22.0

Controls were placed in water for 10 min, then in labelled calcifying solution for 20 h. Non-nucleated preparations were placed in 1 mM/l  $\text{CuCl}_2$  for 10 min and in labelled calcifying solution for 20 h. Preparations were nucleated by placing them in unlabelled calcifying solution (15 mg per cent Ca, 5 mg per cent P) for 3 h. After nucleation, they were placed in 1 mM/l  $\text{CuCl}_2$  for 10 min and in labelled calcifying solution for 20 h.

removed the calcium oxalate formed and saliva sediment. This procedure removed 99 per cent of the calcium in solution as shown by calcium-45 tracer. Our further investigations were carried out with such calcium-free saliva. Unless treated with inactivating agents, the films mineralized with the formation of apatite as shown by X-ray diffraction. This mineralization can be readily shown visually by a decisive increase in opacity.

Since there was the possibility of traces of calcium oxalate remaining in our preparation, the nucleating properties of calcium oxalate prepared by adding oxalic acid in an amount equivalent to the EDTA titration of a  $\text{CaCl}_2$  solution was investigated. Such calcium oxalate induced nucleation, but this nucleation was not inhibited by  $\text{CuCl}_2$  under conditions which inhibit nucleation of our saliva extract films<sup>11,12</sup> (Table 3). Another indication that a nucleating substance is present in saliva is calcification of a fraction obtained by passing decalcified centrifuged saliva through a 'Sephadex G-25' column. These fractions were obviously calcium oxalate free.

The next question was whether the inorganic phosphate present in the film was responsible for the calcification. By adding phosphorus-32 labelled phosphate to the saliva and establishing the initial inorganic phosphate and determining the phosphorus-32 content of the films of several slides, it was found that the film per slide contained 4.0 micrograms inorganic  $\text{PO}_4$ . After soaking for 10 min in water which preceded our calcification, the organic phosphorus was 1.3  $\mu\text{g}$ . The 30 ml. of calcifying solution which we employed contained 1,670  $\mu\text{g}$  of  $\text{PO}_4$  phosphorus. Thus, it may be concluded: (1) that the amount of  $\text{PO}_4$  present cannot account for inducing the calcification; (2) that most of the  $\text{PO}_4$  dried on the film when in air re-dissolved on re-immersion into the saliva, indicating again that the formation of a film represents a concentration of the less soluble portions of saliva.

Table 3 presents typical replicate investigations of films prepared from calcium-free saliva. The saliva film induces mineralization. Prior treatment with 1 mM  $\text{CuCl}_2$  prevents this mineralization. However, if the slides are exposed to the calcifying solution (15 mg per cent calcium  $\times$  5 mg per cent phosphorus) for 3 h, at which time an increase in calcium phosphate is scarcely detectable, the film continues to mineralize almost to the same degree as the water-treated controls. Thus,  $\text{CuCl}_2$  can be used to distinguish between 'nucleated' and 'non-nucleated' saliva extract film, just as was the case for collagen and hypertrophic epiphyseal cartilage<sup>11,12</sup>.

Table 4 shows that pre-nucleated saliva will continue calcifying at products lower than required for initiating calcification, just as was the case for hypertrophic epiphyseal cartilage and collagen<sup>6,14,15</sup>. This is a further indication that the calcium-free film was not pre-nucleated.

The calcium-free nucleating substance(s) mineralizes at a calcium  $\times$  phosphorus product of 35 as seen in Table 5. Using the same calcifying solutions, this minimum is 60 for acid-soluble collagen, 50 for acid-insoluble collagen and 30 for rachitic epiphyseal cartilage<sup>6,12</sup>. Thus, the nucleating catalyst present in saliva appears to be more

Table 4. CALCIFICATION OF 'NUCLEATED' AND 'NON-NUCLEATED' SALIVA MATRIX

Treatment	Calcium incorporated ( $\mu\text{g}$ )
Placed in low-strength calcifying solution	5.47
Placed in low-strength calcifying solution	1.85
Nucleated then placed in low-strength calcifying solution	202.0
Nucleated then placed in low-strength calcifying solution	248.0

Two preparations were placed in the labelled calcifying solution (12 mg per cent Ca, 2.5 mg per cent P) for 20 h. Two other preparations were nucleated by placing them in unlabelled calcifying solution (15 mg per cent Ca, 5 mg per cent P) for 3 h. After nucleation they were placed in the labelled calcifying solution (12 mg per cent Ca, 2.5 mg per cent P) for 20 h.

Table 5. CALCIFICATION OF SALIVA EXTRACT FILMS AT DIFFERENT CA  $\times$  P PRODUCTS

Calcifying solution (mg % Ca)	(mg % P)	Calcium ( $\mu\text{g}$ )	Phosphorus ( $\mu\text{g}$ )
7	5	31.7	16.9
7	5	103.0	53.0
7	5	2.99	1.48
6	5	1.39	0.64
6	5	1.06	0.42
5	5	0.47	0.13
5	5	1.06	0.39
4	5	0.400	0.082
4	5	0.211	0.053
4	5	0.299	0.099

Slides were placed in water for 10 min and then in labelled calcifying solution for 20 h.

effective than collagen, although not quite as good as the complete system present in the matrix of hypertrophic epiphyseal rachitic cartilage.

It is of interest to note that  $\text{Cu}^{++}$  ion uptake by the saliva extract is maximal at the end of 1 min as shown by radioactive tracer studies. A clue of the nature of copper inhibition with nucleation may be present in the investigations of Malik and Salahuddin with gelatin<sup>17</sup>. They found that copper binds  $\alpha$ -amino groups and carboxyl groups. That both amino and carboxyl groups are involved in nucleation is a reasonable hypothesis and will be explored further.

$\text{ZnCl}_2$  behaves similarly in detecting nucleation, but was not investigated exhaustively.

The saliva extract was analysed for total nitrogen and results indicate the value to be 4.8 per cent. The results of amino-acid analysis are shown in Table 6. The nitrogen percentage, the number of amino-acids and amide links in the infra-red, indicate the presence of high amounts of protein or polypeptide in the preparation.

To obtain some preliminary information concerning the mechanism of nucleation, the saliva extract was placed in  $\text{HNO}_3$  at pH 4.5 at room temperature for varying lengths of time. The nitrous acid was prepared by adding sufficient 1.0 molar HCl to 1 molar  $\text{NaNO}_2$  to reach a pH of 4.5. After the material was removed from the  $\text{HNO}_3$ , it was rinsed and placed in calcifying solution (15 mg per cent calcium; 5 mg per cent phosphorus) at 37° C for 20 h. The nitrous acid inactivated calcifiability in as little as 3 sec (Table 7). The control placed in 1.0 molar NaCl adjusted to pH 4.5 with HCl was far less effective than the nitrous acid solution in inactivating

Table 6. AMINO-ACID COMPOSITION OF CALCIFYING SALIVA EXTRACT

Amino-acid	Moles per 2-mg sample
Aspartic	0.180
Threonine	0.078
Serine	0.143
Proline	0.390
Glutamic	0.206
Glycine	0.255
Alanine	0.068
Valine	0.048
Cystine	0.038
Isoleucine	0.035
Leucine	0.078
Tyrosine	0.100
Phenylalanine	0.045
NH <sub>2</sub>	1.000
Lysine	0.067
Histidine	0.035
Arginine	0.067
	8.795

2 mg of material prepared from oxalate decalcified saliva supernate was scraped off glass slides, placed in 6 N HCl in sealed glass tubes at 110° C for 6 h, dried to remove HCl and analysed on an amino-acid analyser (courtesy of Wm. Shoemaker, Michael Reese Hospital, Chicago).

Table 7. SURVIVAL OF CALCIFIABILITY ON EXPOSURE TO NITROUS ACID WITH NaCl:HCl MIXTURE AS A CONTROL

Time of exposure to nitrous acid	Calcium incorporated ( $\mu$ g)	Time of exposure to NaCl:HCl	Calcium incorporated ( $\mu$ g)
Control	200	Control	240
Control	216	Control	208
3 sec	17.2	3 sec	205
3 sec	12	3 sec	174
5 min	3	5 min	197
5 min	2	5 min	109
10 min	4	10 min	94
10 min	2	10 min	121

Saliva extracts were placed in 1.0 molar  $\text{HNO}_3$  solution at pH 4.5 or in 1.0 molar NaCl solution with 1.0 molar HCl added to bring the pH to 4.5 for varying lengths of time. They were then rinsed in water and placed in calcifying solution (15 mg per cent Ca, 5 mg per cent P) containing  $^{45}\text{Ca}$  for 20 h. Controls were placed in water 10 min and in the calcifying solution 20 h.

calcifiability (Table 7). The results indicate that amino-groups may play a part in nucleation by this material.

Further evidence indicating the involvement of amino-groups is the fact that dinitrofluorobenzene treatment also inhibits calcifiability.

The results indicate the importance of testing for pre-existing nuclei before proposing that a substance is capable of inducing the formation of nuclei. For this purpose, methods for detecting nuclei more decisive than chemical analysis are proposed in this paper. The evidence for the presence of a nucleating substance(s) in human saliva is presented and is proposed as a significant factor in the formation of oral calculi. We hope that the properties of the purified nucleating fraction of saliva will shed further light on the mechanism and structural

aspects required for inducing the formation of nuclei of hydroxyapatite and related substances.

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## ACTION OF THYROID-STIMULATING HORMONE ON RIBONUCLEIC ACID SYNTHESIS IN THYROID SLICES AND IN ISOLATED THYROID NUCLEI

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**P**ROLONGED administration of thyroid-stimulating hormone (TSH) causes an increase in the RNA content of the thyroid gland<sup>1,2</sup>, followed by an increment in DNA content due to cell multiplication. Using calf thyroid tissue slices, Hall<sup>3</sup> showed that TSH increases the uptake of  $^{14}\text{C}$ -formate by the purine bases of the tissue RNA after incubation for 3 h, and attributed this effect to stimulation of synthesis of purine ribonucleotides caused by greater formation of ribose in the presence of TSH. In addition, he observed that  $^{14}\text{C}$ -adenine incorporation into the RNA of thyroid slices was raised by addition of TSH to the medium. We have examined the time-course of  $^{14}\text{C}$ -adenine incorporation into the RNA of thyroid slices during incubation with TSH. These experiments demonstrated an effect on nuclear RNA labelling which was confirmed by incubation of isolated nuclei with  $^{14}\text{C}$ -adenine in the presence of TSH.

Slices of sheep thyroid gland were prepared using the Mollwain chopper<sup>4</sup> and were incubated in Krebs-Ringer phosphate medium<sup>5</sup> pH 7.4 containing 100 mg glucose/100 ml. and an amino-acid mixture<sup>6</sup>, together with 100 units penicillin/ml. to eliminate bacterial growth during incubation. Tissue slices of about 400 mg were suspended in 4 ml. medium and 5  $\mu$ c. 8- $^{14}\text{C}$ -adenine and 0.4 units TSH (Armour 'Thytropar') were added to this volume, to give a final concentration of 0.1 units/ml. Incubation was carried out over a 3-h period in an atmosphere of oxygen, samples being taken at 0.5-h intervals. The slices were then washed with fresh Krebs-Ringer medium to remove free isotope, followed by homogenization in 0.25 M sucrose containing 0.001 M  $\text{MgCl}_2$  and 0.002 M  $\text{CaCl}_2$ . After removal of debris and unbroken cells by centrifugation at 100g for 5 min, the supernatant fluid was layered on to 0.35 M sucrose and a crude nuclear fraction was spun down at 600g for 10 min, the cell cyto-

plasm remaining in the lighter fraction. The nuclei were then purified by spinning through 2.2 M sucrose, as described elsewhere<sup>7</sup>. The RNA contained in the nuclear and cytoplasmic fractions was isolated as described in detail elsewhere<sup>7</sup> by the modified Schmidt-Thannhauser procedure of Fleck and Munro<sup>8</sup>. The nuclear and cytoplasmic fractions were then treated with cold 0.2 N  $\text{HClO}_4$  containing 2 mg/ml.  $^{14}\text{C}$ -adenine and were washed three times with 0.2 N  $\text{HClO}_4$  to remove acid-soluble small molecules. Bovine serum albumin (2 mg per sample) was added to the purified nuclei before cold acid precipitation, in order to ensure an adequate precipitate. After treatment with cold  $\text{HClO}_4$ , the residue of protein and nucleic acids was digested for 1 h in 0.3 N KOH at 37° and the DNA and protein removed by acidifying the digest to 0.2 N with  $\text{HClO}_4$ . The activity of the supernatant fraction was measured in a gas-flow counter and compared with its RNA content by ultra-violet absorption at 260 m $\mu$ . The specific activity of the RNA is expressed as c.p.m./mg RNA.

Fig. 1 shows uptake of  $^{14}\text{C}$ -adenine into nuclear and cytoplasmic RNA at various times of incubation of tissue slices for periods up to 3 h. Addition of TSH at the start of incubation had no effect on RNA incorporation until 1 h had elapsed, when an increase in nuclear RNA uptake commenced; the action of TSH on RNA labelling was not apparent in the cytoplasmic fraction until some 2 h of incubation, a finding compatible with transfer of RNA from nucleus to cytoplasm. The effects of inhibitors of protein synthesis (puromycin) and DNA-primed RNA synthesis (actinomycin D) were examined in this system (Table 1). When puromycin was added at the start of incubation, it reduced uptake of  $^{14}\text{C}$ -adenine into nuclear RNA by 60 per cent, and completely eliminated the stimulant action of TSH. When this inhibitor

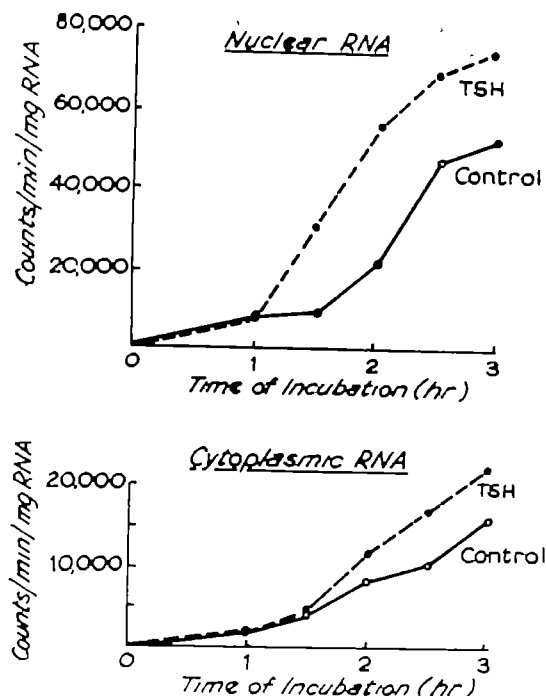


Fig. 1. Uptake of  $^{14}\text{C}$ -adenine by the RNA of thyroid cell nuclei (upper graph) and cytoplasm (lower graph) during *in vitro* incubation of slices of sheep thyroid gland in the presence or absence of thyroid-stimulating hormone (0.1 I.U./ml.). Each point is the mean result of two experiments.

was added after 1 h of incubation, it still reduced  $^{14}\text{C}$ -adenine incorporation, but the action of TSH added at the start of incubation was now fully effective. This suppression of TSH action by treatment with puromycin at the start of incubation suggests that the mechanism involves synthesis of protein during the 1-h period before the increased  $^{14}\text{C}$ -adenine uptake commences (Fig. 1). Addition of actinomycin D at the start of incubation greatly reduced  $^{14}\text{C}$ -adenine incorporation and suppressed the action of TSH (Table 1). When added after 1 h of incubation, the effect of this inhibitor on RNA synthesis was still apparent; a small stimulant action of TSH was, however, obtained.

Table 1. ADENINE UPTAKE BY NUCLEAR RNA DURING *in vitro* INCUBATION OF THYROID SLICES WITH  $^{14}\text{C}$ -ADENINE AND THYROID STIMULATING HORMONE FOR A 3-H PERIOD

Time of adding inhibitor	Control slices	$^{14}\text{C}$ -Adenine uptake (c.m.p./mg RNA) TSH-treated slices	Increase
<b>Puromycin addition.</b>			
None	53,500	75,800	+21,800
At 0 h	21,600	16,800	0
At 1 h	25,400	45,200	+19,800
<b>Actinomycin addition.</b>			
None	14,200	18,200	+4,000
At 0 h	2,300	2,100	0
At 1 h	6,700	7,200	+700

The hormone (0.1 I.U./ml.) was added at the start of incubation. Puromycin (100  $\mu\text{g}/\text{ml}$ . medium) and actinomycin (10  $\mu\text{g}/\text{ml}$ . medium) were added at various times during the course of the 3-h incubation. The figures are the mean results of two experiments with each inhibitor.

Since the stimulant action of TSH was more extensive and occurred at an earlier time in the case of nuclear RNA, we examined the effect of TSH on uptake of  $^{14}\text{C}$ -adenine by isolated thyroid nuclei. Nuclei, prepared from sheep thyroid glands as already described here, were suspended in the nuclear incubation medium described by Allfrey, Mirsky and Osawa<sup>8</sup>, the pH of incubation being 7.1. Thyroid nuclei containing 400  $\mu\text{g}$  DNA were added to 1 ml. medium, which also contained 1  $\mu\text{C}$ .  $^{14}\text{C}$ -adenine. At various times of incubation up to 60 min, the reaction was terminated by plunging the sample tubes into a freezing mixture (ethanol-solid carbon dioxide). They were then treated as described here to extract RNA and to measure its specific activity. Fig. 2

Table 2. EFFECT OF THYROID STIMULATING HORMONE ON INCORPORATION OF DIFFERENT RNA PRECURSORS INTO ISOLATED THYROID NUCLEI INCUBATED IN THE PRESENCE OR ABSENCE OF NUCLEOTIDE TRIPHOSPHATES

Labelled precursor	Addition of triphosphates	Uptake of label into RNA (c.m.p./mg RNA)		
		Control nuclei	TSH-treated nuclei	Difference
$^{14}\text{C}$ -adenine	-	703	1,140	+443 (63%)
$^{14}\text{C}$ -adenine	+	982	1,308	+326 (33%)
$\alpha$ -P-UTP	+	280	473	+193 (69%)

Where indicated, 0.1 I.U./ml. of thyroid stimulating hormone and 0.4  $\mu\text{mole}/\text{ml}$ . of ATP, CTP, GTP and UTP were added to the medium. The  $\alpha$ -P-UTP was chemically synthesised with the label in the  $\alpha$ -phosphorus atom. The figures are the mean results of two experiments, each performed in duplicate.

shows that the uptake of  $^{14}\text{C}$ -adenine by nuclear RNA is sensitive to the presence of TSH in the medium. Increasing levels of TSH resulted in stimulation of RNA incorporation which was roughly proportional to the logarithm of the dose added. Under similar conditions of incubation, uptake of  $^{14}\text{C}$ -adenine by the RNA of isolated liver cell nuclei was not responsive to TSH.

The increased incorporation of  $^{14}\text{C}$ -adenine into the RNA of thyroid slices after addition of TSH has also been observed by Hall<sup>9</sup>, who attributed this effect to more rapid formation of free nucleotides from the base. We therefore incubated thyroid nuclei with UTP labelled with phosphorus-32 in the  $\alpha$ -phosphorus atom; unlabelled ATP, GTP and CTP were also added in equimolar proportions to the medium. Table 2 shows that TSH induced the same increase in uptake of phosphorus-32 from UTP in the presence of the other nucleotide triphosphates as observed when  $^{14}\text{C}$ -adenine was incubated alone. It would thus appear that increased free nucleotide synthesis is not the factor responsible for the stimulant action of TSH on  $^{14}\text{C}$ -adenine uptake into nuclear RNA.

The suppression by puromycin of the action of TSH on RNA synthesis in thyroid slices (Table 1) suggests that the hormone may first affect synthesis of nuclear protein. Purified thyroid nuclei were therefore incubated with  $^{14}\text{C}$ -leucine. The same incubation medium was used, with 1  $\mu\text{C}$ .  $^{14}\text{C}$ -DL-leucine in place of the labelled adenine. At the end of incubation, the samples were treated as for RNA isolation, but the DNA-protein fraction was retained and dissolved in 0.3 N sodium hydroxide for counting and for protein determination<sup>10</sup>. The addition of TSH to the incubation medium had no significant effect on leucine incorporation into mixed nuclear proteins (Fig. 3), although the same dose increased uptake of  $^{14}\text{C}$ -adenine into nuclear RNA by 60 per cent (Fig. 2).

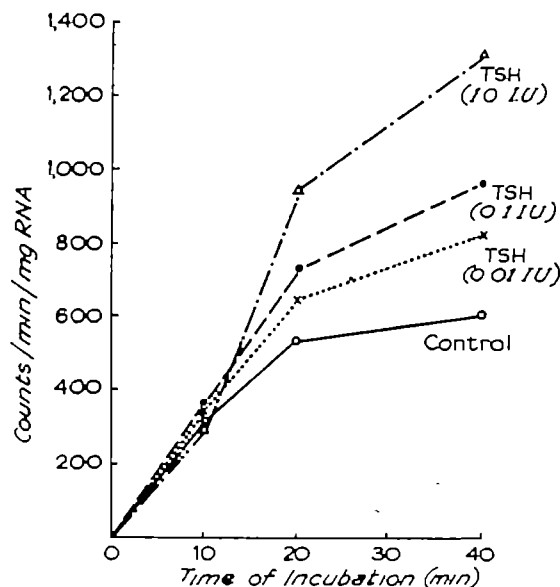


Fig. 2. Uptake of  $^{14}\text{C}$ -adenine by the RNA of thyroid cell nuclei during incubation of isolated nuclei with various concentrations of thyroid stimulating hormone. Each point is the mean result of two experiments.



Table 3. ADENINE UPTAKE BY RNA DURING INCUBATION OF ISOLATED THYROID NUCLEI WITH <sup>14</sup>C-ADENINE AND THYROID-STIMULATING HORMONE

Pre-incuba- tion with inhibitor (min)	Time of incubation (min)	Control nuclei			TSH-treated nuclei			Increase with TSH		
		No inhibitor	Puromycin	Actino- mycin D	No inhibitor	Puromycin	Actino- mycin D	No inhibitor	Puromycin	Actino- mycin D
None	10	400	400	370	890	840	530	+490	+440	+150
	20	1,030	1,240	780	2,900	1,540	1,900	+1,870	+800	+1,120
	30	1,670	1,440	960	3,400	1,650	2,330	+1,730	+210	+1,560
20	10	200	90	200	330	180	200	+130	+40	+25
	20	550	400	200	720	300	230	+170	0	+30

Puromycin (100 µg/ml. medium) and actinomycin D (10 µg/ml. medium) were added either at the time of addition of <sup>14</sup>C-adenine and thyroid stimulating hormone (0.1 I.U./ml.) or 20 min beforehand. Each entry is the mean result from two experiments and is expressed as counts/min/mg RNA.

This finding indicates that under our conditions TSH does not have a general effect on nuclear protein synthesis; however, this does not exclude an action of TSH limited to synthesis of one nuclear protein.

The actions of puromycin and actinomycin on RNA synthesis by isolated thyroid nuclei were investigated under two conditions of incubation. Either inhibitors were added along with <sup>14</sup>C-adenine and TSH, or else the nuclei were incubated with the inhibitors for 20 min before the addition of label and hormone. Table 3 shows that addition of puromycin along with <sup>14</sup>C-adenine did not affect incorporation into the RNA of the control nuclei at any time up to incubation for 30 min; nevertheless, it suppressed the effect of added TSH from 10 min incubation onwards. This confirms the conclusion drawn from the tissue slice experiments (Table 1) that the increased incorporation of <sup>14</sup>C-adenine caused by TSH addition requires preliminary synthesis of protein.

The addition of actinomycin to nuclei at the same time as addition of <sup>14</sup>C-adenine resulted in a depression of adenine uptake throughout incubation. Actinomycin also reduced the increased incorporation of adenine into nuclear RNA caused by addition of TSH, but only in proportion to its effect on adenine uptake by the RNA of the control nuclei. This indicates that actinomycin added to nuclei along with <sup>14</sup>C-adenine and TSH does not affect the synthesis of nuclear protein responsible for the enhancement of adenine uptake by RNA. This lack of effect of actinomycin could be due to a greater rate of penetration and action of TSH added at the same time as the inhibitor. Accordingly, thyroid nuclei were pre-incubated with puromycin or actinomycin for 20 min before the addition of TSH and <sup>14</sup>C-adenine. Table 2 shows that both inhibitors now completely suppressed

the action of TSH on adenine incorporation. These findings suggest that the first effect of TSH on nuclear RNA metabolism is actinomycin-sensitive and thus may involve synthesis of a messenger RNA for a specific nuclear protein. The synthesis of this protein can also be inhibited by puromycin, which interferes with the assembly of amino-acids on this mRNA template. Since this protein is necessary for the stimulant action of TSH on general RNA synthesis, it could be an RNA polymerase or polymerases. The proposed sequence of events following the action of TSH on thyroid nuclei is shown in Fig. 4.

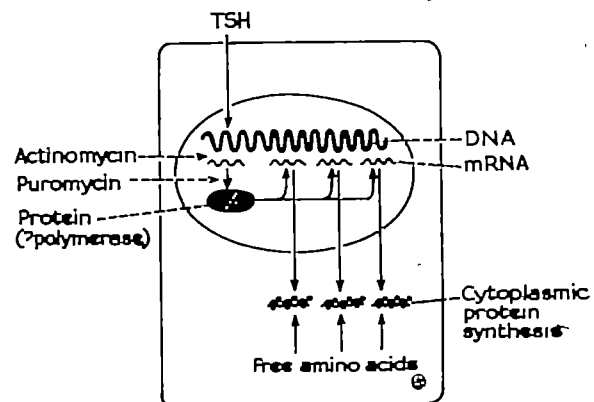


Fig. 4. Diagram showing the mode of action of thyroid stimulating hormone on thyroid nuclear metabolism.

Many effects of TSH on thyroid cell metabolism have been described. These include actions on pyridine nucleotide concentrations<sup>11</sup>, glucose oxidation<sup>12</sup>, sodium uptake<sup>13</sup> and phospholipid turnover<sup>14</sup>, all of which occur within 10 min of adding TSH to thyroid slices. It is not possible at present to relate these reported early actions of TSH on whole cell preparations to the changes we have observed in isolated nuclei.

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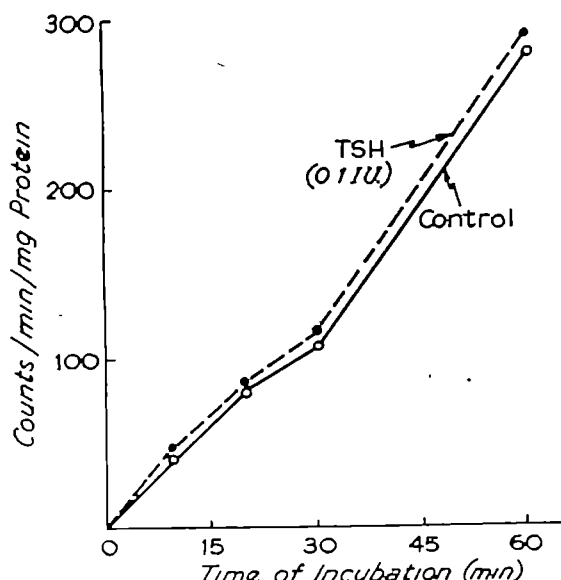


Fig. 3. Uptake of <sup>14</sup>C-leucine by the protein of thyroid cell nuclei during incubation of isolated nuclei with thyroid stimulating hormone at a concentration of 0.1 I.U./ml. Each point is the mean result of two experiments.

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## INDICATIONS OF THE PRESENCE OF ORGANOCHLORINE INSECTICIDES IN RAINWATER IN CENTRAL ENGLAND

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**A**LTHOUGH organochlorine insecticides have been widely used for many years, their exact fate in the environment is still largely unknown. Occasionally, background levels of dieldrin in untreated soil in microplots at Wellesbourne have appeared to increase by amounts too large to be explained solely by variations in sampling and analytical techniques. Appreciable amounts of insecticide are known to be 'lost', presumably into the atmosphere, during spraying operations<sup>1</sup>, and Harris and Lichtenstein considered it evident from their experiments "that volatilization of insecticidal residues from soil is a major factor in their 'disappearance'"<sup>2</sup>. In the spring of 1964, we decided to explore the possibility that insecticides might now be present in the atmosphere and consequently in rainwater at concentrations sufficient to account for apparent residue increases observed in the microplots.

An 8-in. diameter copper rain gauge was set up on short grass at the Agro-Meteorological Station at Wellesbourne on April 1, 1964, and rainwater was collected in a 2.5-l. glass bottle placed inside the body of the rain gauge. The collecting bottle was changed on May 1 and afterwards on the first day of each calendar month so that monthly rainfall samples were obtained during April 1964–February 1965 inclusive. Supplementary samples of rainwater were also collected on occasions during January and March 1965 after rain had begun and it was evident that a prolonged period of rain could be expected. Glass dishes were placed on a wire-mesh grid about 40 cm above the ground in the centre of a grass field at Wellesbourne and, when the rain stopped falling, the water collected in the dishes was emptied into a glass bottle and taken to the laboratory.

Throughout the work scrupulous attention was given to minimizing potential contamination or interference from reagents or other within-laboratory sources. The all-glass apparatus used was washed successively in hot water with detergent, distilled water, acetone and hexane and then heated for several hours at 100°–110° C. It was rinsed again with acetone and hexane prior to use and the purity of the final rinsings was tested by gas/liquid chromatography. Reagent blanks were also processed concurrently with the samples.

The method used to analyse the April–October monthly rainfall samples was of marginal sensitivity, but a simpler, more sensitive method was developed before the later samples were analysed. In the latter method, the rainwater was extracted with hexane and, by maintaining a ratio of 30 ml. hexane to 1 l. of water,  $\gamma$ -BHC, dieldrin and  $p,p'$ -DDT were found to be extracted with an efficiency in excess of 95 per cent. The hexane extract was passed through hexane-washed anhydrous sodium sulphate and concentrated under reduced pressure at 57° C to leave about 0.2 ml., which was subjected to thin-layer chromatography on 'Silica Gel G' (Merck), the adsorbent having first been washed by pre-developing the plates with acetone. The corresponding reagent blank extract and analytical grade specimens of  $\gamma$ -BHC, aldrin, dieldrin and  $p,p'$ -DDT were applied to the same thin-layer plate. After developing with a 4:1 mixture of hexane:benzene, the area occupied by the sample and reagent blank extracts was masked while the plate was being sprayed with silver nitrate/phenoxyethanol reagent before being exposed to ultra-violet light to reveal the reference spots. Adsorbent was removed from the chromatographic axes

of the sample and reagent blank extracts in  $R_f$  positions corresponding to those of  $\gamma$ -BHC, dieldrin and  $p,p'$ -DDT, and it was eluted with a 1:1 mixture of hexane:benzene to give 0.20 ml. extract.

Gas chromatograms of the extracts were obtained using a Shandon FB-4 ionization chromatograph fitted with an electron capture detector operated at 5 V. A stainless steel column, 2 mm internal diam. and 40 cm long, packed with 1.5 per cent w/w of a 6:4:1 mixture of 'E301' silicone elastomer: 'Apiezon L': 'Epikote 1001' resin on 100–120 mesh non-acid-washed 'Chromosorb G' was operated at 157° C with a nitrogen flow of 1.0 ml./s. The injection port temperature was approximately 220° C and 5  $\mu$ l. volumes were usually injected. The quantitative estimates were obtained by comparing the peak heights on the gas chromatograms of the extracts with those given by standards prepared from analytical grade specimens of the insecticides, deducting the value for the appropriate reagent blank and correcting for recovery of the insecticides from the thin-layer plates, found to be 90, 70 and 50 per cent for dieldrin,  $\gamma$ -BHC and  $p,p'$ -DDT, respectively.

All the rainwater samples examined so far have yielded solutes identical in behaviour to  $\gamma$ -BHC and dieldrin in both the thin-layer and gas/liquid chromatographic systems. With the more sensitive method used to analyse the post-October samples, a solute corresponding in behaviour to  $p,p'$ -DDT was also detected. The concentrations of the solutes in the rainwater samples varied from month to month and they appeared to be independent of the total monthly rainfall recorded in a nearby rain gauge. The quantitative results for the April–October and the November–February monthly samples and the direct collections made in January and March 1965 are summarized in Table 1.

Microchemical reactions adapted from those proposed by Hamence<sup>3</sup> were used in an attempt to confirm the identity of the extracted solutes in the post-October samples. None of the solutes was affected by chlorination. Treatment with alcoholic potash removed the apparent  $\gamma$ -BHC peaks from the chromatograms, eliminated the apparent DDT peaks and enhanced the response at the retention time of  $p,p'$ -DDE. It had no observable effect on the apparent dieldrin peaks. Bromination eliminated the apparent dieldrin peaks from the chromatograms, yielding several ill-defined peaks with long retention times. In these microchemical tests, the solutes from the rainwater were indistinguishable in behaviour from comparable nanogram amounts of analytical grade specimens of the insecticides.

An estimated 50 nanograms of apparent  $\gamma$ -BHC was obtained from a sample of rain collected on March 15, 1965, and a simple bioassay test using male *Drosophila*

Table 1. AVERAGE CONCENTRATIONS OF APPARENT ORGANOCHLORINE INSECTICIDES FOUND IN RAINWATER SAMPLES, EXPRESSED AS PARTS PER 10<sup>11</sup> PARTS OF RAINWATER

The ranges of concentrations found are shown in parentheses			
Samples	$\gamma$ -BHC	Dieldrin	$p,p'$ -DDT
Monthly samples			
April–October 1964	97 (77–120)	28 (19–30)	—
November 1964–February 1965	100 (82–164)	20 (10–25)	3 (1–4)
Supplementary samples			
January and March 1965	29 (13–52)*	9 (3–16)*	3 (2–4)†
Reagent blanks	2 (1–3)	3 (1–4)	<0.5

\* Five samples.

† Four samples.

indicated that, within the limitations of the test, the solute from the rainwater was as markedly toxic as analytical grade  $\gamma$ -BHC.

The results of this investigation suggest that the rainwater samples obtained at Wellesbourne contained detectable amounts of  $\gamma$ -BHC, dieldrin and DDT, but, unfortunately, infra-red spectrography could not be used to confirm the identity of the minute amounts of the solutes encountered. Nevertheless, it was clear that the apparent increases in residues in the untreated microplots could not be accounted for by fall-out of insecticide in rain because calculations showed that 1 part of solute in  $10^{12}$  parts of rainwater could deposit only 2.7 mg of solute per acre for a 24-in. annual rainfall. No significant increase in the contamination of agricultural land seems likely to arise from this source, but the presence of organochlorine insecticides in the atmosphere and in rain would aid their dispersion in the environment and might explain the occurrence of residues in unexpected places<sup>4</sup>.

In view of the world-wide use of organochlorine insecticides and the extensive distribution of their residues in soil, together with the foregoing evidence, it is possible that they might now contaminate the atmosphere continuously. We have estimated from data on their usage<sup>5</sup> and rates of loss from soil that residues of aldrin, dieldrin,  $\gamma$ -BHC and DDT amounting to about 1, 8, 2 and 30 tons,

respectively, are probably present in the upper 5 mm of soil of the total cropped acreage in England and Wales, even during the winter period when insecticides are not being extensively used. The emanation of vapours from such extensive superficial residues might give rise to a background level of contamination in the atmosphere to which would be added variable direct contributions from present pesticide applications on both local and possibly global scales.

The samples examined during this work were all collected in a locality in central England and, if the identification of organochlorine insecticides in rainwater is afterwards confirmed, rainwater samples will have to be analysed from many localities before the extent and variations in the degree of atmospheric contamination can be fully assessed.

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## GROWTH OF TRANSPLANTED LEUKAEMIC CELLS IN THYMECTOMIZED MICE

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IT is now clear that the immunological responses of mice thymectomized within 24 h of birth are selectively, not totally, impaired. Such animals may be unable to reject allogeneic skin grafts<sup>1</sup>, immunologically competent lymphoid cells from normal donors with different H2-histocompatibility loci<sup>2,3</sup> or tumour homografts<sup>4</sup>, but their capacity to produce humoral antibodies to certain antigens is unimpaired<sup>5</sup>. In the experiments described here allogeneic (AKR) leukaemic spleen cells were used in preference to skin grafts to test the immunological capabilities of C3H/B<sub>1</sub> mice thymectomized 12 h–25 days after birth. Leukaemic cells grow rapidly, disseminate widely and provoke both humoral antibody and cellular responses<sup>6</sup>. They might, therefore, be expected to stimulate the entire lymphoid system as compared with skin grafts which primarily elicit limited cellular reactions in the local lymph nodes. It is also more practicable to assess fine differences in immunological responsiveness by injecting varying numbers of leukaemic cells than to use multiple skin grafts provided by donors of several strains. Three parameters, age at thymectomy, age at injection and dose of leukaemic cells, were examined. In this investigation only the overall immune responses of the mice were assessed, no distinction being made between the cellular and humoral components.

Thymectomized or intact inbred C3H/B<sub>1</sub> mice were given  $1 \times 10^5$  or  $1 \times 10^6$  AKR leukaemic spleen cells subcutaneously in a volume of 0.1–0.2 ml. Groups of intact inbred AKR mice injected with  $1 \times 10^5$ ,  $1 \times 10^6$  or  $1 \times 10^7$  leukaemic cells were also included for comparison. Animals which did not die of leukaemia were killed for examination at least six weeks after injection. New-born C3H/B<sub>1</sub> mice were thymectomized using anaesthesia produced by cooling<sup>7</sup>, but ether was used for operations on weanlings aged 23–25 days. Wherever practicable, some mice in each litter were left intact. The leukaemic cells used were 117th–170th transplant generations of a lymphatic leukaemia which had originated spontaneously in an 11-month-old female AKR

mouse and had been passaged regularly, since November 1960, in young adult AKR recipients. The inocula were prepared from suspensions of spleen cells in Hanks's solution which were counted and diluted as required in the same medium.

**Response of intact mice to leukaemic cells** (Table 1). Intact AKR mice injected with leukaemic AKR spleen cells at 27–100 days died within 8–16 days. They showed hepatosplenomegaly, gross enlargement of all the lymph nodes and leukaemic infiltration of many other organs, including the thymus. Few tumours were seen at the site of inoculation and then only in mice which had received the smallest dose of one thousand cells. Survival time decreased as the number of leukaemic cells in the inoculum was increased from one thousand to one million, and young mice injected when 28 days old died more quickly than older animals aged 80–100 days.

By contrast, the majority of the intact C3H/B<sub>1</sub> mice rejected the allogeneic leukaemic cells and survived. Even nestlings aged 14–18 days were unaffected by a thousand cells. Some (7/34) animals, less than six weeks old, died of leukaemia when the cell dose was increased, but the older recipients (42–211 days) survived. It is not surprising that a few of the C3H/B<sub>1</sub> mice accepted the allogeneic leukaemic cells since they have the same histocompatibility locus (H2<sup>b</sup>) as the AKR strain.

Table 1. RESPONSE OF INTACT AKR AND C3H/B<sub>1</sub> MICE TO AKR LEUKAEMIC SPLEEN CELLS INJECTED SUBCUTANEOUSLY

Strain	Age at injection (days)	Dose of leukaemic cells	No. mice leukaemic No. mice injected	Mean survival time and range (days)
AKR	27–37	$1 \times 10^4$	13/18	12.0 (12–16)
	27–37	$1 \times 10^5$	8/8	10.1 (10–11)
	27–37	$1 \times 10^6$	8/8	8.1 (8–9)
	28	$1 \times 10^4$	8/8	10.4 (9–11)
	80–100	$1 \times 10^4$	0/12	—
C3H/B <sub>1</sub>	14–18	$1 \times 10^4$	0/6	—
	23–24	$1 \times 10^4$	0/9	—
	35–38	$1 \times 10^4$	2/14	9.4 (8–10)
	27–30	$1 \times 10^5$	5/30	9.8 (8–13)
	36–42	$1 \times 10^5$	0/10	—
	43–49	$1 \times 10^5$	0/15	—
	90–100	$1 \times 10^5$	0/5	—
	184	$1 \times 10^5$	0/6	—
	211	$1 \times 10^5$	0/6	—
	211	$1 \times 10^6$	0/6	—

Table 2. RESPONSE OF THYMECTOMIZED *C3H/Bi* MICE TO  $1 \times 10^6$  *AKR* LEUKAEMIC SPLEEN CELLS INJECTED SUBCUTANEOUSLY

Age at thymectomy (h)	Age at injection (days)	Total No. mice	No. mice leukaemic	Mean survival time and range (days)	No. mice wasting	Mean survival time and range (days)	Mean age at death and range (days)
12-24	35-38	7	2	21.5 (18-25)	5	12.4 (8-10)	48.0 (43-54)
Intact	35-38	9	0	—	—	—	—
12-24	—	20	—	—	20	—	43.5 (30-60)
12-24	16-24	8	4	15.0 (13-18)	4*	27.3 (21-32)	43.3 (39-56)
Intact	16-24	11	0	—	—	—	—
24-48	14-16	6	0	—	—	—	—
Intact	14-16	7	0	—	6*	36.9 (28-55)	53.9 (44-69)
24-48	—	27	—	—	—	—	—
48-72	(a) 52-194	7	0	—	24	—	54.8 (36-89)
Intact	(b) 99-241†	6	0	9.5 (9-12)	1	5.0	190
48-72	(a) 52-194	12	0	—	—	—	—
Intact	(b) 99-241†	12	0	—	—	—	—
48-72	—	28	—	—	16	—	69.8 (41-106)

\* Spleen suspensions from wasting mice passaged—see text.

† Additional dose of  $1 \times 10^6$  leukaemic cells given 47-51 days later.

*Response of thymectomized mice to leukaemic cells.* A number of factors determined the design of these experiments. For example, *C3H/Bi* mice thymectomized 12-48 h after birth develop a wasting syndrome and die prematurely, usually within 12 weeks<sup>2,3,4</sup>, and their immunological responses had, preferably, to be tested before they were six weeks old despite the fact that intact animals of this age were unable completely to resist large numbers of leukaemic cells. Wasting mice can be distinguished from leukaemic mice, *post mortem*, by the normal or small size of their lymphoid organs and, histologically, by a lymphocyte deficit in their tissues and blood, but subsidiary passage experiments were also introduced to check wasting mice for the presence of leukaemic cells. These restrictions did not apply to most mice thymectomized later than 48 h *post partum* because progressive delay in thymectomy minimizes and finally eliminates the wasting syndrome<sup>2,3,4</sup>.

Only 2/7 *C3H/Bi* mice thymectomized within 24 h of birth and none of the intact controls died of leukaemia when injected with one thousand allogeneic leukaemia cells at 35-38 days of age (Table 2). The remainder (5/7) died wasting at the same age (43-54 days) as uninjected thymectomized mice, on average 12.4 days after injection. It was possible that all the thymectomized mice would have afterwards become leukaemic if wasting had not intervened, but it was noted that they wasted at the same time after injection as intact *AKR* mice, given an identical dose of cells, succumbed to leukaemia, namely, 12.0 days. We assumed, therefore, that some of the *C3H/Bi* mice thymectomized on the day of birth had at least offered token resistance to the leukaemic cells.

This assumption was tested by giving the leukaemic inoculum at the much earlier age of 16-24 days (Table 2). In this case 4/8 mice thymectomized within 24 h of birth developed leukaemia 12-18 days after the injection of a thousand cells, but an equal number survived for as long as 21-32 days without becoming leukaemic and wasted at the same age as uninjected thymectomized mice. By delaying thymectomy until 24-48 h the incidence of leukaemia was reduced to nil although all the mice again wasted at the expected time (Table 2).

When wasting was ameliorated by further postponing thymectomy until 48-72 h after birth, none of the seven mice tested when 52-194 days old developed leukaemia. If, however, an additional and greater (one million) number of leukaemic cells was given to six of these animals after an interval of 47-51 days they were unable to make a secondary response and died rapidly of leukaemia 9-12 days later. Intact controls, similarly treated, showed no leukaemic symptoms (Table 2).

Immunological deficiencies in the thymectomized *C3H/Bi* mice became apparent when the number of leukaemic *AKR* cells in the test dose was increased to a million (Table 3). Many more mice (40/50) thymectomized to four days *post partum* died of leukaemia if tested 27-58 days than did intact animals (7/49) of a comparable age. Even mice thymectomized at 50-96 h, showed no wasting symptoms, still died precipi-

tately when the leukaemic inoculum was given as late as five months after the operation. If thymectomy was delayed until weaning, only 5/27 mice developed leukaemia.

Despite the large number of leukaemic cells injected, some of the animals thymectomized less than 24 h or 24-48 h after birth died wasting and were not overtly leukaemic (Table 3). It was evident that 4 of 18 mice thymectomized at 24-48 h wasted at the same time as, or later than, those which showed gross leukaemic infiltration. It seemed that these few mice had either managed to reject the malignant cells or to delay their growth.

*Passage experiments.* Cell suspensions were prepared from the spleens of thymectomized *C3H/Bi* mice which had received a thousand leukaemic cells at 14-16 or 16-24 days and which had been killed wasting but not overtly leukaemic 21-55 days later. These included four mice thymectomized 12-24 h after birth and three animals thymectomized 24-48 h *post partum* (Table 2). Healthy intact litter-mates, similarly treated, were killed at the same time to provide control material.

Concentrated spleen cell suspensions from each donor were then injected subcutaneously into groups of intact two-month-old *AKR* mice. None of the 46 recipients showed any gross or microscopic evidence of leukaemia when they were killed six weeks later, although similar recipients injected with spleen suspensions from three leukaemic thymectomized donors died within one week of passage.

Allogeneic leukaemic cells can be used to test the immunological capabilities of thymectomized mice. The test is stringent but it has the virtues of flexibility, precision and simplicity. The results obtained confirm our previous statements that any assessment of the immunological status of thymectomized mice must take into account the fact that they are capable of some lymphoid development and that their ability to make an immunological response may be determined by the nature and extent of the antigenic stimulus applied and the age at which it is given<sup>2,10</sup>.

The dose of leukaemic cells was the most important of the three parameters examined. If the test inoculum contained only a thousand allogeneic leukaemic spleen cells a considerable number of neonatally (12-72 h) thymectomized *C3H/Bi* mice, like intact controls, were able to marshal their immunological resources and reject

Table 3. RESPONSE OF THYMECTOMIZED *C3H/Bi* MICE TO  $1 \times 10^6$  *AKR* LEUKAEMIC SPLEEN CELLS INJECTED SUBCUTANEOUSLY

Age at thymectomy	Age at injection (days)	Total No. mice	No. mice leukaemic	Mean survival time and range (days)	No. mice wasting	Mean survival time and range (days)
12-24 h	27-30	15	10	10.6 (8-16)	5	7.1 (6-10)
Intact	27-30	14	2	9.4 (8-10)	—	—
24-48 h	42-58	18	14	10.8 (8-13)	4	11.3 (9-17)
Intact	42-49	10	0	—	—	—
50-65 h	35-38	8	7	10.1 (8-13)	1	8.0
Intact	35-42	20	5	9.8 (8-13)	—	—
72-96 h	43-48	9	9	9.7 (8-11)	0	—
Intact	134-147	6	6	10.2 (9-12)	0	—
Intact	134	5	0	—	—	—
23-25 days	34-52	23	5	10.4 (8-15)	0	—
23-25 days	210	4	0	—	0	—
Intact	211	6	0	—	—	—

them. That rejection had occurred was suggested by the passage experiments which showed that no transplantable leukaemic cells were present in thymectomized wasting animals or in their intact litter-mates. When the test dose was small a slight delay in thymectomy of 12-48 h *post partum* enabled the animals to combat the leukaemic cells more effectively. Nevertheless, mice thymectomized 48-72 h after birth were unable to mount a secondary response against a more severe antigenic stimulus.

If the test dose was increased to a million leukaemic cells the majority of the animals, whether thymectomized at 12 h or at 4 days, were unable to react immunologically and died of leukaemia 8-16 days later. The mice even accepted and succumbed precipitately to cells injected 5 months after thymectomy. Only mice thymectomized at weaning and intact controls rejected this number of leukaemic cells and survived. One important fact revealed by the high dosage levels was that mice thymectomized at the comparatively late age of 4 days were immunologically impoverished. Yet such animals, under ordinary laboratory conditions, do not waste but grow normally and live their full life span. Thus, apparent normality may disguise a very real immunological defect.

We consider the ability of neonatally thymectomized C3H/B<sub>6</sub> mice to make a primary response to small numbers

of allogeneic leukaemic cells and their vulnerability to larger doses to be equally striking findings. While no attempt was made to differentiate between cellular and humoral responses to the injected cells, it is possible to speculate that the residual responses of thymectomized mice to small numbers of cells were mediated by cytotoxic antibody and the failure to respond, either primarily or amnestically, to large numbers of cells was due to defective cellular immune mechanisms. This hypothesis must await further experimentation but it must be concluded that an immunological system correctly established by the thymus in very early life is of considerable importance in combating the growth of transplanted malignant cells.

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## STRUCTURE OF THE PIGMENTS OF PRECOOKED IRRADIATED MEATS

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IT has been known for some years that the brown colour of cooked meats is partially converted to red by ionizing radiations<sup>1</sup>. The red colour of pre-cooked irradiated meats has been characterized by Tappel as a denatured globin haemochrome<sup>2</sup>. However, Tappel's work was performed on meats which had received a radiation dose of  $2 \times 10^6$  rads only. Therefore, the character of the pigment in cooked meats treated with various radiation doses is still doubtful.

It has been previously shown that the colour of the iron-porphyrin co-ordination complexes responsible for the colour of meats treated in various ways depends not only on the oxidation state of the iron, but on the electronic configuration of the  $3d$  electrons of the metal ion<sup>3,4</sup>. In turn, the ligands occupying the  $3d$  orbital of the iron directly affect the electronic configuration of the iron<sup>5</sup>, and, thus, the colour of meat<sup>6</sup>. In the case of pre-cooked irradiated meats, both the ligands and the electronic configuration of the iron in the porphyrin co-ordination complex are unknown. In addition, very little can be found in the literature regarding the total cooked meat pigment losses which occur during radiation.

Recent reflectance investigations provided evidence on the electronic configuration of the iron-porphyrins responsible for the colour of cooked and cured meats<sup>7,8</sup>. It is thought that application of the same methods may throw more light in the structure of the pigments of irradiated cooked meats.

The purpose of the work recorded here was, therefore, to provide evidence regarding the identity of the pigments responsible for the colour of the irradiated cooked meats and to evaluate the pigment losses that occur during radiation.

Fresh beef, lamb, veal and pork were purchased at a local market, most of the fat was removed and the meat was minced in a mechanical mincer. After thorough mixing by hand, the meat was packed in No. 301 cans, 175 g of meat per can, sealed under vacuum and cooked

in a retort to an internal temperature of 70° C. Half the cans, packed in dry ice, were shipped to the Australian Atomic Energy Commission radiation facilities at Lucas Heights, New South Wales, where they received doses of ionizing radiation ranging from  $1 \times 10^4$ – $5 \times 10^6$  rads, using a cobalt-60 source. The remaining cans were stored in a freezer, at a temperature of -20° C, to serve as controls. The reflectance spectra of all samples were recorded, after separating the surface layer and mincing the meat again, using a Beckman DK2 recording spectrophotometer with the appropriate attachments, as described in previous publications<sup>9,10</sup>. The total pigment content of the various meats was extracted and estimated according to Hornsey's method<sup>6</sup>.

The reflectance spectra of the irradiated pre-cooked beef, lamb, veal and pork are shown in Figs. 1-4 respec-

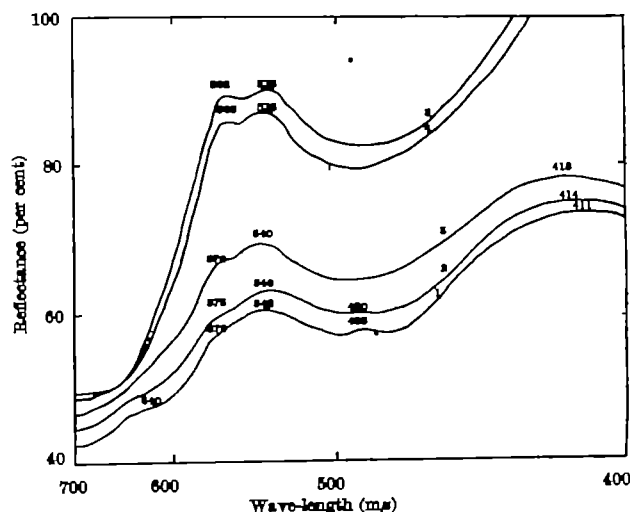


Fig. 1. Reflectance spectra of cooked beef, after irradiation with the following doses (rads): 1, None; 2,  $1 \times 10^4$ ; 3,  $5 \times 10^4$ ; 4,  $2 \times 10^5$ ; 5,  $5 \times 10^6$ .

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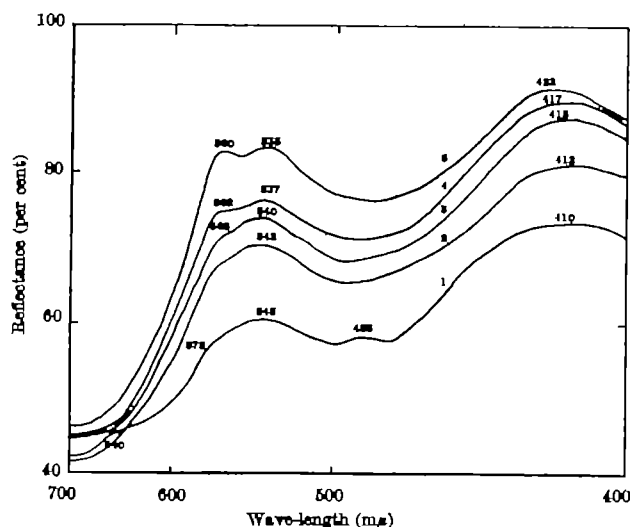


Fig. 2. Reflectance spectra of cooked lamb, after irradiation with the following doses (rads): 1, None; 2,  $1 \times 10^4$ ; 3,  $5 \times 10^4$ ; 4,  $2 \times 10^5$ ; 5,  $5 \times 10^5$ .

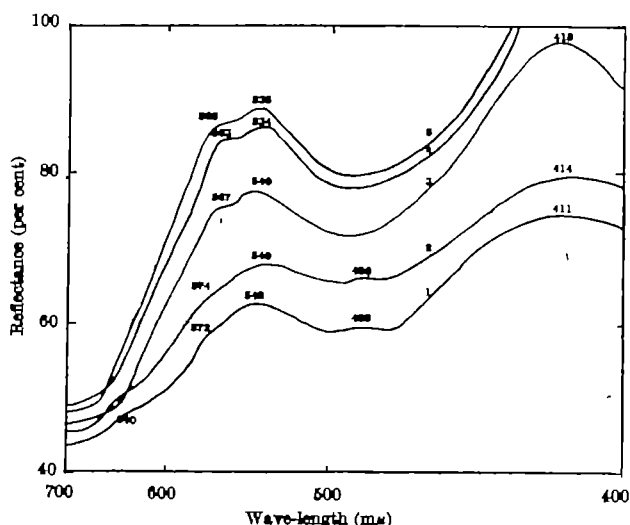


Fig. 3. Reflectance spectra of cooked veal, after irradiation with the following doses (rads): 1, None; 2,  $1 \times 10^4$ ; 3,  $5 \times 10^4$ ; 4,  $2 \times 10^5$ ; 5,  $5 \times 10^5$ .

tively. The spectra of the unirradiated controls are included for comparison.

In general, the reflectance spectra of the various cooked meats and at the same radiation dose-level are very similar. The observed slight differences are to be expected, due to variations in pigment content.

The intensity of all spectra increases with radiation, while the ratio of the reflectance maxima of  $a:b$  bands ( $580:540\text{ m}\mu$ ) rises also, tending to reach unity. At the same time, the maxima of both  $a$  and  $b$  bands shift to shorter wave-lengths with radiation, while those of the  $\gamma$ - (Soret,  $400\text{ m}\mu$ ) band move toward longer wave-lengths (Table 1). In addition, and at radiation doses above  $5 \times 10^4$  rads, the charge transfer bands at  $485$  and  $640\text{ m}\mu$  disappear.

Table 2 shows the total pigment content of the unirradiated controls and that after radiation at the various dose-levels. It is interesting that during radiation at

Table 2. TOTAL PIGMENT IN P.P.M. OF HAEMATEX

	Beef	Lamb	Veal	Pork
Non-irradiated	209	202	98	74
Irradiated (rads):				
$1 \times 10^4$	166	162	75	55
$5 \times 10^4$	166	155	75	54
$2 \times 10^5$	154	143	68	50
$5 \times 10^5$	132	127	61	46

least 50 per cent of the total pigment loss occurs at a dose of  $100,000$  rads.

The reported spectra in Figs. 1-4 and values in Tables 1 and 2 represent averages obtained after several measurements on at least 4 different samples from each treatment.

In interpreting the spectra of irradiated cooked meats, one is faced with the inconsistency that the intensity of the spectra is inversely proportional to the total pigment content of the meat. However, it is well known that the absorption or reflectance spectra of the metal co-ordination compounds arise from electronic transitions initiated by absorption of light<sup>7</sup>. It is equally known that the intensity of a spectrum depends solely on the spin state of the metal ion of the co-ordination complex<sup>8,9</sup>; for example, equal amounts of met-, oxy- and haemo-globin possess much different  $E_{\text{max}}$ <sup>10</sup>. The differences are explained because the  $d-d'$  electronic transition of the high-spin methaemoglobin is a forbidden one, in the low-spin oxyhaemoglobin is a partially allowed one, while in the high-spin haemoglobin is an intermediate of those two<sup>7</sup>.

Close examination of the reflectance spectra of the irradiated cooked meats leads to the conclusion that both the spin and the valency state of the iron is affected by ionizing radiations. The increased intensity of the spectra, accompanied by the appearance of two distinct bands ( $a$  and  $b$  bands), clearly indicates that the high-spin ferri-porphyrin compound of the cooked meats is gradually converted by radiation to the low-spin state<sup>8</sup>.

It is further shown by the spectra that the reflectance maxima of both  $a$  and  $b$  bands shift to shorter wave-lengths with radiation, while those of the  $\gamma$ -band move toward longer wave-lengths. This is interpreted as strong evidence that the ferric ion of the metmyochromogen is gradually reduced by radiation to the ferrous form<sup>8,9</sup>. However, the reduction of the iron does not seem to be quantitative, since even at  $5 \times 10^5$  rads the intensity of the  $a$  band tends to be equal to but not higher than that of the  $b$  band. A complete reduction is indicated by an intensity  $a:b$  band ratio much higher than unity<sup>8</sup>.

The fact that both charge transfer bands ( $485-640\text{ m}\mu$ ) of the metmyochromogen disappear with radiation gives additional evidence supporting the conversion of the high-spin ferric to the low-spin ferrous form<sup>8</sup>. It has been

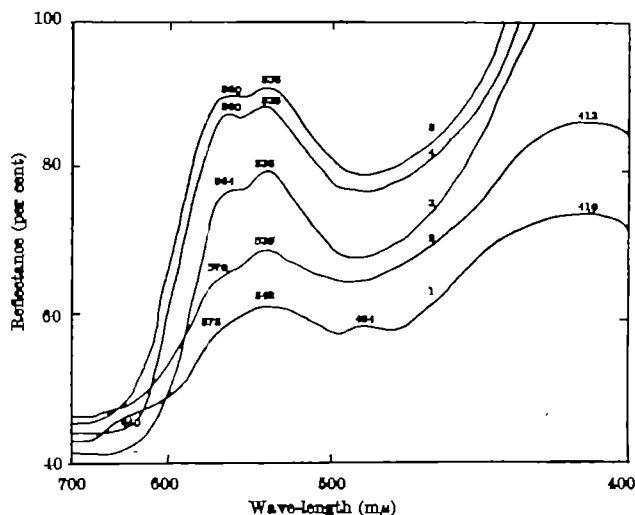


Fig. 4. Reflectance spectra of cooked pork, after irradiation with the following doses (rads): 1, None; 2,  $1 \times 10^4$ ; 3,  $5 \times 10^4$ ; 4,  $2 \times 10^5$ ; 5,  $5 \times 10^5$ .

Table 1. REFLECTANCE MAXIMA OF SORLET ( $\gamma$ ) BAND ( $\text{m}\mu$ )

	Beef	Lamb	Veal	Pork
Non-irradiated	411	410	411	410
Irradiated (rads):				
$1 \times 10^4$	414	412	414	412
$5 \times 10^4$	418	415	418	416
$2 \times 10^5$	420	417	419	421
$5 \times 10^5$	424	422	426	429

generally accepted that the iron of the porphyrins in cooked meats is reduced by ionizing radiation<sup>1</sup>. If reduction was the only effect of radiation on iron, then the high-spin ferric porphyrin compound of cooked meats would have been converted to a high-spin ferrous compound. In this case, the spectrum of such a compound would have shown only one band of medium intensity, located at 545 mμ, and not a two-banded spectrum<sup>2,3,4</sup>.

The spectra of the irradiated cooked meats show that during radiation the spin state of the iron is altered while iron is being reduced. Since the spin state of a metal ion in a co-ordination complex depends on the electro-negativity of the ligands, then it follows that replacement of the ligands of the iron during radiation must precede any changes in spin state. It is, therefore, evident from the presented spectra that the electro-negative ligands of the iron of the high-spin metmyochromogen of the cooked meats are gradually replaced during radiation by electro-positive ligands. Then the latter induce the change in the spin state of the iron.

In view of the foregoing results, the mechanism of colour conversion of the cooked meats by ionizing radiations may be visualized as follows: at low radiation doses, at least one of the ligands of the iron in the metmyochromogen, and most probably water, is dissociated from the complex and is replaced by a less electro-negative group present in the medium. The new ligand is then responsible for the gradual change in the spin state of the iron, from the high- to the low-spin form. At the same time, some of the iron is being reduced. At radiation doses above  $5 \times 10^5$  rads, the dissociation of both ligands of the iron increases, together with their replacement by electro-

positive ones, while more iron is reduced. Thus, at  $5 \times 10^5$  rads, most of the iron is converted to the low-spin ferrous form.

It is thought, therefore, that the compound responsible for the colour of the irradiated cooked meats and at radiation doses up to  $5 \times 10^5$  rads is a mixture of high-spin ferric, high-spin ferrous and low-spin ferric porphyrin co-ordination compounds. At radiation doses above  $5 \times 10^5$  rads, the mixture consists mostly of a low-spin ferrous and a low-spin ferric porphyrin. The contribution to the mixture of the low-spin ferrous form increases with the radiation dose.

Because of the recent interest in radiation pasteurization, it has to be pointed out that cooked meats, subjected to low radiation doses, would be unstable from the lipid oxidation point of view, since most of the pigment would be in the high-spin form<sup>11</sup>. Such oxidative changes, however, may be effectively prevented by the use of suitable antioxidants<sup>12</sup>.

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## ABSENCE OF ANTIBODY PRODUCTION IN THE BURSA OF FABRICIUS

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THE importance of the bursa of Fabricius in the development of humoral immunity in the chicken has become well established since the finding that early bursectomy prevents the production of antibody to a bacterial antigen<sup>1,2</sup>. Furthermore, it has recently been clearly demonstrated that complete bursectomy in the neonatal period, coupled with sub-lethal irradiation, results in both permanent agammaglobulinaemia and absence of germinal follicles and plasma cells from the spleen<sup>3</sup>. However, although the vital role of the bursa in the development of the immunoglobulin-producing system has been established, the precise mechanism by which it exerts its influence has not been fully elucidated and is now the subject of much investigation.

An analogous situation existed with regard to the function of the thymus after it had been demonstrated to be essential for the development of cellular immunity and to a lesser degree for the development of humoral immunity. However, it soon became apparent that the thymus exerts its function in at least two ways. The work of Noessl<sup>4</sup> and Sainte-Marie and Leblond<sup>5</sup> has shown clearly that the thymus can act by distributing lymphoid cells to the peripheral lymphoid organs. The chromosome and immunological marker investigations of Miller<sup>6</sup> and of Dalmaso *et al.*<sup>7</sup>, followed by the diffusion chamber experiments of Levey *et al.*<sup>8</sup> and Osoba and Miller<sup>9</sup>, have established a humoral function for the thymus in mice, and since then evidence for a humoral function in rats has been presented by Aisenberg and Wilkes<sup>10</sup> and MacGillivray *et al.*<sup>11</sup> and in the hamster by Wong *et al.*<sup>12</sup>.

Recently, St. Pierre and Ackerman<sup>13</sup> and Janakovic and Leskowitz<sup>14</sup> have demonstrated that the bursa exerts its effects at least in part by a humoral action. Peterson *et al.*<sup>15</sup> have shown that the bursa of Fabricius is essential to development of lymphoid malignancy in the chicken and more recent investigations indicate that this malignant development is probably dependent on the humoral influence of the bursa<sup>16</sup>. The question of whether the bursa distributes a population of cells with a particular developmental potential remains unanswered.

In other investigations of the role of the thymus in immune mechanisms, Billingham *et al.*<sup>17</sup> and Mathé *et al.*<sup>18</sup> have shown that thymic cell suspensions can exert graft-versus-host reactivity although much less effectively than spleen cell suspensions. It is also known that the reactivity of these cells can be greatly increased by prior immunization *in vivo*<sup>19</sup>. It is thus apparent that the thymus can take part in the expression of cellular immunity as well as being essential for its development. Evidence that bursal cells produce specific antibody *in situ*, as well as being responsible for the development of immunoglobulin production, has been scanty and inconclusive<sup>20</sup>. As part of our investigations of the role of the bursa of Fabricius in humoral immunity, we have found that the bursal cells do not produce specific haemolysins or haemagglutinins to sheep red blood cells.

The technique of Jerne<sup>21</sup> for the detection of haemolysin production by single cells in agar gel and the method described by Zaalberg<sup>22</sup> for the detection of haemagglutinin production by single cells were found to be suitable for this investigation. The former method was used as described by Jerne except that 2 ml. of a 1:1 dilution of fresh-frozen whole chicken plasma was put on

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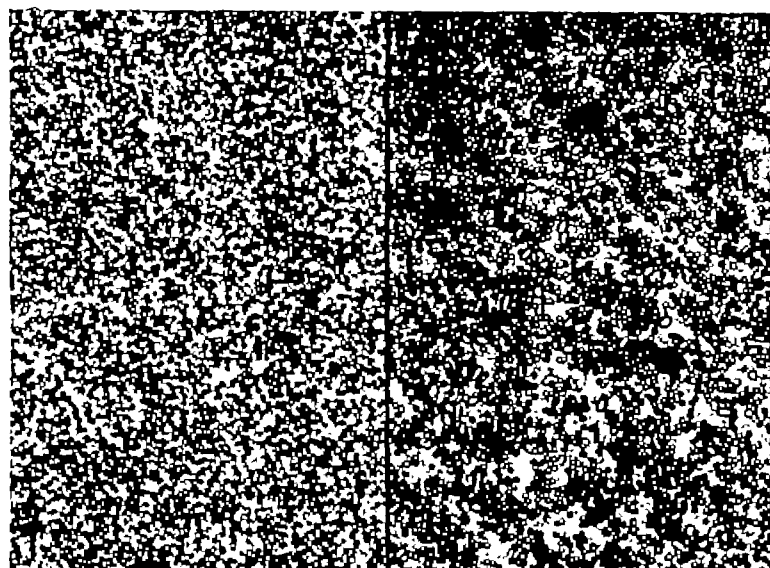


Fig. 1. Left, typical field of bursa cell suspension incubated for 2 hours in 1 per cent suspension of sheep red blood cells ( $\times 4,80$ ); right, typical field of spleen cell suspension incubated for 2 h in 1 per cent suspension of sheep red blood cells showing an abundance of cluster formation ( $\times 4,80$ ).

to the plates for 10 min at  $37^{\circ}\text{C}$  prior to the addition of guinea-pig complement. This was necessary because avian antibody requires the species-specific first component of complement in complement-dependent reactions<sup>14</sup>. For the detection of haemagglutinin-producing cells, approximately  $10^7$  cells were added to a 1 per cent suspension of washed sheep red blood cells in Eagle's basal medium in screw-top 10-ml. test-tubes. These were incubated in a shaker water bath at  $37^{\circ}\text{C}$  for 2 h. The cells producing haemagglutinin were identified by the adherence of sheep red blood cells to them, and these clusters were readily counted (Fig. 1) in a haemocytometer under 100 times magnification.

Five week-old White Leghorn chickens obtained from a commercial hatchery were used in this work. They were immunized three times at three-day intervals with 0.2 ml. of washed packed sheep red blood cells intravenously. Three days after the last injection they were bled and the spleen, bursa and two thymic lobes were removed. A single cell suspension in Eagle's basal medium was made by mincing the tissue with fine scissors and pressing the fragments against the walls of a Potter-Elvehjem homogenizer with the handle of the pestle. The pestle was then placed into the homogenizer and allowed to drop to the bottom by the force of gravity. The overlying fluid was removed and left to stand in the cold for 15 min to allow the larger fragments to sediment out. The cell suspension was then decanted and centrifuged for 10 min at 1,000 r.p.m. at  $4^{\circ}\text{C}$ . The sedimented cells were resuspended in Eagle's medium to give the desired concentration. Viability was determined by the trypan blue dye exclusion method, and cell counts were performed using 2 per cent acetic acid as diluent. As chicken red blood cells are nucleated and are not lysed by acetic acid, the percentage of red blood cells in the spleen cell suspension was determined by doing a differential count and the total cell count was correspondingly adjusted to exclude red blood cells. There were negligible numbers of red blood cells in the thymus and bursa cell suspensions.

The results (Tables 1 and 2) show very clearly that the bursa and thymus cell suspensions contain insignificant numbers of cells producing either haemolysin or haemagglutinin against sheep red blood cells. The few antibody-producing cells found in these organs could be the result of contamination of the organ cell suspensions by antibody-producing cells from the peripheral blood.

The difference between the number of cells producing haemolysin and the number producing haemagglutinin is in accord with Zaalberg's observations in the mouse. Although we realize that the rate of haemagglutinin synthesis may be greater than that of haemolysin synthesis by the individual cells, we feel that the 1,000-fold differences we observed in the number of antibody-producing cells detected by these methods may have been exaggerated for the following reasons. It may be that the figures obtained in the Zaalberg assay are too high, because in some of these clusters one is unable to see the antibody-producing cell (Fig. 2), which, indeed, may not be present—the cells agglutinating simply as a result of liberation of the agglutinin into the medium. On the other hand, the results of the Jerne assay may be too low. The plaques formed in the agar gel by haemolysin-producing cells were quite small and had to be counted under the dissecting microscope at 10 times magnification. We noted at 30 times magnification many very small areas of incomplete haemolysis which may represent cells producing insufficient antibody to form a distinct plaque (Fig. 3) or sub-cellular

particles which have recently been shown to be capable of plaque formation<sup>15</sup>. The value of these techniques in qualitative and semi-quantitative assessment of antibody production by single cells is unquestionable. However, it seems doubtful if their accuracy is adequate for precisely quantitative investigations.

A previous examination of the production of specific antibody by the bursa was made using fluorescent antibody techniques, and it was suggested that bursal cells

Table 1. HAEMAGGLUTININ-PRODUCING CELLS IN THE LYMPHOID ORGANS OF THE CHICKEN

Organ*	Viable cells per culture ( $\times 10^6$ )	Clusters per 0.9 mm.*	Clusters per $10^6$ viable cells
Control chickens			
Spleen <sub>1</sub>	21.5	0	0
Spleen <sub>2</sub>	16.5	0	0
Bursa <sub>1</sub>	9.4	0	0
Bursa <sub>2</sub>	5.1	0	0
Thymus <sub>1</sub>	40.0	0	0
Thymus <sub>2</sub>	25.4	1	50
Immunized chickens			
Spleen <sub>1</sub>	10.9	126	14,102
Spleen <sub>2</sub>	22.5	54	2,372
Spleen <sub>3</sub>	30.0	610	24,766
Bursa <sub>1</sub>	15.4	0	0
Bursa <sub>2</sub>	7.4	0	0
Bursa <sub>3</sub>	12.0	2	22
Thymus <sub>1</sub>	33.8	2	56
Thymus <sub>2</sub>	34.8	0	0
Thymus <sub>3</sub>	43.4	2	56

\* Subscripts refer to the number of the individual chickens.

Table 2. HAEMOLYSIN-PRODUCING CELLS IN THE LYMPHOID ORGANS OF THE CHICKEN

Organ*	Viable cells per culture ( $\times 10^6$ )	Plaques counts per plate†	Plaques counts per $10^6$ viable cells
Control chickens			
Spleen <sub>1</sub>	21.5	0	<1
Spleen <sub>2</sub>	16.5	0	<1
Bursa <sub>1</sub>	9.4	0	<1
Bursa <sub>2</sub>	5.4	0	<1
Thymus <sub>1</sub>	40.0	0	<1
Thymus <sub>2</sub>	25.4	0	<1
Immunized chickens			
Spleen <sub>1</sub>	10.9	54.8	5
Spleen <sub>2</sub>	22.5	941	42
Spleen <sub>3</sub>	30.0	245	8
Bursa <sub>1</sub>	15.4	0	<1
Bursa <sub>2</sub>	7.4	0	<1
Bursa <sub>3</sub>	12.0	1	<1
Thymus <sub>1</sub>	33.8	4	<1
Thymus <sub>2</sub>	34.8	2	<1
Thymus <sub>3</sub>	43.4	2	<1

\* Subscripts refer to the number of the individual chickens.

† Average of 2 or 3 plates.

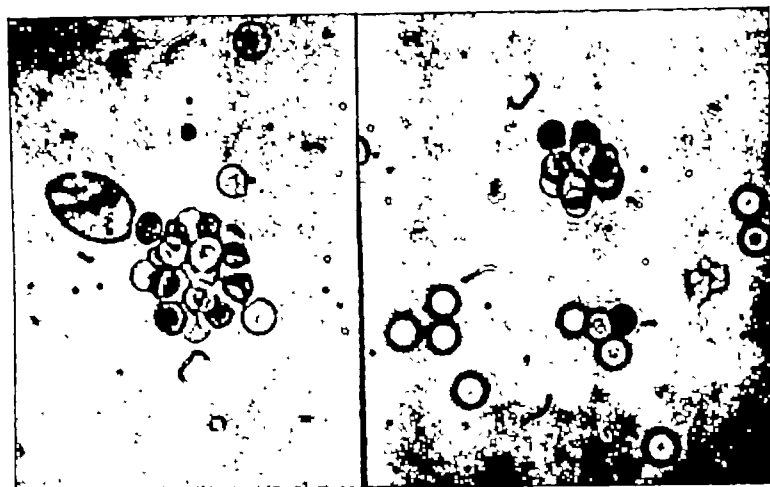


Fig. 2. Left, typical cluster of sheep red blood cells around a central splenic lymphoid cell. The oval-shaped cell is a chicken red blood cell ( $\times c. 660$ ), right, atypical cluster of sheep red blood cells in which the central lymphoid cell is not apparent ( $\times c. 660$ )

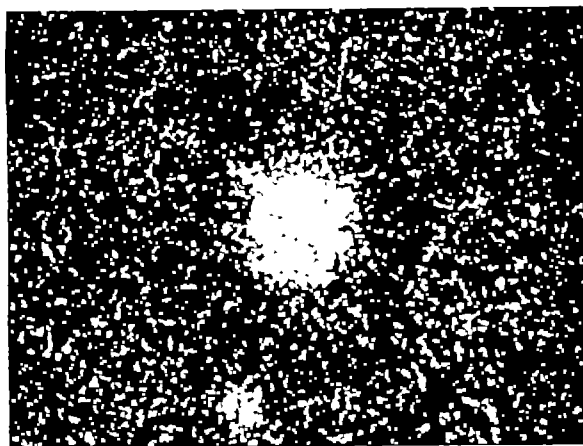


Fig. 3. 50 times magnification of a typical haemolytic plaque and, below it, an area of incomplete haemolysis

did produce specific antibody after immunization with bovine  $\gamma$ -globulin<sup>10</sup>. The validity of this work has been questioned because of the failure to show specificity of the staining. We have shown conclusively that the bursa does not produce antibody to an antigen which elicits a strong antibody response in the cells of the spleen. If it is true that the bursal cells do not produce specific antibody, it now becomes of great importance to discover whether they produce  $\gamma$ -globulin, for if this were so it would provide indirect evidence for the existence of  $\gamma$ -globulin without antibody specificity and would have far-reaching implications regarding the mechanisms of antibody formation<sup>11</sup>. Further investigations along this line are in progress.

The concept of central lymphoid tissues, the prime function of which is to ensure the normal development of immune mechanisms which are, in turn, expressed by the peripheral lymphoid tissues, is a useful one. The notion of the thymus as a source of the lymphocytes in the peripheral lymphoid tissues, originally proposed by Beard<sup>12</sup>, has been modified to take into account the recent developments in the field of immunobiology, particularly the emphasis on hormonal influences and on the analogous 'source tissue' functions of such tissues as the bursa of Fabricius. The experiments reported herein, demonstrating the lack of antibody-producing cells in the bursa of Fabricius, provide further evidence for the distinction between the central and peripheral lymphoid tissues.

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## AN AUTOMATIC DYNAMIC SPECTRUM ANALYSER FOR TAPE-RECORDED SIGNALS

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A DYNAMIC spectrum analyser provides amplitude or intensity as a function of both frequency and time of transient signals such as music, speech, atmospheric 'whistlers', geomagnetic micropulsations, etc. Typically, for such phenomena, one requires a frequency

resolution  $\Delta f$  corresponding to 1 per cent or less of the frequency range of the phenomena and a time resolution limited only by the uncertainty relation  $\Delta f \cdot \Delta t \approx 1$ . Two types of analyser are commercially available. The first uses a bank of several hundred narrow band-pass

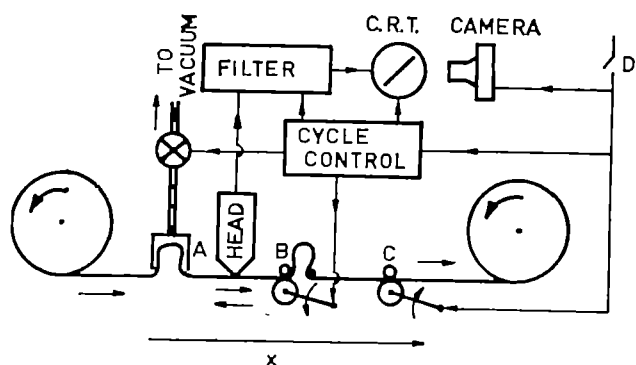


Fig. 1. The complete spectrum analyzer, showing details of the multiple pass system

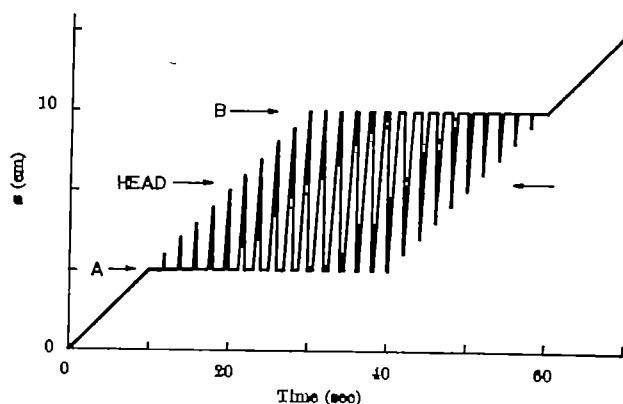


Fig. 2. The locus of a point on the tape as it passes through a 16-pass system. The slopes correspond to the slow capstan, playback and flyback speeds. The  $s$  direction is as shown in Fig. 1

filters. This type has the advantage of 'real-time' analysis but is expensive and inflexible. The second type uses a single narrow band which is tuned (usually by a hetero-

dyne system) over the desired range. However, in order to obtain the maximum available time resolution the signal must be passed several hundred times through the filter. In fact, it is easily shown by the uncertainty principle that the number of passes required is about equal to the number of filters required in a filter bank analyser. Its advantages follow from the use of only one filter. Thus it is relatively inexpensive and highly flexible in that its frequency range and frequency resolution are easily adjusted manually or automatically to follow a pre-set programme. Its obvious disadvantage is related to the large number of passes. Even for high-speed replay, continuous analysis of long signals is very tedious.

Some ingenious methods (for example, ref. 1) have been devised to overcome this, even to the extent of providing 'real-time' analysis. However, in our case the phenomena are already recorded on tape. What we required was an

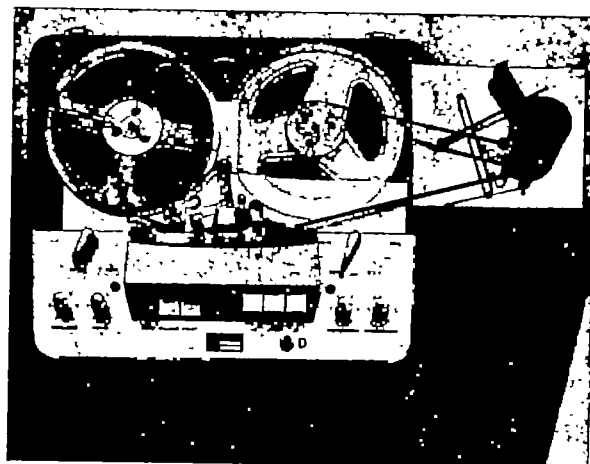


Fig. 3. A photograph of the tape deck showing the modifications. A, B, C and D correspond with the points so marked in Fig. 1. The filter and display are not shown

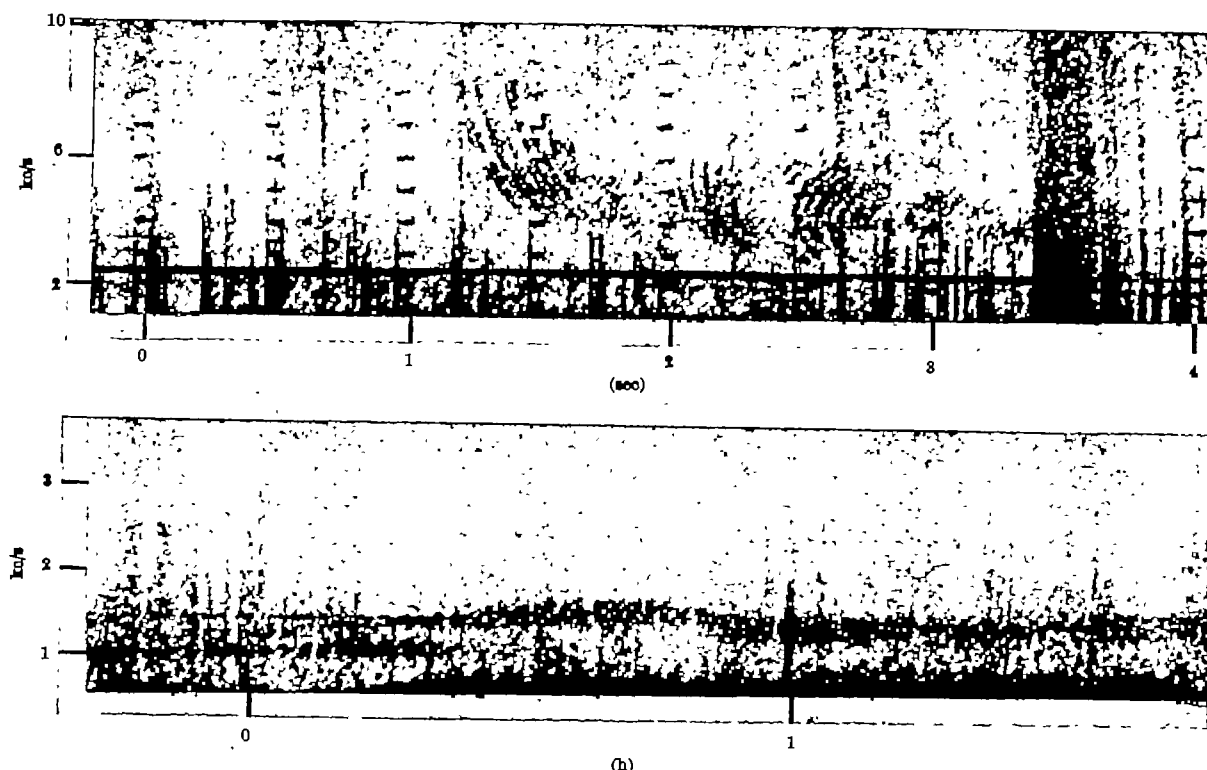


Fig. 4. Continuous spectrograms produced by the analyzer in Fig. 1. The upper one shows a very-low-frequency 'nose whistler' and the lower one a train of 'micropulsation whistlers' (both phenomena were recorded at Hobart)

analyser which would provide continuous analysis of any length of tape completely automatically. A long analysis time, provided the operator's time was not involved, was quite acceptable.

The principle of operation is illustrated in Fig. 1. Between *A* and *B* the tape travels at the normal playback speed (4.75 cm/sec) controlled by the capstan *B*, or very much faster in the reverse direction when vacuum is applied at *A* and the capstan *B* is disengaged. Thus the tape executes an oscillatory 'saw-tooth' motion past the pick-up head. The forward travel is determined by the programmed duration of a pass (since the speed is fixed). The extent of the 'flyback' is determined by the size of the loop at *B*. However, since part of this loop has been pulled out by the slow speed capstan *C* during a pass, the flyback travel is slightly less than the forward travel. Thus the sample of tape passed is changed slightly after each pass. It will be seen that the number of times a given point on the tape passes through the head is equal to the velocity ratio of capstans *B* and *C*. The position  $x$  of such a point as a function of time is shown in Fig. 2, for a capstan velocity ratio of sixteen. A photograph of the tape deck taken during operation is shown in Fig. 3.

A saw-tooth voltage synchronized to the tape motion controls the frequency of the filter and provides the frequency-time base trace for the display cathode ray tube. The detected output of the filter is applied to the cathode-ray tube as intensity modulation. The modulated trace is recorded on continuously moving film. Note, however, that since the analyser scans in both frequency and time, the trace must be inclined at a certain angle relative to the film direction in order to obtain orthogonal fre-

quency and time axes on the resulting spectrogram. The film speed is adjusted for slight overlap of successive traces. The frequency-time aspect ratio is then controlled by the slow capstan (*C*) speed. This is made adjustable so as to provide only the required aspect ratio (and time resolution) with a consequent saving in both film and analysis time.

Examples of dynamic spectrograms made by this analyser are shown in Fig. 4. When referred to the playback speed (4.75 cm/sec) the frequency range in each case was 0–10 kc/s, the filter band width (frequency resolution) 80 c/s, and aspect ratio about 10 kc/s = 1 sec. The real time or record speed values depend on the play-back/record speed ratio, which was very high for the long period phenomena. The capstan velocity ratio (or pass number) was 385 in each case, which was somewhat greater than necessary. Note that the analysis time is less than 'real-time' when the capstan velocity ratio is less than the play-back/record ratio. Note also that tape recordable phenomena in any frequency range can be analysed by suitable choice of the latter ratio.

The method described here provides automatic and continuous spectrum analysis in a relatively simple way. The instrument involved only minor alterations to a standard commercial tape recorder. In fact if the recycling programme is switched off and the slow capstan disengaged it will operate as a 'normal' tape recorder. As an additional refinement our slow capstan is engaged by a solenoid. Thus at any time during 'normal' operation for aural monitoring, analysis can be started immediately by pressing switch *D* shown in Figs. 1 and 3.

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## MULTIPLE TRANSVERSE BREAKAGE OF THE FILAMENTOUS PARTICLES OF TURNIP-MOSAIC VIRUS BY ULTRASONIC VIBRATION

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ATTEMPTS have been made by some workers to use the Ouchterlony agar diffusion precipitin test<sup>1</sup> for serological work on rod and filamentous-shaped viruses. When the length of the virus was comparatively short, as in the case of tobacco mosaic<sup>2</sup> (300 mμ) and barley stripe mosaic virus<sup>3</sup> (126 mμ), successful results have been obtained; but viruses with long filamentous particles appear to diffuse so slowly that satisfactory precipitation lines are rarely formed. Thus Ford<sup>4</sup>, working with clover yellow mosaic virus (539 mμ), obtained sharp precipitation lines only after 1–2 weeks. Purcifull and Shepherd<sup>5</sup>, however, were able to make gel-diffusion tests with four filamentous viruses by chemical degradation of the rods into protein fragments using ethanolamine and carbonate buffers. In some instances, however, the chemical treatments produced several serologically active components resulting in two or more precipitation lines.

In the investigation recorded here the principle of producing small, faster-diffusing fragments of a long filamentous virus particle was also used; but the method chosen was to attempt to fracture the particles by ultrasonic treatment. The use of this method to disrupt the particles of tobacco mosaic virus has been discussed by Bawden<sup>6</sup>. An isolate of the turnip mosaic virus originally obtained from rhubarb (*Rheum rhabontium* L.) was used in the test reported here. It was cultured in the leaves of mustard (*Brassica juncea* Cass.) and infected leaves were homogenized at 2° C in 0.5 M sodium borate

buffer (pH 7.5) containing 0.1 per cent thioglycolic acid. The virus was afterwards purified from the filtered homogenate by the borate-butanol method of Tomlinson<sup>7</sup>. The preparations were infective at dilutions of 10<sup>-4</sup> and they contained filamentous particles, most of which were approximately 720 mμ in length. Rabbit antiserum of the virus, obtained by a series of intravenous injections with purified virus preparations, reacted positively in precipitin tube tests with purified virus diluted in normal saline (titre 1/1024). No comparable reactions were obtained between the virus preparations and normal serum or between the antiserum and clarified healthy mustard leaf sap.

A purified preparation of the virus was treated for various times in a 500-W, 19–25-kc/s M.S.E. ultrasonic cell disintegrator. 5.0 ml. of the preparation in 0.01 M borate buffer (pH 7.5) was placed in a thick-walled, round bottom glass tube (3.2 cm internal diameter) which was cooled in ice water. Using the method described by Hughes<sup>8</sup> a titanium probe (stepdown from 5.8 cm to 2.0 cm) was set with its tip just below the surface and driven at power setting 4, which is just above the cavitation threshold of 0.7 atm/cm<sup>2</sup> for 12 min. 0.5 ml. aliquots were removed from the solution after 1, 3, 6 and 12 min treatment and the remaining solution was treated for 2 min at power-level 6 which is just sufficient to avoid cavitation unloading.

Preparations of the virus before and after ultrasonic treatment were negatively stained with a 2 per cent (w/v)

neutralized solution of phosphotungstic acid and mounted on to 'Formvar' coated grids for examination with a Siemens Elmiskop I electron microscope. Electron micrographs showed that the untreated preparations contained the long, flexuous filaments of the virus (Fig. 1). Ultrasonically treated preparations, however, contained predominantly much shorter particles (Fig. 2). The normal length of the negatively stained particles before the ultrasonic treatment was 722 m $\mu$  and the arithmetical mean length of the treated particles was 121 m $\mu$ . The length distribution of the rod-shaped particles in the untreated and ultrasonically treated virus preparations is shown in Fig. 3. There was little or no aggregation of virus particles in any of the virus preparations examined and preliminary observations have suggested that more of the surface detail structure of the virus was visible after the ultrasonic treatment.

Agar diffusion tests were made in 9-cm glass Petri dishes in 0.75 per cent agar dissolved in 0.9 per cent sodium chloride and 0.04 per cent sodium azide. The antiserum and antigen wells removed from the agar were 5 mm in diameter and spaced 6 mm apart. The pattern of wells used in the tests consisted of a central well containing the antiserum diluted 1/10 in normal saline surrounded by 8 peripheral wells which were filled with the virus preparations. The dishes were incubated at 20° C in a moist chamber.

After 1-2 days single precipitation lines were formed near the antigen wells filled with virus preparations that had received ultrasonic treatment, the intensity of the lines increasing with increasing treatment. By contrast no precipitation line was formed near the wells filled with either the untreated virus preparation or clarified mustard

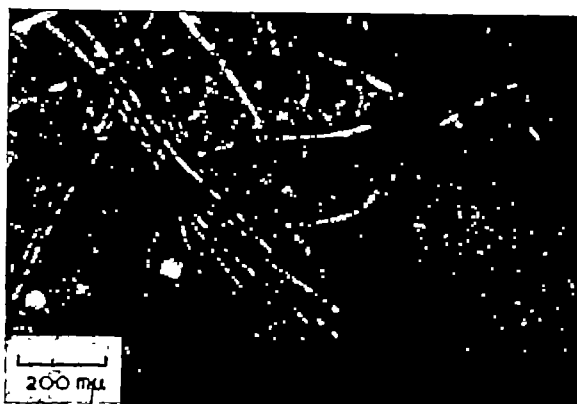


Fig. 1. Electron micrograph of an untreated, partially purified preparation of turnip mosaic virus showing the mainly long filamentous particles

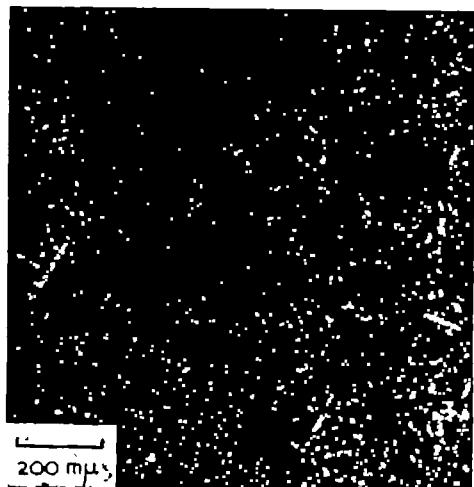


Fig. 2. Electron micrograph of a partially purified preparation of turnip mosaic virus showing the short-lengthed broken virus fragments after ultrasonic treatment

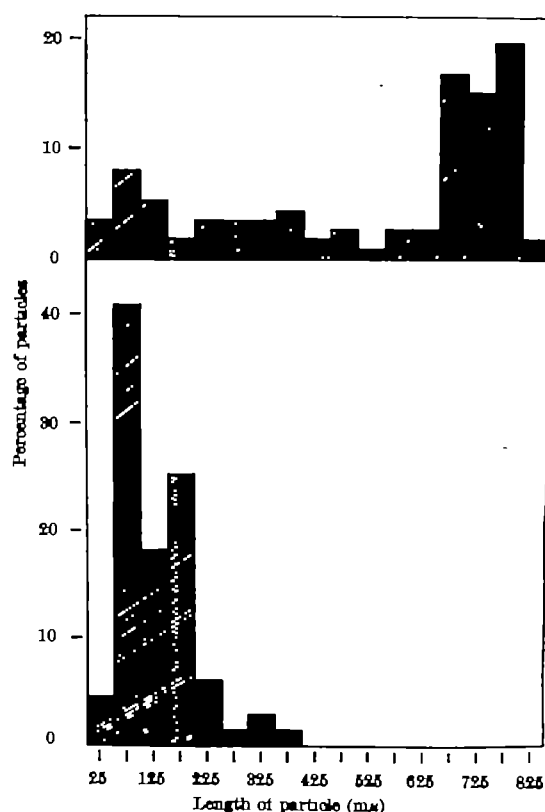


Fig. 3. The length distribution of particles in a preparation of turnip mosaic virus (a) before and (b) after ultrasonic treatment

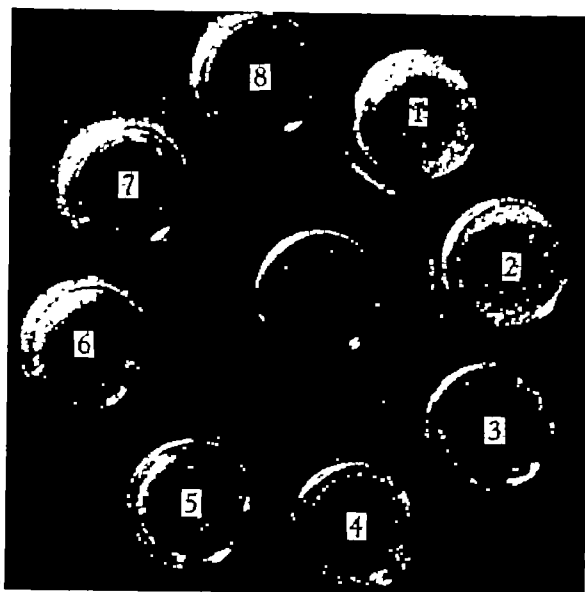


Fig. 4. 36-h agar-diffusion test against turnip mosaic virus antiserum (central well). 1-4, virus treated for 12 min (power 4) plus 2 min (power 6); 5-6, untreated virus; 7-8, clarified mustard sap.

sap. Fig. 4 illustrates the precipitation lines formed after 36 h using virus that had been treated for 12 min at power 4 plus 2 min at power 6. It was concluded that the ultrasonic treatment of the virus particles had produced virus fragments that were still serologically active and which had a rate of diffusion greater than that of the virus particles present in the untreated preparations.

Following this initial experiment the treatments were repeated on a fresh sample of purified virus preparation and the results obtained in the first experiment were confirmed.

It is not known which of the various sizes of particle produced by ultrasonic treatment constituted the principal antigenic component, as the lower limits of fragmentation were not determined. It was noted, however, that in addition to the clearly discernible virus fragments, the electron micrographs of the ultrasonically treated preparations showed a background matrix of smaller particles. These were difficult to resolve but some were roughly circular and of about the same diameter as the width of the intact virus. The particles of turnip mosaic virus may be similar to those of tobacco mosaic virus or beet yellow mosaic virus<sup>8</sup> and consist of hollow helices composed of protein sub-units. If so, the circular particles seen in

the electron micrographs may be composed of one or more of these helices. Some of the virus may have been broken into even smaller units. These and other aspects of the ultrasonic treatment are now under investigation.

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## SPLEEN AND LYMPH NODE FRACTIONS WITH ANTIBODY ACTIVITY

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**T**HERE is substantial evidence that ribosomal particles are connected with protein synthesis. According to this concept, an unstable fraction of ribonucleic acid (RNA), formed through a deoxyribonucleic acid (DNA)-dependent synthesis<sup>1</sup> and acting as a messenger<sup>2-4</sup>, provides the code for protein synthesis to occur on polyribosomal particles<sup>5-7</sup>. A schematic model of this process has been postulated<sup>8,9</sup>.

Despite what is known about the mechanism of protein synthesis, little is known concerning the molecular aspects of antibody synthesis. Uhr<sup>11</sup> has observed that antibody formation by lymph node fragments cultured *in vitro* is inhibited by actinomycin D. This result has been interpreted to mean that antibody synthesis is DNA-dependent. Owing to the general effects of actinomycin D on protein synthesis, it is still difficult to specify the steps involved in antibody synthesis. For example, we cannot say in what manner and at what point during synthesis immunological specificity is conferred on the antibody. Except in a broad sense, we also do not know the molecular role that the antigen takes. We are also uncertain as to whether a messenger RNA is produced that contains the information for carrying out a complete synthesis.

In order to gain an insight into the process, extracts of spleen and lymph node cells from normal and immunized rabbits were examined by differential and sucrose density gradient centrifugations. The antigen used was luciferase, a highly stable enzyme responsible for the blue luminescence of the marine ostracod crustacean, *Cypridina hilgendorfi*. The enzyme catalyzes the oxidation of luciferin (substrate) by molecular oxygen with the emission of a blue light. The activity of luciferase is inhibited by antibodies ( $\gamma$ -globulin fraction) produced against the enzyme. Properties of this luminescent reaction, including methods for isolating luciferin, for measuring light intensity, and for quantitating luciferase activity, have been described previously<sup>12,13</sup>. Luciferase was isolated by fractional precipitation with ammonium sulphate and acetone, followed by gradient elution chromatography on diethylaminoethyl cellulose<sup>14</sup>. Rabbits were immunized with luciferase dissolved in isotonic saline (3 mg/ml.) and clarified at 105,000g for 1 h in a Spinco model L ultracentrifuge before use. In all cases, centrifugation yielded virtually no sediment. Subcutaneous injections were given above each shoulder according to the following daily schedule: 1st and 2nd day, 1.5 mg; 3rd-5th day, 3 mg; 6th-15th day, 0 mg; 16th-19th day, 3 mg. On the 20th day, when the level of anti-luciferase activity in serum approached a maximum, the animal was anaesthetized with ether, bled by heart puncture (blood later used to prepare antiserum), and killed.

The organs were immediately weighed and placed in cold Ringer's solution, containing 0.0056 M glucose.

Unless otherwise specified, all subsequent manipulations were carried out between 0°-4° C. The organs were cut into small pieces with a pair of scissors, placed in 30 ml. of a solution containing 0.14 M NaCl, 0.005 M KCl, and 0.0015 M MgCl<sub>2</sub> (solution 1), and ground by hand in a loosely fitting Potter-Elvehjem grinder to obtain a single-cell suspension (Fraction 1). Fraction 1 was then centrifuged for 10 min at 11,000g in a Servall RC-2 refrigerated centrifuge, to yield a supernatant (Fraction 2) and precipitate. The precipitate was washed again in 30 ml. of solution 1 and centrifuged at 11,000g. The supernatant (Fraction 3) was decanted and the precipitate was suspended in 25 ml. of 0.88 M sucrose containing 0.01 M *tris* (hydroxymethyl) aminomethane hydrochloride buffer, pH 7.4, 0.01 M KCl, and 0.0015 M MgCl<sub>2</sub> (Fraction 4). Fraction 4 was exposed to ultrasound between 2°-4° C in a Raytheon 50-W, 9-ko/s magnetostriction oscillator, model S-102A. Sound was applied as follows: 3 min, 2 min off, and 3 min. Cellular disruption was verified by microscopic examination. The treated suspension (Fraction 5) was diluted with a solution containing 0.01 M *tris*-hydrochloride buffer, pH 7.4, 0.01 M KCl, and 0.0015 M MgCl<sub>2</sub> (solution 2) to give 0.25 M sucrose (Fraction 6, final volume 88 ml.). After centrifuging at 20,000g for 10 min, the supernatant (Fraction 7) was decanted and the precipitate was re-suspended in 7 ml. of solution 2 containing 0.25 M sucrose (Fraction 8). The latter suspension was centrifuged at 780g for 10 min to remove nuclei, cell debris and possibly unbroken cells (Fraction 8B). The resulting supernatant (Fraction 8A) was then ground by hand in a closely fitting Potter-Elvehjem grinder to give Fraction 8AA. Fraction 7 (88 ml.) was further centrifuged at 105,000g for 1 h to give a supernatant (Fraction 9) and a microsomal precipitate<sup>15</sup> which was suspended in 5 ml. of solution 2 containing 0.25 M sucrose (Fraction 10). All Fractions were immediately frozen and stored at -15° C.

The Fractions were analysed in linear sucrose gradients prepared from 15 and 30 per cent, w/w, sucrose solutions containing 0.01 M *tris*-hydrochloride buffer, pH 7.4, 0.01 M KCl, and 0.0015 M MgCl<sub>2</sub> (refs. 16 and 8). Gradients were prepared in Spinco SW 25 tubes, 34 ml. capacity. 1 ml. of the suspension was layered over the surface and run in triplicate at 55,000g for 2 h. The gradient tubes were then immersed in ice-water and a fine stainless-steel needle, controlled by a rack and pinion arrangement, was lowered to the bottom of each tube. Fifty-one equal fractions were collected using a finger pump and fraction collector. From this point on, all manipulations were carried out at room temperature. Any pellet remaining at the bottom was taken up for assay in 0.61 ml. of 0.25 M sucrose. Control experiments showed that even with a highly active antibody fraction at the top, the procedure

did not significantly contaminate the pellet. All gradient fractions were stored at  $-15^{\circ}\text{C}$  for approximately 1 week before assay.

The absorbancy of each fraction was read against water at 280 m $\mu$  (antiseraum fractions were additionally read at 280 m $\mu$ ) in a Beckman DU spectrophotometer equipped with a microcell attachment (Pyrocell Mfg. Co., Westwood, New Jersey). To determine the relative position of gradient fractions from run to run, the refractive indices of selected fractions were read in a single-drop Zeiss model A Abbe refractometer, in sodium light. Sucrose densities calculated from refractive indices of standard mixtures showed the gradient to be linear. The calculated density at the top was 1.0490 g sucrose/cm $^3$  and at the bottom 1.1370 g sucrose/cm $^3$ , both measured at  $20^{\circ}\text{C}$ . The antibody activity in the collected fractions was assayed as follows<sup>12</sup>: To 3 combined gradient fractions, a constant amount of luciferase was added and, after standing for 3 h at  $25^{\circ}\text{C}$ , a saturating concentration of luciferin was added. The initial light intensity, measured with a photomultiplier photometer, was directly proportional to the concentration of unneutralized luciferase present. This value divided by the mean value of all fractions with no apparent antibody activity gave the fraction of initial luciferase activity remaining. The working sensitivity of the method was  $10^{-6}$  of the luciferase concentration used for immunization, with a lower limit of  $10^{-8}$ . Based on the observation that one antibody molecule inactivates a catalytic site<sup>11</sup>, the antibody assay was of the same order of sensitivity. The presence of luciferase was roughly estimated by adding luciferin to 3 combined gradient fractions, as in antibody assay, and measuring the light intensity. Luciferase activity ranged between  $10^{-6}$  and  $10^{-8}$  of the concentration used for immunization.

Table 1. ANTIBODY ACTIVITY OF SPLEEN AND LYMPH NODE FRACTIONS (Expressed arbitrarily as the number of units of luciferase activity neutralized) (Rabbit No. 440)

Fraction No.	Total activity in fraction		Activity/g (wet wt) suspended material	
	Spleen	Lymph node	Spleen	Lymph node
1	84	104	42	116
2	60	92	—	—
3	0	7	—	—
4	40	60	24	118
5	61	92	87	180
6	50	170	—	—
7	28	123	—	—
8	14	17	18	72
9	26	76	—	—
10	6	11	9	28

Table 1 consists of a typical set of data from one of ten rabbits studied. The column at the left shows the total activity of each fraction. The activities of Fraction 1 and 2 may be attributed largely to material released during initial grinding and to residual antiserum. Fraction 3, being a wash, contained negligible activity or none, and additional washings did not release further activity. The activity of Fraction 4 was always relatively high, possibly due to activity associated with partially broken cells and to additional activity released on re-suspension in 0.88 M sucrose. A pronounced increase in activity occurred after exposure to sound, as seen in Fraction 5. Dilution of Fraction 5 by a factor of approximately 3.5 caused lymph node activity to be almost doubled and spleen activity to be moderately reduced. This relative increase in activity occurred with practically every rabbit studied, indicating further release or exposure of new activity. Of five rabbits examined, Fractions 8, 8A, 8AA and 8B all showed activity, the highest total activity being found in Fraction 8B. For both organs, the gradient patterns showed antibody activity near the top in Fraction 8A, but in the lower regions in several separate bands in Fraction 8AA. The sum of the activities of Fraction 9 and 10 was not always found to add up to Fraction 7. Preliminary observations have indicated that the loss of

activity came about from both storage at  $-15^{\circ}\text{C}$  and particle aggregation similar to that observed by Gull-ohriest and Bock<sup>13</sup> for ribonucleoprotein particles in 3-30 per cent sucrose.

The column on the right in Table 1 contains specific activities for which wet weights of suspended material were readily determinable. Wet weights were determined directly in polypropylene centrifuge tubes, after the tubes were decanted and wiped dry. Owing to the large volume of Fraction 7 and to the tube capacity being 8.3 ml. for the Spinco No. 50 rotor, Fraction 7 was divided into approximately 10 equal volumes for the centrifugation. The wet weight of Fraction 10 was obtained by summing the 10 wet weights (making due allowance for the aliquot removed for assay). Control experiments were run as follows: Fraction 5 was exposed to sound, but instead of being diluted to 88 ml., the suspension was centrifuged at 20,000g for 10 min to obtain Fraction 7 (supernatant). Fraction 7 was then centrifuged at 105,000g for 1 h and the pellet was weighed directly in the tube. The wet weights of 'Fraction 10' obtained for 3 different spleens and 3 sets of lymph nodes averaged 14 times smaller than those obtained by the foregoing method. The specific activities of Fraction 10 are therefore undoubtedly too low. They would become the highest, however, if multiplied by any factor greater than 7.

While it might seem attractive to attribute antibody activity to microsomal particles, the results recorded here do not justify such a conclusion. Owing to inhomogeneity of Fraction 10, it would be equally reasonable, for example, to attribute the activity to a particle involved in antibody storage. In order to obtain further understanding, sucrose density gradient analyses were carried out on antiserum and Fractions 2, 7, 9 and 10. Fig. 1 represents the gradient data on antiserum. For all figures the bottom of gradient is shown at the left; antibody activity is represented by a bar-graph (for 3 combined gradient fractions); and pres-

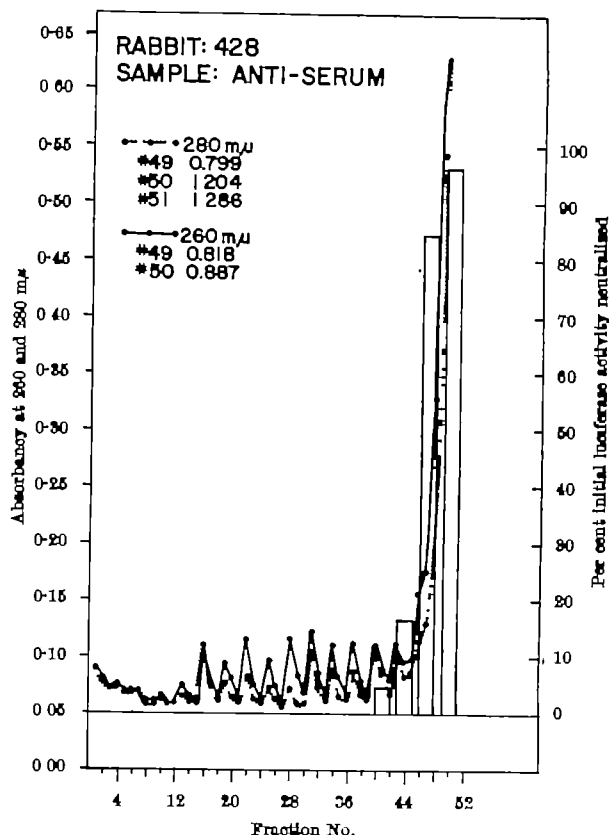


Fig 1 Sucrose density gradient pattern for antiserum, diluted 1:20 with solution 2, 1.0 ml. layered



ence of luciferase is indicated by a horizontal line (below antibody baseline). Fig. 1 shows absence of antibody activity from all fractions except near the top (Fractions 40-51). Luciferase activity was also absent and the pellet showed neither antibody nor luciferase activities. Because of similarities in the gradient patterns, only the spleen pattern for Fraction 2 is presented (Fig. 2). The absorbency curve for lymph node was similar to that of spleen, with a peak at fraction 44. With both organs, the highest antibody activity was found near the top of the gradient (fractions 46-48 and 49-51), being highest in fractions 49-51. Spleen showed some antibody activity in fractions 4-6, 7-9, and 10-12, but lymph node showed only slight activity in this area. Fractions 37-39 of lymph node also possessed additional activity, equal to about one-eighth the total activity in fractions 49-51. When tested with luciferin, luciferase activity was detected in spleen fractions 1-3, 4-6, 7-9, 40-42, 43-45, 46-48, and 49-51, and in lymph node fractions 4-6 and 7-9. Antibody and luciferase activities were absent from the pellets of both organs. A rather surprising finding was the detection of both antibody and luciferase activities in the same fractions, as seen in spleen fractions 4-6, 7-9, 46-48 and 49-51.

Figs. 3, 4 and 5 represent sucrose density gradient patterns for lymph node Fractions 7, 9 and 10, respectively, obtained from a single animal. Antibody activity in Fraction 7 (Fig. 3) was found primarily in fractions 46-48 and 49-51, with small amounts being present in fractions 10-12 and 13-15. Luciferase activity was detected in fractions 7-9, 19-21, 22-24 and 25-27. Antibody and luciferase activities were absent from the pellet. Fraction 9 (Fig. 4) had all its antibody activity in fractions 46-48 and 49-51. No luciferase activity was detected in the gradient fractions, and antibody and luciferase activities were absent from the pellet. As expected, the patterns in Figs. 3 and 4 were similar, since Fraction 9 was the supernatant of Fraction 7. The disappearance of activity from Fraction 7 (fractions 10-12, 13-15 and 46-48) on centrifugation was undoubtedly due to the activity being brought down in

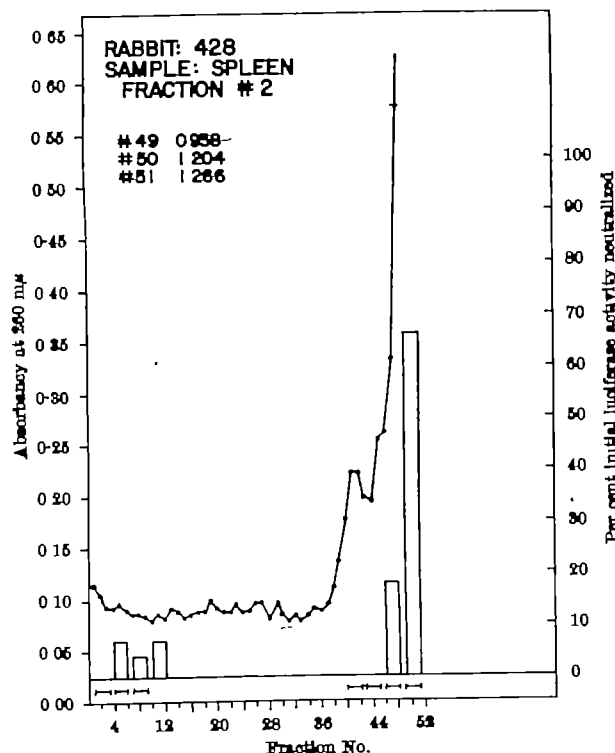


Fig. 2. Sucrose density gradient pattern for spleen Fraction 2

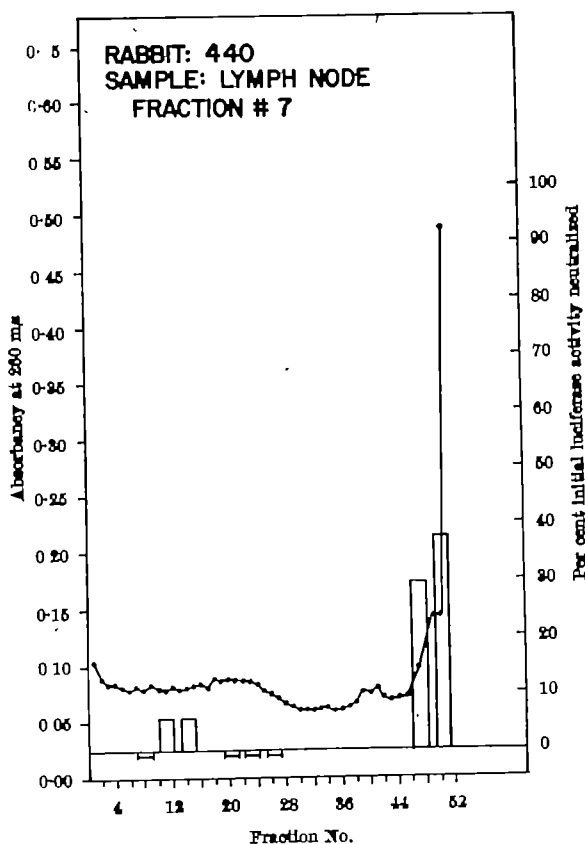


Fig. 3. Sucrose density gradient pattern for lymph node Fraction 7

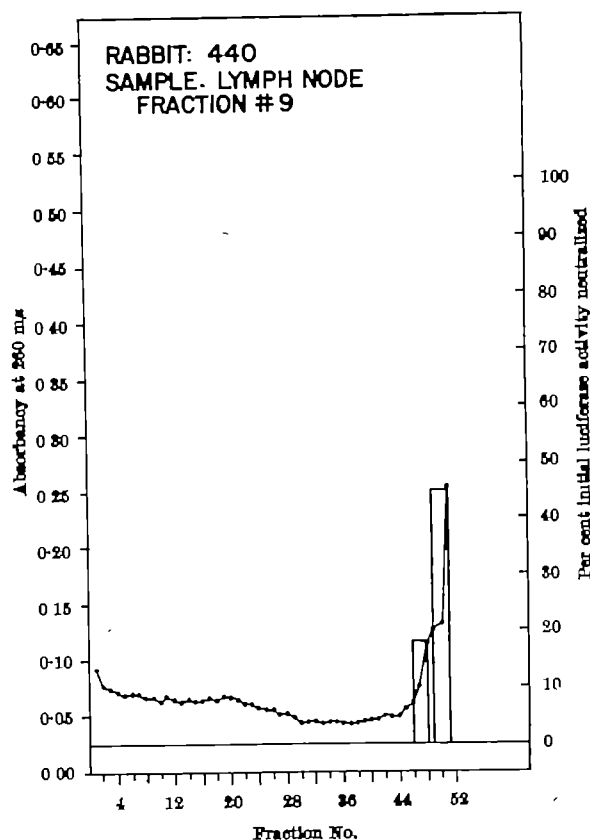


Fig. 4. Sucrose density gradient pattern for lymph node Fraction 9

Fraction 10. The antibody activity of Fraction 10 was concentrated in the upper regions of the gradient (fractions 40-42, 43-45, 46-48 and 49-51). Some antibody activity was present at the bottom, namely, fractions 1-3, 4-6 and 10-12. Luciferase activity was found in fractions 1-8, 4-6, 7-9 and 10-12. Antibody and luciferase activities were absent from the pellet.

The antibody activity of Fraction 10 was always observed near the top of the gradient in both spleen and lymph node. Occasionally, the activity was found in the lower third of the gradient (not shown). Since the gradient analyses were carried out on Fractions frozen prior to use, a series of gradient patterns were also run on fresh material. Fig. 6 shows the results with Fraction 10 of lymph node. The activity was divided between the top and the pellet, suggesting that freezing and thawing either affect the density or cause a release of activity from the microsomal pellet. The absorbancy and antibody activities, however, were determined on fractions stored overnight at  $-15^{\circ}\text{C}$ . No luciferase was detected in either the fractions or the pellet. Differences in absorbancies may also be seen on comparing Figs. 5 and 6. Virtually the same results were obtained with fresh spleen. Evidently, the active material in the pellet is quite labile to re-suspension. The band-width of antibody activity at the top of the gradient for Fraction 10 was also a little wider in about half the patterns examined than diluted antiserum of approximately equal activity.

The finding that antibody activity is associated with the microsomal fraction is not new and has been reported<sup>1-3</sup> by others. More recently, it has been reported<sup>4</sup> that sucrose gradients of crude ribosomal fractions prepared from spleen cells of immunized rabbits show an 80S-120S peak near the meniscus which is absent in unimmunized rabbits. While the evidence clearly indicates the presence of antibody activity in the microsomal fraction, it is still largely presumptive as to the mode of synthesis or identity of the active material. The

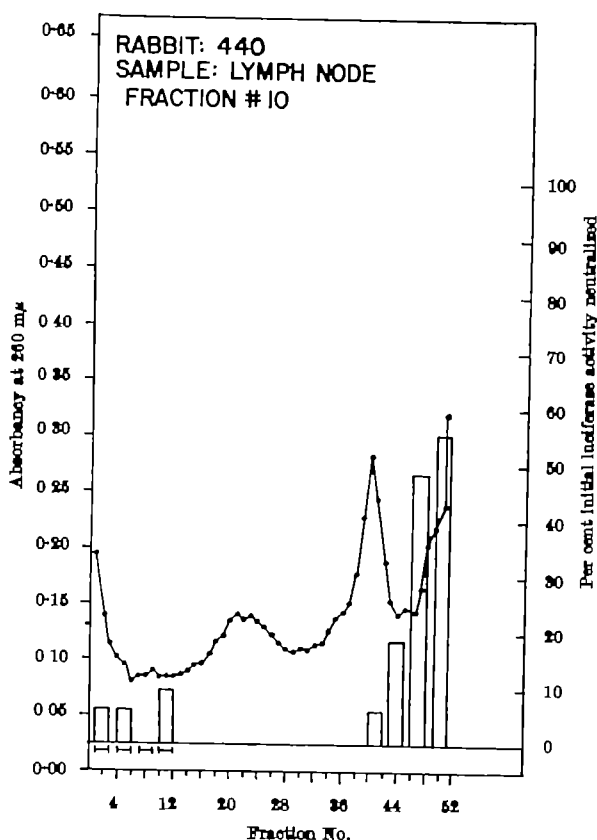


Fig. 5. Sucrose density gradient pattern for lymph node Fraction 10

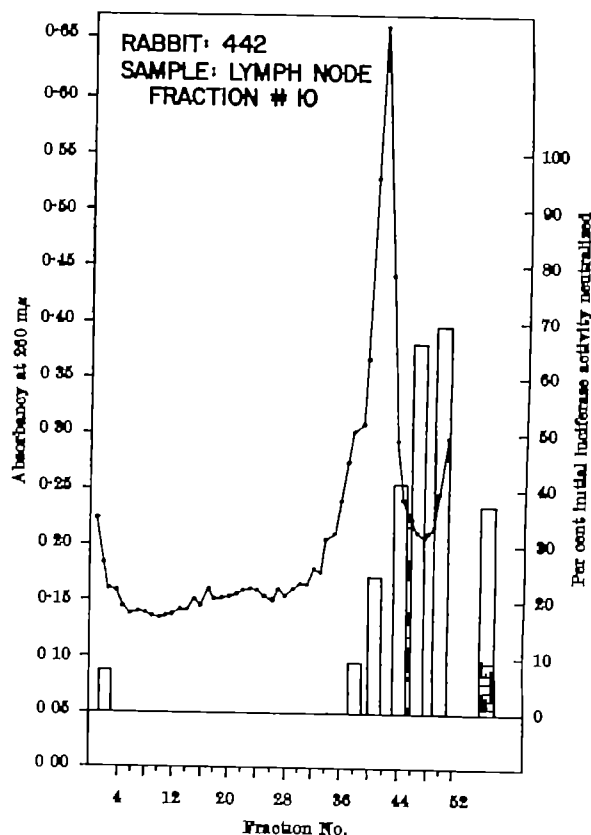


Fig. 6. Sucrose density gradient pattern for unfrozen lymph node Fraction 10

present work suggests the highest specific antibody activity to be in the microsomal fraction. If sonication is not an important factor, it also suggests that a complex distribution pattern of antigenic and antibody activities develops in spleen and lymph node cells in response to immunization.

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# AN IN VIVO CELL SURVIVAL SYSTEM BASED ON THE RECOVERY OF RAT GROWTH CARTILAGE FROM RADIATION INJURY

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THE development by Puck *et al.*<sup>1,2</sup> of a single cell culture technique has provided the basis for extensive *in vitro* studies of mammalian cell survival curves. The work of Hewitt and Wilson<sup>3</sup> and of Till and McCulloch<sup>4</sup> has extended these measurements to *in vivo* systems and in this article a further *in vivo* system will be described which is based on the mode of recovery of the epiphyseal cartilage plate following an acute X-irradiation. A dose of approximately 2,000 rads kills a large proportion of the cells in the cartilage plate, but after 10–15 days the few surviving cells start to divide again and form clones of cells that are readily visible in the matrix (Fig. 1). These clones enlarge and coalesce to repopulate the cartilage plate so that the mechanism of endochondral ossification is restored. This recovery process was first described by Dahl<sup>5</sup> in 1936.

3–4 weeks after irradiation it is possible to count the number of clones that arise from cells surviving a given radiation dose. If it is assumed that each clone arises from a single surviving cell then the fractional cell survival can be calculated from the clone count and an estimate of the number of cells in the original population at risk. It is improbable that clones within cartilage could arise from cells that had migrated in from unirradiated tissues in the rat.

Male Wistar rats of 6 weeks of age were taken for experiment. The animals were not anaesthetized during irradiation but were restrained in moulded plastic holders. Both hind limbs were exposed while the rest of the body was shielded with lead. Irradiations were carried out with

230-kV X-rays (0.5 mm copper + 1.0 mm aluminium filtration, half-value thickness 1.2 mm copper), at about 200 rads per min. The dosimetry was carried out with a Baldwin-Farmer thimble chamber placed in the rat mould in the position of the tibia. A factor of 0.95 was used to convert roentgens to rads. During the exposure the beam was monitored for constancy of dose rate and the animals were rotated on a turntable to average out variations in intensity across the beam.

25 days after irradiation the animals were killed and the tibiae embedded in wax. Sections of 10μ thickness were cut in the sagittal plane and from each bone 10 such sections were taken from a ribbon at 100μ intervals and mounted for counting.

Sections from animals killed at age 6 weeks were used in the calculation of the cell population at risk. Cartilage cells in the growth plate are arranged in columns and earlier studies with tritiated thymidine<sup>6</sup> showed that two-thirds of the cells in each column, that is, all those in the proliferating zone, will incorporate tritiated thymidine. If ability to incorporate thymidine is equated with ability to form clones by repeated division, then the number of cells which are at risk in each column can be measured. The total number of columns in the disk-shaped cartilage plate can be estimated by simple geometry from the count of columns across a diameter of the disk. Using the method outlined here the total cartilage cell population at risk in each epiphyseal plate was estimated at  $5 \times 10^6$  cells with a percentage error of about  $\pm 40$  per cent.

The direct method for counting the total number of clones in an epiphyseal plate is by the intercomparison of serial sections. Since this would involve a detailed survey of about 400 sections per tibia an alternative method was used in which an estimate of the total clone number was derived from measurements on a few sections.

On any given section a clone is seen as a roughly circular disk of which the diameter,  $d$ , can be measured, and the thickness,  $t$ , is known from the microtome setting. This disk is a section cut from a sphere of unknown diameter, but the most probable diameter,  $D$ , of the whole clone-sphere is related to the measured diameter,  $d$ , by the relation:

$$D = 4d/\pi$$

Hence the volume of the disk seen on a section may be expressed as a fraction,  $f$ , of the probable whole clone volume:

$$f = \frac{t \cdot \pi \cdot (d/2)^2}{(4\pi/3) \cdot (2d/\pi)^3}$$

The fractional volume per clone,  $f$ , can be summed for all the clones seen in one section  $\Sigma f$ .

Now on each section the cartilage plate appears as a rectangle of width,  $w$ , height,  $h$ , and thickness,  $t$ , which represents a calculable fraction,  $F$ , of the total volume of the disk-shaped cartilage plate:

$$F = \frac{w \cdot h \cdot t}{\pi \cdot (w/2)^2 \cdot h}$$

provided that the sections are selected so that  $w$  is approximately equal to a diameter of the disk.

The total clone number in the complete cartilage plate is given by the relation  $\Sigma f/F$ .

In practice, measurements were made on ten sections and the value of the total clone number for one tibia was taken as the mean of the ten determinations of  $\Sigma f/F$ .

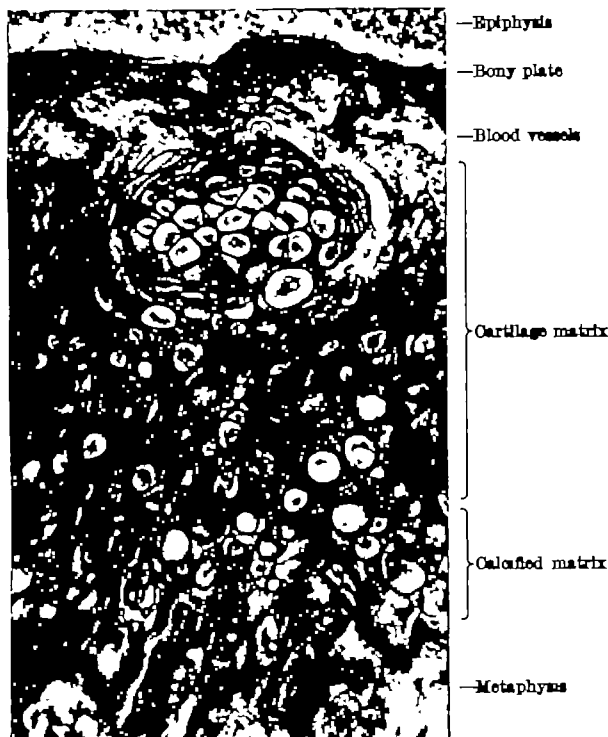


Fig. 1 A recovery clone within the growth cartilage matrix 25 days after 2,000 rads. Longitudinal section of rat tibia, stained haematoxylin and eosin ( $\times 800$ )

One particular difficulty that arises from this method of estimation is the heavy weighting given to small clones. Since the fractional clone volume,  $f$ , varies inversely with the measured diameter of the clone, a small clone contributes proportionally more to the value of  $\Sigma f/F$  than does a large clone. Although this is mathematically correct it introduces large uncertainties into the answer because the smaller a clone is, the more difficult it becomes to recognize as an actively growing group of cells.

Fig. 2 is a semi-logarithmic plot of experimental values for the survival of cartilage plate cells calculated by this method. The points are limited to the dose range of 1,700–2,300 rads since at doses below 1,700 rads there are too many clones to be counted with any accuracy and at doses above 2,300 rads there are too few clones for meaningful analysis. The data are given for experiments on 7 animals at 1,700 rads, 18 at 1,900 rads, 15 at 2,100 rads and 39 animals at 2,300 rads. The standard deviations reveal the wide spread of individual values. Although it cannot be assumed that these points lie on a straight line, in order to make a comparison with other survival data a tentative line has been drawn from an extrapolation number of 6 through the experimental points. The slope of the line,  $D_0 = 190$  rads, is similar to the values obtained from other *in vivo* systems. The value of 6 for the extrapolation number was selected from the results of some split dose experiments of the Elkind and Sutton<sup>6</sup> type.

In theory, this system should be useful for measuring the effect of dose fractionation and experiments were carried out in which one leg of a rat was given a single dose of 2,300 rads while the other leg was given two doses of 1,700 rads and 600 rads with an interval of 24 h between the fractions. The ratio of the clone numbers in the two legs should give the recovery factor for the split dose. Experiments on 6 animals gave a mean value of 6 with individual values varying from 3 to 14. Two other animals had so few clones in the leg given an unfractionated dose that the calculations were unreliable. Fractionation experiments of this type are limited by the narrow range of clone numbers measurable, although the system could be used to measure  $D_0$  (ref. 9), the intercept or quasi-threshold dose, by determining the single dose that produces the same clone number as a split dose. There remains, however, the more fundamental criticism that radiation damage to one leg may well affect the degree of recovery in the other.<sup>10</sup>

The experimental results given in Fig. 2 relate to clone

counts made on animals killed 25 days after irradiation. Clones are seen as early as 10 days after irradiation and an experiment was carried out to discover how the clone number varies with time after irradiation. The results of this investigation are shown in Table 1. There is an increase in clone numbers over the period 21–30 days at 1,900 rads, but this increase is small compared with the variation in individual values. At 2,300 rads no increase in clone number is detectable.

Table 1. VARIATION OF SURVIVING FRACTION WITH TIME AFTER IRRADIATION

Days		21	25	30
Number of animals		9	4	9
Survival after 1,900 rads	Mean	1.4	1.9	2.0
$\times 10^{-4}$	S.D.	$\pm 1.1$	$\pm 0.8$	$\pm 1.4$
Survival after 2,300 rads	Mean	0.25	0.31	0.33
$\times 10^{-4}$	S.D.	$\pm 0.23$	$\pm 0.18$	$\pm 0.30$

It becomes evident when the clones are measured that they vary in type, size and distribution on any one section. The typical clone has an appearance similar to that illustrated in Fig. 1. There is a region of actively dividing cells and a region of hypertrophic cells. In the larger clones the cells are clearly seen to be lined up into the normal columnar arrangement of the cartilage plate. The active process of endochondral ossification is resumed when blood vessels from the metaphysis break through the calcified matrix and invade the hypertrophied cells. Many clones, however, differ from the typical appearance. Some clones contain only dividing cells and others include a large amount of inter-cellular matrix. Some clones contain giant cells and degenerate nuclei and, when clearly recognized, these were classified as abortive clones and excluded from the counts. An abortive clone arises from a cell which divides a few times before latent damage brings division to a stop. Most abortive clones are small in size so that if they are unrecognized and included in the counts they each add an appreciable error to the calculated clone number in a way that has been explained above.

The variations of clone diameter with dose and time after irradiation are analysed in Table 2. The smallest recognizable clones have a diameter of about 40  $\mu$  and clones of this minimum size were found in most of the tibiae. Because the number of clones increases with time (Table 1) the mean clone diameter is not a useful index, but the maximum clone sizes show that there is an increase of diameter with time after irradiation and that clones appear to grow faster after a lower irradiation dose. At any one dose-level and time interval there is a large variation in the sizes of clones measured.

Table 2. VARIATION OF CLONE DIAMETERS WITH TIME AFTER IRRADIATION

Days		21	25	30
1,900 rads	Mean	110 $\mu$	160 $\mu$	170 $\mu$
	Range	40–400	40–600	40–700
2,300 rads	Mean	85 $\mu$	100 $\mu$	180 $\mu$
	Range	40–200	40–400	50–800

The distribution of clones within the volume of the cartilage plate is not random. Looking on the plate as a thin disk, the possibility of variations in distribution within the thickness (200  $\mu$ ) and then across the diameter of the disk (4,000  $\mu$ ) must be considered. The distribution of surviving cells within the thickness of the plate cannot be accurately assessed since many of the clones at 25 days have a diameter of 100  $\mu$  or more and so obscure the site of their origin. An experiment was carried out with 3-week-old rats which have thicker plates and measurements on these animals indicated that clones arose from surviving cells throughout the region of the damaged plate that corresponds to the normal proliferation zone.

A non-homogeneous distribution of clones across the diameter of the cartilage disk was very evident in the sections from animals irradiated with 2,100 and 2,300 rads. The highest density of clones was found at the anterior edge of the plate, and in order to correct for this effect the data at these dose-levels were calculated from counts made over the central 2,000  $\mu$  of the plate. The reason for the apparent increased survival of cells in this

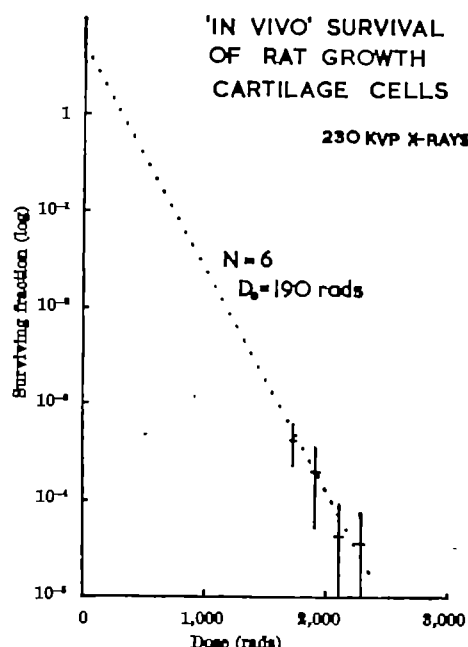


Fig. 2. Cell survival data for rat growth cartilage giving mean and standard deviations of values. Rats breathing air during irradiation

region is not yet clear although three possibilities have been considered.

The first possibility is a local difference in dose compared with the rest of the cartilage plate. This could be produced by variation in the beam across the 4 mm of the plate but such a difference would be small. There could also be an effect at the radiation quality used due to secondary electrons from calcified tissue. The proliferating cartilage cells lie in a 200 $\mu$  thick slab between the epiphyseal bony plate and the partially calcified matrix of the hypertrophied cartilage, but towards the anterior edge the bony plate curves away from the dividing cells. Since few proliferating cartilage cells in any part of the plate are nearer than 30 $\mu$  to calcified tissue it is unlikely that the local dose at the edge could be more than a few per cent lower than the dose at the centre of the cartilage plate. (Data from the International Commission on Radiological Units and Measurements<sup>11</sup> show that the dose beyond 30 $\mu$  from bone is not increased by more than 10 per cent at any quality.)

A further possibility is a local reduction in oxygen tension. Some measurements supported this idea in that some proliferative cells at the anterior edge were as far as 350 $\mu$  distant from their blood supply while the cells in the rest of the plate are no more than 200 $\mu$  distant at maximum.

The third possibility is based on the mechanism by which the cartilage plate normally grows in diameter. Investigations using tritiated thymidine<sup>12</sup> have shown that cells in the fibrous perichondrium differentiate into chondrocytes and add themselves on to the edge of the plate. If these perichondrial cells are more radio-resistant than cartilage cells, or if they can migrate from unirradiated parts of the rat, then there is the possibility that they could form clones at the edge of the irradiated cartilage plate. Further investigations are needed to determine which of these possibilities is effective.

It was stated in the introduction that this method depended on the assumption that each clone arose from a single surviving cell. However, it can be shown from a simple consideration of a Poisson distribution for surviving cells in space that some clones must arise from more than one surviving cell, that is, two or more cells in close proximity give rise to clones that coalesce and are seen as one clone. An analysis of this problem showed that at the maximum clone densities counted this factor was unlikely to introduce an error of more than 20 per cent in the survival values. There remains, however, a possible complication in that adjacent cells in the cartilage plate are partially synchronized in the mitotic cycle since they both arose from the same division. If cells in different stages of the mitotic cycle vary in their sensitivity to radiation then there is an increased possibility of the survival of adjacent synchronous cells.

The recovery of the cartilage plate cannot be considered without some reference to the effects of radiation on the surrounding tissues and the whole animal. For this reason an investigation of the effects of radiation on the vascular supply to the plate was undertaken. Previous work<sup>3,13</sup> on vascular damage to bone has been concentrated on the pronounced changes that occur in the metaphysis, but the cartilage plate is supplied by vessels from the epiphysis. Therefore, these epiphyseal vessels were examined at intervals up to 12 weeks after irradiation by the vascular injection technique using Berlin blue. At doses of 2,000 rads it was found that the supply to the cartilage plate was reduced but not obstructed during this time interval. A parallel approach to the problem of the effect of radiation on the supply of metabolites was made in a brief autoradiographic study with sulphur-35 labelled sulphate; it was found that this metabolite was available to cells in all regions of the plate at times up to 4 weeks after irradiation. It was concluded from these investigations that damage to the vascular supply was not effective in reducing the number of clones.

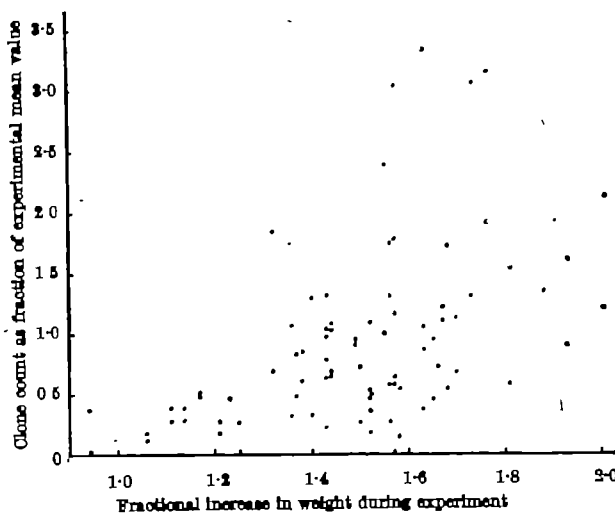


Fig. 3. Variation in the tibial clone count with the increase in weight of the animal during the experiment. The clone count is expressed as a fraction of the mean clone count for all tibiae having the same radiation treatment.

Endochondral growth is regulated by complex hormonal controls. As a first step in the study of such controls on the recovery of cartilage some correlation was sought between the general health of the animal and the clone count in the tibiae. Some analysis was made using increase in the weight of an animal during the experiment as a crude index of health. The data are presented in Fig. 3. The ordinates represent the clone count for each leg expressed as a fraction of the mean clone count for all legs having the same radiation treatment. The abscissae show the corresponding fractional increase in weight of the animal during the period of the experiment. The points do show the trend that animals with a low weight-gain also show a below-average clone count and that health is one of the factors that affect the clone count in a given animal. This reduced count cannot necessarily be equated with a reduced survival of cartilage cells since the effect of general sickness may be to prevent surviving cells from dividing fast enough to produce a detectable clone by 25 days. It was noted that clones in sick animals were smaller than average in diameter.

This investigation of the recovery of growth cartilage shows that the clone system gives valid, if imprecise, results for cell survival from acute irradiation. It is important to note that the results obtained for cells of growth cartilage may not apply to other types of cartilage, that is, articular and structural cartilage, which both have lower proliferation rates. The system is now being used to investigate oxygen effects on the sensitivity of growth cartilage cells to radiation.

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## A STATISTICAL APPROACH TO THE PHILADELPHIA CHROMOSOME

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IN chronic granulocytic leukaemia it has been found that one of the small acrocentric chromosomes of the 'G' group is usually smaller than its partner. This has been called the Philadelphia, or  $Ph^1$ , chromosome and its recognition can be used in the diagnosis of this disorder. During a recent investigation of the karyotypes of two patients with megakaryocytic myelosis, a difference of opinion arose as to whether the  $Ph^1$  chromosome was present or not. One member of the 'G' group appeared to be smaller than the other three in both cases and was thought to be the  $Ph^1$  chromosome. Later the material was submitted to an experienced observer who considered that the  $Ph^1$  chromosome was not present in either case. Re-examination of the material led us to revise our opinion and to consider that only one of the two cases was  $Ph^1$  positive. Another independent observer examined the material and agreed that the  $Ph^1$  chromosome was not present in one case, but the other was reported on as follows: Nine out of ten mitoses examined from direct bone marrow preparations showed the presence of a  $Ph^1$ -like chromosome (ref. 1).

As a result of this experience we thought a statistical approach to the problem would be of value. Twelve cases of chronic granulocytic leukaemia considered to be  $Ph^1$  positive<sup>2</sup>, the two cases of megakaryocytic myelosis and nine normal controls have been studied (Table 1).

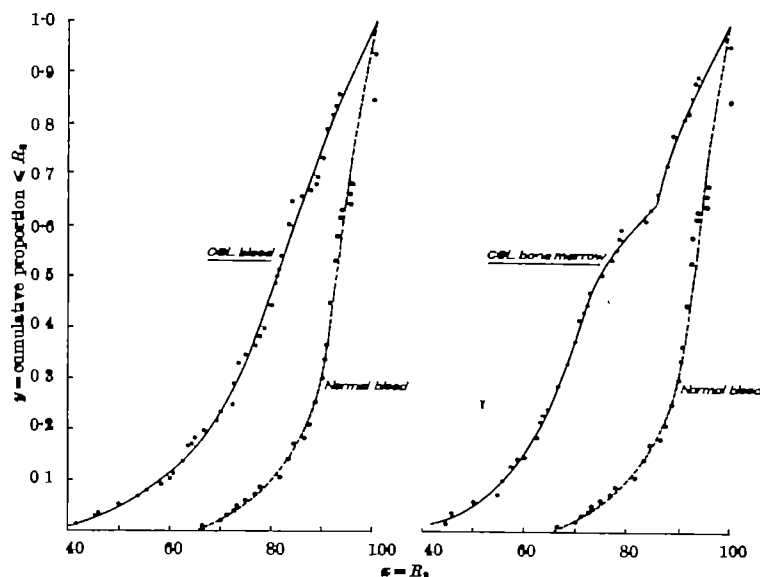
At first photographic negatives of the karyotypes were projected on to paper and the outline of the 'G' group chromosomes traced. Since then direct projections of the metaphases have been used. The area of each of the four chromosomes was then measured in arbitrary units, using squared paper.

A first attempt at statistical treatment involved the use of Grubbs's test for outliers<sup>3</sup>. In other words, the projected areas of the four 'G' chromosomes in each cell were treated as a sample of a normal distribution, and the ratio of variances including and excluding the smallest chromosome was tested. In most cases of chronic granulocytic leukaemia, however, this test failed to give unequivocal results.

Next, attention was directed to the ratio of the area of the smallest chromosome to that of the others. Two such ratios were examined—that of the smallest to the mean of the other three ( $R_1$ ), and that of the smallest to the next smallest ( $R_2$ ). It would be possible to deduce the distribution of these ratios on the assumption of normal distribution of the four area measurements around a common mean. But such an assumption is of doubtful validity, particularly since the process of measurement leads to a small discrete set of values, and the resulting distributions would, in any event, be awkward functions to handle. Accordingly, it was decided to use empirical

Subject	Sex	Age	Preparation	No. of metaphases counted
<b>Table 1</b>				
<i>Chronic Granulocytic Leukaemia</i>				
L.P. 502	F	54	Bone marrow	13
L.P. 501	M	63	Bone marrow	13
L.P. 256	F	55	Bone marrow	7
			Peripheral blood	6
L.P. 419	M	35	Bone marrow	12
			Peripheral blood	11
L.P. 426	F	46	Bone marrow	20
L.P. 478	M	38	Bone marrow	4
L.P. 94	F	42	Peripheral blood	12
L.P. 580	M	72	Peripheral blood	13
L.P. 467	M	51	Peripheral blood	12
L.P. 578	M	34	Peripheral blood	15
L.P. 597	F	37	Peripheral blood	13
L.P. 33	F	46	Peripheral blood	6
<i>Megakaryocytic Myelosis</i>				
L.P. 556 (Case I)	F	51	Bone marrow	12
L.P. 590 (Case II)	F	41	Bone marrow	5
<i>Normal Controls</i>				
J.W.	M	36	Peripheral blood	12
J.M.	F	21	Peripheral blood	16
R.P.	M	22	Peripheral blood	15
J.H.	F	23	Peripheral blood	16
V.S.	F	25	Peripheral blood	17
J.M.	M	20	Peripheral blood	11
A.R.	F	19	Peripheral blood	4
R.R.	F	23	Peripheral blood	5
K.A.	M	22	Peripheral blood	2

distributions of the ratios in known cases of chronic granulocytic leukaemia and in normal individuals for comparison. It was first ascertained that there were no significant differences in distribution between individuals within each of these two comparison groups, so that all the cells recorded in each group could be treated as a single population. Then the ratios for each of the cases of megakaryocytic myelosis were ranked jointly with those

Fig. 1. Cumulative empirical distribution of  $R_2$ .

for the normal group and those for the chronic granulocytic leukaemia in turn, and Wilcoxon's<sup>4</sup> rank test applied. The results were unequivocal. In Case I, the pattern of 'G' chromosome size differed widely from that of normal cells ( $P=0.002$ ), but agreed satisfactorily with that for chronic granulocytic leukaemia ( $P=0.452$ ). In Case II, the reverse was true ( $P=0.447$  and  $0.008$  respectively). Tests with the two ratios led to similar conclusions; the  $R_2$  probabilities are quoted above. Since  $R_2$  is simpler to calculate than  $R_1$  and there is little difference in sensitivity,  $R_2$  seems preferable.

The Wilcoxon rank test would be a little troublesome to apply on a routine basis, so an alternative non-parametric procedure is proposed. The graphs of Fig. 1 give the cumulative empirical distribution of  $R_2$  for normal cells and for cells from the peripheral blood and the bone marrow of chronic granulocytic leukaemia patients. If the ratios obtained for cells of a case of uncertain status are arranged in order, they may be compared with these graphs by the Kolmogorov-Smirnov test. (Strictly speaking, the Kolmogorov-Smirnov test is applicable only to theoretical distributions; as an approximation, however, its use for empirical distributions based on fairly large samples, as in the present case, may be accepted.) This is best explained by an example. The twelve ratios determined are shown in the first column of Table 2. In the second column are given the proportion of the sample which each ratio equals or exceeds, and in the third and fourth columns are the corresponding figures from the two graphs of Fig. 1. One now looks down the columns to find the largest discrepancy between the two sets of figures. In comparison with the normal

series this occurs in the sixth line and is 0.505; in comparison with the leukaemic series it is in the fourth line and amounts to 0.158. These maximum discrepancies are then compared with figures tabulated (for example, by Owen<sup>5</sup>) for different levels of significance. We find that, for a sample of twelve, a value of 0.375 will be exceeded once in twenty times, a value of 0.449 once in one hundred times. Accordingly we conclude that this particular case is most unlikely to be normal but agrees satisfactorily with a diagnosis of chronic granulocytic leukaemia.

It may be noted that the curve for bone marrow in Fig. 1 for leukaemia patients has a zone of reduced slope in the middle. This agrees with the impression from visual observation that two cell populations may be distinguished in these patients—one with and one without a distinct  $PA^1$  chromosome. Similar statements have been made about cells in peripheral blood, but the resulting bimodality cannot be detected with certainty in its curve.

The  $PA^1$  chromosome is usually found in a higher proportion of cells from preparations of bone marrow than peripheral blood. The distribution of the  $R_1$  ratio for leukaemic patients does in fact differ between these two types of preparation (the Wilcoxon rank test gives  $P=0.014$ ), but the difference for  $R_2$  does not reach significance ( $P=0.140$ ). The means are respectively:

	$R_1$	$R_2$
Bone marrow	0.859	0.781
Blood	0.710	0.795

The smallness of the difference may also be seen in comparing the curves in Fig. 1. For the test described in this article there thus seems little advantage in the use of bone marrow preparations.

We thank Dr. A. G. Baikie and Mrs. H. Trowell for help, Mr. P. Onesti and Mr. R. Pozzi for assistance, and Miss N. Wood, Mrs. P. Giroud and Mrs. M. Bulanyi for help with calculations, some of which were performed in the Computing Centre of the University of Western Australia.

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Table 2. KOLMOGOROV-SMIRNOV TEST APPLIED TO RATIO  $R_2$ ,  
Proportion of cells  $\leq R_2$  in

$R_2$	Unknown	Normal	Chronic granulocytic leukaemia
0.500	0.083	0.001	0.045
0.647	0.167	0.005	0.158
0.703	0.250	0.021	0.246
0.714	0.417	0.031	0.261
0.766	0.500	0.063	0.372
0.777	0.583	0.078	0.411
0.866	0.667	0.202	0.670
0.904	0.750	0.325	0.757
0.925	0.833	0.478	0.812
0.937	0.917	0.545	0.841
1.000	1.000	1.000	1.000

## A COMPARISON OF THE GLYCOLIPIDS FOUND IN DIFFERENT STRAINS OF ASCITES TUMOUR CELLS IN MICE

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IN the past few years a number of different complex glycolipids have been found in several mammalian tissues<sup>1-4</sup>. Usually they were detected as very minor components in the total lipid extracts from the tissues though relatively large quantities were found in some diseased tissues<sup>1,4</sup>. Although only a limited number of tissues have been investigated the results have suggested that small amounts of several types of complex glycolipid might be present in most mammalian tissues. All the glycolipids that have been isolated from different tissues contain ceramide (an amide of sphingosine and a long-chain fatty acid) and the differences in their individual physical and chemical properties are due to variations in the size and structure of the carbohydrate moiety of the glycolipid molecule.

The elegant immunochemical studies of Rapport *et al.*<sup>1-3</sup> in recent years showed that certain glycolipids were lipid haptens and that probably all glycolipids possessed hap-

tenic function. The differences in specificity were attributable to differences in the carbohydrate moiety of the glycolipid molecule, and with two compounds, cytolipin H and galactocerebroside (ceramide galactoside), Rapport was able to relate immunological activity to a specific chemical structure. Cytolipin H was first isolated<sup>1</sup> from a human epidermoid carcinoma grown in conditioned rats, and later chemical studies<sup>19</sup> showed that it was a ceramide lactoside. Following up an earlier report<sup>11</sup> on a glycolipid found in ox-spleen, which contained sphingosine, a fatty acid and two sugars, Rapport *et al.*<sup>1</sup> isolated a compound which was chemically and immunologically similar to cytolipin H. Some cytolipin H activity was detected in sheep and dog spleens also, but not in rodent, cat or horse spleens<sup>12</sup>. Although cytolipin H was detected in some normal tissues and was not detected in all the cancer tissues which were examined, Rapport *et al.*<sup>19</sup> stated that it accounted most frequently for the differences observed



between the reactions of lipid extracts of normal tissues and those of cancer tissues on the basis of reactions with antisera against the latter.

Minor amounts of glycolipids were found in the lipid extracts of Landschutz ascites carcinoma cells and BP8 ascites sarcoma cells<sup>13</sup>, and as a result of a more detailed investigation<sup>14</sup> of the glycolipid composition of BP8 ascites sarcoma cells, maintained in C3H mice, four types of glycolipids were isolated and partially characterized as ceramide monohexoside, ceramide dihexoside, ceramide trihexoside and ceramide hexosaminyldihexoside. Similar studies carried out on OL2 ascites leukaemia cells maintained in BALB/c mice showed, however, a very different glycolipid composition (Gray, G. M., and Wells, J. J., unpublished results). The major glycolipid component was identified as a ceramide hexosaminyldihexoside. These findings suggested that tumour specificity and/or mouse specificity might be factors which affected the distribution of the types and the proportions of glycolipids in the tissue. In order to examine further this possibility the glycolipids in several strains of ascites tumour cells were isolated and their compositions compared.

MO1M and SA1 ascites sarcomas were maintained in C3H and A mice respectively, EL4 and OL2 ascites leukaemias were maintained in C57BL and BALB/c mice, respectively, and TA3 ascites carcinoma was maintained in A mice. The mice were bred at the Microbiological Research Establishment, Porton, Wilts., and the growth and recovery of the tumour cells were as described by Davies<sup>15</sup>. The quantities of different freeze-dried cells used ranged from 20 g to 68 g. Each batch of freeze-dried tumour cells was extracted with 3 volumes of chloroform:methanol (1:1, v/v) followed by 3 volumes of chloroform:methanol (2:1, v/v); the two extracts were combined, washed with 0.1 M potassium chloride (0.2 vol.), dried over anhydrous sodium sulphate, filtered and evaporated to dryness *in vacuo*<sup>14</sup>. Each lipid extract was dissolved in light petroleum (b.p. 40°–80°) and dialysed through a rubber membrane<sup>16</sup> against light petroleum to separate the neutral lipids from the phospholipids and glycolipids. The phospholipid/glycolipid fraction was treated with 0.07 N methanolic sodium hydroxide:chloroform (2:1, v/v) to remove the diacyl glycerophospholipids as water soluble phosphate esters<sup>17</sup>. The fatty acid methyl esters formed in the reaction were separated from the alkali-stable phospholipids and glycolipids by dialysis through a rubber membrane. The approximate composition of each alkali-stable lipid extract was determined by chromatographing samples on silicic acid impregnated paper<sup>18</sup> and silica-gel G (Merck) thin-layer plates<sup>14</sup>. At this stage the lipid extracts from all the strains of tumour cells contained at least eight components and in each case the major one was sphingomyelin, the main contaminating phospholipid.

Previous work<sup>14</sup> showed that the standard procedure for the removal of phospholipids from a glycolipid fraction by adsorption on Florisil<sup>19</sup> was unsuitable for lipid extracts from ascites tumours and better results were obtained with alumina<sup>20</sup>. The lipid extract was loaded on to an alumina column (0.5 mg phosphorus/g alumina) in chloroform:methanol (1:1, v/v) and fractions were collected from the column and monitored by thin-layer chromatography. Most of the phospholipid (sphingomyelin) was eluted from the column with the same solvent. The solvent was changed to chloroform:methanol:water (10:10:1, by vol.) and the water content was gradually increased from approximately five up to ten per cent.

The glycolipids were eluted with solvent containing a water concentration within this range. The fractions from the column were combined according to their composition as indicated by thin-layer chromatography and these fractions were analysed for phosphorus and carbohydrate<sup>14</sup>. The glycolipids from each of the five strains of ascites tumour still contained small amounts of phosphorus after alumina chromatography and, with the exception of the glycolipid fraction from SA1 ascites tumour, these were successfully removed by passing each fraction dissolved in chloroform:methanol:water (2:5:2 by vol.) through a small column of Deacidite FF (anion exchange resin) in the same solvent. The yield of glycolipids obtained from each strain of ascites tumour was very small (Table 1) and it was impractical to attempt to separate individual glycolipids on a quantitative basis. However, the glycolipid compositions of the five different ascites tumours were compared after thin-layer chromatography of the total glycolipid fractions on silica-gel G (Merck) with a

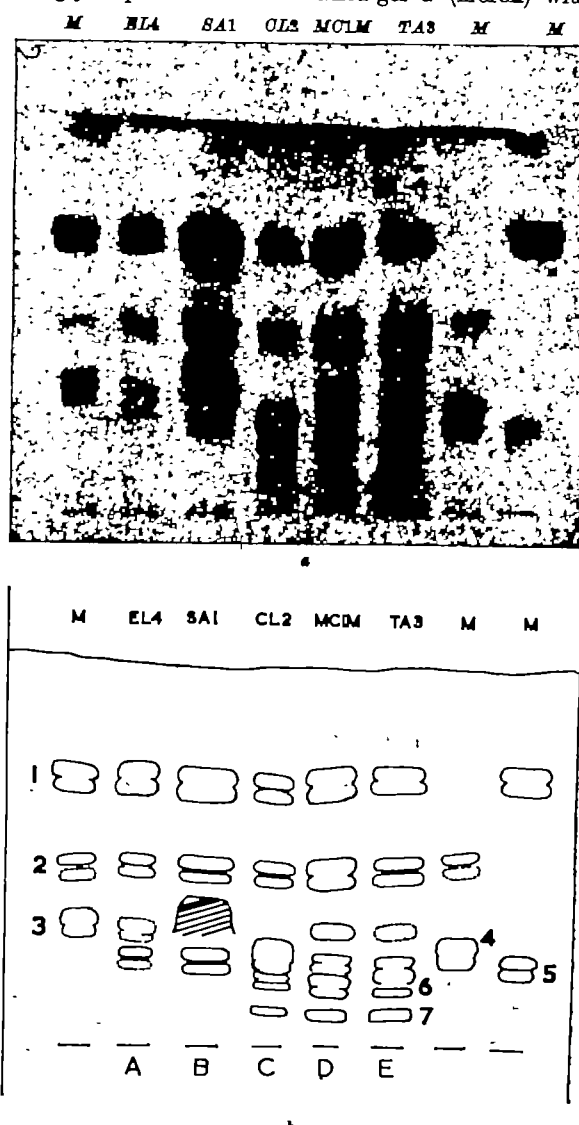


Fig. 1. a, The separation into individual classes of the glycolipids present in different ascites tumours in mice by thin-layer chromatography on silica-gel G (Merck); solvent system, chloroform:methanol:water (65:25:4, by vol.). Plate was sprayed with 5 per cent phenol/60 per cent sulphuric acid (1:1, v/v). b, Identification of components by comparison with known glycolipid markers (A): (1) ceramide glucoside; (2) ceramide lactose; (3) ceramide digalactosylglucoside; (4) ceramide *N*-acetyl-galactosaminyldigalactosylglucoside; (5) ceramide *N*-acetylgalactosaminyldigalactosylglucoside; (6) ceramide *N*-acetylgalactosaminyldigalactosylglucoside; (7) ceramide *N*-acetylgalactosaminyldigalactosylglucoside. A, Glycolipids from EL4/C57BL ascites leukaemia; B, SA1/A ascites sarcoma; C, OL2/BALB/c ascites leukaemia; D, MO1M/C3H ascites sarcoma; E, TA3/A ascites carcinoma. Components 6 and 7 are probably glycolipids containing larger carbohydrate residues than 5 or sialic acid.

Table 1

Ascites tumour/ mouse	Amounts of lipids isolated from ascites tumour cells calculated per 100 g freeze- dried cells			Glycolipids % by wt of total lipids
	Neutral lipids (g)	Phospholipids (g)	Glycolipids (mg)	
MO1M/C3H	8.6	5.5	41	0.20
TA3/A	10.0	6.8	37	0.16
SA1/A	6.0	6.2	7	0.06
EL4/C57BL	5.6	6.7	61	0.50
OL2/BALB/c	6.0	7.2	64	0.48

solvent system of chloroform:methanol:water (85:25:4). Ceramide monohexoside, ceramide dihexoside, ceramide trihexoside, ceramide hexosaminyldihexoside and ceramide hexosaminyltrihexoside were chromatographed at the same time for reference purposes. The solvent front was allowed to travel about four-fifths of the plate height, the plate was dried and sprayed with 5 per cent phenol: 60 per cent sulphuric acid (1:1, v/v) and heated at 120°. The glycolipids showed up as purple-brown spots.

There was a considerable variation in the composition of each strain of tumour (Fig. 1 a, b). The major component in *MO1M*, *EL4* and *SA1* was a ceramide monohexoside, in *TA3* a ceramide dihexoside and in *OL2* a ceramide hexosaminyldihexoside. This particular glycolipid, which was characterized as ceramide *N*-acetyl-galactosaminylgalactosylglucoside (Gray, G. M., and Wells, J. J., unpublished results), occurred only in the *OL2* ascites tumour, but this strain did not contain ceramide trihexoside nor ceramide hexosaminyltrihexoside, both of which were present in the other strains of tumours. (The glycolipid fraction from *SA1* contained a phospholipid contaminant which had similar chromatographic properties (Fig. 1) to ceramide trihexoside and it was not possible, therefore, to confirm the presence of the glycolipid in this strain.) *OL2*, *MO1M* and *TA3* also contained significant amounts of two glycolipids (Fig. 1b (6 and 7)), which, by their chromatographic behaviour, had carbohydrate residues which were either larger than a tetrasaccharide (Fig. 1b (5)) or contained sialic acid.

The 'double spot' obtained for each glycolipid component after thin-layer chromatography on silica-gel *G* (Merck) represented a separation of glycolipids containing normal straight chain fatty acids from those containing hydroxy fatty acids\* (the slower moving component). Most of the glycolipids (Fig. 1) contained hydroxy fatty acids, and in several cases the hydroxy compounds appeared to be present in amounts equalling those of the normal straight chain fatty acids. The apparent proportions of glycolipids containing mono-, di-, tri-, and tetrasaccharide moieties in the *MO1M* ascites sarcoma maintained in *O3H* mice were similar to those reported<sup>14</sup> for *BP8* ascites sarcoma also maintained in *O3H* mice. However, the proportions of the glycolipids in *TA3* ascites carcinoma and *SA1* ascites sarcoma, both maintained in *A* mice, were quite different (Fig. 1), and thus mouse specificity does not seem to be a factor controlling the general composition of the glycolipids. It is important to realize that although the differences in the proportions of glycolipids present in different strains of tumours and mice may not be a function of tumour or mouse specificity small variations in the fine structure of each glycolipid, such as a different linkage between two sugar residues, may be so related. There is no doubt that subtle differences do exist. Rapport (personal communication) tested the lipid extracts from *BP8*, *OL2*, *EL4*, *SA1*, *TA3* and Landschutz ascites tumours for cytolipin *H* activity by the very sensitive immunochemical technique<sup>7</sup> and found that only the *BP8* ascites tumour lipids contained cyto-

lipin *H* (ceramide lactoside; compare Gray<sup>14</sup>). It has been shown that all these tumours contain a ceramide dihexoside (Fig. 1) and so it is concluded that the structures of the dihexosides in the tumours other than *BP8* (*MO1M* was not tested) are different from lactose. A ceramide digalactoside<sup>14</sup>, which was found in the *BP8* ascites tumour as well as ceramide lactoside, may be the dihexoside present in some of the other tumours. Differences within a particular class of glycolipid may be the result of variations in the linkage of one sugar to another. A glycolipid has been isolated from *OL2* ascites tumours and identified as a ceramide galactosylglucoside (Gray, G. M., and Wells, J. J., unpublished results); however, as no cytolipin *H* activity was found in the lipid extract from *OL2* tumours it seems likely that the galactose to glucose linkage is not  $\beta(1\rightarrow4)$ . If the glycolipids containing di-, tri-, and tetrasaccharide residues have related pathways of biosynthesis<sup>14</sup>, the structural differences which appear to exist between the ceramide dihexoside from different ascites tumours may also occur in the glycolipids containing the larger carbohydrate residues.

The isolation of the glycolipids in quantities which are necessary for the identification of their chemical structures has not been possible because of lack of tumour material. The problem of insufficient material may not be so serious in the future if a variety of glycolipids within the classes described in this article can be synthesized<sup>11</sup>; with these compounds of known structure available the immunochemical technique described by Rapport would provide an excellent means of characterizing the glycolipids in very small amounts of tissue.

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## GENETIC PROPERTIES OF EXOGENOUS DEOXYRIBONUCLEIC ACID AT VARIOUS LEVELS OF DEGRADATION IN *Drosophila melanogaster*

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THE injection of deoxyribonucleic acid (DNA) into the haemocoel of adult *Drosophila* males was shown<sup>1</sup> to initiate the induction of small chromosome deletions resulting in the *Minute* phenotype. The same activity was later detected with a diversity of natural<sup>2</sup> and syn-

thetic<sup>3</sup> macromolecules, such as ribonucleic acid (RNA), deoxyribonucleoproteins (DNP), representative of acidic, basic and neutral proteins (albumins, histones and globulins), as well as some anionic polymers (polynucleotides, polyglutamic acid, polymethacrylic acid). None of

these agents possessed any chemical reactivity which could have resulted in direct damage to the genetic material. Their mutagenic action must have accordingly been indirect, and it was possible to infer<sup>3,4</sup> that the underlying mechanism involved disturbances in DNA synthesis during chromosome replication, as a consequence of the inhibition of some of the requisite enzymes.

In contrast to the activity of DNA in the production of chromosome deletions, it was completely ineffective in the mediation of specific locus transformation and gave weak and rather inconsistent activity as regards the induction of sex-linked recessive visibles and lethals<sup>1,2</sup>. The negative results with transformation could have been the outcome of the physiological incompetence of the tested samples of DNA, perhaps as a result of their partial degradation. Conversely, the inconsistent activity as regards the point mutations might have been the outcome of insufficient DNA degradation, if mutagenicity was not due to the polymer itself, but to the nucleotides and nucleosides derived from its intracellular breakdown. Some of the samples of *Drosophila* DNA used in the earlier genetic experiments<sup>1</sup> were extracted<sup>1</sup> from developing embryos (before egg hatching) and were of low viscosity, which suggested a certain degree of degradation. More recently, better methods of DNA extraction from the adult flies were developed<sup>7</sup>, which yielded samples of larger molecular size, thus warranting the repetition of the transformation tests. For the mutagenicity tests, the large DNA molecules were artificially degraded by ultrasonics or high doses of  $\gamma$ -rays. The results with irradiated DNA were of significance not only to the understanding of the polymer's mechanism of action, but also in relation to the problem of genetic hazards to man that might arise as a result of food sterilization by ionizing radiation. The possibility of such hazards has been indicated by a recent report<sup>11</sup> that an irradiated sample of DNA was highly mutagenic in *Drosophila*, particularly as regards the induction of sex-linked recessive lethals. A comparative examination was accordingly undertaken of the mutagenic activity of untreated and degraded DNA (both by sonication and irradiation) as regards the induction of chromosome deletions and point mutations, and the results are presented in this article.

The DNA samples used in the work recorded here were all extracted from the adults of *Drosophila melanogaster* ('Oregon-K' strain) by our colleagues, Hastings and Kirby<sup>7</sup>. In principle, their procedure involved the preliminary separation of RNA, by the use of naphthalene-1,5-disulphonate and phenol/8-hydroxyquinoline<sup>12</sup>, followed by the isolation of DNA by the *p*-aminosalicylate/phenol technique<sup>8</sup>. Purification and concentration of the isolated DNA were undertaken with minimal exposure to low ionic concentration and organic solvents, so as to reduce molecular fragmentation. Physico-chemical measurements (viscosity, sedimentation patterns and melting curves) on the isolated material indicated a homogeneous and satisfactory order of molecular integrity. DNA samples with the largest obtainable molecular size (mol. wt.  $\approx 4 \times 10^6$ , as estimated from sedimentation velocity experiments) were used for the transformation experiments and some of the mutagenicity tests. The material was dissolved at the required concentration in isotonic saline (0.4 per cent sodium chloride) and administered by micro-injection into the haemocoel of adult males, immediately around their testes. Aliquots of the same sample of DNA were then subjected to the degradation procedures and their mutagenic activity was reassayed by the same genetic techniques as with the untreated material. For degradation by ultrasonics, a DNA solution of high concentration (0.5 per cent w/v in isotonic saline) was cooled in an ice bath, and subjected to short-wave sound waves (20 kc/s/60 W) for 3 min; the sonicated material was then heated in a water bath (100° C) for 10 min. After this treatment the initial DNA solution virtually lost its

viscous consistency and was found to contain DNA fragments of fairly small size (mol. wt.  $\approx 200,000$ ). For degradation by radiation, a DNA gel (1.0 per cent, w/v in isotonic saline) was subjected to  $\gamma$ -rays (from a cobalt-60 source) at a dose rate of 1,370 r./min for 2 h 50 min, corresponding to a total dose of 232,900 r. After irradiation, the DNA gel was changed into a somewhat viscous hydrosol, and was found to contain larger fragments of the polymer (mol. wt.  $\approx 500,000$ ) than after sonication and boiling.

The genetical techniques used in the investigation recorded here have already been described in detail elsewhere<sup>1-4</sup>. Transformation was examined at seven recessive loci: *yellow* (*y*), *vermillion* (*v*), *miniature* (*m*) and *forked* (*f*) on the X chromosome; *brown* (*bw*) on the second, *ebony* (*e*) on the third, and *polster* (*pol*) on the fourth chromosomes. The detection of the mutations was undertaken by the attached-X technique for the visibles—both sex-linked recessive and autosomal dominants (especially *Minutes*); and by the Muller-5 method for sex-linked recessive visibles and lethals. In all the mutagenicity tests the treated males were repeatedly mated to an approximately equal number of females every three days, and the mutation rate in each brood was determined separately. Breeding was undertaken in mass cultures, with a maximum of ten pairs per half-pint bottle.

In previous transformation experiments<sup>1</sup>, DNA was extracted from the wild-type flies and injected at various concentrations into males homozygous for the 7-mutant (abbreviated 7-*m*) marker stock. With this technique it would be possible to detect the displacement of the mutant loci by the wild-type alleles from the administered DNA. In the event of successful transformation, homozygosity at the affected site would be changed to heterozygosity, which would lead to reversion to the wild-type expression at the transformation site—because of the recessiveness of the marker genes. In fact, no such revertants were observed, either in earlier experiments with somewhat degraded DNA, or in the more recent ones with better samples of the polymer. The total chromosomes carrying the test markers which were exposed to the various samples of DNA from the wild-type flies were accordingly pooled (+DNA in 7-*m* flies, Table 1) to give an idea about the size of sample which failed to incorporate wild-type DNA instead of the mutant genes. The improved DNA samples were also used to test for the reverse transformation: the incorporation of mutant DNA in place of the wild-type genes. For this purpose, DNA was extracted from the stock homozygous for the 7-mutant loci and injected into wild-type males. These were then crossed to females homozygous for the multiple recessive markers and their progeny were scored for any of the mutant phenotypes. In the event of specific locus transformation, the mutant DNA segment from the extract replaces its wild-type allele in the genome of the treated male and is later rendered homozygous by the back-cross to the marked female, thus revealing the mutant phenotype of the transformed gene. No such transformants were detected (7-*m* DNA in + flies, Table 1) at any of the test loci,

Table 1. ANALYSIS OF THE TRANSFORMATION EXPERIMENTS  
Showing the size of sample observed for the 7-mutant (7-*m*) loci—*y v m f bw e pol*—which failed to exchange with their allelomorphs when supplied as exogenous DNA

Chromosomes and loci exposed	Treatment		Sample observed	Loci
	+DNA in 7- <i>m</i> flies	7- <i>m</i> DNA in + flies	Chromosomes	
X: <i>y, v, m, f</i>				
In gonads and xygotes	46,149	73,213	119,462	477,848
In xygotes only*	85,423	(62,807)†	85,423	341,692
Second, third and fourth:				
<i>bw, e, pol</i>				
In gonads and xygotes	88,309	136,120	224,429	673,287
In xygotes only*	88,309	—	88,309	264,927
Total	308,190	209,433	517,613	1,757,764

\* Assuming passive transfer of DNA by the sperm.

† Male progeny receiving the marked maternal X-chromosome, which precludes scoring for transformants.

thus showing that exogenous DNA (whether normal or mutant) was not readily acceptable for incorporation into metazoan cells—as genetically functional units. On the basis of the negative results so far obtained (last column, Table 1), the probability of transformation per locus in the germ line of *Drosophila* cannot exceed  $2 \times 10^{-6}$  (the upper limit of the Poisson expectation at  $P = 0.025$ ).

The administration of mutant DNA into wild-type males, although ineffective as regards transformation, showed signs of mutagenic activity as regards the induction of point mutation visibles. Among the male progeny from the back-cross of the DNA-treated fathers to the multiple recessive mothers, ten eye-colour mutants were detected through their interaction with *vermillion* (*v*), which resulted in various shades of orange eyes. Genetic analysis of these abnormal males revealed that in four instances the induced mutants were alleles of the sex-linked recessive visible *garnet* (*g*); three others were autosomal dominants, and the remaining three were with large rough eyes and proved sterile. The *g*-mutations were in the multiple marked maternal X-chromosome and could, therefore, have occurred either spontaneously, or indirectly through the passive transfer of DNA—which was injected in the males—via the sperm during fertilization. The four *garnet* instances were recovered from separate cultures and broods, indicating that they arose as independent mutational events. The maternal X-chromosomes (among which the *g*-mutants occurred) would be passed to their sons, the number of which was 62,807 (X-chromosomes exposed in zygotes only, Table 1). The mutation rate at the *g*-locus would, therefore, be  $6.4 \times 10^{-4}$ , which is considerably higher than the spontaneous rate and is equivalent to that occurring after 1,500 r. of X-irradiation<sup>5</sup>. There can be no doubt, therefore, that the mutants observed were not of spontaneous origin, and must accordingly have been induced by DNA conveyed at fertilization with the treated sperm.

Attention has already been directed<sup>1</sup> to the possibility that the mutagenic activity of DNA might depend on the degree of its molecular integrity. For the same sample of DNA, the mutagenic activity—as regards the *Minutes*—was roughly proportional to the injected concentrations. On the other hand, different samples at the same concentration gave various mutagenic activity and there were indications that the larger DNA molecules were less effective. To test this possibility, a comparison was undertaken of the mutagenic activity of untreated DNA relative to that of samples degraded by ultrasonics and  $\gamma$ -radiation. The mutagenic activity, as regards small chromosome deletions (*Minutes*), was significantly above the control level with all the three tested samples, but was markedly higher with the degraded DNA (whether sonicated or irradiated) than with the untreated materials (Table 2). The rate of *Minutes* with the present sample of undegraded DNA was not significantly different from that induced by the same concentration (0.5 per cent) of a previous sample extracted from adults by the same chemical procedure<sup>7</sup> (ref. 2, Table 1). This activity, how-

ever, was considerably lower than that expected for the same concentration of embryonic DNA, which was extracted by a different isolation technique<sup>8</sup>. In fact, the mutagenicity of the embryonic DNA sample was of the same order as that of the sonicated material in the work recorded here, suggesting that it was degraded by the isolation technique then used. This demonstrated the superiority of the recent extraction method from adults, which yielded larger DNA molecules of lower mutagenic effect. It should also be noted that there was no marked difference in the rate of *Minutes* with the sonicated and irradiated DNA, in spite of the higher concentration of the irradiated material. This again might be a function of various degrees of degradation, since the irradiated sample contained larger fragments of DNA than after sonication.

Table 3. THE SEX-LINKED RECESSIVE LETHALS (l) AND VISIBLES (v) INDUCED BY SONICATED OR IRRADIATED DNA DETECTED IN THE F<sub>1</sub> PROGENY FROM MULLER-5 EXPERIMENTS (Mosaic mutants entered in brackets)

Brood	Control				0.5% Sonicated DNA				1% Irradiated DNA			
	Chrm.	l	v		Chrm.	l	v		Chrm.	l	v	
I	8,453	21	1		522	2	0		473	1	0	
II	5,330	5	1		434	0	0		514	(1)	2	
III	2,037	1	0		453	2 (1)	0		280	1	0	
IV	1,542	2	0		480	0	0		501	0	0	
V	982	2	0		161	0	0		10	0	0	
VI	—	—	—		156	0	0		—	—	—	
Total	18,234	31	2		2,256	4 (1)	0		1,777	3	2	
Mutants per 10 <sup>4</sup>					1.70	0.11			1.60		1.13	

In contrast to the decisive activity of DNA in the induction of small chromosome deletions resulting in *Minutes*, our previous work<sup>1,2</sup> yielded no consistent evidence for the polymer's activity in the production of point mutations (sex-linked recessive visibles and lethals). This has now been ascertained for the degraded samples as well, by both the attached-X and Muller-5 techniques. The rate of the sex-linked recessive visibles in the attached-X tests was the same with the undegraded and degraded DNA and did not differ significantly from the control level (Table 2). In the Muller-5 tests, no visibles were recovered with the sonicated DNA; but the irradiated material induced two mutants in 1,777 chromosomes (Table 3), which is above the upper limit of the Poisson expectation ( $P = 0.025$ ) on the basis of the spontaneous visible rate. Comparable positive results as regards the induction of visibles have previously been encountered with unirradiated DNA (ref. 1), which suggested that these mutations could rarely be induced in the presence of the polymer, under appropriate cellular conditions. On the other hand, the sonicated and irradiated DNA (Table 3), like the untreated material<sup>1,2</sup>, were completely ineffective in the induction of sex-linked recessive lethals; the mutation rate in this respect was the same as among the progeny of males injected with isotonic saline.

The results presented here showing the inactivity of degraded homologous DNA, as regards sex-linked recessive lethals, are in clear contradiction with Parkash's report<sup>11</sup>

Table 2. THE *Minutes* AND SEX-LINKED RECESSIVE VISIBLES (v) INDUCED BY UNTREATED AND DEGRADED DNA (SONICATED OR IRRADIATED), DETECTED IN THE F<sub>1</sub> MALES FROM ATTACHED-X EXPERIMENTS, MOSAIC MUTANTS ENTERED IN BRACKETS

Broods	Control				Undegraded DNA				Degraded DNA							
					0.5% Untreated				0.5% Sonicated				1% Irradiated			
	<i>Minutes</i>		v		<i>Minutes</i>		v		<i>Minutes</i>		v		<i>Minutes</i>		v	
	Males	No.			Males	No.			Males	No.			Males	No.		
I	19,768	39	1.97	4 (1)	4,603	8	1.74	0	5,336	27 (3)	5.06	2	3,857	21 (1)	5.44	1
II	19,247	50 (1)	2.60	5 (1)	5,237	19	3.63	1	5,006	23 (2)	5.50	2	3,379	20 (1)	6.10	0
III	17,073	27 (3)	1.51	2 (1)	5,345	19	3.55	0	4,622	18 (1)	3.80	1	840	3	3.57	0
IV	20,201	25 (3)	1.24	0	4,145	16	3.96	0	5,223	23 (2)	4.40	0	—	—	—	—
V	11,513	7 (1)	0.61	0	3,195	6	1.88	0	4,090	24 (2)	5.87	0	—	—	—	—
VI	11,742	23 (3)	1.87	2	1,330	1	0.75	0	3,306	11	3.23	0	—	—	—	—
VII	10,522	11 (1)	1.06	2 (1)	8	0	0.0	0	1,116	8	7.17	0	—	—	—	—
VIII	9,624	12 (1)	1.25	0	—	—	—	—	—	—	—	—	—	—	—	—
Total	120,530	193 (12)	1.60	16 (4)	23,863	69	2.89	1	23,719	139 (10)	4.84	5	7,976	44 (2)	5.52	1

that these mutations were produced at a high rate (5.7 per cent) among the progeny of flies fed with an unspecified sample of irradiated DNA (presumably heterologous). This mutagenic activity was stated to be the direct effect of the irradiation products of DNA, and was attributed to the incorporation of parts of the macromolecule into the nuclei of the fly's germ line. Great care was accordingly taken in the design of the present experiment with irradiated DNA, to favour the induction of recessive lethals, on the basis of Parkash's proposals. Homologous DNA was used, in the hope of favouring exchange between the exogenous material and the genome of the germ cells.

Degradation of DNA was undertaken by its exposure in the gel-state to a massive dose of  $\gamma$ -rays ( $> 230,000$  r.), which was more than double that used by Parkash. The irradiated DNA was immediately injected around the males' testes, in the hope of capturing any metastable mutagenic compounds that might have resulted from irradiation. The injected males were tested throughout the full period of their fertility, so as to ensure the recovery of sperm treated in the spermatocytic and spermatogonial stages. These were the cells predominantly exposed to the treatment (in the larval testes) with Parkash's feeding procedure, and they would be sampled with the brood technique used in the present work on injected adults in the fourth and later broods, more than 9 days after treatment<sup>4</sup>. In spite of these precautions, the irradiated DNA showed no signs of activity—as regards sex-linked recessive lethals—either in the early or in the late progeny (Table 3). The results in the fourth and later broods were of particular interest, since they showed the response of the early sector of the germ line. The progeny in these broods was invariably low with the higher injected doses of all the tested macromolecules<sup>1-3</sup>, because of their preferential cytotoxicity on the metabolically active early germ cells. Nevertheless, in the Muller-5 test with irradiated DNA, it was possible to recover 511 chromosomes after the ninth day of treatment, the majority of which must have been treated in the meiotic and premeiotic stages. If mutagenic response to irradiated DNA was restricted to these stages, the tested X-chromosome sample would be expected to contain a minimum of 19 lethals (the lower limit of the binomial expectation at  $P = 0.025$ ) on the basis of its reported<sup>11</sup> activity (5.7 per cent); instead none was observed. There can be no doubt, therefore, that the recessive lethals which occurred in Parkash's experiment were not the direct outcome of the incorporation of DNA fragments (released by irradiation) into the genome of the fly's germ line. The possibility still remains, however, that the DNA irradiation products might have been metabolically converted into mutagenically active derivatives as a result of their passage through the flies' alimentary canal. This possibility is now under investigation, in view of its implications as regards human genetic hazards after food sterilization by ionizing radiation.

The demonstration of the higher activity of degraded DNA in the induction of *Minutes* suggested that its mutagenicity might—in part at least—be due to its intracellular breakdown to the constituent nucleotides and the corresponding nucleosides after dephosphorylation. In support of this possibility were the recent results<sup>4</sup> with the nucleosides of pyrimidine analogues: the 5-halogen derivatives of deoxyuridine—FUDR, BUDR, IUDR; cytosine arabinoside, 6-azauridine, and 6-azacytidine. All these compounds showed the DNA type of mutagenicity, in so far that they were mainly effective in the induction of *Minutes*, were rarely active for recessive visibles and inactive for lethals.

From a comparison of the biochemical and mutagenic properties of the tested nucleosides, it was possible to deduce that mutagenicity was the outcome of the shortage of particular nucleotides, through the specific inhibition

of the enzymes required in their synthesis. A slight disturbance in this way would be expected to result in the loss of one—or a few—nucleotides during the synthesis of genomic DNA, thus resulting in point mutations (recessive visibles). On the other hand, a drastic metabolic disturbance at the nucleotide level would upset the balance between the four requisite DNA precursors, thus blocking polymerization and resulting in the loss of whole molecules, and the eventual induction of chromosome deletions. A comparable disturbance in the optimal equilibrium between DNA precursors might well arise as a result of intracellular accumulation of the polymer's degradation products, which could lead to the same errors in the polymer's synthesis and the associated mutagenic effects. It seemed, however, that the natural nucleosides (derived from DNA degradation) produced the slight metabolic disturbances (nucleotide losses) more frequently than their synthetic analogues, since there were more point mutation visibles after DNA injection than with the tested pyrimidine nucleosides. It is most unlikely, however, that the whole of the biological activity of DNA is due to its degradation products. An appreciable rate of *Minutes* occurred with fairly undegraded DNA, as well as with various synthetic polymers, some of which (like polymethacrylic acid) were known to be completely resistant to intracellular enzymatic degradation. This suggested that, at least in so far as chromosome deletions were concerned, part of the DNA effect was produced while the molecule was still in some polymeric state. Its mutagenic effect in such a state—as with other polymers—was probably due to generalized inhibition of cellular enzymes, including some of those involved in DNA synthesis.

To conclude, the results presented here with intact (untreated) as well as degraded DNA (treated with ultrasonics or irradiation) confirmed and supplemented our previous results<sup>1-3</sup> concerning the genetic mode of action of this macromolecule in metazoan cells. Specific locus transformation was unsuccessful, both with wild-type and mutant DNA, even with samples of fairly large molecular size. In contrast, DNA at various levels of degradation was decisively active as regards the induction of small chromosome deletions (*Minutes*), weakly active for the recessive visibles, but completely inactive for the recessive lethals. Evidence was found indicating that DNA mutagenicity could be produced indirectly, through its passive conveyance by the sperm during fertilization and the subsequent induction of mutation in the course of chromosome replication at cleavage: mutants occurred in the maternal X-chromosome when treatment was restricted to the paternal germ line. Degradation of DNA, by ultrasonics or  $\gamma$ -radiation, did not alter the type of mutations induced, but only increased the mutagenic efficiency as regards the induction of small chromosome deletions (*Minutes*).

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# LETTERS TO THE EDITOR

## RADIOPHYSICS

### Lunar Occultation of the Radio Source CTA 21

THE radio sources CTA 21 and CTA 102 have been of considerable interest since they were first reported by Harris and Roberts<sup>1</sup> from observations at 958 Mc/s. Later work<sup>2</sup> showed that the radio spectra of these sources differed from those of most other sources and that each source had a maximum of emission near 900 Mc/s. It has been suggested<sup>3,4</sup> that this form of spectrum is produced by self-absorption of synchrotron radiation within a source of small angular size, and from the shape of their spectra the very small angular diameters of  $\sim 0.01$  sec of arc have been predicted for both CTA 21 and CTA 102. CTA 102 has recently been identified<sup>5</sup> as a quasi-stellar radio source, but for CTA 21 no optical object can be detected on the 48-in. Sky Survey plates within the limits of error ( $\pm 6$  sec of arc) of the existing radio source position<sup>6,7</sup>. It appears that an identification of CTA 21 with an optical object can only be made if the radio source position is more precisely known and the region of sky can be examined with more powerful optical techniques.

An opportunity to measure an extremely precise position for the source has been presented by the series of lunar occultations which occurs during 1964-65. No further occultations of this source will then be observable for several years. Two occultations were predicted for the C.S.I.R.O. radio telescope at Parkes, Australia. The first occurred close to the local horizon and could not be observed, and even on the second occasion (February 9, 1965) only the disappearance of the source occurred within the normal working altitude range of the telescope.

This disappearance was observed at 2,650, 416 and 154 Mc/s. The measurements at 2,650 Mc/s showed well-

defined Fresnel diffraction fringes, and preliminary calculations suggest that the source is single and that it has a radio diameter of less than 1.5 sec of arc. This result is consistent with that obtained from some recent interferometer measurements<sup>8</sup> which have shown that the source has an apparent diameter  $< 0.4$  sec of arc. Re-appearance occurred  $8^\circ$  below the altitude limit of the telescope and was observed at 154 Mc/s only with an offset feed. Even at this frequency the measured signal-to-noise ratio was appreciably reduced from the on-axis observations and no useful off-axis observations could have been made at the higher frequencies. The use of off-axis observations and the low flux density of the radio source at 154 Mc/s have meant that the signal-to-noise ratio and hence the accuracy of timing are much poorer at the re-appearance than at the disappearance.

The measured times are given in Table 1.

Table 1	
Disappearance	10h 10m 47s.8 $\pm$ 0s.5 U.T.
Re-appearance	11h 28m 02s.5 $\pm$ 0s.5 U.T.

These times correspond to a position of the radio source of R.A. 03h 16m 9s.11, Dec.  $16^\circ 17' 40''.3$  (1950.0). A value of 33s.5 has been assumed for the difference between ephemeris and universal times in order to bring the deduced position on to the FK3 system<sup>9</sup>. The source position together with the limits of error are shown in Fig. 1.

It is understood that no object can be seen in the new radio position on the best existing plates which have been taken with the 200-in. telescope on Mount Palomar.

We thank Mr. B. F. C. Cooper and many others on the laboratory's staff for their assistance with the observations, and Mr. W. Nicholson of H.M. Nautical Almanac Office for sending details of the circumstances of the occultation.

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## PHYSICS

### A New Role for the Electrophorous

THE electrophorous<sup>1</sup> consists of a flat polymeric plate placed in contact with a flat metal plate. The two surfaces are separated only by the higher asperities of the surfaces and so actual contact is only made at a few points. If the polymer surface has been previously negatively charged by the diffusion of electrons into it, then the metal will be negatively charged at the few places where contact is made and positively charged by induction where there is no contact. If a time-varying dynamic load is now applied to the system so that the plates never lose contact and the variation of the distance between them is due to the elastic deformation of the asperities, then there will be time-dependent variations in the induced electrostatic charge on the metal plate. If the

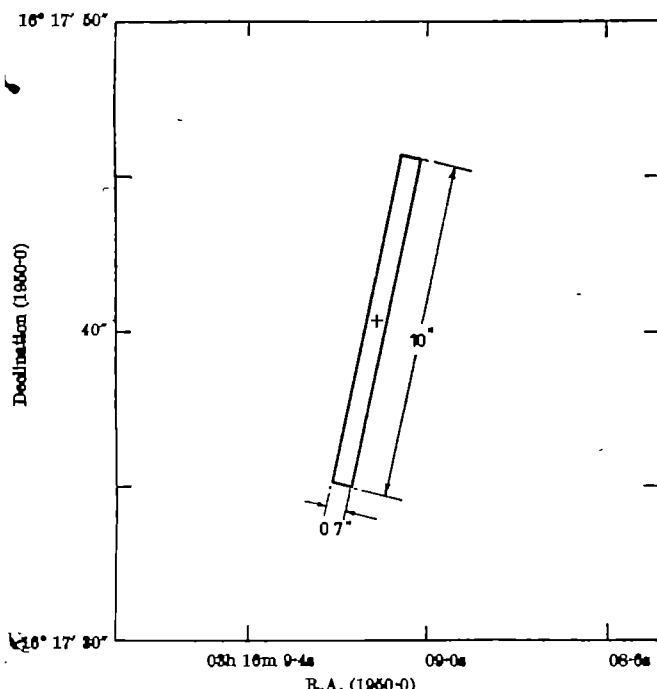


Fig. 1. Diagram showing the mean position (+) of the radio source CTA 21. The limits of error are defined by the range of possible positions of the limb of the Moon allowed by the uncertainties in the measured times of disappearance and re-appearance

induction effects predominate, then it is of interest to measure the current flow to, and from, the plate when it is connected to earth through a high-resistance voltmeter or oscilloscope.

In these investigations a charge is put on the polymer surface by heating in contact with a metal surface at the softening point of the polymer and then applying a static pressure which just deforms the polymer. This process is considered to be the most promising for investigation because the initial electrification action must be electronic diffusion from metal to polymer at the softening temperature.

Although simple to manufacture, it seems likely that the electrical response of the system to a dynamic load may be complicated. This has therefore been subjected to preliminary experimental investigation using the pressure bar method to apply dynamic pressures. A steel rod 2.5 cm diameter and 120 cm long with plate ends is supported horizontally. Longitudinal impact by a 2.5 cm diameter steel sphere, which is suspended as a simple pendulum, produces a stress wave pulse in the rod. The maximum pressure corresponding to a given height of fall of the impactor is obtained from the results of Barton *et al.*<sup>2</sup>

In the present high-pressure experiments the polymer used is polymethylmethacrylate (PMMA). The transducer unit tested using the pressure bar consists of a (PMMA) plate 2.5 cm  $\times$  2.5 cm  $\times$  0.1 cm cemented on to one of the two plane end faces of a short cylindrical steel rod which is 5 cm long and 2.5 cm diameter. Electrostatic charge is then produced on the free PMMA surface as described here. A clean tin foil is next cemented on to the face of a polymer block 2.5 cm  $\times$  2.5 cm  $\times$  1.0 cm. The free tin and charged PMMA surfaces are put in contact and the parts clamped together under a small static compressive force. The unit is then wrung on to the measuring end of the pressure bar and a compressive stress pulse generated by the impactor. The maximum pressure in the stress pulse is much greater than the static pressure. The voltage-time signal recorded from the unit consists of an initial pulse followed by a wave train which decays in amplitude.

The maximum voltage of the initial pulse is compared with the maximum stress pulse pressure for heights of fall,  $h$ , of the impactor over the range 1–15 cm. Up to  $h \approx 10$  cm, corresponding to a maximum pressure of  $70 \times 10^4$  dyn/cm<sup>2</sup>, the experimental observation is that maximum pressures and voltages are directly proportional. Since pressure is proportional to the velocity of the measuring end of the bar<sup>3</sup>, that is, the charged PMMA surface, then it follows that the recorded maximum voltage or current is directly proportional to the velocity with which the charged PMMA surface moves towards the metal surface during dynamic compression.

The value of this experimental result is that it makes possible the application of electrostatic properties to the development of electrical devices which can be used to investigate any continuous mechanical vibration. Various types of dynamic electromechanical transducer, which utilize electrostatic properties in conjunction with the wide range of polymer mechanical properties, are being considered for the examination of the dynamic forces in impact problems, and acoustic vibrations.

Further possibilities for the utilization of electrostatic surface charges initiated by electronic diffusion are being explored.

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<sup>1</sup> Hechtall-Smith, H. W., *Intermediate Electrical Theory* (Dent and Son, London, 1950).

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## Distribution of Near Neighbours in a Random Packing of Spheres

DURING some work on the tensile strength and cohesion of wet particles, it became necessary to have some knowledge of the number of contacts and near contacts existing in a random packing of equal spheres. The range of centre to centre separations from 1.0 to 1.3 diameters was of particular interest to this work because experimental results had shown that liquid bridges existing between spheres separated by these distances were likely to play a major part in the cohesion of a wet bed of spheres<sup>1</sup>.

Both Bernal<sup>2</sup> and Scott<sup>3</sup> have measured radial distributions in packings of spheres for use in their work on liquid structures. However, they were both concerned with a somewhat greater separation range, and their results lack the necessary detail in the narrow range required here. To obtain some additional information in this range the following experiment was performed. One thousand and six good-quality table-tennis balls, diameter 1.5 in., were placed in a polythene bag, and the whole was buried in sand. The sides of the bag had been randomly deformed to prevent systematic packing on the outside of the system. The bag was then filled with a 2 per cent w/v agar solution at 45° C which filled the spaces between the balls. The balls were prevented from floating up in the solution by weighting the top of the assembly. The agar was allowed to set at room temperature, and the complete assembly removed from the bag. The balls were removed one at a time. As each sphere was removed the number of contacts was counted, and the distance of any near neighbours measured through the agar with a simple needle-type probe. This was a direct measurement with an accuracy of 0.25 mm. The total number of contacts and the distribution of near neighbours are counted for the whole bed. The values obtained for the outer layers did not differ significantly from the values for the central core, consequently all the counts are included in the results. These are plotted in the co-ordinates used by Scott in Fig. 1. This illustrates the high incidence of actual contacts, and shows the significant number of non-touching near neighbours lying in the range 1.0–1.3 diameters. An interesting point is the appearance of an apparently significant small peak in the region of 1.22 diameters. The overall packing density of the bed was measured and the limiting value at large values of  $r$  is shown in Fig. 1.

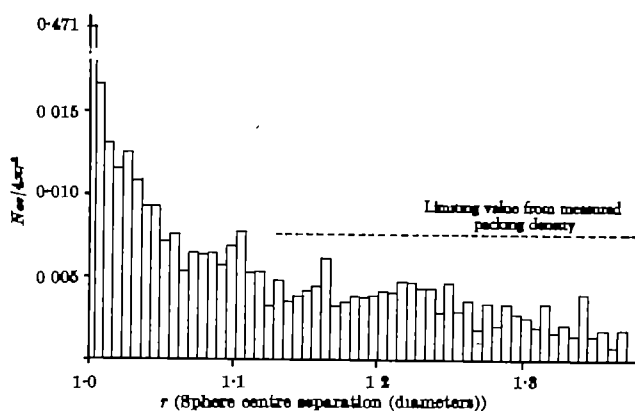


Fig. 1. Histogram of  $N_n/4\pi r^2$  versus  $r$ .  $N_n$  is the average number of spheres in an interval of 0.0656 (0.25 mm) sphere diameters at a distance  $r$  in sphere diameters from the centre of an average sphere.

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## GEOLOGY

## Interpretation of Geological Features on a Satellite Photograph

DURING examination of automatic picture transmission system photographs taken by *Nimbus 1*, a photograph exposed from 308 miles above Lake Ontario, during orbit 254, on September 14, 1964, clearly showed the complex folded pattern of the Appalachian Mountains in the Pennsylvania region. The photograph, produced by a slow scan kinescope method, designed and developed by the National Research Council of Canada, specifically to record and reproduce the full capabilities of the 800-line high-resolution automatic picture transmission vidicon of this American meteorological satellite, was one of many received at Ottawa and Frobisher Bay ground stations.

Detailed examination of the photographic pattern as to size, shape and tone of these Appalachian features showed close correlation with the rock-type units as depicted by the 1/250,000 Geologic Map of Pennsylvania, published by the State Geological Survey, 1960. By projecting a photographically reduced map (Fig. 1) on to the photograph (Fig. 2), variations in tonal pattern agreed in fine detail with the rock groups. The rock groups which were predominantly shales showed in light tones, sandstones and conglomerates were dark, and the boundaries of each distinctive group were clearly delineated on the photograph.

The well-known Pennsylvania anthracite coal region in the Wilkes-Barre-Scranton area is a good example. On the map this area is shown as a crescent-shaped rock group, 50 x 8 miles in extent, known as the Post-Pottsville formation (No. 2 of Fig. 1). It consists of brown or grey sandstone and shales with some conglomerate and numerous minable coals. This group is completely ringed by a border, about 2 miles wide, of the Pocono group (No. 1 of Fig. 1), a predominantly grey massive cross-bedded conglomerate and sandstone with some shale. On the photograph we find an exact duplication in shape and size, a crescent light-toned area for the Post-Pottsville

Group (No. 2 of Fig. 2) bordered by a dark-toned strip corresponding to the Pocono Group (No. 1 of Fig. 2) in spite of the fact that this area is shown on a land-use map as forest-covered.

Again, in the Great Valley running through Harrisburg, we find the Martinsburg Formation (No. 3 of Fig. 1) comprising grey to dark grey, light grey to olive weathering shale with interbedded sandstone appearing on the photograph in a light tone (No. 3 of Fig. 2), corresponding in shape and size to that formation on the map. Along the northern edge is a narrow 1-2 mile wide border of Shawangunk Formation (No. 4 of Fig. 1), a grey to tan thick bedded impure quartzite sandstone conglomerate with interbed of shale. This border is duplicated on the photograph as a narrow dark-toned strip of shape and extent corresponding to the formation. Other strips of this formation occur to the north and west, and also appear on the photograph (No. 4 of Fig. 2). Along the southern edge of the Martinsburg Formation, west of the Susquehanna River, is the Beekmantown Group (No. 5 of Fig. 1) of white to grey medium thick-bedded fine-grained quartzite sandstone conglomerate showing on the photograph as a dark-toned area (No. 5 of Fig. 2). East of the Schuylkill River, the southern border is Pre-Cambrian granite gneiss (No. 9 of Fig. 1). On the photograph its identical pattern is repeated in dark tone (No. 9 of Fig. 2). Similar correlation is repeated across the map area with the various geological groups (Nos. 1-12 of Fig. 1) closely delineated on the photograph by corresponding tonal patterns (Nos. 1-12 of Fig. 2).

It is appreciated that the tonal variations can be linked to the forest cover, and forest boundaries are also known to mark changes in geological formations, particularly as is evidenced to the north-east in the Adirondack Mountain area. However, in the Pennsylvania area, forest cover alone is not sufficient to account for the very distinct and uninterrupted sizes and shapes of the tonal pattern that relate to the rock group areas on the map. Large portions of these individual group areas are known to be under intense varying cultivation, with other areas of considerable magnitude given over to industrial com-

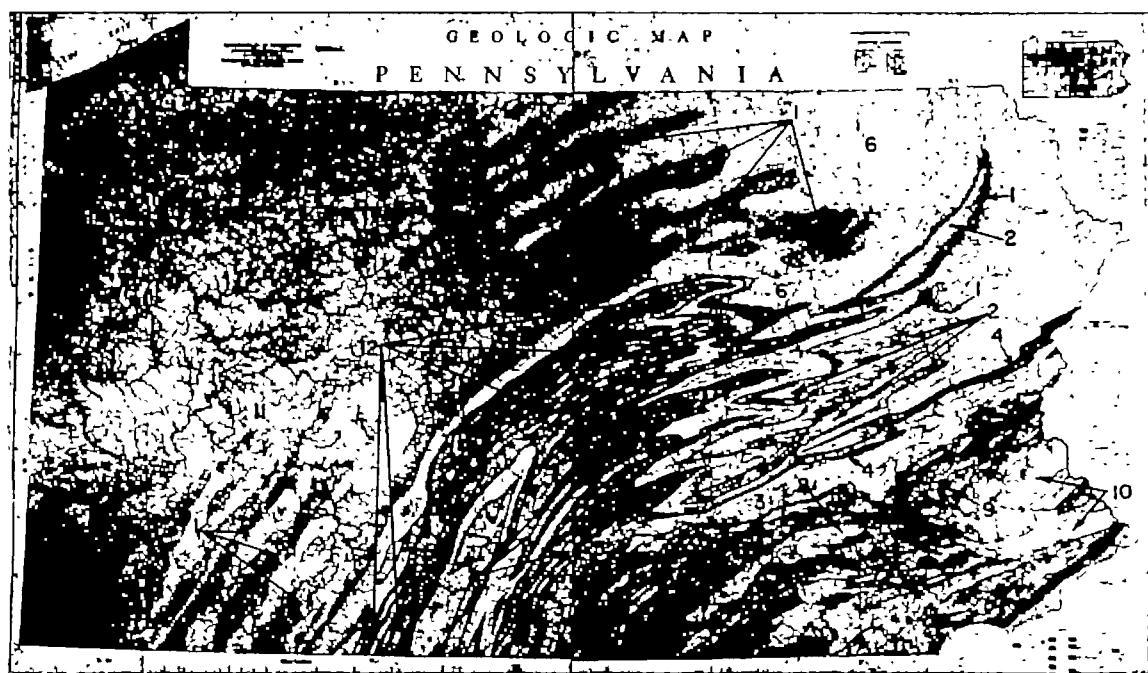


Fig. 1. 1. Mississippian-Pocono Group. 2. Pennsylvanian-Post-Pottsville Group. 3. Ordovician-Martinsburg Formation. 4. Silurian-Shawangunk and Tuscarora Formations. 5. Ordovician-Beekmantown Group. 6. Devonian-Susquehanna Group. 7. Ordovician-Onondaga Formation. 8. Ordovician-Ocoee Formation. 9. Pre-Cambrian-Granite gneiss. 10. Triassic-Brunswick, Lockatong and Stockton. 11. Pennsylvanian-Conemaugh Formation. 12. Ordovician-Juniata, Bald Eagle Formations.

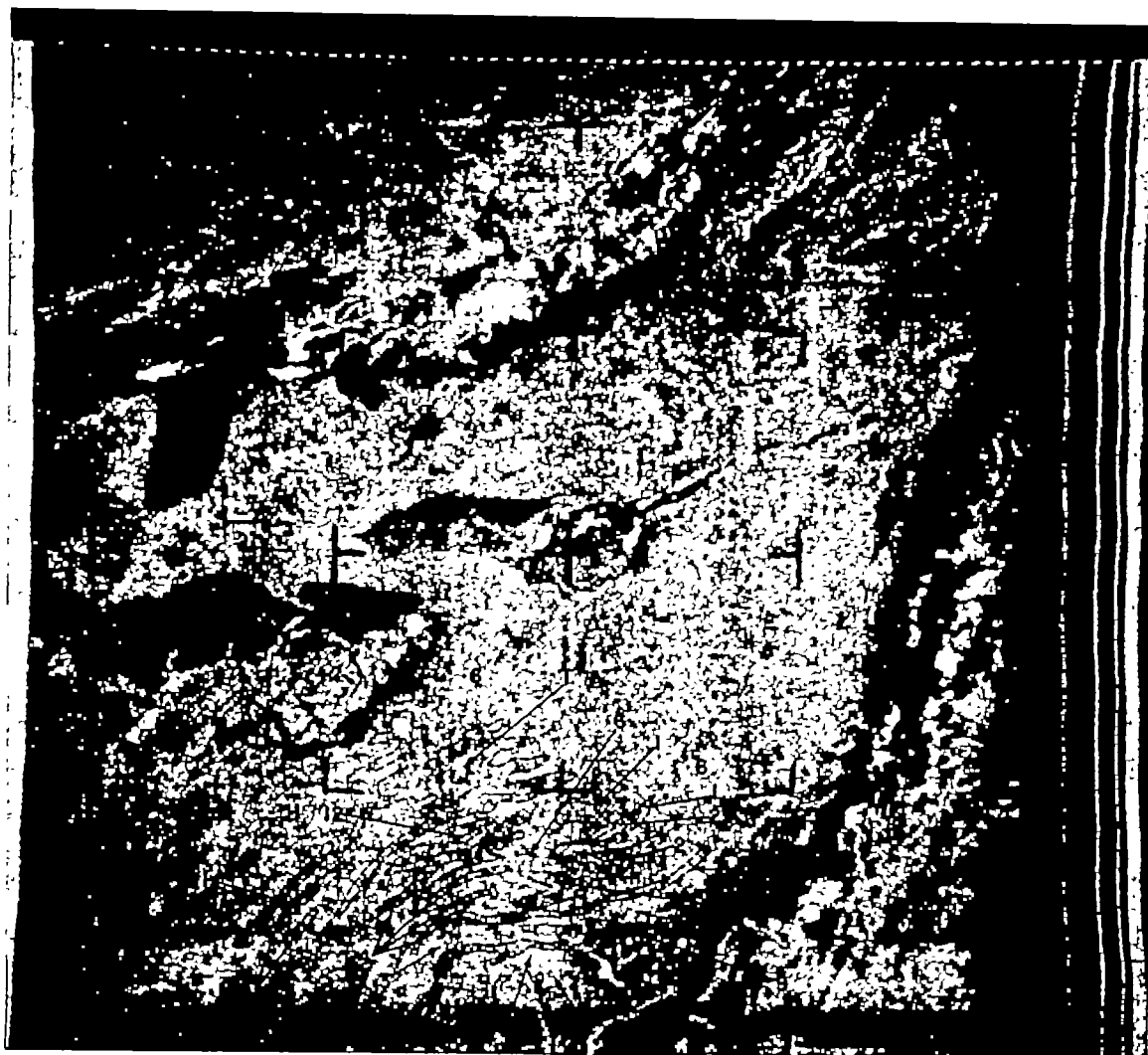


Fig. 2. Dark-toned features: 1. Mississippian—Pocono Group. 4. Silurian—Shawangunk and Tuscarora Formations. 5. Ordovician—Beekmantown Group. 9. Pre-Cambrian—Granite gneiss. 10. Triassic—Brunswick, Lockatong and Stockton. 12. Ordovician—Juniata, Bald Eagle Formations. Light-toned features: 2. Pennsylvanian—Post-Pottsville Group. 3. Ordovician—Martinsburg Formation. 6. Devonian—Susquehanna Group. 7. Ordovician—Conestoga Formation. 8. Ordovician—Cocalico Formation. 11. Pennsylvanian—Conemaugh Formation.

plexes and large urban developments. These, of course, influence but do not upset or disrupt the shapes, sizes or tones of the geological patterns seen on the satellite photograph.

This particular photograph is not a peculiar freak of lighting, camera position, etc., for the same features are also reproduced in an earlier *Nimbus* automatic picture transmission, from orbit 137, taken on September 6. On it, the same features appear in similar shapes, sizes and tones as on the photograph taken eight days later, but they are not so easily seen due to the presence of haze and some cloud over the area.

It is therefore felt that the variations of tones shown in this study are specifically related to the rock types of a detailed geological map, and are greatly influenced by the different reflectivity of the various basic rock-soil textures. Further, it would appear that these textures have a greater influence on the tonal rendition in a very high altitude photograph than when their pattern is cluttered with identifiable and directly visible features such as vegetation and man's developments of the Earth's surface—as is the case with conventional aerial photography.

Additionally, it has also been noted in the original photograph that a faint line runs in a slight arc across the picture from where the Susquehanna River enters the

Appalachian Mountains to Chautauqua Lake, which is barely visible through thin cumulus cloud along the south shore of Lake Erie. This linear may possibly be explained by a deep-seated crustal disturbance, such as a buried 'graben' suggested in this area by Prof. J. Tuzo Wilson<sup>1</sup>.

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<sup>1</sup> Wilson, J. Tuzo, *Amer. Sci.*, 47 (1959).

### Eocene-Oligocene Boundary in New Zealand

THE planktonic foraminifera *Hanikoenina*, *Globigeropsis* and *Pseudohastigerina micra* are widely distributed throughout the world and their last appearance has generally been considered to mark the top of the Eocene<sup>1-3</sup>. Hornibrook stated that *H. alabamensis*, *G. index* and *P. micra* make their last appearance in the Runangan Stage, and consequently regarded the Runangan-Whaingaroan boundary as the Eocene-Oligocene boundary in New Zealand<sup>4</sup>.

Blow and Banner gave ranges of planktonic species in the Lindi area of Tanganyika and showed that the genera *Hanikoenina* and *Globigeropsis* make their last appearance

well below the top of the Eocene, the Eocene-Oligocene boundary there being based on the ranges of larger foraminifera<sup>1</sup>. The larger foraminifera of this age do not occur in New Zealand, but from the ranges of planktonic species given by Blow and Banner, Jenkins concluded that the extinction of *Hanikenina* and *Globigerapsis* does not mark the end of the Eocene in New Zealand and that the Eocene-Oligocene boundary lies within the Whaingaroan stage<sup>2</sup>, which contains most of the 'Oligocene' species mentioned by Blow and Banner.

Finlay and Marwick defined the top of the Runangan Stage at the type locality on the west coast of the South Island as marked by the last appearance of *G. index*<sup>3</sup>. I have found that the last appearance of *G. index* there coincides with that of *Globigerina linaperta* and *G. pseudocampliapertura*, and that *G. euapertura* and *G. ampliapertura* make their first appearance immediately above, at the base of the Whaingaroan stage. *H. alabamensis* extends only up to the lower part of the Runangan and this is consistent with its range at Oamaru on the east coast of the South Island (Hornibrook, personal communication). On the other hand, *P. micra* extends up to about the middle of the Whaingaroan stage.

LINDI, TANGANYIKA		CRITICAL PELAGIC FORAMINIFERA	NEW ZEALAND		
UPPER EOCENE	LOWER OLILOCENE		SAMTAI	WHANGAROA	WHANGAROA
		<i>Buccella</i>	+		
		<i>Cheloniceras</i>			
		<i>Pseudohoplites</i>			
		<i>Globorotalia centralis</i>			
		<i>Cheloniceras</i>			
		<i>G. pseudocampliapertura</i>			
		<i>G. ampliapertura</i>			
		<i>G. euapertura</i>			

Fig. 1. Chart comparing the stratigraphic ranges of some of the Upper Eocene-Lower Oligocene planktonic Foraminifera in New Zealand and the Lindi area of Tanganyika. Arrows indicate greater range than shown on the diagram

The stratigraphic ranges of the significant planktonic species common to New Zealand and the Lindi area are compared in Fig. 1. The only range which is markedly inconsistent is that of the genus *Globigerapsis*, which makes its last appearance earlier than *H. alabamensis* and *Globorotalia centralis* in the Lindi area but later than *H. alabamensis* and *G. centralis* in New Zealand.

Consequently *Globigerapsis* must be regarded as unreliable for correlation between the Lindi area and New Zealand, and from the other planktonic species the Runangan-Whaingaroan boundary appears to be equivalent to the Eocene-Oligocene boundary as delineated by Blow and Banner.

I thank Dr. Paul Vella and Prof. H. W. Wellman for their advice.

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## CHEMISTRY

### Photochemical Degradation of Paraquat

THE establishment of world-wide and varied uses of 'Gramoxone'<sup>1</sup> herbicide requires a complete understanding of the behaviour of its active constituent, paraquat, in all circumstances. The present report is concerned with the photochemical degradation of the chemical.

When paraquat dichloride (I) in aqueous solution is irradiated with ultra-violet light in the presence of oxygen, rapid decomposition occurs. The reaction has been conveniently examined on a small scale (10-20 ml.) by stirring a 0.1 per cent aqueous solution of <sup>14</sup>C-methyl paraquat dichloride with a magnetic stirrer in a beaker covered with a sheet of quartz glass and shining a Hanovia model 18 lamp (used without a filter) on it. Fifty micro-litre aliquots were removed at intervals and applied as spots at the top of a strip of chromatography paper, which was afterwards developed using a mixture of *n*-butanol, acetic acid and water (4:1:2). An autoradiograph (Fig. 1) of the resulting chromatograph (prepared by exposing the chromatograph to X-ray film) showed that only two major radioactive decomposition products were formed.

Compound A in Fig. 1 is paraquat itself (shown by comparison of its *R<sub>F</sub>* value with that of an authentic specimen, and by the grey colour it produced when sprayed with potassium iodoplatinate<sup>2</sup>). After three days' irradiation with ultra-violet light, no paraquat remained. The concentration of compound B increased to a maximum after irradiation for 1 day, but itself decomposed until eventually only compound C remained.

Compound B quenched the induced fluorescence of the paper when the chromatograph was observed under ultra-violet light (whereas compound C did not): this provided a useful means of studying the formation of the compound when non-radioactive paraquat was used. Paper chromatography of an irradiated solution on a larger scale yielded sufficient compound B for measurement of its ultra-violet spectrum. In aqueous solution, this showed maximum absorption at 285 mμ; when the solution was acidified, the absorption band underwent a bathochromic shift. A large-scale reaction was performed so that sufficient compound B could be isolated for its identification. One litre of 0.1 per cent aqueous paraquat was irradiated using a Hanovia 10L photochemical

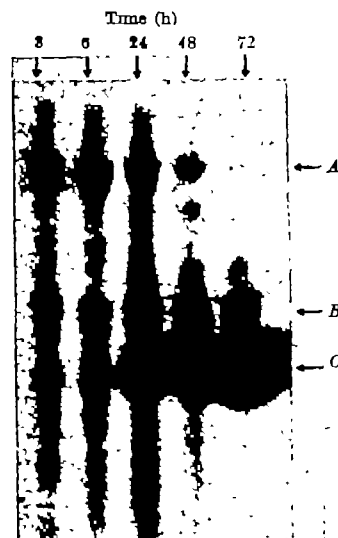


Fig. 1. Autoradiograph of chromatograph of ultra-violet-irradiated <sup>14</sup>C-methyl paraquat

reactor with quartz down-tube which contained a medium pressure mercury vapour arc tube. Oxygen was passed through the irradiated solution, and irradiation was continued until more than 95 per cent of the paraquat had decomposed (judged by paper chromatography of aliquots). This occurred after 4 days and the resulting solution was passed through a column of 'Dowex 50W-X8' cation-exchange resin. Compound *B* was eluted from the column with 2 per cent formic acid (its elution was followed by observing the ultra-violet absorption of the eluate at 270 mμ). Removal of the solvent gave a white solid, which was crystallized from ethanol.

The nuclear magnetic resonance spectrum of the compound was consistent with a methyl quaternary pyridinium compound substituted at the 4-position ( $\delta = 4.43$  (3 protons); 8.13, 8.23 (2 protons); 8.74, 8.85 (2 protons)), and elemental analysis and comparison of its infra-red and ultra-violet spectra with those of an authentic sample showed that the compound was the *N*-methyl betaine of isonicotinic acid (III). The bathochromic shift in the ultra-violet spectrum of III on acidification has been noted before<sup>3</sup>. Compound *B* also had the same *R<sub>F</sub>* value as authentic III on paper chromatography in three solvent systems (4:1:2 *n*-butanol, acetic acid and water; 8:3:4 *n*-butanol, formic acid and water; and 4:1 isopropanol and water).

Ultra-violet irradiation of paraquat labelled with carbon-14 in the  $\alpha$ - and  $\beta$ -positions of the pyridine rings followed by paper chromatography showed that only one major radioactive compound was formed, namely, compound *B*. Thus compound *C* must have been formed from the methyl group of paraquat, and could not have contained any carbon atoms originating from the  $\alpha$ - and  $\beta$ -positions of the pyridine rings.

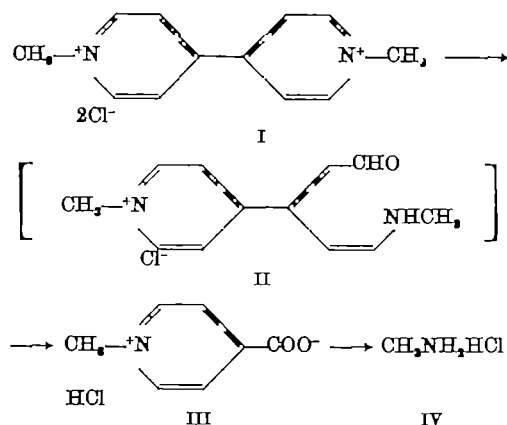


Fig. 2

When the hydrochloride of the *N*-<sup>14</sup>C-methyl betaine of isonicotinic acid in aqueous solution was irradiated with ultra-violet light it decomposed to yield compound *C* as the only isolable radioactive product. This reaction was repeated on a large scale using non-radioactive methyl quaternary isonicotinic acid, and the solution was irradiated until no starting material remained. Removal of solvent yielded compound *C* and comparison of its infra-red spectrum with that of an authentic sample showed it to be methylamine hydrochloride (IV). This was confirmed by examination of its paper chromatographic behaviour in the three solvent systems already described here.

Carbonyl compounds are probably formed as transitory intermediates in the decomposition since addition of 2,4-dinitrophenylhydrazine to an irradiated solution precipitated a small amount of an orange derivative. Thin-layer chromatography on silica gel showed that this was a mixture of at least six components.

A likely early intermediate is the unsaturated amino-aldehyde II (similar compounds have been obtained from

the reaction of bases with pyridinium compounds<sup>4</sup>), which must be rapidly oxidized. The fragments resulting from this oxidation or a similar one include carbon dioxide, since <sup>14</sup>CO<sub>2</sub> is evolved from paraquat labelled with carbon-14 in the  $\alpha$ - and  $\beta$ -positions of the pyridine rings. When aqueous paraquat is irradiated with ultra-violet light in the absence of oxygen, it decomposes, but compounds III and IV are not formed. Instead, a brown resin, presumably polymeric, remains when all the paraquat has decomposed and the solution is evaporated to dryness. This could result from polymerization of compound II or similar materials.

The same decomposition products (III and IV) are formed when paraquat adsorbed on filter paper or thin layers of silica gel is exposed to ultra-violet light from a mercury vapour lamp, or to sunlight. This decomposition in sunlight seems to occur only when the paraquat is adsorbed on a surface, since paraquat in aqueous solution does not decompose in sunlight to any appreciable extent.

More importantly, paraquat which has been sprayed on plants can be photochemically decomposed on the surface of leaves by sunlight, and compounds III and IV are formed (both of which, incidentally, have very low oral toxicities in mammals). Details of work on the decomposition of paraquat on plants will be published elsewhere.

I thank Dr. J. A. Farrington for assistance in the interpretation of the nuclear magnetic resonance spectrum.

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<sup>1</sup> Registered Trade Mark of Plant Protection, Ltd.

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### Autoradiographic Electrophoresis of Tritium-labelled Compounds in a Thin Layer of Buffered Photographic Gelatine

GEL electrophoresis allows the detection of radioactive compounds by contact with a photographic film. I have tried to separate radioactive ions by electrophoresis, using a thin layer of radiosensitive photographic gelatin as supporting medium. I hoped thus to locate smaller amounts of radioactive substances within a shorter time and, above all, to achieve higher efficiency in detection and location of tritium-labelled molecules through autoradiography. This communication gives a brief account of the first runs of autoradiographic electrophoresis in a thin layer of buffered photographic gelatine and affords a glimpse of the possibilities of this new type of supporting medium.

A strip of double-lead 'Kodirex' X-ray film, 10-15 cm long and of the proper width, is immersed for about 20 min at room temperature in a suitable buffer, then quickly blotted between two sheets of filter paper. The film strip is laid on a glass plate on which it sticks tightly. The sample (1-5 μl.) is directly applied on the gelatine layer, without scratching the damp surface. The glass plate used as a support is set on the cover of a Petri dish full of crushed ice, in the central compartment of an electrophoresis apparatus. Two filter-paper wicks, soaked with buffer, ensure the electric junction between the upper surface of the ends of the film and the compartment next to the electrode chambers. A similar glass plate is set on top of the whole system, as a means of reducing evaporation; its weight ensures adequate electric connexion between the filter paper and the gelatine; the thickness of the wicks prevents any contact between the

photographic emulsion and the glass plate used as a cover. When the separation process lasts more than 1 h, evaporation is prevented by the use of another system: the strip is sandwiched between two cooled glass plates, so that the upper glass plate supporting a second cooling Petri dish comes into close contact with the film.

The electrophoresis is carried out at potential gradients of 15–30 V/cm applied for 10 min to 1 h, as the case may be. The film strip is quickly dried in a stream of warm air and stored in the dark until its development through the usual processing.

The entire experiment is carried out in the dark room, with the help of properly filtered light.

(A) *Electrophoretic migrations.* (1) The first runs dealt with the electrophoretic migration, at pH 7.25 (tris/HCl buffer, 0.01 M, containing 5 per cent of glycerol as a means to reduce evaporation), of various substances alone or mixed together: sulphate and taurine-<sup>35</sup>S, taurocholate-<sup>14</sup>C, tritiated succinic acid, a strongly radiolysed tritiated methionine, etc. Fig. 1a shows that one can thus separate and detect histidine-<sup>14</sup>C, glutamic acid-<sup>14</sup>C and tritiated valine from a given mixture of these three amino-acids. The same can be carried out with a mixture of sulphate, cysteine acid and taurine-<sup>35</sup>S. Adsorption is definitely negligible, separation quite sharp, diffusion is weak and, unless the studied compounds' specific activities are very high, radioactive ions have no time to leave any track as they move in the photographic gelatine.

(2) The electrophoretic separation of the three above-named amino-acids was also achieved at pH 5.25 using a volatile buffer (acetic acid/pyridine/water 20/9.5/970 (v/v) containing 5 per cent of glycerol) (Fig. 1b).

(B) *Non-electrophoretic migrations.* The importance of phenomena due to electro-osmosis and evaporation was assessed with the help of a non-ionized compound: *D*-glucose-<sup>14</sup>C solution was regularly applied in small drops 1 cm apart all along the film strip and 17 V/cm potential gradient applied for 1 h. The tracks of Fig. 2 show that at pH 7.25 the radioactive molecules have not been moving except for radial diffusion. Similar results were found at pH 5.25 (pyridine/acetic acid buffer).

(C) *Sensitivity of the method.* Radioactive compounds being in close contact with the silver bromide grains

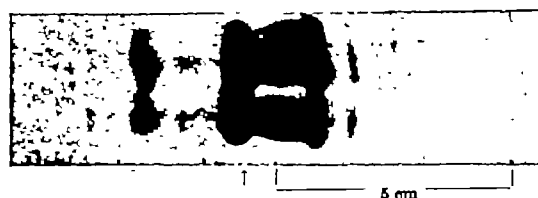


Fig. 3. Autoradiographic electrophoresis of a strongly tritium-labelled methionine: radiolysis and tracks due to high activity of the substance (tris/HCl buffer, 0.01 M; 5% glycerol) 25 V/cm for 60 min

within the very photographic gelatine layer in which they have been moving, this technique offers a high degree of sensitivity as is shown by the shortness of required exposure and especially by the ability of detecting tritiated substances. Thus, nothing came out of a contact autoradiography between a 'Kodirex' X-ray film and a paper electrophoregram supporting tritiated methionine, whereas in the case of a direct autoradiographic electrophoresis and with ten times less tritiated methionine, a definite darkening was achieved after exposure for 2 days. The method allows the detection of 0.001  $\mu$ c. of tritiated valine after exposure for 6 days.

This technique gives quick detection of ionized radioactive impurities in the course of preparing or storing labelled molecules, especially when they have been tritiated. Fig. 3, for example, shows the result of the autoradiographic electrophoresis of a strongly tritiated methionine, which had undergone radiolysis within the course of storage for three years.

The use, as supporting medium for autoradiographic electrophoresis, of glass plates coated with a thin layer of buffered nuclear emulsion should allow, after development, the microscopic detection of the silver grains or the individual tracks left by the radioactive ions after their migration. A still higher degree of sensitivity would thus be achieved.

Autoradiographic electrophoresis offers an opportunity of estimating by densitometry or grain count the relative amounts of tritium in the ionized compounds of a mixture.

I thank Dr. P. Fromageot of the Department of Biologie, Commissariat à l'Énergie Atomique, Saclay, for his advice.

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### Nitrous Oxide as a Scavenger in the Radiolysis of Water

NITROUS oxide has been widely used as a specific scavenger for the solvated electron in the radiolysis of liquid water<sup>1-3</sup>; the rate constant for reaction (1) being greater than that for reaction (2) by a factor of 10<sup>3</sup> or more:

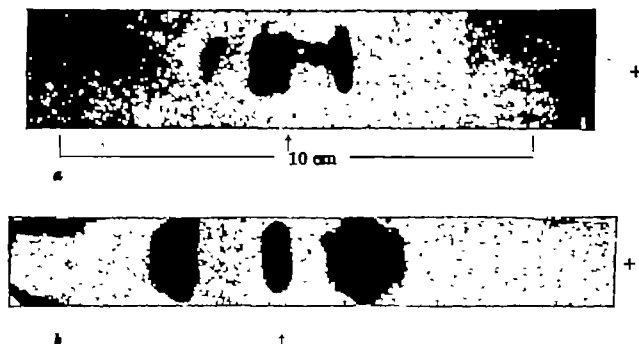
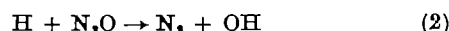
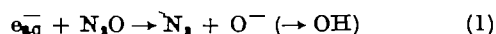


Fig. 1. Separation of histidine-<sup>14</sup>C, H<sup>3</sup>-valine and glutamic acid-<sup>14</sup>C (a), at pH 7.25 (tris/HCl buffer 0.01 M; 5% glycerol), 20 V/cm for 45 min; (b), at pH 5.25 (pyridine/acetic acid/water buffer, 5% glycerol), 20 V/cm for 60 min. The application points are indicated by an arrow

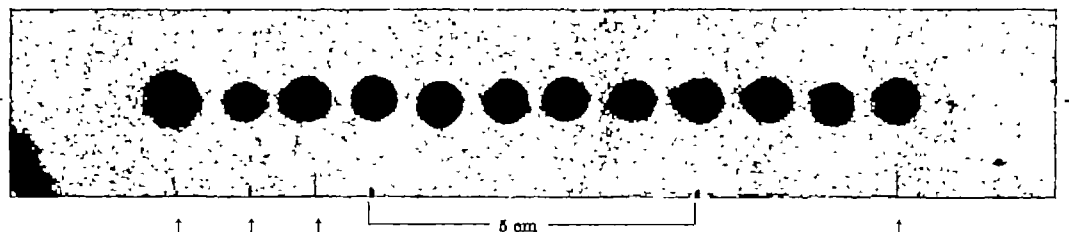


Fig. 2. Glucose-<sup>14</sup>C deposits 1 cm apart; 17 V/cm for 60 min. The radioactive glucose has radially diffused, but there is no non-electrophoretic migration

$\text{N}_2\text{O}$  concentrations  $> 1.2 \times 10^{-3} \text{ M}$  have invariably been found necessary to obtain values of  $G(\text{N}_2)$  independent of  $[\text{N}_2\text{O}]$  despite the large magnitude of  $k_1$ . Values of  $G(\text{N}_2) = 3.0 \pm 0.1$  obtained with this high concentration of  $\text{N}_2\text{O}$  have been equated to  $G(e_{aq}^-)$ .

Using an experimental technique similar to that reported previously<sup>1</sup>, we have examined the variation of  $G(\text{N}_2)$  as a function of  $[\text{N}_2\text{O}]$  in pure water at natural pH irradiated with cobalt-60  $\gamma$ -rays. Contrary to the findings of Dainton and Peterson<sup>1</sup>, who used larger doses, we obtained the results shown in Fig. 1 for doses of  $7 \times 10^{17} \text{ eV ml}^{-1}$ . The curve for  $G(\text{N}_2)$  consists of two distinct steps: first, a very rapid increase in  $G(\text{N}_2)$  from 0 to  $2.45 \pm 0.1$  with increasing  $[\text{N}_2\text{O}]$  from 0 to  $4 \times 10^{-3} \text{ M}$ ; secondly, a slow increase from  $2.45$  to  $3.1 \pm 0.1$  with increasing  $[\text{N}_2\text{O}]$  from  $4 \times 10^{-3}$  to  $1.2 \times 10^{-2} \text{ M}$ , above which  $G(\text{N}_2)$  was independent of  $[\text{N}_2\text{O}]$  up to  $2.5 \times 10^{-2} \text{ M}$ . Values of  $G(\text{N}_2) < 2.45$  were obtained at  $[\text{N}_2\text{O}] < 4 \times 10^{-3} \text{ M}$  but these are not shown in Fig. 1 because with a total dose of  $7 \times 10^{17} \text{ eV ml}^{-1}$  depletion of  $\text{N}_2\text{O}$  occurred. However, we have shown at smaller doses, where very similar curves to that given in Fig. 1 are obtained, that  $G(\text{N}_2) = 1.9$  at  $2 \times 10^{-3} \text{ M N}_2\text{O}$ .

We interpret this curve as indicating that the very efficient scavenging step represents reaction (1) and hence that  $G(\text{N}_2) = 2.45 \pm 0.1 = G(e_{aq}^-)$ . This is more in accord with the values of  $G(e_{aq}^-)$  obtained using other techniques than is the value of  $3.1 \pm 0.1$  found at  $1.2 \times 10^{-2} \text{ M}$ . The efficient scavenging by  $4 \times 10^{-3} \text{ M N}_2\text{O}$ , when competing for  $e_{aq}^-$  with radiation products only is also more consistent with the high value of  $k_1 = 0.86 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$  (ref. 4).

The second step in the curve seems to be the scavenging by  $\text{N}_2\text{O}$  of a second radiation produced species, rather than a consequence of a back reaction or intra-spur scavenging of  $e_{aq}^-$ , because the plot of  $\{G(\text{N}_2) - G(e_{aq}^-)\}^{-1}$  versus  $[\text{N}_2\text{O}]^{-1}$  is linear. The identity of this species having a yield of  $0.65 \pm 0.1$  is at present uncertain but it may be equivalent to the hydrogen atom or to the entity designated  $\text{H}_2$  by Dainton and Peterson<sup>1</sup>. The increase in  $G(\text{N}_2)$  at  $(0.4 \text{ to } 1) \times 10^{-3} \text{ M N}_2\text{O}$  cannot be attributed to intra-spur scavenging of  $e_{aq}^-$  because no region where  $G(\text{N}_2)$  was independent of  $[\text{N}_2\text{O}]$  was observed and at  $10^{-4} \text{ M N}_2\text{O}$  where the second step was  $> 30$  per cent complete the lifetime of  $e_{aq}^-$  would need to be an order of magnitude longer than the  $10^{-7} \text{ sec}$  predicted for intra-spur processes to be completed. Furthermore, although  $G(\text{H}_2)$  falls from  $0.5$  to  $0.1$  due to the addition of  $4 \times 10^{-3} \text{ M N}_2\text{O}$ , further addition of  $\text{N}_2\text{O}$ , up to  $2.5 \times 10^{-2} \text{ M}$ , does not affect  $G(\text{H}_2) = 0.1$ , so that the species scavenged by  $\text{N}_2\text{O}$  in the slower step does not normally lead to the formation of  $\text{H}_2$ . It can be noted also that, bearing in mind the possible alternative reactions of this species, the  $1.2 \times 10^{-2} \text{ M N}_2\text{O}$  necessary to scavenge it

completely is consistent with its rate constant for reaction with  $\text{N}_2\text{O}$  being  $10^4\text{--}10^7 \text{ M}^{-1} \text{ s}^{-1}$ , the probable order of magnitude of  $k_2$  (ref. 2).

Fig. 1 also indicates that  $G(\text{H}_2) + G(\text{O}_2)$  is constant at  $\sim 0.5$  over the full range of  $[\text{N}_2\text{O}]$ . In fact,  $G(\text{O}_2)$  increases as  $G(\text{H}_2)$  falls and both processes are markedly dependent on dose in contrast to the yields of  $G(\text{N}_2)$ . This dose dependence and certain competition investigations will be discussed elsewhere. Values of  $G(\text{N}_2)$  shown in Fig. 1 were corrected for small 'background' yields of  $\text{N}_2$ . Plots of  $G(\text{N}_2)$  versus dose showed positive intercepts as others have reported<sup>1,2</sup>. We attribute these to the fact that when added to an apparently thoroughly de-aerated sample of water (de-aerated by freezing-pumping-thawing cycles or by boiling and pumping)  $\text{N}_2\text{O}$  is able to release traces of dissolved nitrogen (and probably oxygen) from the liquid—amounts up to  $0.2 \mu\text{moles}$  from  $25 \text{ ml.}$  of water, depending on the  $\text{N}_2\text{O}$  pressure.

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## Spectroscopic Evidence of Chemical Interactions at the Gold Film-Silica Substrate Interface

CERTAIN 'intermediates', when placed between a substrate and an evaporated gold film, promote adhesion between the film and the substrate<sup>1</sup>. As a preliminary part of a systematic study of the nature of the chemical interactions in such systems, it was necessary to examine the effect of varying the temperature of the substrate during deposition of gold in the absence of intermediates. The interactions at the ceramic substrate-gold interface were examined with the potassium bromide pellet infrared spectroscopy technique.

Silica powder ('Cabosil') was heated at different temperatures up to  $1,100^\circ \text{C}$  on a tantalum heater strip within an evaporation vessel at a pressure of  $10^{-4}\text{--}10^{-5}$  torr. The amount of powder used was approximately  $3 \text{ mg.}$  and it was maintained at a given temperature for  $10 \text{ min.}$  before evaporation of the gold at that temperature. The sample was allowed to cool in vacuum, then thoroughly mixed with potassium bromide (spectroscopic grade), and pressed at  $80,000 \text{ lb./in.}^2$  to yield a pellet containing  $0.25 \text{ mg.}$  of the silica per  $400 \text{ mg.}$  of potassium bromide. The potassium powder was stored in an oven at  $50^\circ \text{C}$  and the pellets stored in a vacuum desiccator at room temperature before scanning.

The thickness of the deposited layer, calculated from the cosine distribution equation and verified gravimetrically, was approximately  $150 \text{ \AA.}$  At temperatures above  $900^\circ \text{C}$ , the high rate of re-evaporation of gold from the surface and the high partial pressure of gold at such temperatures close to the melting point of gold ( $1,083^\circ \text{C}$ ) introduced additional complicating factors which will be considered in later reports. The temperature of the exposed surface of the powder was measured with a chromel-alumel thermocouple to a precision of  $\pm 3$  per cent at all temperatures.

The pellets were scanned using a Beckman IR-4 spectrophotometer in the range  $1\text{--}16 \mu$ . The reproducibility of scans of different samples of the same system was approximately  $1\text{--}2$  per cent (transmittance) throughout most of the  $1\text{--}16 \mu$  range.

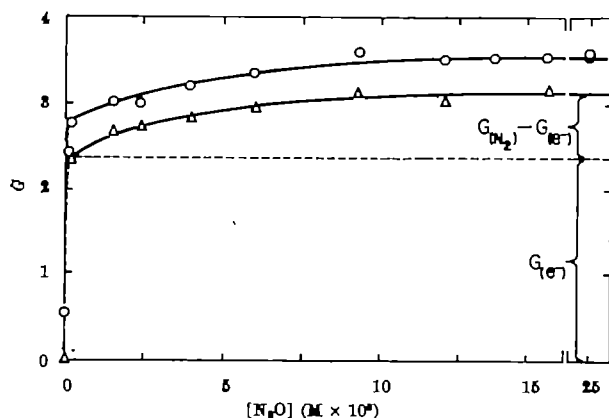


Fig. 1. Variation of  $\Delta G(\text{N}_2)$  and  $\bigcirc G(\text{N}_2) + G(\text{H}_2) + G(\text{O}_2)$  with  $\text{N}_2\text{O}$  concentration. Total dose in each experiment =  $7 \times 10^{17} \text{ eV ml}^{-1}$ .

Silica powder ('Cabosil') exhibits absorption bands at 8.86, 12.12, and 14.15  $\mu$  (ref. 2). Other weaker bands at about 2.8 and 6.1  $\mu$  correspond to the —OH vibrations of the silanol surface groups or to the different forms of water associated with the silica powder\*. We have found that the evaporation of gold on to silica at room temperature increases the transmittance of the 'flat regions' at the sides of the band, thus improving the sharpness of all three absorption bands.

Fig. 1 presents the transmittance spectra between 6.0 and 13.0  $\mu$  of pure silica powder that had been heat-treated at different temperatures and made into pellets about 2 h after the heat treatment. The spectra corresponding to the different samples superimpose, and no distortion of the band is apparent. The spectra of silica powder in Fig. 2 are for samples which were subjected to identical heat treatment procedures and were held at the heat treatment temperature while being plated with gold. The potassium bromide pellets were made about 2 h after the gold deposition.

In Fig. 3 are shown the spectra for the same silica powder as in Fig. 2, with the exception that the potassium bromide pellets were made about a week after the gold evaporation. The spectra of the different samples fall right on top of each other once again.

The decrease in transmittance at the short-wave-length side of the 8.86  $\mu$  band with increase in temperature of the silica during gold deposition (Fig. 2) was also observed to

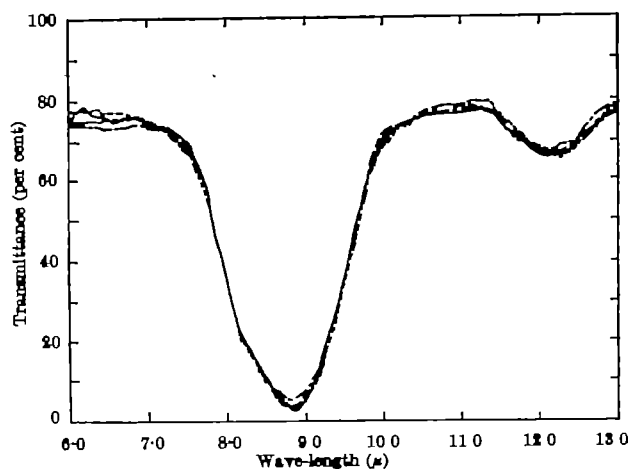


Fig. 3. Infra-red spectra of the same silica samples as in Fig. 2, but scanned one week after the heat treatment-gold filming operations. Heat-treatment: —, 25° C; ---, 500° C; — · —, 800° C; — — —, 1,000° C.

occur when certain metals were evaporated on to the silica substrate at room temperature. However, the spectra corresponding to these metal-silica systems did not change with time. In contrast, it is apparent from Fig. 3 that for silica-gold the effect causing the decrease in transmittance with increasing temperature is reversible.

'Intermediates' such as germanium, bismuth and chromium in the form of the oxide have been found to increase the adherence of gold films to a silica substrate. If we postulate that excitation of the silicon-oxygen energy-levels is related to bonding at the silica-metal interface then the excitation caused by the pure gold when deposited at high substrate temperatures would indicate, by analogy, formation of an oxygen-gold bond at the silica-gold interface. The formation of a surface gold oxide at high temperatures has been suggested previously<sup>4</sup>. The oxide when formed was found to decompose rapidly. This is corroborated by the present results where the instability of the surface gold oxide could account for the de-excitation with time shown in Fig. 3.

The reversibility of the proposed formation of the surface gold oxide may be expressed in equivalent form as either the oxide is unstable or that the silicon-oxygen-water interaction is energetically more stable than silicon-oxygen-gold.

For the purpose of this preliminary analysis, we can assume that the vibrations of the silicon-oxygen group are approximated by those of an anharmonic oscillator<sup>5</sup>. In the absence of rotational interactions, the broadening of a fundamental infra-red absorption band may be attributed to a delocalization of either the ground-state-level or of higher levels or a combined delocalization of both. In liquids, for example, Brownian motion may delocalize the ground state to cause symmetrical broadening<sup>6</sup>.

For the present system, we may postulate that the interaction between gold atoms and silicon-oxygen surface sites at high temperatures produces, on cooling to room temperature, a delocalization of the silicon-oxygen vibrational levels. This could either be in the form of a lowering of the ground-state-level or of a lowering of all levels. In either case, the delocalization will result in a broadening of only the short-wave-length side of the absorption band, as in fact is observed (Fig. 2). Since the silicon-oxygen-gold interaction is unstable, relaxation of the delocalized levels occurs with time. Apparently, for the interaction with other metals such as bismuth the delocalization is stable, indicating strong bonding to the silicon-oxygen surface sites.

Further research is in progress to clarify the details of the probable formation of surface gold oxide and to quantify the interactions of silica surfaces with gold and other metals.

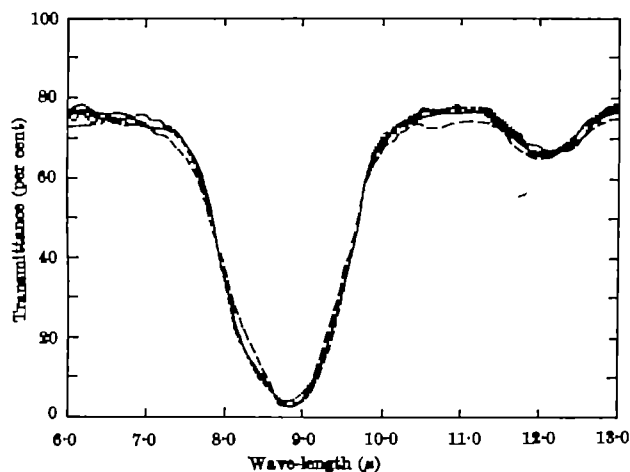


Fig. 1. Infra-red spectra of pure silica samples heat-treated at various temperatures and scanned 2 h afterwards. Heat-treatment: —, 25° C; ---, 400° C; — · —, 600° C; — — —, 1,000° C.

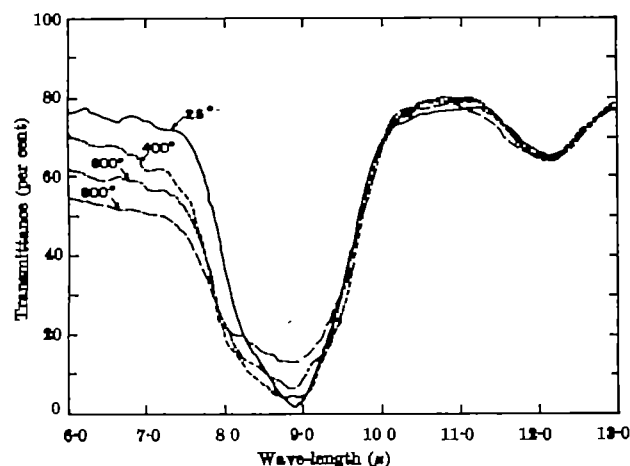


Fig. 2. Infra-red spectra of silica samples heat-treated at various temperatures and filmed with gold while held at each heat-treatment temperature. Samples scanned 2 h after the heat-treatment-gold filming operations. Heat-treatment: —, 25° C; ---, 400° C; — · —, 600° C; — — —, 800° C.



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### $\alpha$ -Dehydro Dimer—a Major Product in Irradiated Methyl Laurate

DURING investigations of the effect of radiation on lipid components of food we have examined the effect of  $\gamma$ -radiation on methyl laurate. There is little information in the literature on the effect of  $\gamma$ -radiation on higher saturated fatty acid esters<sup>1</sup>. Here we report the constitution of the major product in the fractions of higher boiling point than methyl laurate.

The methyl laurate used was Eastman Organic Chemicals practical grade which was further purified by vacuum distillation through a packed column. Only the middle fractions from the distillation were used for irradiations. Using gas chromatography with a flame ionization detector, purity was estimated to be > 99.9 per cent. The methyl laurate was thoroughly degassed and then sealed under vacuum into an all-glass ampoule before irradiation to 40 Mrads with  $\gamma$ -radiation from spent fuel elements. The dose rate was 0.22 Mrads/h, estimated by using the ferrous sulphate dosimeter, taking  $G_{Fe^{+++}} = 15.6$ .

Most of the unchanged methyl laurate from the irradiated material was removed by vacuum distillation, and the residue was fractionated using column chromatography with silica gel as adsorbent, and gradient elution by a petroleum ether-diethyl ether mixture (Fig. 1).

Using gas chromatography, the fractions in peak A (Fig. 1) were found to be almost entirely unchanged

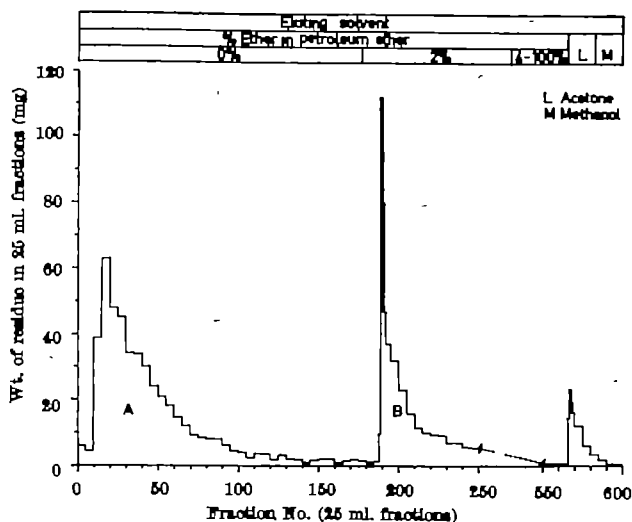


Fig. 1. Column chromatogram of the distillation residue from methyl laurate irradiated to 40 Mrads. Column load, 4.533 g; material recovered, 4.458 g

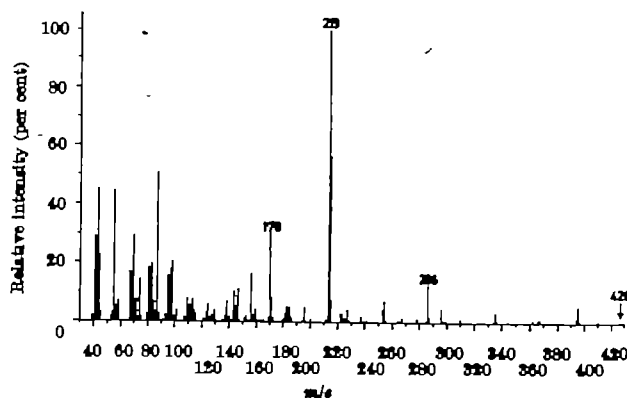


Fig. 2. Mass spectrum of  $\alpha$ -dodecane 11, 12 dimethyl carboxylate

methyl laurate. The first three fractions in peak B were crystalline, and examination of this material by gas chromatography using a column packed with 1.5 per cent SE. 30 on silanized 'Chromosorb W' indicated that it had one major component. The component was found to be the  $\alpha$ -dehydro dimer,  $\alpha$ -dodecane 11, 12 dimethyl carboxylate (DDMC),  $\text{CH}_3(\text{CH}_2)_9\text{CH}(\text{COOMe})\text{CH}(\text{COOMe})$  ( $\text{CH}_3$ )<sub>2</sub>CH, on the basis of the evidence which follows. After recrystallization from ethyl alcohol, the melting point of the compound was found to be 54°–54.5° C uncorrected. The subsequent fractions contained the compound in diminishing amounts up to fraction 200; these and all other fractions were found to be complex mixtures. Although the retention times of some components were possibly too great to be detected by the gas chromatograph, it can be seen from Fig. 1 that the individual amounts of all other components are likely to be relatively small.

The infra-red spectrum closely resembled that for methyl laurate, showing a strong absorption at 1,737  $\text{cm}^{-1}$  in the ester carbonyl region. The relative intensity of the carbonyl to the  $\text{CH}_2$  absorption in carbon tetrachloride solution was similar to that for methyl laurate. Absorption at 718  $\text{cm}^{-1}$  indicated that the  $(\text{CH}_2)_9$  grouping was present, although the irregular spacing of a series of absorptions in the spectrum of the crystalline material over the region 1,190–1,275  $\text{cm}^{-1}$  suggested that the  $\text{CH}_2$  groups were not in a simple straight chain.

The parent peak in the mass spectrum (Fig. 2) corresponded to a molecular weight of 426, which suggested that it was a dehydro dimer of methyl laurate (mol. wt. = 214). The absence of peaks at  $M-73$  (loss of  $\text{CH}_3\text{COOCH}_2$ ),  $M-105$  (loss of  $\text{CH}_3\text{COOCH}_2 + \text{OCH}_2 + 2\text{H}$ ), which are characteristic peaks in the mass spectrum of methyl esters of normal chain dibasic acids<sup>2</sup>, indicated that no  $\alpha$ -OH<sub>2</sub> groups were present and therefore both methyl laurate moieties were linked at the  $\alpha$  position. Further evidence for this structure was the presence of strong peaks at  $M/e = 213$  and 214, and peaks at  $M/e = 286$  and 170, all of which corresponded with those reported by Harrison *et al.*<sup>3</sup> for the related compound which they synthesized, the  $\alpha$ -linked dehydro dimer of methyl stearate.

The nuclear magnetic resonance spectrum of the compound (Fig. 3) gave further evidence that the linkage was between the  $\alpha$ -carbon atoms. The hydrogen atoms attached to the  $\alpha$ -carbon atom in methyl laurate gave a triplet as expected, with a chemical shift,  $\delta = 2.3$  p.p.m. approximately and  $J = 6$  c.p.s. approximately; the ratio from the integral of the absorption due to the chain methylene groups to that due to the  $\alpha$ -hydrogens was 8.5:1, in good agreement with the expected ratio of 9:1. Absorption attributable to an  $\alpha$ -CH<sub>2</sub> grouping was absent from the spectrum of the crystalline compound, but an unresolved multiplet centred at  $\delta = 2.64$  p.p.m. is consistent with an  $\alpha$ -methine grouping, linked

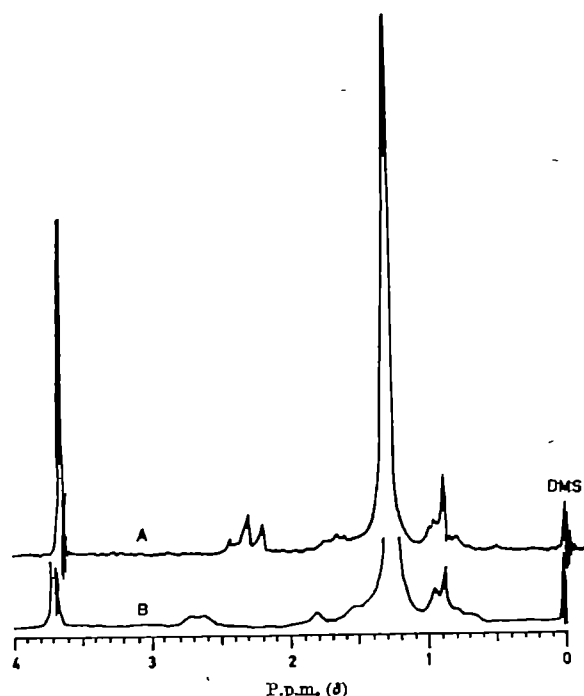


Fig. 3. Nuclear magnetic resonance spectrum of methyl laurate (curve A) and *n*-docosane 11, 12 dimethyl carboxylate (curve B). Solvent, deuterated chloroform.

as in DDMC. The additional complexity of the  $\alpha$ -proton signals was expected because of the coupling of the methine protons with each other as well as with the  $\beta$ -methylene groups. The shift downfield was due to the influence of a second carbonyl group, together with the usual downfield displacement of methine protons versus methylene protons. The ratio of the intensity of this band to that of the chain methylene groups was approximately 1:20, in reasonable agreement with the expected ratio of 1:18.

The *G*-value for the formation of DDMC from methyl laurate was estimated at 0.19 using gas chromatography to determine the proportion of this compound present in the fractions constituting peak B, Fig. 1. The gas chromatogram of the distillation residue from methyl laurate irradiated to 5 Mrads reveals that DDMC is still the major reaction product.

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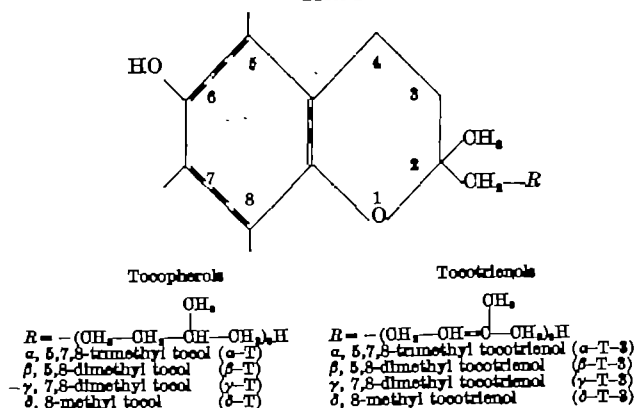
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## BIOCHEMISTRY

### Identification and Estimation of Tocotrienols in Hevea Latex

THE four tocotrienols related to  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherols have been reported to be present in palm oil<sup>1</sup> (Table 1). Some of these compounds are not distributed widely; in fact, palm oil has hitherto been the only known

Table 1



source of  $\delta$ -tocotrienol. This communication reports the presence in a sample of latex from the rubber tree, *Hevea brasiliensis*, of a surprisingly large amount of free and esterified  $\alpha$ -,  $\gamma$ - and  $\delta$ -tocotrienols together with a much smaller amount of  $\alpha$ -tocopherol.

Concentrated latex containing about 60 per cent of rubber and stabilized with ammonia was kindly provided by the Natural Rubber Producers Research Association through Dr. E. G. Cookbain. The latex was extracted by the Folch method<sup>2</sup>, giving 1-1.2 per cent of an oily, slightly yellow lipid mixture. An extract from 20 g of latex was chromatographed on a column of alumina (acid-washed and weakened to Brockmann Grade 3) and fractions eluted by ether/light petroleum mixtures were collected. The 2 per cent (v/v) ether in light petroleum (*E/P*) fraction had an ultra-violet absorption spectrum closely resembling that of  $\alpha$ -tocopheryl acetate (Fig. 1), while the spectra of the 4 per cent and 10 per cent *E/P* fractions indicated the presence of free tocopherol (Fig. 2). The 4 per cent *E/P* fraction showed an absorption maximum of 298 m $\mu$  and an inflexion at 292 m $\mu$  (in cyclohexane) similar to the spectrum of  $\alpha$ -tocopherol, and the 10 per cent *E/P* fraction had peaks at 296 m $\mu$  and 301 m $\mu$  and so resembled both  $\gamma$ - and  $\delta$ -tocopherol.

Thin-layer chromatography of the 4 per cent and 10 per cent fractions by the two-dimensional method of Pennock, Hemming and Kerr<sup>3</sup> showed four tocopherols to be

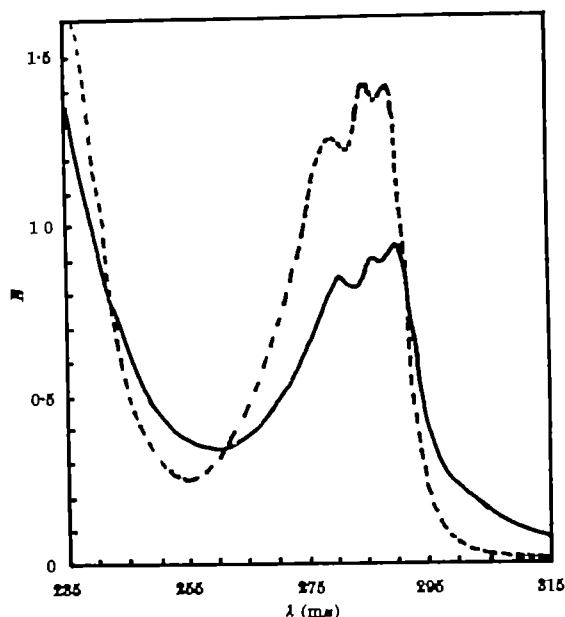


Fig. 1. Ultra-violet absorption of tocopheryl esters. 2 per cent *E/P* fraction—27.7 mg per cent in cyclohexane (—);  $\alpha$ -tocopheryl acetate—81.6 mg per cent in cyclohexane (---).

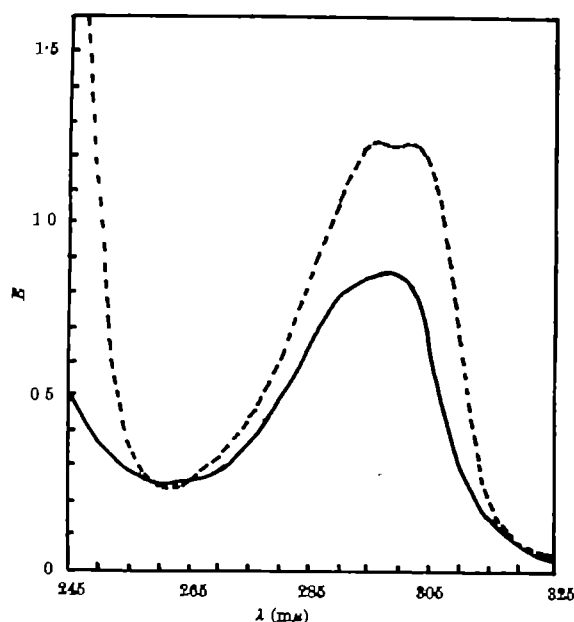


Fig. 2. Ultra-violet absorption of free tocopherols. 4 per cent E/P fraction—59.6 mg per cent in cyclohexane (—), 10 per cent E/P fraction—74.4 mg per cent in cyclohexane (----).

present. Chromatographic comparisons with authentic tocopherols, together with colours given by diazotized *o*-dianisidine, indicated that  $\alpha$ -,  $\gamma$ - and  $\delta$ -tocotrienols and a little  $\alpha$ -tocopherol were present. Larger amounts of the tocotrienols were obtained by preparative thin-layer chromatography and each substance was hydrogenated in the presence of platinum oxide catalyst. The products had thin-layer chromatographic properties similar to those of  $\alpha$ -,  $\gamma$ - and  $\delta$ -tocopherols.

Portions of the ester fractions were saponified<sup>3</sup> or hydrolysed with acid<sup>4</sup> and the products examined by two-dimensional thin-layer chromatography. After alkaline hydrolysis the main surviving product was  $\gamma$ -tocotrienol with smaller amounts of  $\alpha$ - and  $\delta$ -tocotrienols, while after acid hydrolysis there was more  $\delta$ -tocotrienol and less  $\gamma$ -tocotrienol (Table 2). Two reducing spots with lower  $R_F$  values than  $\delta$ -tocotrienol were evident after acid hydrolysis. Whether these materials, which had ultra-violet absorption spectra resembling those of both  $\gamma$ - and  $\delta$ -tocopherols and gave stains with *o*-dianisidine, were artefacts of acid hydrolysis remains to be decided.

Table 2. TOCOTRIENOLS FROM THE ESTER FRACTION OF LATEX LIPID

Method of hydrolysis	Total tocopherol-like compounds ( $\mu\text{g/g}$ latex)	Tocotrienol	$\mu\text{g/g}$ latex	% Composition
Acid	514*	$\alpha$ -T-3	trace	—
		$\gamma$ -T-3	290	83.3
		$\delta$ -T-3	52	16.7
		$\alpha$ -T-3	13	2.6
Alkali	508†	$\gamma$ -T-3	474	93.2
		$\delta$ -T-3	21	4.2

\* Estimated by Emmerie-Engel colour test.

† Estimated from ultra-violet absorption spectrum based on the  $E_{1\%}^{1\text{cm}}$  of  $\gamma$ -tocopherol (that is, 294 m $\mu$  0.64).

Quantitative determinations were made of free and esterified tocotrienols.  $\alpha$ -Tocopherol was present but in concentrations too low to be measured. Latex (5.6 g) was extracted by the Folch method and the resulting lipid (67 mg) was chromatographed on alumina. The ester fraction, eluted by 2 per cent E/P, was hydrolysed by refluxing with ethanolic sulphuric acid for 3 h (ref. 4). Tocotrienols were estimated by the Emmerie-Engel reaction following two-dimensional thin-layer chromatography according to Whittle<sup>5</sup> (Table 2). Tocotrienols were also obtained from another 2 per cent E/P fraction by alkaline hydrolysis and estimated (Table 2). Neither method of hydrolysis seemed to give full recovery. Free

Table 3. FREE TOCOTRIENOL CONTENT OF LATEX LIPID

Tocotrienol*	$\mu\text{g/gm}$ Latex	% Composition
$\alpha$ -T-3	124	30.9
$\gamma$ -T-3	253	62.9
$\delta$ -T-3	25	6.2

Total reducing substance† 436

\*  $\alpha$ -Tocopherol was also present but only in trace amounts.

† In the 10 per cent E/P fraction.

tocopherols were eluted from the column by 10 per cent E/P and estimated as already mentioned (Table 3).

Thus  $\alpha$ -,  $\gamma$ - and  $\delta$ -tocotrienols occur both free and esterified in the lipid fraction of *Hevea* latex, and together these tocotrienols make up about 8.2 per cent of the lipid or 0.9 per cent of the latex.

Rubber latex is collected from the tree every other day and so synthesis of its components must be quite rapid. If latex were collected from a tree which had not been tapped for some time the tocotrienol/tocopherol pattern might be quite different. It was suggested earlier<sup>1</sup> that tocopherol synthesis could occur by successive methylation of  $\delta$ -tocotrienol followed by hydrogenation of the side-chain. The presence of mono-, di- and tri-methyl tocotrienols and of only the trimethyl tocol ( $\alpha$ -tocopherol) in *Hevea* latex agrees with the scheme.

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## Automated Peptide Synthesis

SOLID-PHASE peptide synthesis was conceived and developed with the view of eventually automating the synthesis of polypeptides<sup>1,2</sup>. In principle the process has certain characteristics which lend themselves to mechanization, and by exploitation of these properties an apparatus has now been constructed which performs automatically all the manipulations involved in the synthesis of a polypeptide chain.

Solid-phase peptide synthesis depends on the stepwise assembly of the peptide chain while it is covalently anchored at one end to an insoluble solid particle, followed by liberation of the peptide from the solid support after completion of the synthesis. This technique provides a number of advantages over conventional methods. One important advantage is that the peptide chain, while firmly attached to a totally insoluble particle, can be carried without loss through the many separate steps to which it must be subjected during the synthesis. In addition, the solid support provides important physical properties which greatly simplify the mechanical manipulations involved in mixing, filtering and washing of the peptide. Thus it becomes possible to carry out all the synthetic steps in a single reaction vessel without the need for repeated transfer of material from one container to another, and without the usual losses associated with such manipulations. After any synthetic reaction an effective purification procedure is necessary. Classically this has been recrystallization. In the solid-phase method the purification procedure is simply thorough and efficient washing. Effective washing is readily carried out mechanically while recrystallization is not.

The polypeptide chain has one inherent property which lends itself to automated synthesis—the repeating peptide bond which forms the backbone of the molecule. In principle, the addition of each amino-acid can be done in

exactly the same way. Thus the chain can be assembled in a stepwise manner by a relatively simple series of reactions which is repeated for each peptide bond, using the appropriate amino-acid derivative according to the sequence desired in the peptide.

Utilization of the combined advantages afforded by peptide structure and solid-phase synthesis has led to the construction of the instrument to be described. While the instrument has so far been operated with one particular set of amino-acid derivatives and reaction conditions, it was designed with sufficient flexibility to be adaptable to the use of a wide range of protecting groups and coupling reactions. The apparatus can be divided into two main parts, the first being the reaction vessel with the components required to store and select reagents and to transfer them into and out of the vessel, and the second being the programmer which automatically controls and sequences the operation of the various components.

The reaction vessel is similar to one previously described<sup>1</sup>, but modified to contain a small fritted disk at the top in addition to the large disk at the bottom. Reagents and solvents are stored in suitable reservoirs which are connected by 'Teflon' tubing to a motor-driven 12-port circular 'Teflon' selector valve. The proper solution is then pumped into the bottom of the reaction vessel for a specified time by an all-'Teflon' diaphragm metering pump. Air is displaced at the top through a capillary tube which is then closed by a solenoid-operated valve. The contents of the vessel are mixed by rotation of the vessel through 180° at a rate of 10 cycles per min. After a suitable period the vessel is stopped in a vertical position and the solvent removed by filtration under vacuum through the fritted disk at the bottom, while dry air is admitted at the top. The rotary valve then selects the next reagent or solvent, and the pumping, mixing and filtering steps are repeated. During one complete cycle of the synthesis the rotary valve makes one complete revolution and thus selects in sequence each of the reagents and solvents required for the lengthening of the peptide chain by one amino-acid residue. The correct amino-acid solution for each succeeding cycle is selected by a second rotary valve which advances one position for each cycle of the synthesis.

In order to control these events a stepping-drum programmer is used. Properly positioned pins on the drum activate micro-switches which in turn operate individual components in the apparatus. At the end of each operation the drum is stepped to the next position by a time-controlled signal. The programme can be readily changed by resetting the pins and timers to conform to the conditions required by the particular reactions which are being used for the synthesis.

The apparatus was programmed specifically to carry out the synthesis of peptides using *t*-butoxycarbonyl-amino-acids and the dicyclohexylcarbodiimide-mediated coupling reaction. Under these conditions 80 separate drum steps were needed for the addition of one amino-acid residue to the peptide chain. This cycle was completed in 4 h, after which the programme was automatically repeated in exactly the same way except that a new amino-acid was introduced. In the present model, provision has been made for sequential addition of six amino-acids during a 24-h period without manual attention.

The operation of the instrument thus programmed was tested by repetition of the recent solid-phase synthesis of bradykinin<sup>2</sup>. The reaction vessel was loaded with 10 g of *t*-butoxycarbonyl-nitro-L-arginine-resin. The coupling of the eight additional amino-acids to complete the protected nona-peptide chain of bradykinin on the resin was accomplished in 32 h of continuous operation of the machine. The peptide was cleaved from the resin, hydrogenated, and purified by countercurrent distribution as previously described. The results, based on yield,

purity, and biological activity, were comparable with those obtained by the manual solid-phase synthesis, while the time and effort were significantly reduced. The improvements in overall time and effort are expected to be major benefits when syntheses of long-chain polypeptides are undertaken.

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### Malic Dehydrogenase Isozymes

It has been shown previously<sup>1-3</sup> that at least two isozymes of malic dehydrogenase are present in the extracts of various tissues. In addition, the distinct characteristics of these isozymes have been demonstrated by the recent reports<sup>4-6</sup>.

In 1963 Thorne, Grossman and Kaplan<sup>7</sup>, using electrophoresis on starch gel, separated six fractions from malic dehydrogenase in the pig heart mitochondria. We recently reported<sup>8</sup> that two or more isozymes of malic dehydrogenase in the separable form (NAD, oxidoreductase EC 1.1.1.37) were found in the extracts of rat and human tissues on the cellulose acetate paper according to the method of Barnett<sup>9</sup>. The present investigations are concerned with the separations of malic dehydrogenase isozymes (NAD, NADP, oxidoreductase EC 1.1.1.40) in rat tissues. Rat tissues were homogenized in distilled water, frozen and thawed twice, and then these solutions were cleared by high-speed centrifugation (for 30 min at 15,000g). The separation was electrophoresed on cellulose acetate paper at 4° C. After electrophoresis the resulting papers were incubated with NAD, NADP, phenazine methosulphate, malic acid and nitro blue-tetrazolium or INT (for 10 min at 37° C in the dark). On separation by electrophoresis three bands were obtained, dependent on the presence of the specific substrate. The results obtained with rat tissues are shown in Fig. 1 and Fig. 2. It has been shown that the first and third bands

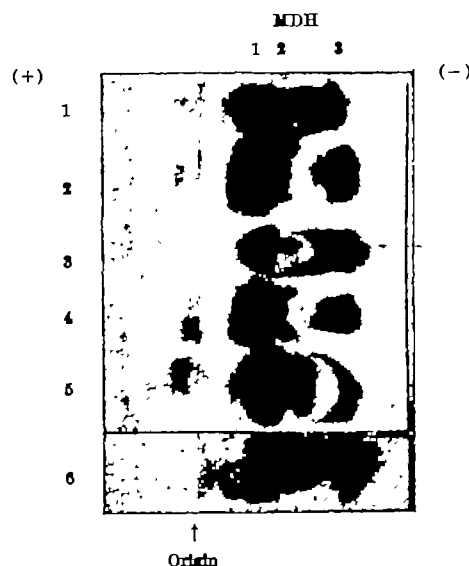


Fig. 1. Isozyme patterns of malic dehydrogenase (NAD, NADP) in rat tissue extracts: 1, brain; 2, liver; 3, heart; 4, kidney; 5, skeletal muscle; 6, testis.

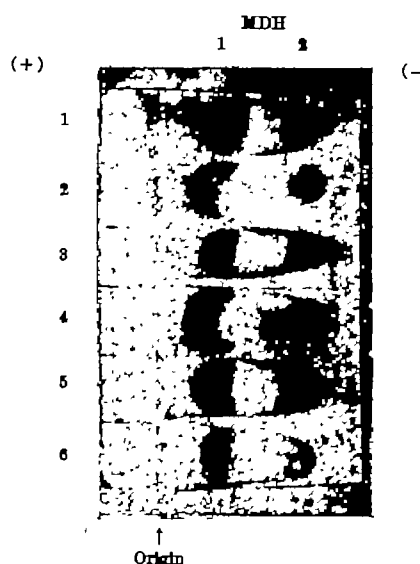


Fig. 2. Isozyme patterns of malic dehydrogenase (NAD) in rat tissue extracts: 1, brain; 2, liver; 3, heart; 4, kidney; 5, skeletal muscle; 6, testis

were of malic dehydrogenase (NAD) and the second band was of malic dehydrogenase (NADP) enzyme respectively. The former was inhibited completely by the increasing concentration of oxaloacetate, but the latter was not inhibited by oxaloacetate or pyruvate. It was suggested that the latter is malic enzyme established by Ochoa<sup>10,11</sup>. The same number of bands were present in the different extracts. However, the significance of the presence of the isozymes is not known. We are now trying to elucidate the significance of their function.

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### Short Pseudo-repeat in Paramyosin

PARAMYOSIN, a fibrous protein which has so far only been found in the muscles of invertebrates, gives an X-ray pattern with sharp 1.5 and 5.1 Å meridional and diffuse 10 Å near-equatorial reflexions and so is classified with α-keratin, myosin and fibrinogen as an α-fibrous protein. The X-ray patterns of these proteins differ, however, in the low-angle region where the pattern may be indexed on a repeat of 198 Å for α-keratin<sup>1</sup>, 420 Å for striated muscle<sup>2</sup>, 226 Å for fibrinogen<sup>3</sup> and 725 Å for paramyosin<sup>4</sup>. The only one of these low-angle patterns which is clearly understood is that of paramyosin which may be interpreted in terms of either the two-dimensional net shown in Fig. 1(a) or a helix<sup>5</sup>. Electron micrographs have shown a two-dimensional pattern of spots<sup>6</sup> similar to the lattice in Fig. 1(a) where the axial repeat is 725 Å, and this is reduced to 145 Å in axial projection (Fig. 1(b)). This communication reports a pseudo-repeat of 29 Å in axial projection which shows up when the paramyosin is treated with silver.

Table 1. INTENSITIES OF MERIDIONAL REFLEXIONS ON X-RAY PATTERNS FROM NATIVE AND SILVER-TREATED PARAMYOSIN

Reflexion (order of 145 Å)	Native	Silver-treated
1	vs	—
2	vs	vs
3	vs	vs
4	m	m
5	ms	vvs
6	s	s
7	ms	m
8	s	s

It is well known that if atoms of high atomic number can be isomorphously incorporated into organic crystals, the positions of these atoms are readily determined by X-ray methods<sup>7,8</sup>, and information can often be obtained about the structure of the rest of the molecule. This technique has been successfully applied in the complete structure determination of globular proteins<sup>9</sup>. In the case of fibrous proteins, metal treatment of collagen<sup>10</sup>, α-keratin<sup>11</sup> and feather keratin<sup>12</sup> leads to changes in the intensities but not the positions of low-angle X-ray reflexions, and this may be taken as an indication that these proteins have taken up metal atoms isomorphously. Although complete structure determination of these fibrous proteins has not been achieved by this method, it is clear that when isomorphous replacement occurs, the possibility of obtaining information about the molecular structure is considerably increased, especially in the case of paramyosin where the low-angle pattern is readily interpreted.

Paramyosin-containing fibres from the Sydney rock oyster (*Crassostrea commercialis*) adductor muscle were dried *in situ* over phosphorus pentoxide and then removed and soaked for four days in a 1 per cent aqueous solution of silver nitrate at room temperature. The fibres were then washed for one day in distilled water, and air-dried. The X-ray diffraction pattern (Fig. 2) was obtained by exposing the fibres for 2 days in a 5-cm microcamera using a lead glass capillary; the camera was evacuated during exposure. In Table 1, the intensities of the low-angle meridional reflexions from the native and the treated fibres are compared. The axial period was the same in both cases and the only striking difference in the relative intensities was the intensification in the silver-treated case of the meridional reflexion corresponding to a spacing of 29 Å which

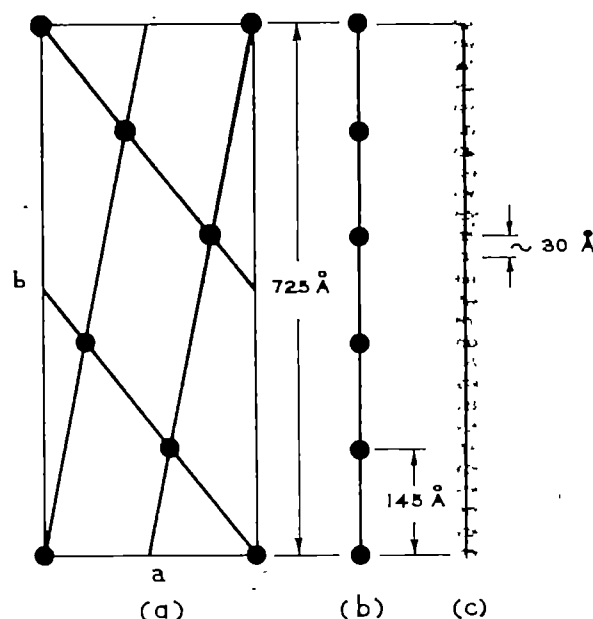


Fig. 1. (a) Two-dimensional lattice derived from low-angle X-ray pattern of dry native paramyosin.  $a = 250$  Å,  $b = 725$  Å,  $\gamma = 90.5^\circ$ . (b) Axial projection of (a). (c) Axial projection of the distribution of silver atoms in the treated protein

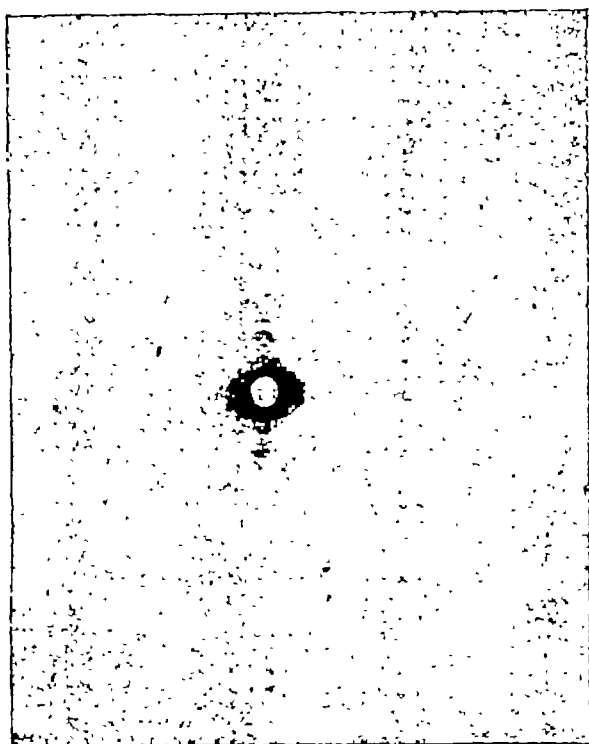


Fig. 2. Low-angle X-ray pattern of silver-treated paramyosin

was one-fifth of the 145 Å projected periodicity in the native material. Since no new reflexions appeared this means that the metal atoms had entered the protein in such a way as to preserve the original lattice and also to show up a pseudo-repeat of 29 Å in axial projection illustrated in Fig. 1(c).

It is difficult to give a precise interpretation of this pseudo-repeat. Periods of 1400 Å have been observed in electron microscopy of reprecipitated fibrils<sup>13</sup> and this has been associated with molecules lying parallel to the fibre axis in the native material and separated by an axial translation of 725 Å (ref. 14). The 725-Å axial spacing could also arise from anti-parallel packing of molecules 1400 Å long and the spot pattern observed in electron micrographs of native material, since it corresponds so closely to the lattice predicted by the X-ray pattern, may be due to the fact that neighbouring molecules are displaced by 290 Å relative to each other along the fibre axis (Fig. 2) and, in addition, the striations in the electron micrograph spaced 145 Å apart may indicate a sub-unit of this length in the molecule. The 29 Å pseudo-repeat would indicate a regular distribution within this sub-unit of amino-acids which bind silver atoms. It may be noted that paramyosin has a high helical content<sup>15</sup> (about 90 per cent) and the distance 145 Å corresponds to an integral number (98) of amino-acid residues along the fibre axis. The distance 29 Å does not correspond to an integral number of residues and this draws attention to the fact that the X-ray evidence requires only a pseudo-repeat and not a true repeat of this length. The actual distance along the fibre axis between any two silver atoms could lie between 28 and 31 Å and all such distances need not be the same.

It is possible, as suggested by Petruska and Hodge<sup>16</sup>, that the situation in paramyosin is similar to that in collagen where the tropocollagen molecule is thought to consist of sub-units of different length, and it is likely that the pseudo-repeat described here will help to fix some of the sub-unit parameters. A more precise description will await detailed analysis with the electron microscope and further investigation on the effects of metals on the X-ray pattern.

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### Molecular Weight of Acid Proteinase of *Aspergillus saitoi*

THE recent development in this laboratory of methods for producing acid proteinase of *Aspergillus saitoi* (EC, 3.4.4.17, aspergillopeptidase A) from culture filtrate has made available sufficient material for a correlative study of its enzymatic properties<sup>1,2</sup>. The optimal pH for milk casein digestion is in the pH range of 2.5–3.0 and the proteinase is fairly stable over the pH range of 2.5–6.0 (ref. 3). Aspergillopeptidase A is capable of activating trypsinogen and chymotrypsinogen A at pH 4.5 (ref. 4). The further purification method for separating acid proteinase has been improved by chromatographic procedures with the use of ion exchange materials, 'Duolite OS-101', DEAE-cellulose and 'SE-Sephadex'<sup>5</sup>. The purified aspergillopeptidase A appears to be homogeneous on free boundary electrophoresis over a pH range of 2.0–10.0 and on ultracentrifugation at pH 4.1. The iso-electric point has been found to be at pH 3.65 in Sørensen's citrate buffer. In the investigation reported here, the molecular weight of aspergillopeptidase A was found to be 34,900 from sedimentation and viscosity, according to Scheraga–Mandelkern's formula<sup>6,7</sup>, and was also determined to be 34,200 according to Yphantis's treatment<sup>8</sup>.

For determination of the sedimentation constant<sup>9</sup> at zero concentration, runs were made at 59,780 r.p.m. with 0.438, 0.876, 1.313 and 1.751 per cent solution of aspergillopeptidase A in 0.1 M acetate buffer, pH 4.1, using a model 'E' Spinco analytical ultracentrifuge. All runs were made at room temperature. Five photographs were taken at 15-min intervals. The extrapolation has led to a value  $[S]_{0,w}$  of 3.33 S at zero concentration. The partial specific volume ( $\bar{V}$ ) of aspergillopeptidase A calculated from the specific volumes of constituent amino-acid residues<sup>10</sup> was calculated to be 0.722 ml./g. The specific volume values of the amino-acid residues are the values given by Meekin *et al.*<sup>11</sup>, and by Cohn and Edsall<sup>12</sup>. The buffer used for viscosity measurement contained 0.1 M acetate buffer, pH 4.1. Viscosity was checked at five different concentrations at 20° C. A value for intrinsic viscosity,  $[\eta]$ , of 0.032 dl./g was obtained.

The molecular weight was computed from the Scheraga–Mandelkern's formula<sup>7</sup>,

$$M = \frac{4690 [S]_{0,w}^2 [\eta]^{1/2}}{(1 - \bar{V} \rho)^{1/2}} \quad (1)$$

In equation 1 a value of  $\beta$  equal to  $2.16 \times 10^4$  has been used. With  $S = 3.33 \times 10^{-18}$ ,  $[\eta] = 0.032$  and  $\bar{V} = 0.722$ , the molecular weight is calculated to be 34,900.

The second method used for determination of the molecular weight of aspergillopeptidase A was Yphantis's procedure of sedimentation in the ultracentrifuge<sup>8</sup>. The molecular weight was calculated from:

$$M = \frac{1}{f c_s} \left( \frac{dc}{dr} \right)_{r=r_0} \frac{RT}{\omega^2 (1 - \bar{v} \rho)} \quad (2)$$

The molecular weight value of aspergillopeptidase A was checked at four concentrations using circular channel cells, and the individual values were obtained from the run at 12,500 r.p.m. in 0.1 M acetate buffer, pH 4.1, at 22° C. The time required to attain equilibrium was seen to be about 43 min. The extrapolation to zero concentration has led to  $M = 34,200$  with  $\bar{v} = 0.722$ , which is in fairly good agreement with the  $M$  value determined from sedimentation-viscosity measurement.

We thank Dr. K. Suzuki, National Institute of Radiological Science, for the operation of sedimentation analyses, and Dr. S. Yagisawa, University of Tokyo, for the measurement of molecular weight according to Yphantis's procedure.

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## Quantitative Determination of Piperine with the Labat Reagent

In the presence of concentrated sulphuric acid and gallic acid, methylene-dioxyphenyl compounds produce a green colour which changes to blue when the solution is heated<sup>1</sup>. This reaction has been used to determine small amounts of methylenedioxyphenyl-containing pyrethrum synergists<sup>2</sup> and safrole<sup>3</sup>. Its application to the determination of the piperine content of pepper is described here.

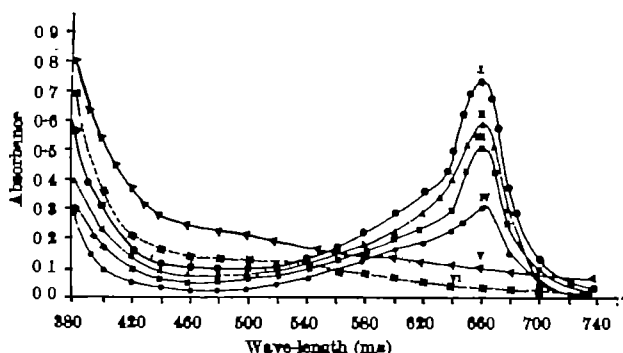


Fig. 1. Absorption spectra of colours produced by formaldehyde and piperine and its degradation products after reaction with the Labat reagent. I, Piperine; II, Piperone acid; III, Piperonal; IV, Formaldehyde; V, Piperidine; VI, Reagent blank

The colour exhibits maximum absorption at 660 mμ (Fig. 1).

The reagent used consisted of 50 mg gallic acid (recrystallized 3 times from hot water) dissolved in 100 ml. of concentrated  $H_2SO_4$ . It was stored in an amber bottle under refrigeration and a fresh batch was made up every 2 weeks.

For colorimetric analysis, a standard curve was constructed as follows: 1 ml. containing 0.0–0.1 μM of piperine, m.p. 129°–130° C (K and K Laboratories, New York), dissolved in absolute ethanol, was placed in consecutive glass-stoppered borosilicate test-tubes. Nine ml. of the reagent was added, the tubes were loosely stoppered, heated in a boiling water bath for 60 min., and then cooled in an ice-water bath and allowed to stand at room temperature  $20^\circ \pm 1^\circ$  C for 30 min. The absorbance of the colour developed was then measured at 660 mμ against a reagent blank, similarly treated.

For the determination of the piperine content of pepper an alcoholic extract of the pepper (black or white) was prepared according to the method of Lee<sup>4</sup>. One ml. of an appropriate dilution was then used for assay.

Optimum conditions for reproducible assay are as follows: a heating time of 60 min at  $99^\circ \pm 1^\circ$  C, a level of 0.5 mg of gallic acid per ml. of the reagent and 36 N  $H_2SO_4$ . The reagent, stored in an amber bottle under refrigeration, is stable for 2 weeks and the colour developed is stable for at least 4 h. The method is highly reproducible and very sensitive when compared with other methods (Table 1). The standard deviation for 10 different determinations of piperine was found to be  $\pm 1.12$  per cent. Over three levels of 10, 20, and 30 μM of piperine added to pepper samples, average recoveries of 99.4–100.6 per cent were obtained. The molar extinction coefficient of the chromogen was found to be  $2.2985 \times 10^4$ . Beer's law held good over the range of 0.002–0.01 μM of added piperine per ml. of reagent. The standard curve may be described by the following equation:

$$x = 0.16415 y - 0.00118$$

where  $x$  = concentration of piperine per ml. of reagent,  $y$  = absorbance at 660 mμ.

Per cent piperine in sample =

$$\frac{x (0.028533) (\text{dilution factor})}{\text{Sample weight (milligrams)}} \times 100$$

Table 1. COMPARISON OF DETERMINATION OF PIPERINE CONTENT OF PEPPER SAMPLES BY DIFFERENT METHODS

Method used	Piperine content of peppers analysed*		
	A	B	C
	(white pepper)		
Ultra-violet spectrophotometric	6.70	5.70	5.00
Chromotropic acid	7.80	7.40	6.85
Labat's reaction	7.76	7.45	6.25

\*Average of five determinations

The mechanism involves the cleavage of the methylenedioxy group in the presence of strong sulphuric acid to produce formaldehyde which couples with gallic acid to form a coloured product. Therefore, piperic acid, piperetine and chavicine<sup>5,6</sup> will respond. As a result, the method measures "the total bite principles of pepper", as does the chromotropic acid method.

All compounds which contain the methylenedioxy group or which can produce formaldehyde in the presence of concentrated  $H_2SO_4$  as well as the ethylenedioxy group<sup>7</sup> glycolic, tartaric, glyceric and glyoxylic acids will also respond to the Labat test<sup>8</sup>. However, under the conditions of extraction of the piperine with ethanol<sup>4</sup> it is highly unlikely that interference from these sources will be encountered, since such substances are either present in pepper at rather low levels or will not be readily extracted. Other possible precursors of formaldehyde listed by Berova<sup>7</sup> can likewise be ignored. The possible contribution of glucose due to the  $-CH_2OH$  grouping as shown by



Beroza is much lower in this procedure than in the chromotropic acid method<sup>1</sup>. This is due largely to the fact that with chromotropic acid the wave-length of maximum absorption of the glucose colour is the same as for piperine (580 mμ), whereas with the Labat reagent the glucose colour has maximum absorption at 540 mμ, which is appreciably removed from the absorption maximum (660 mμ) for the piperine colour.

This method, in conjunction with the development of colorimetric methods based on colour complexes formed by piperine with alkaloid reagents, and coloured salts formed with some of the concentrated acids<sup>2,3</sup>, can provide useful and easily executed alternatives to the methods now being used for the determination of the piperine content of pepper. The reagent as described is more stable than the chromotropic acid reagent which is usually prepared daily.

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## Use of Methylene Blue for Staining Sialomucoproteins on Paper

FOR some time we have used in our laboratory a simple and sensitive method for the location of sialomucoproteins separated by electrophoresis on paper.

Whatman No. 3 MM paper strip (10 cm wide × 34 cm long) was moistened with a volatile buffer solution such as pyridine-acetic acid-water (10:0.4:90, by volume) buffer of pH 6.5 (ref. 1) and then pressed between two sheets of filter paper to remove the excess of the buffer. The strip was then placed in an electrophoretic apparatus. Sample solution containing sialomucoprotein was applied at the origin. Electrophoresis was carried out in the buffer by the horizontal open strip method at 200 V for 7 h and platinum electrodes were used. After electrophoresis the strip was allowed to dry overnight at room temperature. The dry strip was immersed in a solution of methylene blue (100 mg of Merck's methylene blue 'B' was dissolved in 200 ml. of 90 per cent methanol) for 20 min, and excess dye was removed by washing with 90 per cent methanol until the strip had only a light blue tint. The strip was then dried at room temperature. The sialomucoprotein gave a blue, while acid mucopolysaccharides such as chondroitin, chondroitin sulphate, hyaluronic acid and heparin gave each a blue violet, metachromatic spot under the same conditions.

Methylene blue gave better results than toluidine blue and alcian blue for the staining of sialomucoproteins under the same conditions.

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## Portal Venous Transport of Free Pelargonic Acid following Intestinal Instillation of Tripelargonin

SATURATED odd-chain fatty acids such as valerate (C5) and pelargonate (C9) are metabolized differently from even-numbered saturated fatty acids in the same range of carbon-chain length, caproate (C6) and caprylate (C8)<sup>1-4</sup>. The three terminal carbons of the odd-chain acids appear to be metabolized as a unit, which can serve as a carbohydrate precursor, in contrast to the behaviour of the terminal two-carbon units of even-chain acids. Digestion of glycerides of odd-chain fatty acids and absorption and transport of the digestion products appear to have been little studied.

This communication describes experiments with tripelargonin suggesting that, in the dog, this triglyceride is hydrolysed in the gut with the resulting C9 acids transported in the portal vein in the free fatty acid (FFA) form.

Dogs were lightly anaesthetized with sodium pentobarbital and a polyethylene catheter was implanted in the portal vein. Thirty-five g tripelargonin homogenized with 20 ml. of a 2.5 per cent aqueous solution of sodium taurocholate was injected directly into the duodenal lumen. The pylorus was occluded by a non-traumatic clamp during the 5-min instillation period. After the injection the portal vein catheter was exteriorized and the abdomen closed. The catheter was kept open by means of a slow infusion of 0.9 per cent saline solution. Samples of blood were obtained from the portal vein before, and at frequent intervals for 4 h after, intraduodenal instillation of the glyceride. The lipids were extracted from each plasma sample and the lipid residue separated by thin-layer silicic acid chromatography into phospholipid, free cholesterol, FFA, triglyceride and cholesterol ester. The lipid esters and FFA were eluted with chloroform-methanol and the fatty acid moieties were identified by gas-liquid chromatography (GLC) following methylation. To provide a sufficient quantity of lipid ester for fatty acid analysis, the post-prandial samples were pooled by lipid ester class. Samples were run at both high (175°C) and low (100°C) column temperatures in order to obtain a more complete spectrum of fatty acids.

Results in one experiment are shown in Figs. 1 and 2. In Fig. 1 are shown the fatty acid patterns by GLC of the FFA from portal venous plasma in the post-absorptive state and 2 h after intraduodenal instillation of tripelargonin. Under both high and low column temperatures, post-absorptive portal plasma FFA did not contain any fatty acid below C<sub>14</sub>. However, in the post-prandial sample, pelargonic acid was readily demonstrated in the FFA fraction. In contrast, the lipid ester fractions from the post-prandial plasma did not contain any detectable amount of pelargonic acid under either high or low temperature column conditions (Fig. 2).

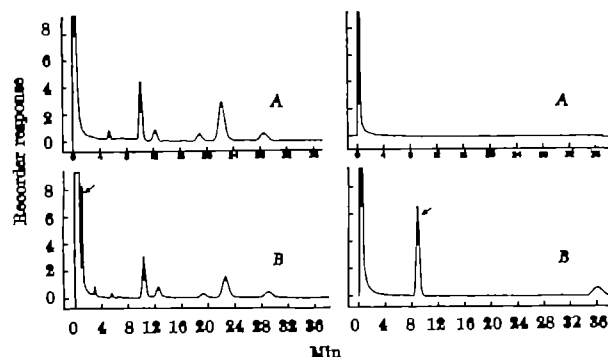


Fig. 1. Gas-liquid chromatograms of free fatty acids in canine portal venous plasma before (A) and 2 h after intraduodenal instillation of tripelargonin (B). Pelargonic acid is readily identified (arrow) in the post-prandial plasma at both high (left) and low (right) column temperatures.

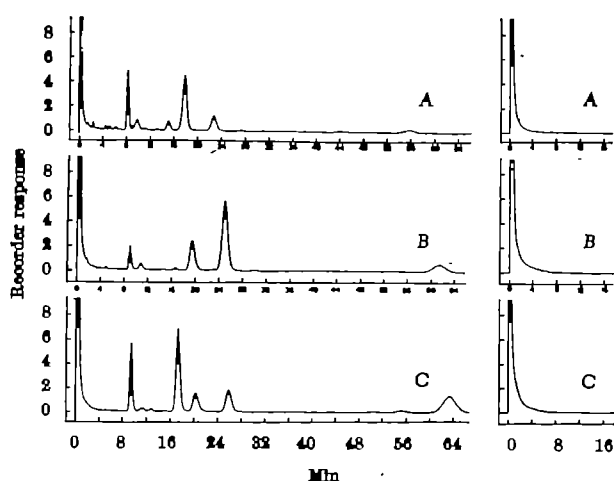


Fig. 2. Gas-liquid chromatograms of the fatty acid moieties of three lipid ester fractions in canine portal venous plasma during a four-hour period following intraduodenal injection of tripelargonin: A, Triglyceride; B, Cholesterol ester; C, Phospholipid. Pelargonio acid cannot be identified at either high (left) or low (right) column temperatures.

Since only non-esterified pelargonio acid appeared in the portal venous plasma following the intraduodenal administration of tripelargonin, it must be inferred that the triglyceride was first hydrolyzed in the intestine. Indeed, the mechanism of absorption and portal venous transport of pelargonate appears to be similar to that reported for caprylate<sup>4,5</sup>. It seems unlikely that any significant portion of the pelargonate travelled by the lacteal-thoracic duct system since our own (unpublished) experiments in rats have shown that pelargonio acid comprised less than 5 per cent of the total fatty acids recovered from thoracic duct lymph during a 24-h collection period after oral administration of tripelargonin.

It is concluded that after intestinal hydrolysis of the parent triglyceride, the absorbed pelargonate is transported principally in the portal vein as a free acid.

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### Heterogeneity of the Protein Moiety of the Human Serum High-density Lipoproteins

THE possibility of preparing a water-soluble, essentially lipid-free protein,  $\alpha P$  (ref. 1), from human serum high-density lipoproteins (HDL) of  $d$  1.063–1.21 gm/ml. has provided a means for studying its physical and chemical properties and the reason for its high avidity for lipids<sup>2</sup>. As a partial aid to the elucidation of these problems a comparative analysis of the antigenic properties of HDL and  $\alpha P$  was conducted. Normal human serum HDL was separated by flotation at density between 1.063 and 1.21 gm/ml. (NaCl–NaBr) in a Spinco model L ultra-

centrifuge (40.3 rotor, 114,480g, 18° C, 24 h) and re-spun for an additional 48 h under the same conditions to remove serum protein contaminants.

$\alpha P$  was prepared from HDL by a modification of the procedure of Soanu *et al.*<sup>1</sup> and dissolved in buffer (tris hydroxymethylamino methane), pH 8.6, 0.1 ionic strength. For immunization, rabbits were injected intramuscularly with HDL or  $\alpha P$  suspended in complete Freund's adjuvant, at weekly intervals for a total of 4 weeks. Blood was taken 8 days after the last injection. Antibody titres were determined according to Kabat and Meyer<sup>3</sup>. Immunodiffusion studies in agar gel (Bacto Agar, Difco Laboratories, Detroit) were conducted according to Ouchterlony<sup>4</sup> and Scheidegger<sup>5</sup>. By agar electrophoresis (Fig. 1A) HDL showed a major fast band stainable for protein (Amido Schwartz) and lipid (oil red O) and a minor slow one detected only by protein stain. With  $\alpha P$ , the major band was close to the origin, while a faint one moved anodically in the  $\alpha_1$ -globulin area. Immuno-electrophoretic analysis of HDL using anti-HDL or anti- $\alpha P$  sera showed three protein stained arcs of precipitation: only the major one (Figs. 1B and C) was detected by lipid stain. Three arcs of precipitation, lipid-fast, were noted in reactions between  $\alpha P$  and anti- $\alpha P$  or anti-HDL sera (Figs. 1B and C). The position of the major arc, as compared with HDL, was closer to the origin, an expression of the low mobility of  $\alpha P$  in agar gel<sup>6</sup>. Antigenic heterogeneity of either HDL or  $\alpha P$  was also detected by the Ouchterlony technique (Fig. 1). Anti-HDL sera adsorbed with  $\alpha P$  failed to react with HDL. Similarly, no reaction was noted between  $\alpha P$  and anti- $\alpha P$  sera, after adsorption with HDL. Immuno-electrophoretic analysis of mixtures of HDL and  $\alpha P$  (HDL:  $\alpha P$ ; 2.0:1; 1:1 and 0.5:1) against anti-HDL sera gave results similar to those seen with whole HDL. However, the intensity of the arc of precipitation closest to the origin varied according to the concentration of  $\alpha P$  in the mixture. Both HDL and  $\alpha P$  exhibited a high electrophoretic mobility in a neutral gel medium ('Agarose, J-2404-S', Fisher Scientific Co., Chicago). The immunodiffusion patterns were the same as those in plain agar.

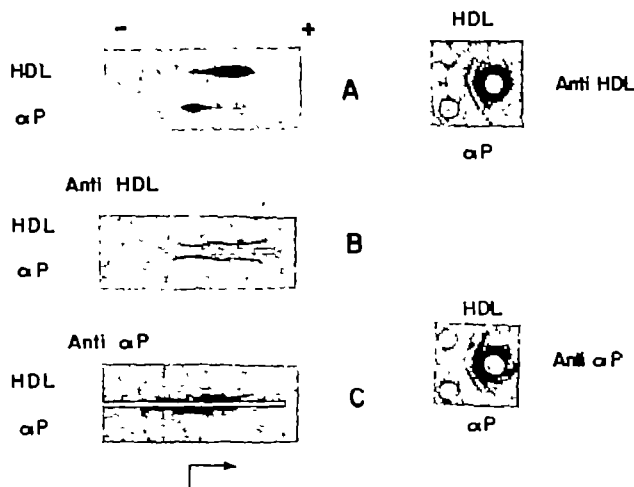


Fig. 1. Electrophoresis and immunodiffusion patterns in agar gel of HDL or  $\alpha P$  against anti-HDL or anti- $\alpha P$  sera. Conditions of electrophoresis: 'LKB Immaphor' apparatus (Stockholm, Sweden), veronal buffer, pH 8.6, ionic strength 0.04, 6 V/cm, 40 m. amp, 2 h, 25° C. Protein stain: amido Schwartz.

From these investigations it would appear that HDL, separated by ultracentrifugation, exists as a mixture of lipid-rich ( $\alpha LP$ ) and lipid poor ( $\alpha P$ ) forms, readily dissociable by agar electrophoresis. This conclusion, which is in agreement with that derived from preliminary findings by Levy and Fredrickson<sup>7</sup>, may not apply to circulating HDL.

The fact that HDL and  $\alpha P$  produced in rabbits an identical qualitative immunological response seems to favour the concept that the antigenicity of HDL resides in its protein moiety. However, quantitative immunoprecipitation experiments in the presence of either HDL or  $\alpha P$  showed a significantly higher activity of anti-HDL sera as compared with anti- $\alpha P$ , thus suggesting that HDL lipid, which is inert by itself<sup>8</sup>, has an enhancing effect on the antigenic activity of the protein moiety,  $\alpha P$ .

Antigenic homogeneity of  $\alpha P$  was previously noted by Scann *et al.*<sup>6</sup> during investigations using horse anti-normal human sera. Discrepancy between previous and present findings was related to the difference in antibody potency, weaker antisera being able to detect only the major of the three antigenic determinants. The reasons for the antigenic heterogeneity of HDL, recently reported also by Ayrault-Jarrier *et al.*<sup>9</sup> and Burstein and Fine<sup>10</sup>, are not clear. The position of the area of precipitation in the agar plates suggests that the three antigenic determinants of  $\alpha P$  are different in size and/or charge. That  $\alpha P$  may exist in solution as molecular forms containing a various number of monomeric units is indicated by the work of Shore<sup>11</sup>. It is possible, however, that  $\alpha P$  heterogeneity is related to changes of the primary amino-acid sequence of its polypeptide chains or of their carbohydrate content<sup>12</sup>. Work in progress on  $\alpha P$  fragments obtained by chemical or enzymatic cleavage of HDL protein may shed light on this problem.

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Note added in proof. Since submission of the manuscript for publication, the work cited in ref. 7 has appeared *in extenso* (Levy, R. I., and Fredrickson, D. S., *J. Clin. Invest.*, 44, 426; 1965).

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### Effect of Freezing and Thawing on the Stability of Double Helix of DNA

INCREASING attention has been directed in recent years to the effects of freezing and thawing on the structural and functional integrity of various biological materials, especially from the point of view of low-temperature storage. At the level of isolated enzyme proteins, it has been shown that there exists a critical region of temperature in which some proteins are denatured during freezing and thawing. The range in which the maximal level of denaturation was produced is, for example, from  $-12^{\circ}\text{C}$  to  $-75^{\circ}\text{C}$  for catalase, and from  $-20^{\circ}\text{C}$  to  $-72^{\circ}\text{C}$  for myosin<sup>1,2</sup>.

On the other hand, there is little information concerning the effects of freezing and thawing on the stability of deoxyribonucleic acid, that is, the genetic material.

This communication deals with the effects of freezing and thawing on the stability of double-stranded helical

structure of calf-thymus DNA in terms of the hyperchromic effects at 259 m $\mu$  on denaturation. Calf-thymus deoxyribonucleic acid used was a preparation from Sigma Chemical Co. (sodium salt, type 1). This preparation was characterized by its extinction per gram-atom of phosphorus at 259 m $\mu$ , the value of which  $\epsilon(p)$  was 6650. The 'melting' temperature ( $T_m$ ) for this preparation was also determined as follows: 3.5 ml. samples of DNA solution (30  $\mu\text{g}/\text{ml}$ .) were heated in stoppered tubes in a thermostat set to each required temperature. After 10 min the heated samples were removed and cooled rapidly to  $0^{\circ}\text{C}$  in a few seconds, and the spectroscopic measurements were carried out at room temperature. In 0.15 M sodium chloride plus 0.5 mM phosphate buffer (pH 7.0), the 'melting' temperature was  $91.5^{\circ}\text{C}$  and it was decreased to  $59.0^{\circ}\text{C}$  in 0.5 mM phosphate buffer, pH 7.0. These results for  $T_m$  were almost similar to those reported by Lee *et al.*<sup>3</sup>.

In my experiments, the effects of freezing and thawing on the ultra-violet absorption of DNA were investigated under the following conditions: 3.5 ml. samples of DNA solution (30  $\mu\text{g}/\text{ml}$ .,  $5.5 \times 10^{-4}$  M in phosphorus) were placed in test-tubes and frozen by immersion in a low-temperature bath for a given time. The frozen samples were then removed and thawed rapidly in a bath at  $20^{\circ}\text{C}$ , and the spectroscopic measurements were carried out at room temperature ( $20^{\circ}\text{C}$ ). All control samples were immersed without freezing in an ice bath at  $0^{\circ}\text{C}$ .

Table 1 shows the percentage increase in the absorbance at 259 m $\mu$  on freezing DNA for 10 min at each temperature. In 0.15 M sodium chloride plus 0.5 mM phosphate buffer, pH 7.0 ( $T_m = 91.5^{\circ}\text{C}$ ), no increase in the ultra-violet absorbance of DNA by freezing and thawing was observed. In 0.5 mM phosphate buffer ( $T_m = 59.0^{\circ}\text{C}$ ), on the other hand, there occurred a slight decrease, rather than some increase, in the absorbance at 259 m $\mu$  by freezing and thawing. These circumstances were the same with the time of freezing for 5 min to the next few hours, and also the same with the concentrations of DNA of 3  $\mu\text{g}/\text{ml}$ –30  $\mu\text{g}/\text{ml}$ . The slight decrease (less than 5 per cent) in the ultra-violet absorbance which occurred by freezing DNA in the low ionic strength may be attributed to the possibility that some partial loosening in the helical structure of the DNA used has become tighter by freezing.

In the range of temperature that has been investigated, down to  $-192^{\circ}\text{C}$ , it can be concluded, therefore, that the double-stranded helical structure of DNA would not be broken down by freezing and thawing, in terms of the hyperchromic effect of denaturation.

As compared with the fact that there exists a definite critical region of temperature for denaturation of proteins by freezing and thawing, the remarkable stability of the

Table 1. CHANGES IN THE ULTRA-VIOLET ABSORPTION OF DNA BY FREEZING AND THAWING

Temperature ( $^{\circ}\text{C}$ )	$\Delta A_{259}$ (per cent)*	
	0.5 mM phosphate buffer $T_m = 59.0^{\circ}\text{C}$	0.15 M NaCl plus 0.5 mM phosphate buffer $T_m = 91.5^{\circ}\text{C}$
$0^{\circ}\dagger$	0 (0.378)	0 (0.318)
$-10^{\circ}\dagger$	-2.1	0
-20	-2.6	0
-30	-2.4	0
-40	-2.1	0
-50	-2.6	0
-60	-3.4	0
-70	-3.9	0
-80	-4.5	0
-90	-4.7	0
-100	-4.2	0
-110	-4.7	0
-120	-4.7	0
-130	-4.7	0
-140	-4.7	0
-150	-4.7	0
-160	-4.7	0
-170	-4.7	0
-180	-4.7	0
-192	0	0

\*  $\Delta A_{259}$  is the percentage increase in absorbance at 259 m $\mu$ . DNA solutions were frozen by immersion in each required low-temperature bath for 10 min, and then thawed rapidly in a bath at  $20^{\circ}\text{C}$ . DNA concentration; 30  $\mu\text{g}/\text{ml}$ .

$\dagger$  Control samples were at  $0^{\circ}\text{C}$  without freezing. The absorbance of each sample at 259 m $\mu$  is shown in parentheses.

$\ddagger$  Inoculated with ice crystal.

helical structure of DNA during freezing and thawing suggests that some different types of interaction with water are involved in these biological macromolecules.

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## PHYSIOLOGY

### Heart Valve Lesions following Papillary Muscle Damage

THE genesis of rheumatic valvular disease in man has been difficult to establish owing to the scarcity of human material in the early stages and the absence of any comparable condition in experimental animals. On the evidence at present available it has been stated that the calcific lesions found in human rheumatic valvular disease are unlikely to be the direct result of the rheumatic process in the valve cusps<sup>1</sup>. In recent experiments we have been studying the effects of papillary muscle damage and inactivation on the function of the mitral valve and the left ventricle of the dog<sup>2</sup>. In these experiments either the anterior or the posterior papillary muscle was injected with 0.5–1.0 ml. ethanol and the animal allowed to recover. A surprising finding was that when the animals were killed three months later, four out of twelve dogs in addition to papillary muscle damage also had fibrotic, calcific or chondritic lesions of the mitral valve cusps (Fig. 1). In all four animals the valvular lesion was in the segment of the aortic cusp of the mitral valve which was attached to the damaged papillary muscle and there was no continuity of the valvular lesions with either the papillary muscle lesion through the chordae tendinae or the atriotomy scar in the three animals in which this had been carried out. In ten dogs used as controls in which the ethanol injection was made into the lateral wall of the left ventricle between the papillary muscles, no valvular lesions were found. Histological examination of the lesions showed a fibrous plaque on the atrial surface of the valve cusp with replacement by calcification and chondrification (Fig. 2). There was no evidence of an infective lesion.

Chondrification of endothelial vascular tissue in response to trauma has been reported previously in both dogs and



Fig. 1. Open left ventricle of dog, 10 weeks after injection of papillary muscle. Area of papillary muscle damage and lesion on aortic cusp of mitral valve outlined.



Fig. 2. Longitudinal section of aortic cusp of mitral valve in dog, 15 weeks after papillary muscle injection. The plaque on the atrial aspect of the cusp is composed of fibro-cartilage with patchy calcification.

chickens<sup>3-5</sup>. Cartilaginous tissue was found in the chicken beyond an artificial coarctation<sup>4</sup> and it was suggested that it was the consequence of abnormal compression forces; but invagination of the left atrial appendage over a silastic ball, although producing papilliform excrescences on the heart valves, did not produce chondrification within the heart<sup>5</sup>.

Endoscopic photography in these animals has shown excessive 'flutter' in the valve cusps and mitral incompetence<sup>7</sup>. Our present results suggest that the absence of normal papillary muscle action may give rise to abnormal stresses within the valve cusps which may produce calcific and chondritic lesions. It is therefore possible that in human valvular disease, also, calcification is the consequence of traumatic stress.

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### Some Behavioural Correlates of the Transcephalic d.c. Potential in Cats

NEURAL direct current (d.c.) potentials have recently received much attention<sup>1</sup>. A steady d.c. potential across amphibian cortex in a fronto-occipital plane has been observed<sup>2</sup>. These measurable exterior surface potentials are correlated with anaesthesia and sleep<sup>3,4</sup>. High correlations have been reported between transcephalic d.c. potential (measured between mid-forehead to low union), level of anaesthesia and electro-encephalograph (EEG)<sup>1</sup>. In the awake state the surface-recorded steady potentials are frontally negative, while in anaesthesia the frontal

negativity decreases, that is, the forehead becomes more positive with respect to the occiput. The general relationship between EEG activity and behaviour (sleep-wakefulness) of humans and animals is, of course, well known<sup>6,7</sup>. This correlation of behaviour and EEG breaks down following the administration of anticholinergics such as atropine<sup>8</sup>. The EEG activity is slowed, whereas the animal appears excited. Such dissociation of EEG and behaviour under atropine has been observed in cat<sup>8</sup>, dog<sup>10</sup> and rabbit<sup>11</sup>.

Becker *et al.*<sup>12</sup> have suggested that the organized d.c. activity of the brain functions as a controlling mechanism by influencing the rate of message-flow action potentials, that is, that this potential represents a primitive form of control system. They raise the possibility that this d.c. potential state was the original functioning system in the first primitive nerve nets. It seems reasonable to assume that if the organized d.c. activity of the brain represents a sort of primitive control system, such steady potentials should be a more stable (less easily modified) reflexion of observed behaviour under certain basic physiological conditions than the EEG. This examination attempted to investigate the preceding assumption by recording EEG and d.c. fronto-occipital potentials from atropine-treated cats. Four adult male cats with food and water available *ad lib.* were used.

A Hewlett Packard d.c. meter in conjunction with large surface carbon saline electrodes<sup>13</sup> was used to determine bioelectric d.c. potentials and a Grass model 5 polygraph to record EEG. A commercial shielded observation box was used to contain the animal.

Five stainless-steel wire electrodes were placed on the calvarium of each of four adult male cats anaesthetized with pentobarbital under aseptic conditions. Each wire was soldered to a contact on a miniature socket which was anchored to the calvarium of each cat with dental cement. Five weeks of convalescence were allowed before the first individual testing. Each cat was extensively pre-handled and given five successive days of 0.5 h unrestrained exploration (adaptation sessions) in the observation box. On the initial test day parietal-occipital EEG was recorded for several minutes, and the fronto-occipital d.c. potential (mV) measured. Each cat was then immediately injected intraperitoneally with either 2 mg/kg atropine in 5.0 c.c. 0.9 per cent saline or 5.0 c.c. saline alone. Half the cats received atropine with saline and the other half only saline. Following a 30-min observation period of gross behaviour, EEG and d.c. potential were again recorded. Five to seven days later the foregoing procedure was repeated, but those animals that had received atropine with saline were given saline alone and vice versa. Approximately two weeks later each cat was placed in the observation box, the d.c. fronto-occipital potential measured and 2 mg/kg atropine injected intraperitoneally. Measurements of d.c. potential were taken 30 sec after the injection and again after a 30-min interval.

Prior to atropine or saline injection, parietal-occipital EEG recordings associated with semi-alertness were obtained from each of the four cats tested. Thirty min after atropine injection (2 mg/kg) the EEG recordings were of typical sleep pattern. The usual manifestations of atropine were observed following injection, that is, pupil dilation, tachycardia, etc. Thus, under atropine the classical behavioural dissociation occurred. No changes were found in observed behaviour or EEG activity after saline administration compared to the pre-injection measurement. No appreciable differences occurred in the d.c. potentials of cats receiving saline alone as compared with those receiving atropine, that is, no d.c. potential shift occurred. The d.c. potential reflected observable behaviour, the EEG did not. These data are summarized in Table 1.

When d.c. potentials were measured immediately before atropine injection, 30 sec after injection and again at

Table 1. EEG, BEHAVIOUR AND FRONTO-OCCIPITAL D.C. CHANGES UNDER ATROPINE AND SALINE INJECTION

Cat	Pre-injection			30 min post-injection		
	EEG pattern	Behaviour pattern	d.c. Potential (mV)	EEG	Behaviour	d.c. Potential (mV)
Atropine						
1	Awake	Alert	-12	Sleep	Alert	-12
2	Awake	Alert	+6	Sleep	Alert	+4
3	Awake	Alert	-2	Sleep	Alert	-2
4	Awake	Alert	-10	Sleep	Alert	-10
Saline						
1	Awake	Alert	+4	Awake	Alert	-4
2	Awake	Alert	+1	Awake	Alert	0
3	Awake	Alert	-11	Awake	Alert	-14
4	Awake	Alert	+8	Awake	Alert	+4

30 min, a positive d.c. shift occurred at the 30-sec measurement. The forehead of each cat, compared with the pre-injection measurement, became more positive with respect to the occiput 30 sec after injection. The relative positivity was transient and 30 min later the standing d.c. potential no longer showed any consistent pattern compared with the pre-injection level for each animal. These data are given in Table 2.

Table 2. EFFECT OF ATROPINE ON THE FRONTO-OCCIPITAL D.C. POTENTIAL (mV)

Cat	Pre-injection	30 sec Post-injection	30 min Post-injection
1	+0.5	0.0	0.0
2	-10.5	+4.5	-2.0
3	-4.0	+1.0	+2.0
4	+1.0	+8.0	+2.0

Previous investigations have indicated high correlation between sleep-wakefulness cycles, EEG and transecephalic d.c. potentials. This work has demonstrated a dissociability of EEG and fronto-occipital d.c. potentials with this standing potential retaining a much closer relationship with observable behaviour. The d.c. potential was not correlated with atropine administration. Atropine produced EEG sleep patterns but did not produce a uniform positive shift of the d.c. potential. Therefore, the d.c. potential was a more accurate representation of observable behaviour. It may be speculated, then, that the transecephalic d.c. potential underlies EEG activity and represents the more fundamental parameter of nervous activity.

The transient positive d.c. shift obtained immediately (30 sec) after injection may suggest the momentary internal stimuli orientation of a primitive control system. To evaluate this internal stimuli orientation a preliminary food deprivation investigation was carried out. Water was available *ad lib.* during this food deprivation period. Fronto-occipital d.c. potential measurements (mV) were taken on ten individually caged adult male cats deprived of food for 24 h. Following these initial d.c. measurements, the cats were fed commercial cat food, and 30 min after eating the standing d.c. potential for each animal was again determined. In nine of the ten cases: (a) the forehead was negative with respect to occiput prior to food intake; (b) 30 min after eating the fronto-occipital potential had become more positive. As evaluated by the Mann-Whitney *U* test, these positive d.c. potential shifts are statistically significant ( $P < 0.05$ ). These data again tend to support the suggestion of Becker *et al.* that the organized d.c. activity of the brain represents some primitive form of control system inasmuch as nutrient intake may logically be considered an essential function. Speculatively, the relative frontal negativity noted before eating may represent a generalized activation of the organism toward external stimuli, while the positive d.c. shift after food intake may be ascribed to a different orientation, that is, toward internal stimuli. The measurements obtained 30 min after atropine injection offer little information on the external-internal stimuli hypothesis as atropine modified both exteroceptive input (pupil dilation) and interoceptive input (tachycardia).

The data obtained following atropine administration tend to support the suggestion of Becker *et al.*<sup>12</sup> that the organized d.c. activity of the brain represents a primitive

form of control system inasmuch as such steady potentials were dissociable from EEG under atropine and yet paralleled observable behaviour. If the d.c. potential shift immediately following enteric stimulation represents a primitive parameter of system arousal then it may be of interest to investigate EEG manifestations associated with various d.c. potential patterns.

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### Underwater Breathing Device utilizing Oxygen dissolved in Water

THE respiration of an animal can be supported if its expired air is re-oxygenated, freed of carbon dioxide, and re-inhaled. At the water's surface dissolved gases equilibrate with the air above, providing a partial pressure of oxygen ( $pO_2$ ) of approximately 150 mm Hg. The thickness of the water layer with an oxygen concentration approximating that at the surface varies widely in the oceans; in the Atlantic south of 50° S. it extends to about 50 m depth, and at 100 m the water is still 90–95 per cent saturated<sup>1</sup>. In expired air of man  $pO_2$  is 116 mm Hg (ref. 2). Thus, for a considerable distance from the surface there exists a  $pO_2$  gradient of roughly 35 mm Hg from water to expired air. Since the partial pressure of carbon dioxide ( $pCO_2$ ) in water is negligible, a gradient exists for the diffusion of  $CO_2$  from expired air to water.

An apparatus was constructed to allow a guinea-pig to maintain respiratory exchange by means of these diffusion gradients (Fig. 1). A 'Teflon' membrane was interposed

between the guinea-pig's expired air and the water; the air above the water was at atmospheric pressure. Oxygen diffused into the expired air and carbon dioxide diffused out. The gas mixture was then re-breathed by the guinea-pig.

Fifteen membrane units were supported on an open frame and had a combined surface area of 5.9 m<sup>2</sup>. Each unit was made by placing screening between two 'Teflon' membranes 12.5  $\mu$  thick. Expired air flowed between the two membranes, which were sealed by autoclavable masking tape. A manifold at each end of the apparatus led to one side of a respiratory valve attached to the trachea of an anaesthetized guinea-pig. All membranes were submerged but the apparatus was kept close to the surface by two buoyant pieces of plastic. The animal was secured to the apparatus above the water. The membrane array moved through the water at approximately 6 m/min. Gases entered at the back and left at the front relative to movement through the water, the purpose being to aid diffusion by countercurrent. It was realized that the effect might be small in this case.

The pool was 3.5 m by 1.9 m. It was filled to a depth of 0.7 m with tap-water aerated for 12–16 h. The aeration was stopped and the water was allowed to stand from 2 to 24 h. The  $pO_2$  of the water at the time of experimentation was within 4 mm Hg of that of the room air. The  $pO_2$  and  $pCO_2$  of water and gases were measured with modified Clark and Severinghaus electrodes using a 'Model 105' Instrumentation Laboratory meter.

In each of two experiments a guinea-pig was anaesthetized, a tracheotomy performed, and a tracheal cannula connected to a respiratory valve. The valve allowed exhaled gas to enter one end of the membrane array and the animal to re-breathe gas from the other end. Respiratory frequency,  $pO_2$  and  $pCO_2$  of expired and inspired gases were measured. Nitrogen equal in volume to the withdrawn gas samples was added to the closed circuit.

The possibility existed that room air would leak into the system through the tracheotomy or valve. Consequently, at the conclusion of each experiment the animal and valve were disconnected from the membrane array and connected to a 'control apparatus', consisting of a closed circuit of Erlenmeyer flasks with volume totalling that of the membrane array. If there were no air leaks one would expect that within the control apparatus oxygen would quickly be used up and carbon dioxide would accumulate.

An animal weighing 306 g was used in the first experiment. There was a rise in  $pO_2$  as expired air passed through the apparatus. The rise is seen as the difference between the expired  $pO_2$  and inspired  $pO_2$  (Fig. 2) and resulted primarily from oxygen diffusion from the water but to a lesser extent from oxygen trapped within the apparatus at the beginning of the experiment. For the first 3.5 h the  $pO_2$  of the inspired and expired gases fell, thereby causing an increase in the diffusion gradient for oxygen from water to expired air. As a result, the quantity of oxygen that diffused across the membrane increased until it equalled the amount of oxygen utilized by the animal. This occurred after 3.5 h; at that time the  $pO_2$  of inspired and expired gas were constant and remained so for an hour. The respiratory frequency rose, however, presumably because of increasing hypoxia and fatigue. Shortly after disconnecting the animal from the apparatus its respiratory rate was 32 and the minute volume was 100 ml. From the respiratory parameters it was calculated that the apparatus furnished approximately 4.0 ml. oxygen/min or transferred 0.68 ml. oxygen/m<sup>2</sup>/min.

At the end of the experiment the animal was attached to the control apparatus. Within the first 15 min  $pO_2$  went from 146 to 52 mm Hg and  $pCO_2$  from nearly zero to 107 mm Hg. We conclude that there was no significant air leakage through the valves or tracheotomy.

In the second experiment (Fig. 3) a guinea-pig weighing 211 g was used, but the membrane apparatus was first

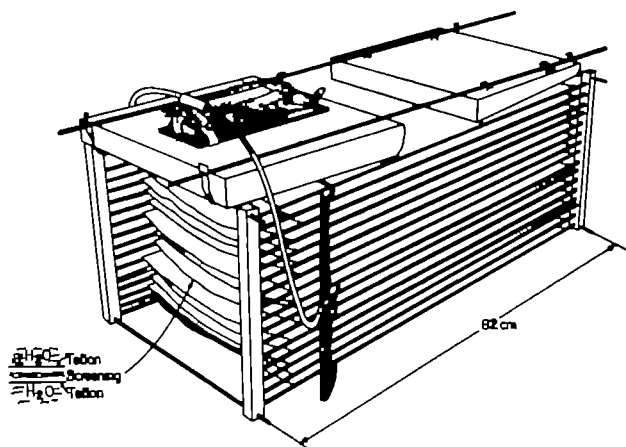


Fig. 1. Underwater breathing apparatus consisting of 15 'Teflon' membrane units. The exhaled air of a guinea-pig enters the membrane units; oxygen diffuses in and carbon dioxide diffuses out. The air is then re-breathed. Cross-section of a membrane unit is shown.

flushed with an air-nitrogen mixture of  $pO_2$  108 mm Hg. The inspiratory  $pO_2$  this time rose for the first half-hour and thereafter the inspiratory  $pO_2$ , expiratory  $pO_2$ , and expiratory  $pCO_2$  showed little change. After 5 h the animal was connected to the control apparatus containing gas of initial  $pO_2$  of 128 mm Hg and a  $pCO_2$  close to zero. After 10 min  $pO_2$  was 85 mm Hg,  $pCO_2$  55 mm Hg, and the animal's respiratory rate was 50.

In both experiments the  $pCO_2$  of the inspired gas remained below 10 mm Hg. A control experiment showed that in a membrane unit with comparable gas flow per unit area (no animal attached) the  $pCO_2$  fell from an initial 23 mm Hg to 2 mm Hg. Also, decreasing the gas flow into the apparatus led to a higher  $pO_2$  of gas leaving since more time was allowed for equilibration. It was not, therefore, surprising that the smaller animal had the higher inspiratory  $pO_2$ , one which remained within physiological range.

The membrane apparatus allowed transmembrane diffusion of oxygen and carbon dioxide at the rates at which the animal utilised oxygen and gave off carbon dioxide. We conclude that respiratory gas exchange can be maintained with the apparatus for an indefinite period of time.

The apparatus described has, however, certain inherent limitations. Sea-water contains at best only 9 ml. of oxygen per litre<sup>1</sup>. Thus, a large quantity of water as well as a large membrane surface area are necessary to support the respiration of even a small animal. More significantly, the apparatus described can function only within a metre of the water's surface. As a diver goes deeper the air pressure within his chest must equal the increasing water pressure. Nitrogen furnishes four-fifths of air pressure. As total intrathoracic pressure is increased the partial pressure of nitrogen within the system is also increased. Nitrogen would then diffuse from expired air to water; therefore nitrogen cannot be used to maintain a high pressure within the system. However, if a physiologically inert gas could be found to which a given membrane were impermeable, a small amount of the inert gas would last indefinitely to maintain a pressure equal to hydrostatic pressure. The membrane, if it were permeable to oxygen and carbon dioxide while impermeable to the inert gas,

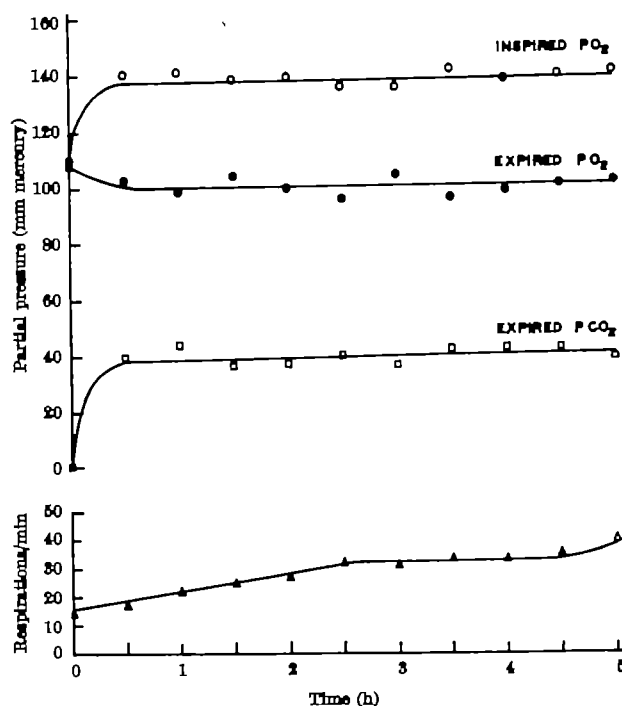


Fig. 3. Respiratory parameters as a function of time after attaching a 211-g animal to the membrane apparatus which was first flushed with an air-nitrogen mixture

would then allow oxygen flow regardless of hydrostatic pressure.

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## Hypophysectomy and Blood Sugar Regulation in a Cyclostome, *Myxine glutinosa*

THE removal of the pituitary has contributed to the understanding of the endocrinology of many groups of vertebrates. However, ablation of what is probably the most primitive extant pituitary<sup>1,2</sup>, namely that of a myxinoide cyclostome, has, to the best of our knowledge, not yet been reported.

This operation has been successfully performed recently on the hagfish, *Myxine glutinosa*, at Kristineberg Zoological Station, Sweden. The animals were anaesthetized by immersion in 0.25 per cent propylene phenoxetol (Nipa Laboratories Ltd.) for 10–15 min<sup>3</sup>. A ventral approach was used, a median incision being made in the skin just behind the mouth for a distance of about half an inch. The protractor muscles, that is, the copulo-glossus profundus and superficialis muscles and the copulo-palatinus muscles, were retracted back from the centre line. The retraction exposed the basal plate which was medially bisected with a sharp scalpel. The fore-gut in the region of the lingual teeth thus exposed was retracted to one side to reveal the caudal termination of the cartilaginous floor of the nasohypophyseal canal. This cartilage was then bisected back from the junction of the nasohypophyseal canal with the fore-gut to expose the pituitary lying embedded in the cartilage forming the roof of the nasohypophyseal canal

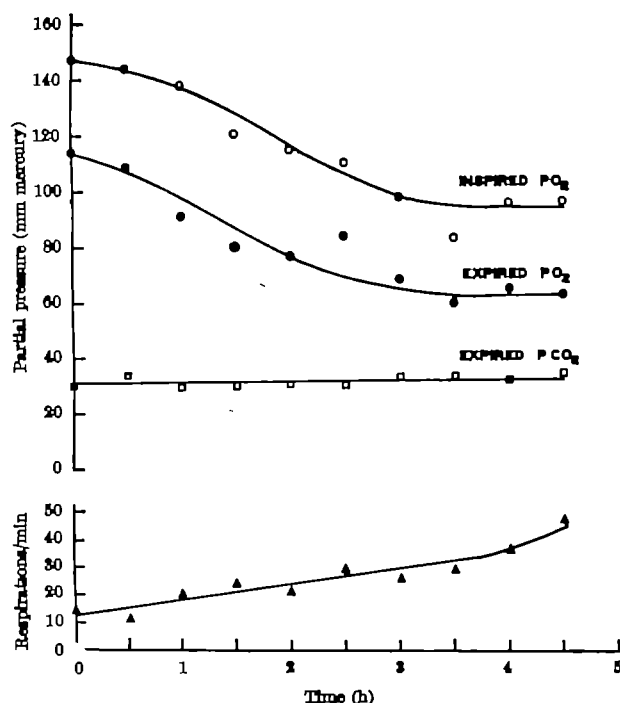


Fig. 2. Respiratory parameters as a function of time after attaching a 306-g animal to the membrane apparatus



and floor of the brain. Using oblique light the oval patch marking the position of the adenohypophysis could be seen. With the aid of a fine scalpel and a binocular dissecting microscope a rectangle of cartilage containing the pituitary was removed, exposing the hypothalamus. The cavity was plugged with sterile gelatine sponge. The wound was ligatured in the usual manner. On return to water the hypophysectomized animals resumed active respiratory movements within 10–20 min and were kept in aquaria for periods up to three weeks. As control experiments, isolated mechanical lesions were made in the hypothalamic region. Sham operations included exposure of the pituitary without its removal. Microscopic examinations were carried out at autopsy to ascertain the completeness of pituitary removal and the extent of the hypothalamic lesions. Repeated blood samplings were performed *in vivo* via the large caudal venous sinuses and the blood glucose-level was assayed by the glucose oxidase method as described previously<sup>4</sup>. Altogether 23 hagfish were successfully hypophysectomized and 7 were used as controls.

The most surprising feature of the operation was that, unlike the lamprey<sup>5,6</sup>, the hagfish did not become strikingly pale after the operation; no change in pigmentation could be observed. This might be taken to imply the absence of melanocyte-stimulating hormone, although Adam<sup>7</sup> has quoted unpublished data reporting that extracts made from the ventral hypothalamus and adenohypophysis of hagfish produce skin colour changes in *Bufo viridis*.

Both hypophysectomy and hypothalamic lesions, and to some extent also the sham operations, caused an increase in blood glucose values, from about 16 mg/100 ml. to about 60 mg/100 ml., one or two days after the operations. After 10–20 days these elevated values returned to normal. No continued hyperglycaemia was observed in spite of force-feeding the hagfish. Thus, it must be concluded that the elevated blood sugar was a stress response and not due to the presence in the pituitary gland of a factor influencing blood sugar values.

Having provided a technique for hypophysectomy of *Myxine* it is hoped that the equivocal situation of pituitary regulation of adrenal cortex, thyroid, and gonads in myxinoidea might be investigated.

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## PHARMACOLOGY

### A Highly Selective Carcinolytic Agent Isolated from Cobra Venom

CRUDE cobra venom contains a number of proteins which can be demonstrated by agar electrophoresis (pH 8.3) (Fig. 1a). By the use of column chromatography in carboxymethyl cellulose and ammonium sulphate fractionation of cobra venom, it has been possible to

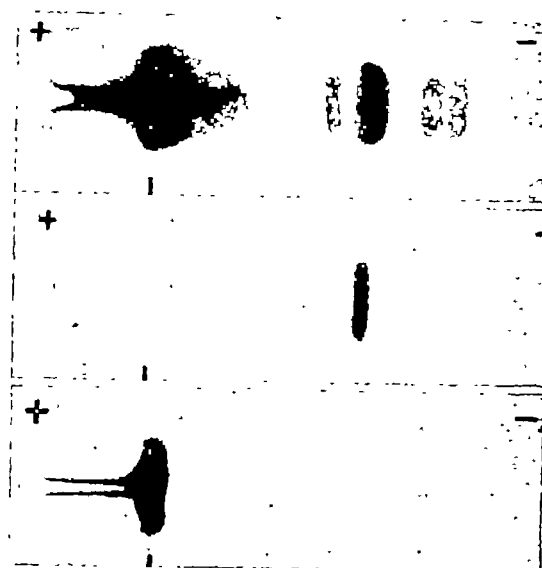


Fig. 1. Agar electrophoresis of cobra venom. a, Whole venom; b, the neurotoxin; c, the fraction of low toxicity which produces lysis of tumour cells.

separate the protein fraction which is responsible for the toxicity of the venom in whole animals (Fig. 1b) from another fraction which has low toxicity and remains close to the origin in agar electrophoresis, using the method of Wieme and Rabeys<sup>1</sup>. This is shown in Fig. 1c. The *MLD* for this fraction was 100, whereas the *MLD* for the crude venom and the neurotoxin was 17 and 4, respectively (the *MLD* minimum lethal dose in  $\mu$ g of protein of the material required to kill a mouse weighing 20–25 g within 18 h). This comparatively non-toxic fraction still has a remarkable action on Yoshida sarcoma cells.

The interference microscope has been used to observe changes in cell membrane permeability leading to loss of cytoplasm from these cells and from various normal cells. The effects are similar to those examined in the familiar phenomenon of lysis of erythrocytes leading to the loss of haemoglobin and appearance of cell ghosts. Interference microscopy, because it is so sensitive to small mass changes, makes it possible to examine the lysis of tissue cells<sup>2</sup>. Results obtained using Yoshida rat sarcoma cells and on various normal rat cells suspended in Tyrode's solution and in rat serum are shown in Table 1. The erythrocytes, lymphocytes from lymph nodes and bone marrow cells were all observed as suspensions prepared without the use of chelating agents or trypsin. The intestinal mucosa cells were obtained as small areas of epithelial sheets containing 20–50 cells, also prepared without the use of chelating agents or trypsin. The mucosa cells were therefore obtained in their natural sheet-like state and would be in a very similar situation to that occurring *in vivo*. It can be seen from Table 1 that this fraction of low animal toxicity is extremely toxic for tumour cells and is highly selective in its action. Serum has a protective action on the normal cells but has little effect on the sensitivity of the tumour cells.

These observations support the view that changes in the cell membrane of tumour cells are of a characteristic nature. The low adhesiveness and high mitotic rate of the tumour have not been brought about by membrane

Table 1. CYTOTOXICITY OF A COBRA VENOM FRACTION

Cell type (Rat cells)	Dilution of a solution of 1 mg/ml. protein required to produce 50% of cell lysis in 0.5 h	
	Tyrode	Rat serum
Y. sarcoma	1/128	1/128–1/64
Red blood cells	1/64–1/32	1/4
Lymphocytes	1/16–1/8	1/4
Bone marrow	1/32	1/2
Intestinal mucosa	1/2	1/2

properties similar to rapidly dividing normal cells such as intestinal mucosa, nor are they similar to those of relatively non-adhesive erythrocytes, lymphocytes or bone marrow cells. Ambrose and Easty<sup>2</sup> obtained evidence for slight selectivity using whole venom (Russell's viper) in comparative investigations of tumour and normal cells in culture.

Preliminary *in vivo* investigations using this fraction have been quite encouraging, and several animals have been cured of Yoshida ascites tumours; but *in vivo* effects involve problems connected with blood supply to the tumour, rate of elimination of the agent, etc. Whatever the final success obtained with this fraction *in vivo*, the experiments described here on lytic action indicate that the necessary specificity of a structure is present on the tumour cell surface in this case. It may be possible to exploit this by the use of biological macromolecules containing the required properties in their amino-acid, sugar or nucleotide sequence as carcinolytic agents.

We thank Prof. A. Haddow and Dr. A. R. Gopal-Ayengar for their advice. One of us (E. J. A.) also thanks the U.K. Ministry of Overseas Development and the Atomic Energy Commission, Government of India, for their support (Colombo Plan).

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## Reversal of Selective Toxicity of (-)- $\alpha$ -Lipoic Acid by Thiamine in Thiamine-deficient Rats

INTRAPERITONEAL injection of ( $\pm$ )- $\alpha$ -lipoic acid has been reported to be toxic to thiamine-deficient rats<sup>1</sup>. Development of the toxic effect was prevented by simultaneous injection of 100  $\mu$ g of thiamine hydrochloride. It was also noticed that ( $\pm$ )- $\alpha$ -lipoic acid, unlike neopyrithiamine, had no effect on thiamine excretion. The toxicity of ( $\pm$ )- $\alpha$ -lipoic acid to thiamine-deficient rats therefore could not be related to the depletion of the thiamine reserves present in the organs of thiamine-deficient animals. Obviously, the results obtained from the use of the racemic form of lipoic acid could not reveal whether both or one of the isomers had been responsible for the toxic effects in the deficient animals. In order to clarify the relative toxicity of the isomers of lipoic acid in thiamine-deficient rats, the experiments were repeated with the natural and unnatural isomers of  $\alpha$ -lipoic acid. The preparation of pure (+)- $\alpha$ -lipoic acid was accomplished from (+)-6,8-dichlorooctanoic acid (-)-ephedrine salt<sup>2</sup>. However, the (-)- $\alpha$ -lipoic acid was found to be contaminated with 15-20 per cent of the dextro-rotatory enantiomorph<sup>3</sup>. Therefore, the pure (-)- $\alpha$ -lipoic acid was synthesized from the purified (-)-6,8-dichlorooctanoic acid (+)-ephedrine salt. This was obtained after converting a sample of (-)-6,8-dichlorooctanoic acid (-)-ephedrine salt into the (+)-ephedrine salt. (The (-)-6,8-dichlorooctanoic acid (-)-ephedrine salt was generously supplied by Dr. W. J. Wayne of Du Pont.)

In the experiments with the various forms of lipoic acid, 20 mg/kg of  $\alpha$ -lipoic acid was administered intraperitoneally to thiamine-deficient Sprague-Dawley rats of 100-150 g average weight. When the animals developed the 'acute stage' of polyneuritis<sup>4</sup>, they were divided into

Table 1. TOXICITY OF (-)- $\alpha$ -LIPIC ACID AND ITS EFFECT ON URINARY THIAMINE EXCRETION IN THIAMINE-DEFICIENT RATS

Compound	Mortality of deficient rats (%)	Urinary thiamine* in 3 days $\mu$ g	%
( $\pm$ )- $\alpha$ -Lipoic acid	75 (18)	—	—
( $\pm$ )- $\alpha$ -Lipoic acid + Thiamine (250 $\mu$ g)	0 (10)	69	28
(+)- $\alpha$ -Lipoic acid	40 (16)	—	—
(+)- $\alpha$ -Lipoic acid + Thiamine (250 $\mu$ g)	10 (10)	64	26
(-)- $\alpha$ -Lipoic acid	80 (16)	—	—
(-)- $\alpha$ -Lipoic acid + Thiamine (250 $\mu$ g)	20 (10)	70	28
Neopyrithiamine	27 (10)	—	—
Neopyrithiamine + Thiamine (250 $\mu$ g)	0 (6)	150	60
Isotonic saline + Thiamine (250 $\mu$ g)	0 (6)	60	24

The animals in the various groups received i.p. injection of 20 mg/kg of the different lipoic acids or 5 mg/kg of neopyrithiamine. \*In some groups when the urinary thiamine excretion fell below detection, the animals received 250  $\mu$ g of thiamine hydrochloride followed by injection of lipoic acids, or neopyrithiamine or saline. No. of animals is given in brackets.

subgroups as shown in Table 1. After the daily collection of urine, the animals were fed in the morning and the food was withdrawn. When the urinary thiamine values fell below detection, some of the animals in each group received 250  $\mu$ g of thiamine HCl intraperitoneally, followed by i.p. injection of lipoic acid or neopyrithiamine. The urine samples were 24-h collections. They were collected for three days and were daily analysed for thiamine excretion. The values in Table 1 represent the total thiamine excreted in three days. Earlier it was established<sup>1</sup> that the 20 mg/kg ( $\pm$ )- $\alpha$ -lipoic acid corresponded to 1/4 of the  $LD_{50}$  dose. Since intraperitoneal injection of isotonic saline was not lethal to the thiamine-deficient animals, it is apparent that the mechanical effect of injection *per se* is of no consequence. Again it is obvious from Table 1 that the simultaneous injection of thiamine and lipoic acid prevented death, which ordinarily occurred when lipoic acid was administered alone to the deficient rats. Interestingly, (-)- $\alpha$ -lipoic acid seemed to be extremely toxic to thiamine-sufficient animals had not produced any undue physiological effects. As a further confirmation of earlier findings<sup>1</sup>, it has been demonstrated that a single intraperitoneal injection of 250  $\mu$ g of thiamine hydrochloride followed by the injection of any of the lipoic acids does not lead to increased urinary thiamine excretion during the succeeding 72 h. The results show that neither the racemate nor either of the isomers of  $\alpha$ -lipoic acid had any effect on thiamine mobilization while intraperitoneally administered neopyrithiamine (5 mg/kg) produced significant increase in thiamine excretion. No significant difference between the amounts of thiamine excretion before and after lipoic acid treatment could be observed in the experiments with thiamine-sufficient rats. At present the marked toxicity of (-)- $\alpha$ -lipoic acid is interpreted as an interaction between the thiaminethiol of the thiamine and (-)- $\alpha$ -lipoic acid, yielding a toxic intermediate in the brain possibly through a heteromeric interaction between thiamine and sulphydryl groups as suggested by Banhidi<sup>4</sup>. Such interaction could yield a toxic intermediate which would not only tie up thiamine reserves in the brain but also could cause the displacement of the natural isomer of  $\alpha$ -lipoic acid from the sites of enzymatic decarboxylation of  $\alpha$ -keto acids. The search for existence of such an intermediate is proposed to be undertaken with the aid of labelled isomers of  $\alpha$ -lipoic acid.

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## HAEMATOLOGY

# Role of Properdin, 0° Factor, Polyinosinic Acid, and Thrombin in the Haemolysis of Paroxysmal Nocturnal Haemoglobinuria Erythrocytes

ABSORPTION of serum at 15° C with zymosan renders the serum deficient in properdin and destroys the capacity of such RP serum to haemolyse paroxysmal nocturnal haemoglobinuria erythrocytes (PNHE)<sup>1</sup>. Absorption of serum with zymosan at 0° C does not remove properdin, but does remove a zymosan specific fraction ('0° factor'), so that on incubation with fresh zymosan at 15° C properdin is no longer affected<sup>2</sup>. Recent work in this laboratory has defined the PNHE haemolytic system as one dependent on fluid phase complement (C') activation resulting in red cell membrane destruction as a result of direct attack by C'3 sub-components<sup>3</sup>. C'1 esterase or C'1 activators have been shown to enhance PNHE lysis and in addition certain C'1 activators such as polyinosinic acid (poly I) may initiate the haemolysis of normal human erythrocytes (NHE), presumably by similar fluid phase complement reactions<sup>4</sup>. Thrombin has also been shown to enhance PNHE lysis, but the mechanism of this enhancement has been the subject of considerable controversy. The observations shown in Table 1 were designed to assess the relative role of properdin and '0° factor' in native and poly I enhanced PNHE lysis, and to compare the stimulating effect of poly I on PNHE haemolysis with the ability of thrombin ('Thrombin', bovine, Upjohn Co., Kalamazoo, Mich.), both absorbed with NHE and unabsorbed, to enhance PNHE lysis.

Table 1. EFFECT OF PROPERDIN AND '0° FACTOR' DEPLETION ON POLY I-ENHANCED HAEMOLYSIS OF PNHE RED CELLS; COMPARISON OF POLY I EFFECT WITH THROMBIN EFFECT

Addition to test serum	Percentage haemolysis					
	Normal serum	pH 7.6 0° super-natant serum	RP serum	Normal serum	pH 6.5 0° super-natant serum	RP serum
Saline (0.1 ml.)	14	14	1	51	54	2
Poly I—1 $\mu$ mole P	56	56	15	81	81	64
Thrombin—50 U	66	66	63	73	73	68
Absorbed thrombin—50 U	27	24	1	53	53	28

The haemolytic test system consisted of 1 ml. of the appropriate serum, acidified where indicated with 0.3 N HCl, 0.05 ml. of a 20 per cent suspension of PNHE in 0.15 M NaCl, and 0.1 ml. of 0.15 M NaCl or 0.1 ml. of the appropriate material dissolved in 0.15 M NaCl, added in the order mentioned. After incubation at 37° for 30 min under oil, the tubes were centrifuged and read at 540 m $\mu$  in a Zeiss PMQ II spectrophotometer against the appropriate serum blank.

Removal of '0° factor' from serum does not affect the ability of serum to support PNHE haemolysis under any of the experimental conditions cited. Removal of properdin, on the other hand, does inhibit the capacity of serum to support PNHE haemolysis at both pH 7.6 and 6.5. The ability to haemolyse PNHE can be restored to RP serum by the addition of poly I; the latter stimulates PNHE haemolysis in RP serum much more effectively at pH 6.5 than at pH 7.6, but at neither pH does poly I-enhanced haemolysis proceed as well as in normal serum. Absorption of thrombin with NHE diminishes greatly its ability to stimulate PNHE cell lysis. Addition of NHE-absorbed thrombin to normal serum at pH 6.5 does not result in significant enhancement of PNHE haemolysis. The latter observation agrees with those of Hinz<sup>5</sup>, but is at variance with the experience of two other groups<sup>6,7</sup>, who have found residual capacity for PNHE acid haemolysis stimulation in thrombin which has been exhaustively absorbed by NHE. More in keeping with these latter reports, however, we have found that absorbed thrombin can provoke moderate enhancement of PNHE haemolysis in normal serum at pH 7.6, and can partially restore haemolytic capacity to RP serum at pH 6.5. Removal of

properdin from serum diminishes, but does not completely abolish, the ability of poly I to provoke haemolysis of NHE at pH 6.5 (ref. 4).

Properdin and zymosan-specific '0° factor' are not required for poly I-induced haemolysis, although poly I-induced haemolysis is reduced somewhat in RP serum. '0° factor' is also not required for native PNHE haemolysis. Viewing properdin as related intimately to the recognized components of C' (ref. 8), and recognizing the peculiar affinity of zymosan for C'3 (ref. 9), one could speculate that properdin is, in fact, a sub-component of C'3. Such a possibility has been reinforced by our recent observations that the intermediate complex, PNHE<sub>1</sub>, formed by exposure of PNHE to partially purified human  $\beta_{12}$  globulin (C'3a), is highly susceptible to haemolysis in RP serum in the presence of Na<sub>2</sub>HEDTA (ref. 3). Bearing in mind the concepts recently developed on the special relationship of late-acting C' components to PNHE haemolysis<sup>8,9</sup>, it is conceivable that partial depletion of such a C'3 sub-component might critically affect a haemolytic system dependent on threshold amounts of activated fluid phase, late-acting C' components (native PNHE haemolysis); might partially, but not completely, inhibit a haemolytic system capable of activating more efficiently residual fluid phase late-acting C' components (poly I-induced haemolysis); and might inhibit only slightly, if at all, a haemolytic system capable of activating and utilizing the residue of such a factor with great efficiency at the cell membrane (classical immune haemolysis).

Most observers agree that much of the ability of commercial bovine thrombin to enhance PNHE haemolysis is attributable to the presence of red cell hetero-antibodies<sup>8-10</sup>. However, our observations, in agreement with those of Jenkins *et al.*<sup>6</sup> and Crosby and Benjamin<sup>7</sup>, demonstrate that even after extensive absorption with human red cells, such bovine thrombin is still capable, under certain conditions, of enhancing PNHE haemolysis. Jenkins *et al.* have clearly shown that thrombin *per se* is not involved in this enhancement; since the factor in question is heat stable, they conclude that it is non-absorbable antibody<sup>6</sup>. On the basis of the mechanisms discussed in this communication, however, this enhancing factor could be either a substance which, like poly I, is capable of activating C'1, or the bovine counterpart of one of the heat stable C'3 sub-components of guinea-pig serum (C'3c, C'3b) (ref. 10). That at least one of the bovine C'3 sub-components can function in a heterospecific C' system has been demonstrated by Lincoff and Nishioka, who used bovine plasma as a rich source of C'3d (ref. 10).

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# PATHOLOGY

## Function of Collagen: Blood Pressure in Lathyrotic Rats

COLLAGEN constitutes approximately one-third of the body protein in mammals. While its significance in maintaining form is obvious, little is known of its possible role in physiological processes. Discovering functions of collagen would be of particular importance in regard to ageing. Physical properties of collagen change markedly with age, presumably because of increased cross-linkages, but these changes have not been related to the decrease in efficiency of physiological processes characteristic of ageing. The changes in collagen with age and their possible significance have recently been reviewed<sup>1</sup>.

Because of its distribution in and around blood vessels, collagen would be expected to influence haemodynamics. The production of lathyrism in laboratory animals by the administration of certain nitriles provides a system in which the role of collagen in blood vessel reactivity can be studied. In lathyrism, maturation of collagen by cross-linking is defective, and affected animals have less than the normal amount of mature collagen<sup>2</sup>. The present work was based on the supposition that if mature collagen influences the reactivity of blood vessels, differences in blood pressure between control and lathyrotic animals should be detected when animals are treated with norepinephrine.

Male Holtzman rats weighing approximately 110 g were used. Three animals were kept as controls and four were given  $\beta$ -amino-propionitrile fumarate intraperitoneally, one mg per g body wt. per day, as a 20 per cent solution in 0.15 M NaCl. After 8 days treatment the animals were anaesthetized with 0.2 ml. 'Nembutal' per 100 g body wt. and heparinized polyethylene catheters (gauge No. 50) were secured in femoral arteries and veins. Each arterial catheter was attached to a mercury manometer and mean blood pressures were read directly. After baselines were established, each animal was given, in the venous catheter, 0.1 ml. 5 per cent dextrose containing 0.1  $\mu$ g norepinephrine. All the control rats showed a greater rise in blood pressure than the lathyrotics (Fig. 1). The mean maximum blood pressure rise for the controls was 49.3 mm Hg; that for the lathyrotics, 35.0.

A possible explanation for this observation is that mature collagen fibres are oriented in such a way that, when arterioles constrict, they stabilize the vessels in their new configuration and maintain pressure. Without collagen support, the smooth muscle cells cannot remain adequately contracted in opposition to the force exerted by the blood. Similar conclusions concerning the role of

collagen fibres in the aorta were arrived at on morphological grounds by Wolinsky and Glagov<sup>3</sup>. If future work indicates that lathyrism causes significant changes in smooth muscle cells, other explanations may be more reasonable.

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## Growth-promoting Effect of Chondroitin Sulphate on Solid Ehrlich Ascites Tumour

In the course of studying the interaction between connective tissue and cancer cells, I found that chondroitin sulphate promoted the growth of solid Ehrlich ascites tumour. Tumour cells used in this study were of Ehrlich hypotetraploid stock (Kawiwara<sup>1</sup> 4N) which was maintained intraperitoneally in SM male mice.

The first series of experiments was made with the chorioallantoic membrane (CAM) of the White Leghorn chick embryo. A disk, 8 mm in diameter and 3 mg in weight, made of 'Spongel' (Yamanouchi Seiyaku Co. Ltd., Tokyo), was soaked with solutions of various concentrations of chondroitin sulphate *O* (av. mol. wt., 50,000; Kaken Yakukako Co. Ltd., Tokyo). For controls, 0.85 per cent saline was used. 0.01 ml. of Ehrlich tumour ascitic fluid containing  $5 \times 10^4$  cells was dropped on to the disk placed in a Petri dish. Then the disk, which contained both tumour cells and the solution, was implanted on the CAM of the chick, on the 6-7th day of its incubation. The embryos were killed on the 8th day after tumour implantation, and the solid tumour developed in and around the 'Spongel' disk on CAM was excised and weighed.

The effect of chondroitin sulphate on tumour growth on CAM is shown in Table 1. A statistically significant increase in weight of tumour tissue was noted in all the chondroitin sulphate-treated groups. The chondroitin sulphate absorbed in the 'Spongel' disk was demonstrated in the excised tumour mass as a metachromatic spot on the filter paper.

The second series of experiments were made with SM male mice about 100 days old, fed with standard pellet diet and water *ad libitum*. One ml. of 2 per cent chondroitin sulphate *O* solution was subcutaneously injected into the right flanks of the mice, immediately followed by inoculation of 0.1 ml. of the Ehrlich tumour ascitic fluid

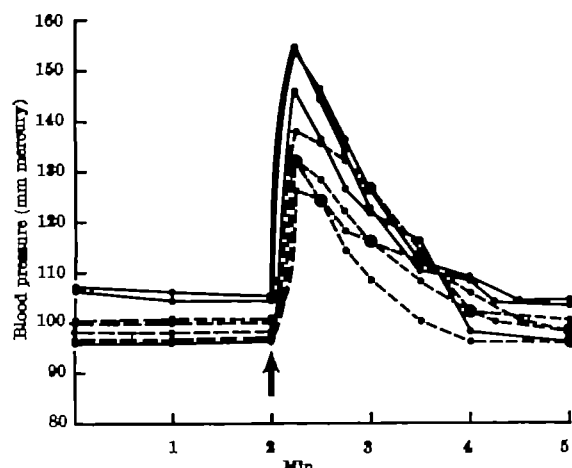


Fig. 1. Mean blood pressures of control and lathyrotic rats as a function of time. Arrow indicates time of intravenous injection of norepinephrine. —, Controls; ----, lathyrotic rats

Table 1. EFFECT OF CHONDROITIN SULPHATE ON GROWTH OF SOLID EHRlich ASCITES TUMOUR ON CAM OF CHICK EMBRYO

Exp. No.	1	2	3	4	5	6	7	Totals
No. tumour	10	7	8	10	6	10	6	57
Control								
No. tumour	9	7	8			6	4	34
2% Chondr. S.								
Tumour weight	112	127	130			123	123	123
Control % 100								
No. tumour				6	8	2	8	30
0.8% Chondr. S.								
Tumour weight				177	144	235	128	129
Control % 100								
No. tumour				4	8	4	9	31
0.08% Chondr. S.								
Tumour weight				111	153	146	154	127
Control % 100								

Average tumour weight  $\pm$  S.E. based on: (n)

Control	70.5 $\pm$ 3.8 mg	(57)	
2% Chondroitin sulphate	91.9 $\pm$ 4.1 mg	(34)	$P < 0.001$
0.8% Chondroitin sulphate	91.1 $\pm$ 4.0 mg	(30)	$P < 0.001$
0.08% Chondroitin sulphate	90.0 $\pm$ 3.7 mg	(31)	$0.001 < P < 0.006$

Table 2. EFFECT OF CHONDROITIN SULPHATE ON GROWTH OF SOLID HEMILICH ASCITES TUMOUR IN SAM-MALE MICE ON THE 8TH DAY AFTER TUMOUR INOCULATION

Exp. No.	Control	No. mice	Exper.	Tumour weight (Control = 100)
1	8	8	8	173
2	15	15	15	184
3	12	12	12	206
Totals	35	35	35	188
Average tumour weight $\pm$ S.E.	1,067 $\pm$ 73 mg	2,008 $\pm$ 120 mg		$P < 0.001$

containing  $5 \times 10^6$  cells into the same site. In the control group, 1 ml. of 0.85 per cent saline was injected before the tumour inoculation. The animals were killed on the 8th day after tumour inoculation, and solid tumours developed in the subcutaneous space of mice were excised and weighed. As shown in Table 2, the average tumour weight in the chondroitin sulphate-treated groups was  $2,008 \pm 120$  mg, whereas it was  $1,067 \pm 73$  mg in the control, and the difference is statistically significant.

In other experiments, the effects of heparin and collagen were tested on the OAM of chick embryos, but no significant promoting effect was observed. Experiments are now being made on the effect of human umbilical cord extract and hyaluronic acid.

Ozzello *et al.*<sup>3</sup> demonstrated the growth-promoting activity of acid-mucopolysaccharides on a strain of human mammary carcinoma cells *in vitro*, and I<sup>4</sup> have also reported that chondroitin sulphate solution enhanced the growth of subcutaneously inoculated Brown-Pearce rabbit carcinoma.

Rogers<sup>5</sup> reported "water retention, rate of diffusion, macroionic function, lubrication and others" as the physical functions of acid-mucopolysaccharides. It is my opinion that they may have some action which preserves the inoculated tumour cells, which may be due to the negative electric charge of acid-mucopolysaccharide, and its viscosity.

Vasiliev<sup>6</sup> showed that formation of new connective tissue around neoplasms may be important in many cases for invasive growth of malignant cells, and the present experiments, I believe, appear to indicate that the proliferation of new connective tissue which produces acid-mucopolysaccharides is favourable to the growth of cancer cells.

I thank Prof. H. Tauchi for his advice.

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### Effect of Aerobic Pre-Incubation on Glycolysis in Resting and Proliferating Liver Cells

A SHORT aerobic pre-incubation period before the onset of anaerobiosis greatly stimulates the rate of anaerobic glycolysis in slices of tissue. This fact, discovered by Rosenthal and Lesnitsky many years ago<sup>1</sup>, has later been confirmed<sup>2-4</sup>; but the mechanism of action of the previous incubation remains up to now largely unexplained.

In the course of work on the events connected with the glycolytic stimulation by previous aerobiosis, we have examined the occurrence of this effect in various tumours, as well as in diverse injured tissues; in this communication, work on Yoshida ascites hepatoma cells, non-tumour actively proliferating liver cells, and normal liver is described.

Glycolysis has been measured manometrically in a conventional Warburg apparatus at 37° C. The compo-

Table 1. Glycolysis of normal, regenerating, foetal rat liver slices and Yoshida ascites hepatoma cells; not pre-incubated (basal) or pre-incubated (stimulated) in oxygen.  $\mu$ l. CO<sub>2</sub>/mg dry weight/h (medium: NaCl,  $1.19 \times 10^{-4}$  M; KCl,  $4.7 \times 10^{-4}$  M; CaCl<sub>2</sub>,  $2.5 \times 10^{-4}$  M; KH<sub>2</sub>PO<sub>4</sub>,  $1.18 \times 10^{-4}$  M; MgSO<sub>4</sub>,  $1.18 \times 10^{-4}$  M; NaHCO<sub>3</sub>,  $2.5 \times 10^{-4}$  M; glucose,  $8 \times 10^{-4}$  M)

Normal rat liver			Regenerating rat liver		
Basal	Stimulated	Stimulation	Basal	Stimulated	Stimulation
(1) 3.6	9.6	+6.0	(1) 4.6	7.5	+2.9
(2) 4.8	9.6	+4.8	(2) 5.1	8.0	+2.9
(3) 5.2	9.0	+3.8	(3) 5.1	9.3	+4.2
(4) 5.6	9.8	+4.2	(4) 5.6	5.3	+1.8
(5) 6.6	10.7	+4.1	(5) 4.6	6.3	+1.8

Foetal rat liver			Hepatoma ascites cells		
Basal	Stimulated	Stimulation	Basal	Stimulated	Stimulation
(1) 15.9	16.6	+0.5	(1) 45.2	44.1	-1.1
(2) 17.3	16.7	-0.6	(2) 57.9	38.6	-19.3
(3) 13.7	15.1	+1.4	(3) 45.0	43.5	-1.5
(4) 14.7	15.6	+0.9	(4) 41.6	38.9	-2.7
(5) 17.6	17.3	-0.3	(5) 44.1	44.2	+0.1

sition of the medium is given in Table 1. For the sake of uniformity, glucose was added in all cases, since glycolysis in tumour cells is strictly dependent on the presence of glucose in the medium.

Pre-incubation was carried out in 95 per cent oxygen + 5 per cent carbon dioxide, for 15 min; the gas was then replaced with 95 per cent nitrogen + 5 per cent carbon dioxide and readings were begun 10 min after the end of aerobic pre-incubation. Glycolysis after pre-incubation is defined as 'stimulated glycolysis', while glycolysis without pre-incubation is called simply glycolysis or 'basal glycolysis'.

Yoshida ascites hepatoma cells were collected from albino rats 8 days after intraperitoneal transplantation. The ascitic fluid was diluted with cold saline and centrifuged for 5 min at 500 g in the cold; the cells were then washed twice in cold saline and finally suspended in the medium used for the experiments.

The amount of cells in each Warburg vessel gave a dry residue (corrected) of about 10 mg.

Livers were sliced by hand with a razor. Foetal livers were taken during the last four days before birth; the slices from several foetuses of the same litter were pooled together, and distributed at random in the vessels. Regenerating livers were taken from fed rats, 6 days after partial hepatectomy. Normal livers were from fed rats.

In normal livers from fed adult rats the pre-incubation in oxygen brings about a consistent increase in the rate of glycolysis. The stimulation, defined as the difference between stimulated and basal glycolysis, is rather variable, but occurs in all cases. The particular features of stimulated glycolysis in normal liver cells are now under investigation.

In regenerating rat liver slices, 6 days after hepatectomy, basal glycolysis is not particularly high, but stimulation is reduced, in partial agreement with previous results<sup>5</sup>.

On the contrary, foetal rat liver displays a high basal glycolysis; but this is in no way increased by aerobic pre-incubation. The same holds true for hepatoma cells, which show an even higher rate of basal glycolysis.

The comparison of the behaviour of the glycolytic stimulation in these various tissues rests on the assumption that they are basically composed of the same cellular types, living and growing in their own peculiar ways. However, slices of normal, regenerating and foetal livers can contain different amounts of connective tissue, while Yoshida ascites hepatoma is a population of pure cells. Besides, in foetal liver slices, cells involved in haemopoiesis are found side-by-side with parenchymal liver cells<sup>6</sup>. If, as is generally done, the tissues are considered sufficiently comparable, the problem of the failure of aerobic stimulation in foetal and neoplastic liver cells can be discussed.

It may be suggested that a higher glycolytic activity in liver cells is attained only under the influence of some factors formed in the normal tissue—but not in foetal or tumour cells—during aerobiosis. Alternatively, it could be said that aerobiosis removes some limiting factors which are present in normal tissue but not in foetal and tumour cells, the glycolysis of which is already fully 'released'.

In view of our present knowledge on the glycolysis of tumours, and on the basis of results which will be fully reported elsewhere, the second hypothesis seems more probable. The existence of mitochondrial factors which interfere with glycolysis in normal cells and which are lacking in cancer cells has been shown by Neifakh *et al.*<sup>7</sup>. Moreover, it should be noted that deficiency in the number of mitochondria seems to be a feature common to foetal and cancer cells.

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## IMMUNOLOGY

### Potentiating Effect of Neonatal Thymectomy on X-ray-Induced Intercapillary Glomerulosclerosis

X-IRRADIATION (150–900 rads) accelerates the course of intercapillary glomerulosclerosis (IGS), a naturally occurring progressive ageing process in mice, rats and hamsters<sup>1</sup>. This acceleration effect occurs only in kidney tissue which is directly irradiated and is preceded by a latent period, the duration of which is dependent on age of the animal at exposure.

There is some reason to think that an immune mechanism might be involved in the pathogenesis of IGS. This is suggested by the progressive nature of the renal lesions, by the presence of  $\gamma$ -globulin at the site of the glomerular lesions<sup>2</sup>, its similarity to changes observed in experimental immune disease of the kidney<sup>3</sup>, and the occurrence of a latent period before cytological changes are demonstrable. The results of neonatal thymectomy on the course of X-ray-induced IGS are reported in this communication.

Swiss-Webster mice were taken from their mothers within 24 h of birth, kept at room temperature for at least 30 min, and then given a single whole-body dose of 375 rads of 250-kV X-ray (42–45 rads/min). Groups of five animals, selected at random, were then returned to mothers chosen at random. In the case of animals to be thymectomized, the returned litter size was eight to allow for the large mortality following operation. Thymectomy was done within 24 h of irradiation by means of sternal exposure and suction. The wound was sutured and the animals placed under a heat lamp for 1 h before they were returned to foster mothers. Cannibalism was largely prevented by trimming the teeth of the mothers just prior to return of offspring. Animals were weaned at age 28 days, then housed in small aluminium pens with wood shavings, 1–4 animals per pen, and fed a commercial pellet ration.

All survivors at age 28 days were skin grafted. Tail-skin from a one-month-old (OSTBL/8By  $\times$  BALB/cBy)  $F_1$  female mouse was transplanted to the tail of the host as described by Bailey and Usama<sup>4</sup>, but modified by eliminating antibiotics and substituting several folds of masking tape for collodion and wound clips to hold the glass tube in position. Recorded observation of graft survival was begun on the fifth day after grafting and continued at two-day intervals.

Animals were killed by cervical dislocation. The histological methods for preparing the kidneys, involving the use of Tellysniak's fixative and the Rinehart-Abdul-Haj colloidal iron-cochineal stain<sup>5</sup>, and for grading the

Table 1. MEAN GRADE OF IGS AND SURVIVAL OF HOMOLOGOUS SKIN GRAFTS FOR ANIMALS KILLED AT AVERAGE OF 47 AND 67 DAYS OF AGE

Group	Age 47 days No. of mice	Mean grades	Age 67 days No. of mice	Mean grades	No. of grafts surviving over 13 days/total
I Sham X-ray	8	5.8 $\pm$ 0.6*	12	7.5 $\pm$ 0.6*	8/21
II Sham X-ray and sham operation	—	—	6	6.8 $\pm$ 1.1	0/6
III 375 rads	10	37.5 $\pm$ 2.1	12	45.8 $\pm$ 2.8	6/21
IV Complete thymectomy	5	5.6 $\pm$ 0.4	18	9.5 $\pm$ 0.8	17/22
V 375 rads and complete thymectomy	8	40.0 $\pm$ 4.1	14	63.9 $\pm$ 2.6	17/21

\* S.E.

degree of IGS were as described previously<sup>6</sup>. Lymphatic and thymic tissues were also stained with haematoxylin and eosin, pyronin-methyl green and Giemsa. All residual tissue in the thymic area of operated animals was examined microscopically; unsuccessfully thymectomized animals were discarded. The animals were killed in two series; the first, an exploratory one, examined at an average of 47 days of age and the second at an average of 67 days. Grades were established to represent the mean values of all animals in each group. For each kidney, the grades were derived by totalling values of 0–4 for each of 25 glomeruli based on histological criteria of degree of change.

In agreement with previous findings<sup>6</sup>, there was a decided effect of X-irradiation on the grade of IGS. The difference of mean IGS grades of irradiated and non-irradiated groups with or without thymectomy was highly significant ( $P < 0.005$ ). The difference between the X-irradiated group and the X-irradiated-thymectomized group was also statistically significant in both the first ( $P < 0.025$ ) and the second ( $P < 0.005$ ) series killed. Histological characteristics of the kidney lesions in these latter two groups were the same, differing only in degree. The difference between the non-irradiated-thymectomized group and either of the sham-control groups was not significant.

Examination of the spleen showed lymphoid depletion of the white pulp in all thymectomized animals. Irradiation alone or combined with thymectomy did not result in measurable effect on the degree of lymphoid depletion. Of significance was the presence of marked concentrations of pyroninophilic cells in the reticulum about the central arteries of the white pulp and their finer branches in all animals which were completely thymectomized at birth. Many of these showed characteristics of mature plasma cells. The lymph nodes also showed large numbers of pyroninophilic cells in the lymph cords and marked depletion of lymphocytes in the cortex.

The number of mice graft-tested in each group is given in Table 1. There were three technical failures. Thymectomy with or without X-irradiation significantly increased the number of surviving grafts ( $P < 0.001$ ) in  $\chi^2$  test. X-irradiation with or without thymectomy had no apparent effect.

From these observations it is clear that neonatal thymectomy potentiates the effect of X-rays on the development of IGS. The nature of the lesion was unchanged and there was no indication of a superimposed inflammatory process either in the irradiated or non-irradiated thymectomized animals. Wasting disease with associated renal disease, such as described by DeVries *et al.*<sup>7</sup>, had not developed in our strain of animals at the time of killing. One may speculate whether the thymus has some tissue regulatory function, a deficiency of which may lead to acceleration of degenerative changes, or whether IGS is a manifestation of an immune reaction. With respect to the former, no pertinent evidence appears to be available. With respect to the latter, there is indication that the immune mechanism is altered in our animals following neonatal thymectomy, as shown by increased tolerance to skin grafts in confirmation of results of other investigators<sup>8–10</sup>. There are some recent data<sup>11</sup> indicating that neonatal thymectomy may also evoke

auto-immunity. The presence of large aggregates of pyroninophilic cells in the spleen and lymph nodes in our neonatally thymectomized animals, and similar observations in rats by Wakeman *et al.*<sup>12</sup>, also suggest this possibility, although as yet we have no proof of immune competence of these cells. Whether auto-immunity plays a part in the pathogenesis of radiation-induced lesions of the kidney remains to be demonstrated by definitive immunological examinations. The present findings warrant further investigations of this possibility.

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### Antigenic Properties of Burnt Skin

ON surveying the literature pertaining to this subject, it was found that workers in the Eastern European countries<sup>1</sup> maintain that the use of convalescent serum in burnt patients is of considerable therapeutic value due to a hypothetical antibody to burnt skin<sup>2</sup>. Workers in the Western world<sup>3-7</sup> in the same field have been unable as yet to prove the existence of such an immunological mechanism and have claimed that any antibody demonstrated so far has been one produced by bacteria, thus not specific to burnt tissue.

Table 1. ANTIGEN-ANTIBODY REACTIONS WITH SERA OF RABBITS IMMUNIZED WITH VARIOUS PORTIONS OF BURNED SKIN, BY DIFFERENT IMMUNOLOGICAL SEROLOGICAL PROCEDURES

Samples of sera	Antigen*	Precipitin test†	BDB haemagglutination test‡	Tannic acid haemagglutination test‡	Inhibition test (BDB)	Passive cutaneous anaphylaxis (PCA)
A	1	0	0	0	Not done	0
B	1	0	0	0	Not done	0
C	2	1:2	1:1280	1:1280	Specific	+
D	Conc.	1:2	1:1280	1:1280	Not done	+
E	3	1:2	1:1280	1:1280	Not done	+
F	3	1:2	1:1280	1:1280	Specific	+
Controls N.R.S.	0	0	0	0	Specific	0

\* Antigens: No. 1, sterile skin; No. 2, skin exposed in oven to 120° C for 20 min; No. 3, skin exposed to burning over gas flame.  
† The highest dilution of immune sera which gave a visible precipitation.  
‡ The highest dilution of immune sera which gave haemagglutination sensitized with various antigens by tanned erythrocytes or erythrocytes linked with 4a-diazotized-benzidine (BDB).

A pilot experiment on this subject was conducted, using several immunological procedures. Three groups of rabbits were immunized with antigens, prepared from: (1) a portion of sterile skin from a freshly killed rabbit; (2) a portion of skin of the same rabbit after having been exposed to a temperature of 120° C in an oven for 20 min; (3) a portion of skin of the same rabbit, after having been exposed to burning over a gas flame. Six rabbits were immunized; A and B, with antigen 1; C and D, with antigen 2; E and F, with antigen 3.

The antigens were prepared by homogenizing the three portions of skin in a Waring blender, with subsequent extraction in normal saline. The antigens were defatted with ether during 24 h. Sterility was confirmed by culture.

The sera obtained from the rabbits after repeated immunization for a period of about 6 weeks were studied by several methods, such as haemagglutination and haemagglutination inhibition tests (BDB and tannic acid method), precipitin tests, gel-diffusion and passive cutaneous anaphylaxis in the guinea-pig. The results are summarized in Tables 1 and 2. These results show that burnt skin has antigenic properties, producing antibodies in rabbits, different from normal skin. It has to be pointed out that there are still considerable difficulties as to the reproducibility of the experiments, their cross-reactivity among the various portions of the skin, etc. Although antibodies in rabbits have been produced from burnt skin, the specificity of these antibodies, and their relationship to human burns, has to be investigated in greater detail.

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### HISTOLOGY

#### Venous Output of Lymphocytes from the Thymus

FOLLOWING the extensive quantitative investigations of Kindred<sup>1,2</sup> on the frequency of mitosis in different lymphatic organs, it has been known that intense lymphocytopoiesis occurs in the thymus. This observation has repeatedly been confirmed by other workers, using

Table 2. CROSS-REACTIONS OF IMMUNE SERA WITH VARIOUS SKIN ANTIGENS

Rabbit sera	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
Antigens*																		
Precipitin test	0	0	0	1	0	0	0	+	+	+	+	±	0	±	+	+	+	+
BDB haemagglutination test†	0	0	0	0	0	0	1:40	1:60	1:160	1:80	1:80	1:320	1:80	1:320	1:2560	1:1280	1:1280	1:1280
Tannic acid haemagglutination test‡	0	0	0	0	0	0	1:20	1:80	1:160	1:80	1:160	1:160	Not done	1:640	1:320	Not done		1:1280
Passive cutaneous anaphylaxis (PCA)	Questionable reaction (±)						±	±	+	+	+	+	0	0	±	±	±	±
Controls N.R.S.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

\* †, See Table 1



the same as well as other methods for demonstration of lymphocytopoiesis<sup>3</sup>.

The fate of these lymphocytes has been a matter of controversy. Kindred suggested that they leave the thymus, but he presented no direct proof of this assumption. Some indirect evidence has since accumulated, suggesting migration of lymphocytes from the thymus into the blood, and seeding of these thymic lymphocytes into other lymphatic organs<sup>4</sup>. Among this indirect evidence can be mentioned observations in sections of the thymus, suggesting diapedesis of lymphocytes into blood vessels<sup>5</sup>. The direction of such diapedesis cannot, however, be judged from sections and, in a recent review, Sainte-Marie and Leblond<sup>6</sup> pointed out "that no route has been demonstrated by which thymic lymphocytes might leave the organ". Gowans, Metcalf and others summarized the question in the same way<sup>7</sup>, that is that it has not been conclusively demonstrated that thymic lymphocytes do, in fact, migrate from the thymus. The lack of direct evidence proving that lymphocytes leave the thymus, is also consistent with the theory that the lymphocytes formed in this organ remain there, die, undergo cytotoxicity and perhaps release nucleic acid or some other non-cellular factors into the circulation.

Three routes for migration of lymphocytes from the thymus are possible—lymphatic channels, perivascular lymphatic spaces and blood vessels. It is generally claimed that the thymus contains few lymph vessels. The role of the perivascular lymphatic channels is not known, and it may be that they do not continue outside the organ. The most probable route is the blood vessels, and these have, in fact, most often been presumed to receive migrating thymic lymphocytes. No studies on the lymphocyte content of the efferent blood vessels from the thymus seem to exist in the literature.

Growing male guinea-pigs (200–300 g) were anaesthetized with sodium nembutal, i.p., and their thymus glands dissected out. Blood samples were taken from the carotid artery near the origin of the thymic arteries, and from the efferent thymic veins. In some animals, blood was also taken from the femoral veins. 25 mm<sup>3</sup> of blood was diluted with 475 mm<sup>3</sup> of Tolisson's solution (containing methyl violet, for staining the white blood cells). Blood smears were prepared simultaneously, and stained with Giemsa stain. The number of white cells per mm<sup>3</sup> was counted in a Bürker counting chamber—288 squares (corresponding to 0.00825 mm<sup>2</sup> each) being counted for each sample. The percentage of lymphocytes was calculated from the smears, 200 white cells being counted.

The number of lymphocytes per mm<sup>3</sup> and the percentage of lymphocytes were (mean  $\pm$  S.E.):

	% Lympho- cytes	No.	Lympho- cytes/mm <sup>3</sup>	No.
Afferent thymus blood (carotid artery)	60.7 $\pm$ 2.5	25	2,323 $\pm$ 233	25
Efferent thymus blood (thymic veins)	69.0 $\pm$ 2.2	25	3,496 $\pm$ 567	10

The difference between the percentage of lymphocytes in afferent and efferent thymic blood is significant at the 0.05 level ( $t = 2.55$ ). The difference between the absolute number of lymphocytes per mm<sup>3</sup> blood is not significant ( $t = 1.82$ ). The percentage of lymphocytes and the number of lymphocytes per mm<sup>3</sup> in the femoral vein blood were approximately the same as in the blood from the carotid artery. This implies that similar increases in percentage and number of lymphocytes were demonstrated in efferent thymic blood also when compared with femoral vein blood.

In view of differences between the animals with respect to white cell count and percentage of lymphocytes, less variation is obtained if the calculations are based on mean differences between the lymphocyte content of samples from the thymic veins and from the carotid artery or femoral vein in the same animal. Such calculations gave the following mean differences (abbreviations:

TV, efferent thymic blood (from thymic veins); CA, blood from the carotid artery; FV, blood from the femoral vein):

Differences in percentage of lymphocytes (mean $\pm$ S.E.):	
TV-CA:	8.36 $\pm$ 2.14, No. = 25, $P < 0.001$
TV-FV:	11.85 $\pm$ 3.14, No. = 18, $P < 0.001$
Differences in number of lymphocytes per mm <sup>3</sup> (mean $\pm$ S.E.):	
TV-CA:	1,025 $\pm$ 233, No. = 9, $P < 0.01$
TV-FV:	784 $\pm$ 204, No. = 8, $P < 0.05$

The results demonstrated an increased content of lymphocytes in the blood leaving the thymus.

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## RADIOBIOLOGY

### Effect of Cysteamine, Cyanide and High-pressure Oxygen on Splenic Oxygen Tension and Sulphydryl Content

The mechanism of action of cysteamine, one of the most potent compounds which protects against ionizing radiation, remains obscure.

Many of the less effective radioprotective compounds have now been reported to exert their action in the intact animal by causing tissue hypoxia. Thus adrenaline, histamine, chlorpromazine and dinitrophenol all lower tissue oxygen tension<sup>1,2</sup> and their effect is potentiated by lowering the oxygen concentration in the gas breathed by the animal, and inhibited by breathing oxygen at pressures above normal<sup>3,4</sup>.

Cysteamine, however, produces little or no change in tissue oxygenation in the spleen of animals breathing air<sup>5</sup> and the radioprotection shown by this compound is only slightly reduced when rats are irradiated during exposure to 5 atmospheres of oxygen<sup>6</sup>. The combination of CN<sup>-</sup> plus cysteamine pretreatment slightly reduces the radioprotection of cysteamine alone and the combined treatment with cyanide plus oxygen at 4–5 atmospheres further counteracts cysteamine protection against X-rays (van den Brank, unpublished observations). Similar effects are seen with 5-hydroxytryptamine and cysteamine<sup>4</sup>.

It was thought that CN<sup>-</sup>, by reducing oxygen consumption by the cells, may potentiate the action of high oxygen pressures in raising tissue oxygen tensions. This was tested using the oxygen electrode technique for the measurement of tissue pO<sub>2</sub> in rat spleen, the latter tissue being a radiosensitive locus. Canberra black male rats, 160–190 g weight, were used in all experiments. The estimation of splenic pO<sub>2</sub> with 60  $\mu$  bare-tipped gold electrodes has been described in detail<sup>7</sup> and the apparatus for pressurization was the same as that used in previous experiments<sup>8</sup>.

The results of splenic pO<sub>2</sub> measurements in control rats breathing air at 1 atmosphere or oxygen at 5 atmospheres are shown in Table 1 and compared with similar values obtained from rats pretreated with cysteamine 180 mg/kg 10 min before compression of the animal, and cysteamine plus CN<sup>-</sup> (2 mg/kg), the cyanide being given 2 min before cysteamine.

Table 1. SPLENIC  $pO_2$  (mm Hg  $\pm$  S.E.M.) IN RATS IN AIR AT 1 ATMOSPHERE OR IN OXYGEN AT 5 ATMOSPHERES ABSOLUTE

Treatment (No. trials)	Air	Maximum $pO_2$ at 5 atm.	$pO_2$ after 20 min at 5 atm.
Controls (85)	16 $\pm$ 2	344 $\pm$ 40	247 $\pm$ 37
Cysteamine (180 mg/kg) (39)	18 $\pm$ 2	251 $\pm$ 26 ( $P < 0.1$ )*	138 $\pm$ 26 ( $P < 0.05$ )*
Cysteamine (180 mg/kg) and cyanide (3 mg/kg) (23)	20 $\pm$ 2	246 $\pm$ 23 ( $P < 0.1$ )*	141 $\pm$ 37 ( $P < 0.1$ )*

\*  $P$  values compared with controls.

It can be seen that in rats breathing air cysteamine had no significant effects on splenic  $pO_2$ , as reported previously by van der Meer *et al.*<sup>1</sup>. Cysteamine plus  $CN^-$  also did not significantly affect normal splenic  $pO_2$ . At 5 atmospheres cysteamine did somewhat decrease splenic  $pO_2$  compared with control rats. This decrease was due in part to the increased number and severity of 'hump' responses after cysteamine: this type of response in spleen on compression of rats in oxygen has been described previously in detail<sup>2</sup>. The 'hump' response is characterized by an initial rise of tissue oxygen tension following compression, then a secondary fall in  $pO_2$ , although the external oxygen pressure is maintained constant. Hump responses were thought to be mediated through the adrenergic nervous system. Cysteamine has been shown to have an action in releasing catecholamines *per se*<sup>3</sup>, so it is possible that its potentiation of the 'hump' response is by this mechanism. In Fig. 1 an example of a marked 'hump' response to pressurization after cysteamine is shown.

Whatever the explanation for the form of the  $pO_2$  response in the spleen to pressurization, it is obvious that cysteamine has some action in lowering splenic  $pO_2$  during pressurization, but the splenic  $pO_2$  remains too high for the radioprotective effect of cysteamine to be explained as a result of hypoxia. The combined effect of cysteamine plus cyanide on rat splenic  $pO_2$  was almost exactly the same as cysteamine alone, both in air or 5 atmospheres of oxygen (Table 1). Thus cyanide plus high-pressure oxygen does not appear able to inhibit cysteamine radioprotection simply by altering tissue oxygen tensions.

It was thought that perhaps high-pressure oxygen reduced the number of active  $-SH$  groups through increased auto-oxidation, as occurs *in vitro*<sup>4</sup>. Thus total sulphhydryl and disulphide contents of the spleens of rats were measured, after exposure to 5 atmospheres of oxygen, and after combinations of high-pressure oxygen with cysteamine and cysteamine plus cyanide. The amperometric titration method with methylmercuric iodide was used as described by Jamieson, Ladner and van den Brenk<sup>11</sup> for lung tissue of rats, but in the present experiments 3 spleens were pooled for each determination.

It is seen from Table 2 that cysteamine raised the sulphhydryl level in the spleen ( $P < 0.001$ ), while the disulphide level remained constant. There is evidence that the spleen accumulated  $-SH$  groups, as the  $-SH$  content after cysteamine is higher than expected from equal distribution among tissues, and this result agrees well with the results of radioactive tracer experiment by Eldjarn and Nygaard<sup>12</sup>. Five atmospheres of oxygen had no effect on the endogenous  $-SH$  or  $-SS-$  content of the rat spleen. However, exposure to high-pressure oxygen did significantly reduce the increase of total splenic  $-SH$  groups produced by cysteamine (Group III v

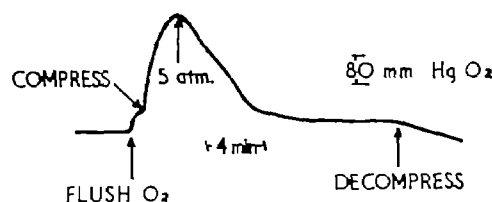


Fig. 1. Splenic  $pO_2$ . Cysteamine (180 mg/kg) was injected intraperitoneally 5 min before pressurization. This figure illustrates the most pronounced version of the 'hump' response with a rapid increase in tissue oxygen tension followed by a severe secondary fall even though the chamber pressure is maintained at 5 atm.

Table 2. SULPHYDRYL ( $-SH$ ) AND DISULPHIDE ( $-SS-$ ) CONTENT OF RAT SPLEEN AFTER HIGH-PRESSURE OXYGEN (5 ATMOSPHERES) TREATMENT OF THE WHOLE RAT AND AFTER INTRAPERITONEAL CYSTEAMINE (180 mg/kg), AND CYSTEAMINE AND CYANIDE (3 mg/kg)

Group	Treatment (No. trials)	Total $-SH$ content $\mu$ moles/g wet tissue $\pm$ S.E.M.	Total $-SS-$ content $\mu$ moles/g wet tissue $\pm$ S.E.M.
I	Controls (9)	19.0 $\pm$ 0.6	3.4 $\pm$ 0.6
II	Oxygen, 5 atmospheres for 15 min (8)	18.5 $\pm$ 0.6	3.4 $\pm$ 0.8
III	Cysteamine, 30 min before killing (8)	22.0 $\pm$ 0.3	3.7 $\pm$ 0.5
IV	Oxygen, 5 atmospheres and cysteamine (10)	21.3 $\pm$ 0.4	3.5 $\pm$ 0.3
V	Oxygen, 5 atmospheres and cysteamine + $CN^-$ (8)	21.7 $\pm$ 0.4	4.0 $\pm$ 0.6

Group IV,  $P < 0.01$ , in Table 2), suggesting that oxygen administered under pressure *in vivo* produces faster oxidation of  $-SH$  groups from cysteamine. The administration of  $CN^-$  to cysteamine-treated rats failed to alter the changes in splenic  $-SH$  or  $-SS-$  content produced by cysteamine.

Thus the slight reduction of cysteamine radioprotection by high-pressure oxygen may possibly be due to a reduction by the latter of total  $-SH$  groups present during irradiation. However,  $CN^-$ , which appeared to act synergistically with high-pressure oxygen in partially inhibiting the radioprotective action of cysteamine, does not appear to exert this action by raising tissue oxygen tension in spleen, or by altering the  $-SH$  level of this tissue.

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### Restoration of Irradiated Algae after a Period of Darkness

It has been found<sup>1</sup> that illumination applied between fractionated doses of X-rays slightly increases survival in the alga *Chlamydomonas*. Illumination during X-irradiation decreases survival in the same alga<sup>2</sup>. According to Godward<sup>3</sup>, visible light, if administered sufficiently soon after irradiation, has some restorative effect on X-irradiated algae.

In view of these findings, the effect of light and darkness on the survival of the unicellular desmid *Microsterias truncata* (Corda) Bréb. was investigated after  $\gamma$ -irradiation with a 350-c. cobalt-60 source. The algae were grown on mineral agar prepared according to Pringsheim<sup>4</sup>. For irradiation, young growing cultures were used. The cultures were illuminated with white fluorescent tubes for 15 h daily at 90  $\pm$  10 ft.-candles (two experiments were repeated at about 220 ft.-candles, but there was no perceptible difference in the results). Under these conditions the cells did not divide during the first part of the light period, that is, at the time when the irradiation was carried out. In order to follow the development of each cell after irradiation, irradiated cells were isolated with micropipettes and put on an agar surface in 10-cm Petri dishes (100–200 cells per dish).

A net consisting of 100 square fields drawn on a 'Cellophane' sheet was mounted outside on the bottom of the Petri dishes to facilitate counting.

Post-irradiation treatments consisted of: (a) incubation in 15 h light followed by 9 h darkness daily for 30 days; or (b) incubation in continuous darkness for various periods (from 1 to 4 days), after which the algae were exposed to the dark and light cycles as under (a).

Cells which grew into colonies with more than 10 cells in 30 days were designated as survivors.

The dose-response curve obtained after irradiation is a sigmoid, and in this respect corresponds to the curves obtained with other algae (*Oedogonium*<sup>1</sup>, *Chlamydomonas*<sup>2</sup>, *Stichococcus*<sup>3</sup>). This curve, plotted on a semi-logarithmic scale (Fig. 1), exhibits a marked shoulder up to doses of 4 kr., after which it descends approximately linearly. The straight portion of the curve (between 4 and 8 kr.) has a slope corresponding to a value of  $D_0 = 2$  kr. (dose required to reduce the surviving fraction  $S$  to 0.37  $S$ ). At doses higher than 8 kr. the curve is not reliable, as more cells should be used than permitted by our method. The extrapolation number<sup>4</sup> is approximately 5. Each point represents means of values of 300–600 cells which were examined in 3–5 experiments.

Post-irradiation incubation in darkness for 24–48 h had no significant effect on survival. An increase in the period of darkness following irradiation up to 3 and especially 4 days resulted in a visible increase in survival in the experiments with 5–11 kr. The survival of the irradiated algae increased after 3 days incubation in the dark following irradiation by a factor of 1.4–1.9, and after 4 days by a factor of 1.5–3.2 (for doses from 5 to 8 kr.). For lower doses the effect was less marked, as the surviving fraction was much higher. For this reason, with 4 days of darkness only doses from 5 to 9 kr. were used. The results are shown in Fig. 2.

Survival is not changed significantly and may even be improved if some light is admitted before the dark period (for example, if the irradiated algae are plated in light and afterwards put in darkness). It seems to be

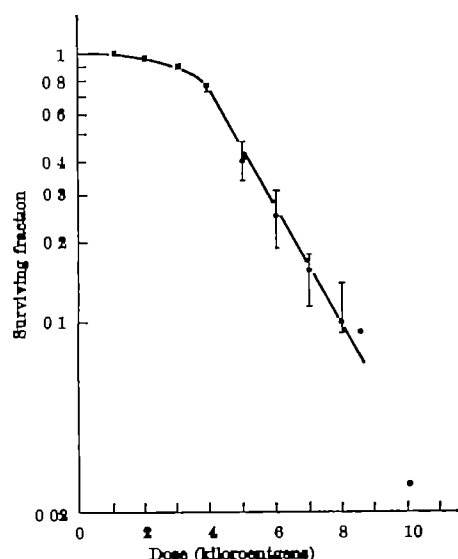


Fig. 1. Survival of *Microcystis aeruginosa* after  $\gamma$ -irradiation. Vertical lines represent  $\pm 1$  standard deviation.

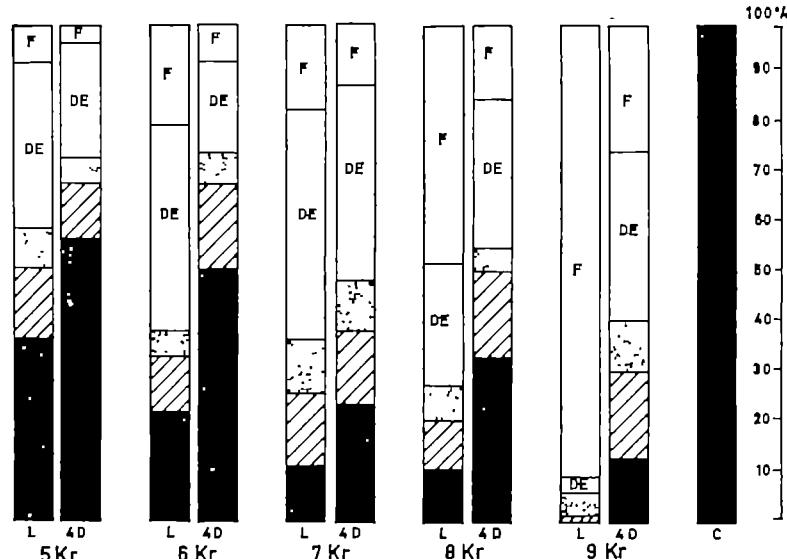


Fig. 2. Effect of light and darkness on the survival and cell divisions of irradiated algae *Microcystis aeruginosa*. Vertical columns represent the distribution of different colony-sizes of irradiated algae after 30 days incubation: L, incubation in light and dark cycles; 4D, 4 days incubation in the dark followed by 26 days in light and dark cycles; C, unirradiated control; DE, dead cells; F, cells that failed to divide; black, colonies of more than 10 cells; oblique lines, colonies with 2–9 cells; dotted, colonies with all cells dead after division except for one that survived. (The experiment with 8 kr. was carried out only once with 200 cells and, therefore, the discrepancy in the number of surviving cells does not seem to be of importance)

important that the algae should be placed in the dark before any cell duplication takes place.

No visible change in the cells could be microscopically detected before some development in the light takes place. During the period of darkness no growth or division occurs. It seems that the best results were obtained when the dark period exceeded the generation times of the control, which is  $77 \pm 4$  h.

Our results could not be compared with the findings of Jacobson on *Chlamydomonas*<sup>1</sup> as the duration of illumination period in his experiments was much shorter and was applied between fractionated doses. However, they agree with the fact shown by various authors (see, for example, refs. 8, 9) that different treatments which retard growth during the period immediately after irradiation increase survival.

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### Fixation of Carbon-11 in the Cells of Proton-irradiated Blood

WHEN considering the possibility of marking cell populations with a short-lived, positron-emitting nuclide, carbon-11 ( $^{11}\text{C}$ ,  $T_{1/2} = 20.5$  min) appears to be suitable because, when produced through nuclear reactions in proton-irradiation of cells, it may be expected to react chemically with the intracellular material in the same way as it does with simpler organic systems<sup>1</sup>. Therefore it was decided to study to what extent  $^{11}\text{C}$  produced during irradiation of citrate blood is fixed in the cellular

fraction of the target. This report describes observations of the localization of  $^{14}\text{C}$  in human blood irradiated with 185-MeV protons. The radioactivity of whole blood, blood corpuscles and supernatant was followed during the course of a step-wise washing procedure after irradiation.

Three blood specimens, A, B and C, each containing 1.5 ml. of freshly drawn venous blood and 0.5 ml. 'Acodex' (for blood preservation), were irradiated individually with 5,000 (A), 5,000 (B) and 11,000 (C) rads in a broad parallel beam of 185-MeV protons from the synchrocyclotron at Uppsala<sup>1</sup>. After irradiation for five minutes the radioactivity was followed through intermittent measurement of the positron annihilation radiation under fixed geometry in a NaI (Tl) well scintillation crystal counter (efficiency for detection of one  $^{14}\text{C}$  disintegration  $\approx 0.6$ ). Results are given in Fig. 1 for 'whole blood' = specimen A and for the 'blood corpuscles' of specimen B collected after ten-fold washing in Ringer-phosphate at pH 7.4 (each volume of washing fluid = each volume of removed supernatant = 2 ml.). Fig. 2 shows the radioactivity in the 'blood corpuscles' and supernatant for specimen C at various intervals during the washing procedure (washing conditions as above). In the latter case the blood corpuscles, after eleven washings in Ringer-phosphate, were stored in 'Acodex' for 1 h before washing was started again.

It can be concluded from the curves shown in Fig. 1 that the radioactivities measured 50 min after irradiation or later in specimens of whole blood and of thoroughly washed blood corpuscles, respectively, are in both cases to a dominating extent due to disintegration of  $^{14}\text{C}$ . Both curves reveal an effective half-life of 20 min, which is, within the statistical uncertainty, identical with that of  $^{14}\text{C}$ . The ratio between the specific radioactivity of the washed erythrocytes and that of the whole blood is seen to be 0.47 and thus within the normal range of the venous haematocrit of man. Fig. 2 reveals that the  $^{14}\text{C}$  of the blood corpuscles at the employed dose of 11,000 rads has been strongly attached to the cells, since, after about ten centrifugations, neither subsequent washing nor incubation in 'Acodex' is able to remove any appreciable amount of radioactivity (less than 1 per cent for each of the two procedures). In no specimen were signs of haemolysis noted visually.

We conclude from the present findings that  $^{14}\text{C}$  may be fixed in the body of the blood cells simply through irradiation of blood with high-energy protons and this to an extent that seems to be of practical importance for

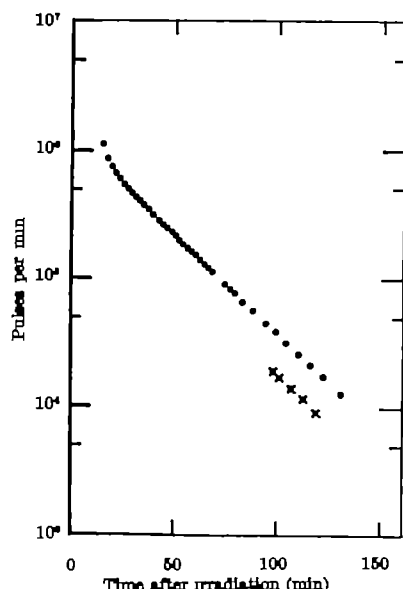


Fig. 1. Counting rate from whole blood (●) of specimen A and blood corpuscles (×) of specimen B. The blood samples were irradiated simultaneously and under identical conditions with 5 krad for 5 min.

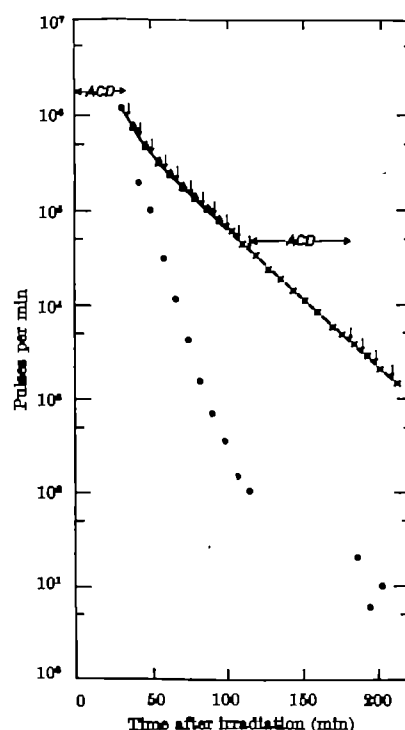


Fig. 2. Counting rate from blood corpuscles and supernatant in specimen C during the procedure of washing following irradiation with 11 krad for 5 min. During time intervals 'ACD' the blood was preserved in 'Acodex'. 'Washing' indicates suspension in Ringer-phosphate with subsequent centrifugation and decantation. ×, Corpuscles; ●, supernatant; Δ, corpuscles with plasma in supernatant; ↓, washing.

labelling purposes. The site of fixation and the possible influence of varying conditions during and after irradiation, including the effect of systemic circulation, will be tested in further experiments.

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## BIOLOGY

### Rhythmic Activity in Littoral Fish

TIDAL rhythms in invertebrates have been known for some time<sup>1-4</sup>. Recently, Williams<sup>5</sup> has described, by means of under-water observations, a tidal cycle of movement in *Olinocottus analis* (Girard) and *Girella nigricans* (Ayres). The tidally controlled movements of *Louresius tenuis*<sup>6-8</sup> and *Enchelyopus cimbrius* (L.)<sup>9</sup> are restricted entirely to breeding.

Preliminary investigations have been made on the activity patterns of several littoral fish using a modification of the apparatus designed by Harder and Hempel<sup>10</sup>. These have shown a very strongly marked tidal rhythm of activity in *Blennius pholis* (L.), peaks of activity occurring during the three to four hours around high water (Fig. 1).

This periodicity continues under conditions of constant light and temperature for at least five days, after which time the relative amplitude difference between active and inactive phases rapidly decreases. For the first 48 h the peak occurs approximately 1 h before high water, but

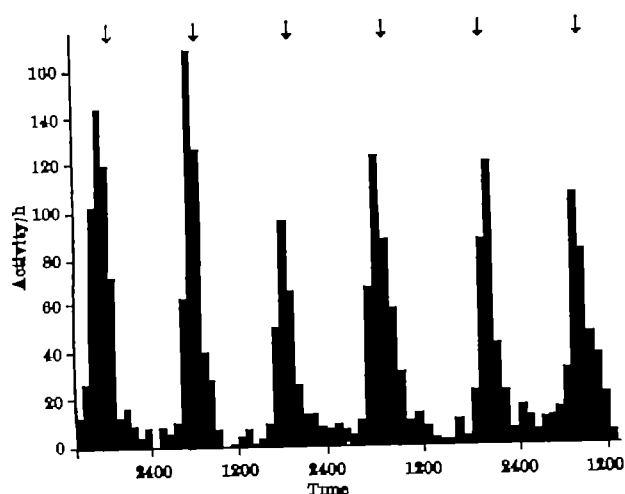


Fig. 1. Histogram illustrating the tidal rhythm in *Blennius pholis* (L.) over a period of 72 h. The vertical arrows represent the predicted time of high water.

gradually the period length tends to increase, so that the highest level of activity occurs nearer the predicted time of high water.

Table 1 gives the results of nine experiments performed in conditions of constant light (Nos. 5-10) and darkness (Nos. 16-19)

Table 1

Experiment No.	Mean period (h)	Phase difference Start (h)	Phase difference Finish (h)	Duration of experiments (h)
5	12.5	-1.1	-0.7	30.5
6	12.5	-2.3	0.0	44
8	12.4	-1.5	-0.7	74
9	12.6	-0.2	0.0	72
10	12.8	-0.7	+3.0	115
16	12.4	+0.2	+0.7	64
17	12.4	-1.6	-1.1	64
18	12.3	-0.4	-1.1	88
19	12.9	-1.5	+1.8	75

The mean periods for the experiments in darkness (12.56 h) and in light (12.5 h) are only slightly greater than the period of the lunar cycle.

There is no diurnal component evident in the rhythm, as in *Carcinus maenas* (L.)<sup>4</sup>, although in conditions of constant illumination the fish have a mean level of activity which is greater than that in constant darkness.

Records obtained in alternating periods of light and darkness showed that the fish respond directly to light by increased activity. This results in a different pattern of activity in light and in darkness. Peak activity in the light is sustained at a higher level for a longer period than in darkness.

Other fish studied were *Acanthocottus bubalis* (Euphrasen) and *Oiliata mustela* (L.). *A. bubalis* showed a short-lived (24 h) tidal cycle after which activity remained at a constant level. *O. mustela* when studied in the same apparatus appears to be arrhythmic in its activity.

The functional significance of the tidal rhythm in *Blennius pholis* is as yet unknown, although there are indications that it is connected with feeding migrations outside the tide pools in which it rests at low tide.

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## Micropores in the Cross-wall of *Geotrichum candidum*

Two types of septal pores have so far been conclusively demonstrated in fungi, the simple pore of the ascomycete type<sup>1,2</sup> and the more complicated pore found in the basidiomycetes<sup>3,4</sup>.

It was therefore most interesting to read of pores in the imperfect fungus *Geotrichum candidum* as shown by a shadowing technique. These did not correspond to either of the foregoing types of pore<sup>5</sup>. Hashimoto *et al.*<sup>6</sup> stated that they were not as yet able to demonstrate these pores by the conventional method of fixation, embedding and ultrathin sectioning, but we have now confirmed their results by this method. A 24-hour-old culture of *Geotrichum candidum* was fixed in 2 per cent KMnO<sub>4</sub> for 30 min, and embedded in epoxy resin<sup>7</sup>. After being cut, the sections were stained with lead hydroxide to increase contrast<sup>8</sup>. The material was viewed with a Siemens Elmiskop electron microscope operating at 80 kV.

Fig. 1 shows a cross-wall in section with two pores in median section and two other pores. These pores widen towards the cell interior, having a minimum diameter of approximately 300 Å and a maximum opening into the cell of about 600 Å. The cross-wall is composed of a thin electron-transparent central layer, between two thicker, more electron-dense, layers. The ectoplasm cannot be resolved clearly. The dense nature of the micropore indicates a dense cytoplasmic connexion between the cells. Fig. 2 shows that the micropores often occur in groups.

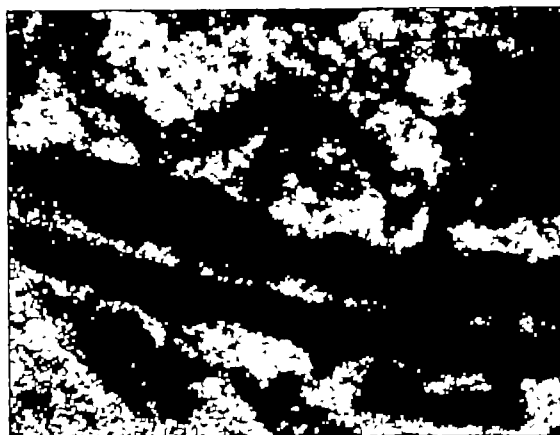


Fig. 1. Electron micrograph of part of the cross-wall of *Geotrichum candidum* showing four micropore connections between adjacent cells. (x 64,000)



Fig. 2. Electron micrograph of the same cross-wall in the next serial section. Some of the pores appearing in Fig. 1 are already out of the section in this figure. (x 64,000)

The term 'septal pore' proposed by Hashimoto *et al.*<sup>4</sup> is not accepted in this case since this term has been applied to the much larger pores of the ascomycete and basidiomycete type. The term 'septal micropore' is proposed for the pore type seen in *Geotrichum candidum*.

Hashimoto *et al.*<sup>4</sup> have likened the small pores observed to the pits in higher plant cell walls. This interpretation should not be accepted because the pit of a higher plant is an unthickened part of a secondary wall, and plasmodesmata in higher plants are the cytoplasmic connexions between adjacent cells. In the ascomycete and basidiomycete type of pore the intercellular connexion is not considered a plasmodesmata by virtue of the way in which they are formed<sup>5</sup>. The 'septal micropores' shown here, however, are of the nature of plasmodesmata.

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### Retarded Embryo Sac Development in Irradiated Clones of *Malus sylvestris* Hort. cv. 'Golden Delicious'

SEVEN 'Golden Delicious' apple clones propagated from buds irradiated at 5,000 r. in a cobalt-60 source were selected for high, intermediate, and low pollen abortion. Pollen abortion was 4.9 per cent in a typical check clone, and varied in the irradiated clones from 10.4 per cent to 84.8 per cent. Blossoms collected at anthesis were sectioned serially at 10 $\mu$  thickness and stained with Heidenhain's iron alum haematoxylin for embryo sac examinations.

Increased pollen abortion was associated with decreased proportions of differentiated (migrating polar nuclei) 8-nucleate embryo sacs. The check clone had 52.2 per cent of its embryo sacs at this stage; the treated clones with 52.9 per cent or less pollen abortion (group 1) had 36.2 per cent at this stage; the intermediate clone (group 2) with 68.0 per cent aborted pollen had 24.3 per cent of its embryo sacs at this stage; and the clones with 70.6 per cent or more pollen abortion (group 3) contained only 9.6 per cent differentiated 8-nucleate embryo sacs. There was a highly significant negative rank correlation ( $r_s = -0.952$ ) between the percentage pollen abortion and the percentage of differentiated 8-nucleate embryo sacs.

As pollen abortion increased, relatively more embryo sacs proceeded an 8-nucleate stage. The check clone had

14.6 per cent of its embryo sacs at the 2- or 4-nucleate stage; group 1 had 20.5 per cent; group 2 had 31.4 per cent; and group 3 had 36.4 per cent of its embryo sacs at this stage. Megaspore mother cells and megaspores were found only in clones with 70.6 per cent or more pollen abortion, except that one megaspore was found in clone 23-26. There was a highly significant positive correlation ( $r_s = 0.952$ ) between pollen abortion and the total percentage of megaspore mother cells, megaspores, and 2- or 4-nucleate embryo sacs. The average number of ovules per pistil was fairly uniform from clone to clone and showed no clear relationship to pollen abortion (Table 1). Irradiation did not materially reduce the proportion of normally developing embryo sacs, or influence the relatively infrequent occurrence of supernumerary or degenerate embryo sacs.

The results of investigations with this cultivar suggest that irradiation resulted in retardation of female gametophyte development. Furthermore, there was a close association between pollen abortion and retarded embryo sac development.

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### Effect of Light Intensity on the Metabolic Pathways in Photosynthesis

TRACER experiments indicate that different substances (carbohydrates, proteins, lipids) are synthesized in plants, even after a short exposure to <sup>14</sup>CO<sub>2</sub> (ref. 1). Therefore, although photosynthesis starts with a single primary reaction, a number of photosynthetic pathways lead to various photosynthetic products. We have investigated the effect of light intensity on these alternative pathways.

The investigations were made in a light thermostat<sup>2</sup>, using *Phaseolus vulgaris* L. (var. 'Sugar Bean') plants grown in sand culture. Low light intensities were applied because the effect of the light factor (near the minimum) is in this way more pronounced. Carbohydrates<sup>3</sup> and nitrogen<sup>4</sup> were estimated from three-week-old plants.

It was found that an increasing light intensity proportionally raises the relative carbohydrate contents of the plants (Fig. 1). This rise seemed to be steady in the starch and soluble carbohydrate fractions up to 2,000 lux; however, at 4,000 lux there was a dramatic increase in the starch fraction of the leaf and in the soluble carbohydrate fraction of the stem.

Table 1. CONDITION OF EMBRYO SACS IN CHECK AND IRRADIATED 'GOLDEN DELICIOUS' APPLE CLONES ARRANGED IN ORDER OF INCREASING POLLEN ABORTION

	'Golden Delicious'							
	Check	Irradiated						
Clone identification No	31-0	23-26	21-26	21-23 N	26-18 S	25-19 S	25-6 S	24-7 N
Abortive pollen (per cent)	4.9	10.4	45.3	52.9	69.0	70.6	76.8	84.8
Average number of ovules per pistil	14.4	13.3	13.9	13.3	14.0	11.9	13.0	16.7
Number of embryo sacs examined	178	101	131	105	70	112	61	160
Percent of embryo sacs in each category								
Megaspore mother-cell	—	—	—	—	—	6.3	8.2	1.9
Megaspore	—	1.0	—	—	—	7.1	—	6.2
2- and 4-nucleate embryo sacs	14.6	13.9	26.7	19.1	31.4	24.1	52.5	33.8
8-nucleate embryo sacs								
undifferentiated*	23.0	22.8	20.6	30.5	23.6	19.6	19.7	15.1
differentiated*	52.2	38.6	35.1	35.2	24.3	16.1	1.6	8.1
Multiple embryo sacs	2.2	6.9	4.6	5.7	—	2.7	3.3	—
Degenerate embryo sac	1.2	3.9	0.8	0.9	—	4.5	1.6	8.1
Embryo sac too irregular to classify	6.7	13.9	12.2	8.6	5.7	19.6	13.1	18.8

\* 8-nucleate sacs were classified as differentiated when the polar nuclei had begun to migrate.

The relative nitrogen contents of the plants decreased in proportion to increasing light intensities (Fig. 1). The decrease appeared in the soluble nitrogen fraction. The level of protein nitrogen remained constant at different light intensities.

The results indicate that at low light intensities photosynthesis results in a higher proportion of nitrogen compounds, while at higher light intensities the formation of carbohydrates is dominant. Referring to Katchman's investigations<sup>4</sup>, we interpret our findings to show that the metabolic pathways of the production of photosynthetic carbohydrate and nitrogen compounds are interlinked through pyruvic acid. In consequence of its lower energy content the low light intensities allow photosynthesis to follow pathways utilizing less energy. During photosynthesis a considerable amount of energy is utilized for the fixation of  $\text{CO}_2$  and for the reduction of phosphoglyceric acid. This second energy-requiring step may be missed when phosphoglyceric acid is transformed, without reduction, into alanine through pyruvic acid (Fig. 2). In this case amino-acids are formed rather than carbohydrates. The former way continues to build up proteins; this process, however, requires energy because of the synthesis of peptide bonds and because the activation of amino-acids requires ATP<sup>5,6</sup>. The lower level of soluble nitrogen and unchanged level of protein nitrogen, evoked by increasing light intensity, are in line with the foregoing statement.

During the carbohydrate-producing pathway of photosynthesis phosphoglyceric acid is reduced to the triose-phosphate level (Fig. 2). It is reasonable to suppose that this reduction may prevail over the other pathway of photosynthesis only at high light intensities<sup>8</sup>. In our experiments it was observed at 4,000 lux. This light intensity is nearest to those under which photosynthesis takes place under natural conditions, when primarily carbohydrates are produced, accumulated in the leaf, and transported to other parts of the plant. The sudden increase in the starch content of the leaf and in the soluble carbohydrates in the stem, measured at 4,000 lux, confirms these statements.

The energy necessary for these energy-consuming processes is available to the plant as ATP and NADPH. These energy carriers are produced during photophos-

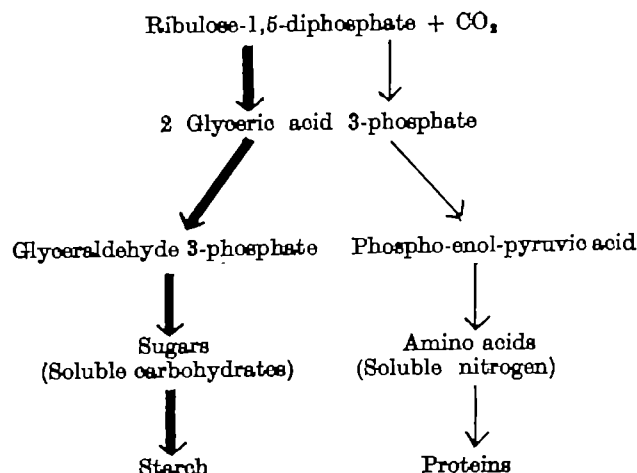


Fig. 2. Alternative metabolic pathways in photosynthesis. At low light intensities reactions marked — are more active, but at high light intensities reactions marked - - - are dominant

phorylation. Studying the dependence of photophosphorylation upon the light intensity, several authors<sup>9,11</sup> have established that it shows a lag phase below 1,000 lux and then a linear relationship up to 10,000–20,000 lux. These statements are in line with our findings about the prevalence of energy requiring carbohydrate synthesis above 2,000 lux.

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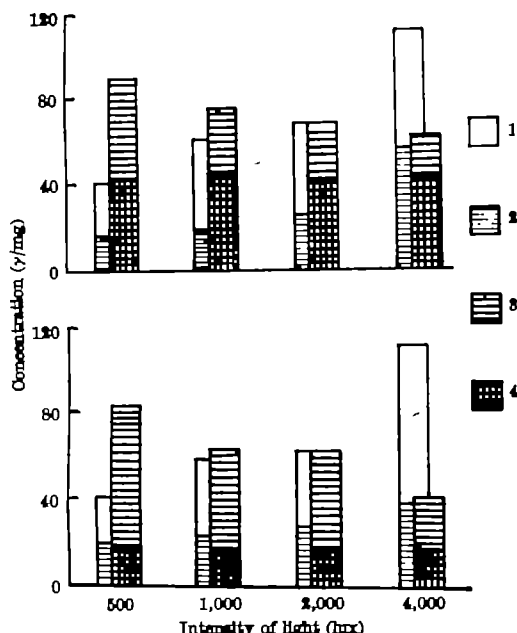


Fig. 1. Effect of light intensity on carbohydrate and nitrogen production in photosynthesis. Above are data from the leaf; below, data from the stem. 1, Soluble carbohydrates; 2, starch; 3, soluble nitrogen; 4, protein nitrogen

## Chromatic Adaptation in Benthic Marine Algae

In rocky coastal waters of the North Atlantic the well-defined zonation with depth of green, brown and red algae is still frequently ascribed to changes in the quantity and quality of light reaching the different habitats. There is little direct evidence that this 'chromatic adaptation' in fact occurs, and reports (for example ref. 1) from other parts of the world indicate that this zonation may not be universal.

To determine whether light is a primary factor in controlling the standing population of attached marine algae, a site is needed where as few other factors as possible change with depth. It seemed desirable to select a locality where the currents and the amplitude of the tide are small and the water is clear, and to investigate a site with a vertical rock face. These requirements are met on the south coast of Malta where there are rock faces falling sheer to sixty metres below sea level. During the summer of 1963 an expedition was organized to study the vertical distribution of the algal species. Aqua-lung diving equipment was used to sample the algal population, and a fixed vertical line, suitably marked, indicated the depth.



To ascertain what should be the area of a sample, samples of varying size were collected and the number of species in each was recorded. When this number was plotted against area it was found that 400 cm<sup>2</sup> approximated to the minimal area of the community. The method of collection was to remove the algae by means of a chisel, and collect them in a hessian bag attached to the frame of the sampling quadrat. Four collections were made at each selected depth and were later sorted into species, dried at 80° C and weighed. In addition, heats of combustion of the dried samples were determined using a Galenkamp 'O.B.370' bomb calorimeter. Light measurements were made with a Weston 'Universal' exposure meter (S461-4): a comparison of the data with those of Jerlov<sup>2</sup> indicated that the transparency of the water was between his types 1 and 2.

Of 40 algal species present, the following formed more than 95 per cent by weight of the standing population:

Chlorophyceae—*Udotea petiolata* (Trev.) Boerg., *Halimeda tuna* (Ellis and Sol.) Lam. Phaeophyceae—*Diclyotoma dichotoma* (Huds.) Lam., *Padina pavonia* (L.) Gail., *Cystoseira* spp., *Sargassum vulgare* Ag. Rhodophyceae—*Polysiphonia subulifera* (Ag.) Harv.

From Fig. 1 it is at once apparent that there is no clear-cut zonation of green, brown and red algae with depth. Near the surface the stand consists very largely of Phaeophyceae, whereas at 45–60 m the proportion is very small. The proportion of Chlorophyceae becomes significant at 15 m, and the trend is for the proportion to increase with

depth. Although a few Rhodophyceae were observed at depths of 15 and 60 m, appreciable amounts were confined to 30–45 m. In Fig. 2 the total weight collected per quadrat is plotted against depth, together with the changes in light intensity and temperature.

Two facts emerge quite clearly: first, that the pigmentation of the dominant algae seems to be unrelated to their distribution with depth; secondly, that the standing population as measured by dry weight (and by calorific value) bears no simple relationship to light quantity, temperature or depth, down to 60 m.

Although the work of Haxo and Blinks<sup>3</sup> on the enhancement of photosynthesis in red algae at certain light wave-lengths has been taken as evidence for chromatic adaptation, the link between photosynthetic efficiency and standing population is tenuous. The standing population represents a balance between complexes of synthesis and attrition, and Blinks<sup>4</sup> quotes data which show that the net yield of a variety of algal species bears little relation to the mass of the standing population. Perhaps more important is the inability of some species to establish themselves in a particular habitat<sup>5,6</sup>. It is possible that a more efficient growth in the sporeling stage accounts for the increase in the number of red algal species with depth<sup>7</sup> despite the dominance of the biomass by the other algal species, such as the green algae *Halimeda* and *Udotea*, which seem to propagate mainly by vegetative means.

It is concluded that the theory of chromatic adaptation is inapplicable to the results of the present investigation. Field observations on the causation of zonation in the marine environment can only be adequately interpreted in the light of long-term studies on establishment, environment, competition and productivity.

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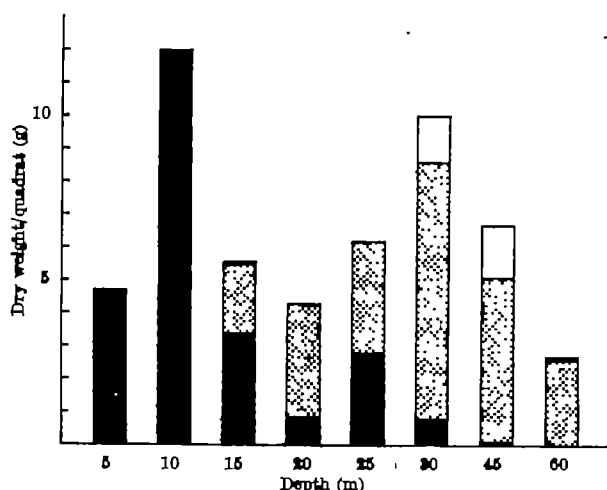


Fig. 1. The composition, in terms of pigmentation, of the benthic marine algae down to a depth of 60 m. Stippling, Chlorophyceae; black, Phaeophyceae; white, Rhodophyceae.

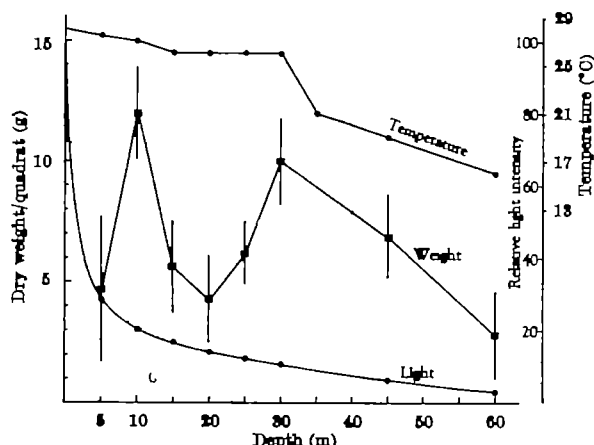


Fig. 2. Variation with depth of temperature, light intensity and dry weight of benthic algae. The standard deviations for the weight measurements are indicated.

## ENTOMOLOGY

### Photoperiodic Control of the Maintenance and Termination of Larval Diapause in *Chironomus tentans*

DURING recent years, dipterous larvae possessing giant polytene chromosomes have provided important new insight into developmental physiology at the sub-cellular level. This is in large measure attributable to the phenomenon of chromosomal 'puffing', which has afforded the opportunity for visualizing the changing pattern of genetic activity within individual tissues during the course of development<sup>1</sup>. Clever<sup>2</sup> has reported significant alterations in the puffing pattern of salivary gland chromosomes from a laboratory population of *Chironomus tentans* which experienced a state of spontaneously arrested development under conditions seemingly favourable for continued growth and metamorphosis. Although not previously described for this organism, the developmental arrest resembles the diapause which typifies the life-histories of many insect species. Diapause is commonly induced or terminated by extrinsic environmental factors, particularly temperature and photoperiod, which exert their effect by

influencing the activity of the endocrine system which regulates insect growth and metamorphosis<sup>6</sup>. In view of the paucity of information on these matters in the case of *C. tentans*, coupled with the general utility of diapausing insects and the special cytological attractions of this species for investigations of biochemical mechanisms underlying growth and differentiation, we have examined the natural occurrence of a larval diapause and its photoperiodic control under laboratory conditions.

The insects used in the work recorded here were collected among mud and debris from the bottom of a shallow woodland pond in Ann Arbor, Michigan. They were transferred to the laboratory and housed at 18°–22° C in covered dishes of pond water contained within closed incubator cabinets. Small pieces of filter paper were added to aid the larvae in constructing their dwelling-tubes; and food was provided regularly in the form of a powder prepared from dried nettle leaves<sup>7</sup>. Illumination was supplied in each cabinet by a 20-W 'cool-white' fluorescent lamp located at a distance of 35–45 cm, and set to provide either 8 h of light alternating with 16 h of darkness (henceforth termed short-day conditions), or 16 h of light alternating with 8 h of darkness (long-day conditions).

Two groups of larvae were examined. The first group included 50 larvae of apparently mature size collected on October 14. These were maintained under short-day conditions. During the next 40 days none exhibited any substantial growth or showed any sign of commencing the sequence of developmental changes which commences with formation of the prepupa and which, at 20° C, culminates about one week later with the emergence of the fully formed adult insect. A total of 12 larvae were then transferred to long-day conditions. In the next 9 days, 3 prepupae and 2 pupae had already formed from among the insects exposed to long-day conditions, while none of those under short-day conditions initiated development. A total of 9 larvae died under short-day conditions, while 2 of those under long-day conditions died, all without initiating development. The experiment was terminated at this point to provide material for other investigations.

The second group of larvae also consisted of 50 individuals of mature size, collected on December 16 from beneath the pond's ice cover of 10 cm. Under short-day conditions during the next 36 days, the majority failed to exhibit any developmental progress and remained as larvae. A total of 20 larvae were then transferred to long-day conditions. Of these, 13 initiated development shortly thereafter, yielding 6 prepupae, 5 pupae, and 2 adults in a period of 2 weeks; while 2 larvae died and 5 remained alive without initiating development. This prompt resumption of development in the majority of individuals transferred to long-day conditions contrasts sharply with the behaviour of the larvae maintained under short-day conditions, among which only 5 individuals sporadically initiated development from the time of their collection until termination of the experiment. Under short-day conditions, 17 larvae died during the 7 weeks' duration of the experiment.

On the basis of the foregoing findings, it is likely that the arrest of development in *C. tentans* is not simply a quiescence enforced directly by temperatures too low to permit development, since the majority of larvae did not develop when maintained at temperatures suitable for rapid growth and metamorphosis. In terms of the criteria summarized by Lees<sup>8</sup>, a genuine diapause appears to be involved. Short-day conditions favour the maintenance of diapause, while its termination is favoured by exposure of larvae to long-day conditions. To the extent that our population of *C. tentans* is representative, the larval diapause reported here resembles that of the pitcher plant midge, *Metricnemus knabi*, and the other Diptera summarized by Paris and Jenner<sup>4</sup>, also the larval diapause of the European corn borer, *Ostrinia nubilalis*<sup>9</sup>, as well as the pupal diapause of the Chinese oak silkworm, *Antheraea pernyi*<sup>10</sup> to mention but a few examples.

The fact that larvae of *C. tentans* and related species do not continue to develop under natural conditions in autumn and winter has previously been reported<sup>7</sup>; but little attention has been directed to the underlying physiological basis. Based on his findings during the mass rearing of *C. tentans* 30 years ago, Sadler<sup>7</sup> suggested that the arrest of development was a direct effect of low temperature, because metamorphosis was observed when the temperature was maintained above the prevailing outdoor level. However, these conclusions now seem open to re-examination since the effect of photoperiod was not considered at the time. Indeed, Prof. Sadler has informed us in a personal communication that his cultures were in fact routinely exposed to daylight supplemented by at least several hours of artificial light. The net result appears to have been illumination almost equivalent to the long-day conditions used in the work recorded here. While the critical day-length has not yet been determined, our findings appear to be reconcilable with those of Sadler as soon as photoperiod is taken into account.

Although the induction of diapause by appropriate photoperiodic stimuli is known to occur in many insects<sup>8,9</sup>, reports on the photoperiodic control of its maintenance and termination have been relatively scarce<sup>1</sup>. Together with those of the recent investigations already cited<sup>4–6</sup>, the findings reported here provide increasing evidence that photoperiod is of widespread significance in regulating the maintenance and termination of diapause as well as its induction. The prompt response of *C. tentans* in initiating development when exposed to long-day conditions, coupled with its rather stable diapause when exposed to a short day-length, suggests that this species may provide favourable material for examining the physiological processes that intervene between imposition of a particular photoperiod and its developmental consequences. Of particular interest in this connexion is the endocrine basis of the diapause, the means whereby the photoperiodic stimulus is integrated in a manner capable of controlling the endocrine system, and the cytological manifestations in the endocrine and target organs. Experiments are now in progress to elucidate these and other matters which remain unanswered by present results. Meanwhile, in view of the characteristic differences in the pattern of chromosomal puffing in tissues of non-developing as compared with developing larvae of *C. tentans*<sup>8</sup>, the results of this work suggest that control of photoperiod is desirable during investigations of chromosomal function in this insect, and on the action of the insect's endocrine system thereupon.

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## MICROBIOLOGY

Non-conservation of  $H^3$ -Thymidine Label in the DNA of Growing Yeast Cells

WHILE investigating the kinetics of transfer of DNA from the parent to the progeny in yeast cells with the help of tritium autoradiography, we found that there occurs considerable degradation of the labelled DNA at the onset of the stationary phase of growth. The relevant experiments are described and the underlying possibilities are discussed.

A small inoculum of *S. cerevisiae* was grown overnight in 1.5 ml. of a nutrient medium containing 1 per cent peptone and 4 per cent maltose in distilled water (pH 5.0) supplemented with 3.3  $\mu$ Ci/ml. of  $H^3$ -thymidine (specific activity: 360 mCi/mM, Schwarz BioResearch, Inc.). The cells were then centrifuged down, washed thoroughly and resuspended in 10 ml. of 'cold' medium without  $H^3$ -thymidine. At specified intervals, haemocytometer counts were taken and smears were prepared on gelatinized glass slides. The cells were fixed for 3 h in the following fixative: 1 per cent chromic acid, 25 ml.; 2 per cent acetic acid, 5 ml.; 2 per cent mercuric chloride to make 100 ml. Fixed cells were then treated with cold 2 per cent perchloric acid (PCA) to remove all unincorporated nucleotides.

After thorough washing and dehydration, the slides were covered with autoradiographic stripping film (Kodak 'AR 10') in the usual manner, air-dried and exposed in an atmosphere of dry  $CO_2$  at 4° C for 16 days. Exposed slides were then developed in Kodak developer 'D19b' for 10 min and fixed in Kodak acid fixer—all at 20° C. After washing, the slides were air-dried and briefly stained with toluidine blue (0.05 per cent w/v in distilled water).

Autoradiographic grain counts were done at a magnification of 1,250 using an oil-immersion objective. A representative autoradiograph is shown in Fig. 1. The background varied between 0.1 and 0.8 grains/25 $\mu^2$ , which is the average area of a yeast cell. To check that the grains observed were due to  $H^3$ -thymidine incorporated into the DNA, a few slides containing labelled cells were treated, prior to autoradiography, with 100  $\gamma$ /ml. of crystalline DNase I (Worthington Biochemicals Corporation) in 0.005 M  $Mg^{++}$ , pH 6.8, at 37° C; 15- and 30-min treatments removed 50 per cent and 70 per cent of the grains, respectively.

The total number of grains that would have been produced by the labelled cells contained in 1 ml. of culture (column 4, Table 1) is proportional to the amount of labelled DNA/ml. and should therefore remain constant unless dispersion and/or degradation occurs during the growth of the cells. It will be seen from Fig. 2 that between 2.5 h and 4 h of growth, that is, while the percentage of budding cells in the population is constant and high at ~80 per cent, the amount of the labelled DNA/ml. of culture remains constant. During this period, the cell number also increases exponentially (column 2, Table 1). Thus during the exponential phase of growth there is no degradation of DNA, which is in accord with the generally accepted view that nuclear DNA is metabolically stable.

However, after 5 h, when the rate of formation of buds begins to slow down, that is, when the cells enter the early stationary phase, the total number of grains or the amount of labelled DNA/ml. falls sharply to about half



Fig. 1. Autoradiograph of yeast cells showing grains due to  $H^3$ -thymidine incorporated into DNA. Fully labelled cells were allowed to grow in 'cold' medium for 4 h before fixation. ( $\times 1,200$ )

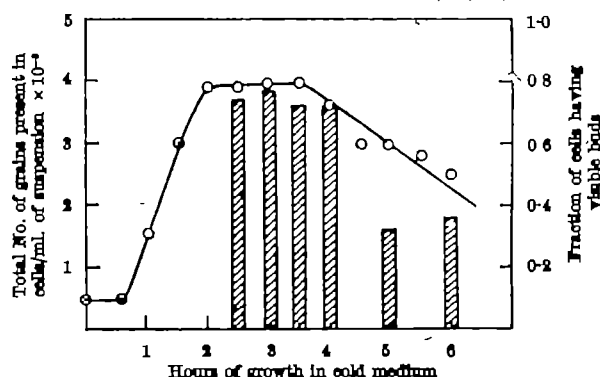


Fig. 2. Plot of the total number of grains that would have been produced by the  $H^3$ -thymidine labelled yeast cells per ml. of culture (hatched bars) at different periods of growth in 'cold' medium. The solid curve shows the fraction of viable buds in the cell population.

its value in the exponential phase. The sharpness of the fall in the amount of the label, as well as its coincidence with the onset of the stationary phase, suggests that it is not due to some dilution process arising out of asymmetric distribution of the labelled DNA during cell growth and that an actual release of incorporated  $H^3$ -thymidine must have taken place.

Schmidt *et al.*<sup>1</sup> have shown that starving yeast cells release nucleotides in a cold TCA-soluble fraction, thereby showing the instability of nucleic acid under these conditions. Halvorsen<sup>2,3</sup> claimed that intracellular turnover of proteins and nucleic acids is characteristic of resting yeast cells, while it is almost absent during the period of active growth. Using  $^{14}C$ -adenine as nucleic acid precursor, he also showed<sup>2</sup> that when the cells are incubated under conditions favouring protein degradation, the radioactive label is released into the TCA-soluble fraction and to a lesser extent into the medium. However, chromatographic analyses by other workers<sup>4,5</sup> of materials released into the suspending medium did not reveal the presence of DNA or its degradation products such as thymidylic acid, deoxyribose, etc.

In the light of the above findings, it is plausible to assume from the present data that DNA begins to be degraded at the onset of the stationary phase, then is released into the cytoplasm and finally removed by the treatment with PCA prior to autoradiography. In this connexion it will be interesting to ascertain the genesis of a small single-stranded polydeoxyribonucleotide which has recently been found associated with the crystalline L(+) lactic dehydrogenase (YLDH) cytochrome  $b_5$  of dry baker's yeast<sup>6</sup>.

Another possibility is that there is no actual degradation of DNA during any stage of growth, but it is rendered more susceptible to cold PCA treatment as the cells enter the stationary phase due to some unknown alteration in the structure of DNA or of the DNA-protein complex.

We thank Prof. N. N. Das Gupta for his advice. One of us (S. K. S.) is supported by a senior research fellow-

Table 1. GRAIN COUNT DATA

(1) Time of growth in cold medium (h)	(2) No. yeast cells/ml. of culture ( $\times 10^{-7}$ )	(3) Average No. grains/cell ( $\pm S.D.$ )	(4) Total No. grains/ ml of medium ( $\times 10^{-7}$ ) ((2) $\times$ (3))
2.5	1.74	21.33 $\pm$ 0.46	36.94 $\pm$ 0.80
3.0	2.00	19.18 $\pm$ 0.44	38.36 $\pm$ 0.88
3.5	2.01	17.76 $\pm$ 0.42	35.70 $\pm$ 0.84
4.0	2.85	12.60 $\pm$ 0.36	35.81 $\pm$ 1.00
5.0	4.74	3.87 $\pm$ 0.18	18.37 $\pm$ 1.00
6.0	5.76	3.43 $\pm$ 0.18	19.64 $\pm$ 1.00

ship of the Council of Scientific and Industrial Research, India. We thank Dr. J. Chakraborty for suggesting the fixative, and Mr. J. C. Mondal for assistance.

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### Plaque-formation on the Chorioallantoic Membrane, by Isologous Spleen Cells, under the Influence of Homologous Bursa of Fabricius Cells

THE thymus and the bursa of Fabricius are both essential for the development of immunological competence in birds. The bursa of Fabricius seems to be responsible for the development of the cells producing serum antibodies<sup>1</sup> and delayed hypersensitivity reactions<sup>2</sup>, whereas the thymus is more particularly involved in homograft reactions<sup>3,4</sup>. The fact that the thymus shows evidence of at least partial activity when implanted in a 'Millipore' chamber impermeable to cells<sup>5</sup> suggests that its influence is humoral.

If the bursa is instrumental in inducing cells to become immunologically competent it could extend this function either (a) by creating conditions which enable inactive cells to start making antibody, according to their predetermined capacity, or (b) by inducing responsive cells to produce antibody in answer to any antigenic stimulus.

To test this, we used the phenomenon of production of plaques (nodules) on the chorioallantoic membrane (CAM) of developing chick embryo by inoculation of a suspension of blood or spleen cells<sup>6,7</sup>. When the recipient embryo and the donor of the blood or spleen cells belong to different strains, the intensity of the plaque reaction is determined by the genetic differences between the strains<sup>8</sup>.

We found that with highly inbred strains which are comparable with pure lines of mice, very few plaques appear, whereas with crossbred strains the embryos show plaques in great numbers. Numerous plaques appeared at 95-98 per cent of the embryos when cells of a foreign strain such as Australorp - (A), White-Rock or New-Hampshire were inoculated on the CAM of Leghorn Schatz Israel (LSI) embryos. In contrast to that when LSI embryos were inoculated with cells of an adult hen of the same inbred strain, only 2 per cent of the embryos reacted, with the appearance of occasional plaques<sup>9,10</sup>.

The experiments that we report here consist of the inoculation of a combination of isologous spleen cells of adult (8-8 month) hens ( $10^4$  cells per inoculation) with the same quantity of homologous bursa cells. This combination caused the appearance of plaques. Bursa and spleen cells alone were ineffective. The ability of the bursa cells to induce plaque formation was dependent on the age of the bursa donor.

Inoculations on the CAM were performed on the 9th day of incubation and the results were examined after another 5 days. Of 112 LSI embryos inoculated with bursa cells ( $2 \times 10^4$  cells per inoculation) of A-chicken (5-16 weeks) none showed the appearance of plaques. Only 5 out of 103 LSI embryos inoculated with LSI spleen cells ( $2 \times 10^4$  cells per inoculation) showed plaques. In 66-84.6 per cent of the embryos inoculated with the combination of spleen and bursa cells of this age-group, plaques appeared in significantly great numbers in contrast to inoculations with spleen and bursa cells alone. When the bursa donor

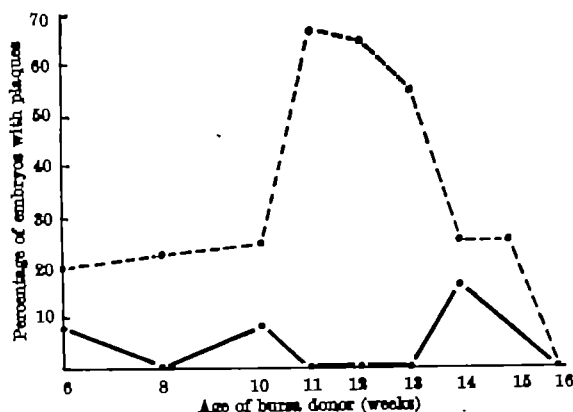


Fig. 1. Effect of inoculation with bursa cells and spleen cells. Bursa cells alone caused no formation of plaques. —, Spleen cells alone; —, bursa cells + spleen cells.

was younger or older, the number of positive results diminished, as seen in Table 1 and Fig. 1. Maximum reactivity of the bursa cells was observed at the age of 11-13 weeks.

Table 1. APPEARANCE OF PLAQUES ON THE CAM OF LSI EMBRYOS INOCULATED ON THE 9TH DAY, 5 DAYS AFTER INOCULATION WITH SPLEEN CELLS OF AN ADULT LSI, AND BURSA CELLS OF STRAIN A

Age of bursa donor (weeks)	A bursa cells alone	LSI spleen cells alone	A bursa cells + LSI spleen cells
6	0/9	1/11	2/10
8	0/10	0/10	3/13
10	0/16	1/13	2/8
11	0/12	0/8	6/9
12	0/14	0/8	9/14
13	0/12	0/12	6/11
14	0/12	2/12	3/12
15	0/12	1/11	3/12
16	0/14	0/17	0/14
		%	%
		8	20
		0	23
		8	25
		0	66
		0	64
		0	54.6
		16	25
		8	25
		0	0

Number of cells inoculated was  $2 \times 10^4$  per inoculation; numerator = the number of embryos with plaques; denominator = total number of embryos inoculated; % = percentage of positive results obtained.

These experiments confirm the fact that isologous spleen cells are unable to react in the CAM of their own strain. The small number of plaques in a low percentage of embryos could be explained through some residual antigenic heterogeneity of the 'pure' strain, possibly because of sex differences, although we tried to use only hens and no cocks as donors.

The results of the experiments show that isogenic cells cannot react against their own antigens present in the embryo; in the terminology of Burnet<sup>11</sup>, these spleen cells are tolerant to isologous cells, but are induced to recognize the same antigen as foreign when under the influence of homologous (allogenic) bursa cells.

**Addendum.** Attempts to reproduce the results with other strain combinations were not fully successful. The strain Leghorn Schatz has now lost its purity through cross-breeding.

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## VETERINARY SCIENCE

## Tolerance and Excretion of Mimosine in the Fowl

Hegarty, Schinokel and Court<sup>1</sup> have reported the use of isolated mimosine ( $\beta$ -[N-(3-hydroxypyridone-4)]- $\alpha$ -aminopropionic acid) in metabolism investigations with sheep. They reported that the major metabolite 3,4-dihydroxypyridine (DHP) was present in the urine, and that degradation of mimosine to DHP occurred in the rumen, the animal being unable to detoxicate the material after absorption. No comparable work on the fowl has been located in the literature.

As a continuation of toxicity investigations of *Leucaena leucocephala* in the fowl, an experiment was designed to determine the route of excretion and possible degradation of mimosine. Since urine and faeces are excreted through a common opening (cloaca) in the fowl, it was necessary to effect a separation of the excreta by surgical means. This was accomplished through a modification of the method of Ariyoshi and Morimoto<sup>2</sup> on a 6-month-old cross-bred cockerel.

Mimosine was isolated from the seed of *L. leucocephala* and purified according to the method of Hegarty, Schinokel and Court<sup>1</sup>. The sample of mimosine used was analysed for purity and its breakdown product, according to the method of Hegarty, Court and Thorne<sup>3</sup>. The purity was found to be 98 per cent mimosine. DHP was not present in the sample.

After the bird had sufficiently recovered from the operation, 1 g of mimosine was administered *per os* in gelatine capsules, and the urine and faeces were collected in polythene bags supported by a harness around the bird's neck. Urine and faecal samples were collected hourly over a 24-h period and analysed for mimosine and DHP. No mimosine or DHP was found in any of the faecal samples. This is contrary to the results obtained by Yoshida<sup>4</sup>, who reported that 32-42 per cent of ingested mimosine was excreted in the faeces of rats fed 0.89 per cent of mimosine in the diet for 3 weeks.

Of the mimosine excreted in the urine, approximately 85 per cent appeared in the first 5 h following administration and thereafter only minor amounts or traces appeared. No trace of any DHP was found in any of the urine samples. Of the 1 g of mimosine administered, a total of 42 mg, or 4.2 per cent, was accounted for in the urine over the entire 24-h collection period. Since there was never more than 0.2 mg excreted during any 1 h after the first 10 h of collection, it would appear that most of the ingested mimosine was rapidly metabolized by the fowl.

It is difficult to compare these results with those of Yoshida<sup>4</sup>, since her rats received mimosine as a part of their diet for 2 or 3 weeks. However, the 1 g of mimosine that the fowl received would have been approximately equal to the amount that it would have consumed in a 24-h period if the mimosine had been added to the diet at the rate of 0.89 per cent. On this basis it would appear that the fowl is better able to detoxify and metabolize mimosine than is the rat: 96 per cent as compared with 15-28 per cent<sup>4</sup>.

In order further to test the apparent tolerance of the fowl for mimosine, a 2-month-old White Leghorn cockerel was injected intraperitoneally with a sterile 1.5 per cent solution of mimosine, prepared as a sodium salt<sup>5</sup>. The bird was injected with 5.8 ml. of mimosine solution a day for a total of 9 days, then with 11.6 ml. a day for the next 6 days.

The bird suffered no apparent untoward ill-effects from the mimosine injections. It remained bright throughout the period, and continued to thrive and to gain weight. There was no apparent increase in the number of feathers shed, nor was there shedding of the toenails.

The cockerel was able to withstand the administration of almost 2 g of mimosine in 15 days, that is, at the rate

of 2.7 g/kg body-weight. Hegarty, Schinokel and Court<sup>1</sup> administered a similar solution to sheep for 8 days, at the rate of 0.68 g/kg, which resulted in shedding of the fleece and hooves, and finally death. It can therefore be assumed that the bird is far more tolerant to injections of mimosine than the sheep.

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## GENETICS

## Autoradiographic Studies of the Chromosomes in Chronic Granulocytic Leukaemia

THE work to be described here was carried out with the particular aim of investigating the labelling pattern of the  $Ph^1$  chromosome in chronic granulocytic leukaemia<sup>1,2</sup>. Blood and bone marrow samples were obtained from one male patient (A. C.) and blood from two female patients (J. S. and V. C.).

**Bone marrow.** Preliminary experiments with bone marrow indicated that direct *in vitro* labelling with tritiated thymidine was not possible. 6 h after aspiration, which was the minimum time necessary to obtain labelled mitoses, the mitotic index was very low. Bone marrow cultures were therefore set up and collected at 24, 48 and 72 h respectively. Tritiated thymidine (specific activity 14.8 c./mmole) was added to a final concentration of 1  $\mu$ Ci/10 ml. of medium at 6 h and colcemid, 0.04 ml./10 ml. of medium, 2 h before collecting.

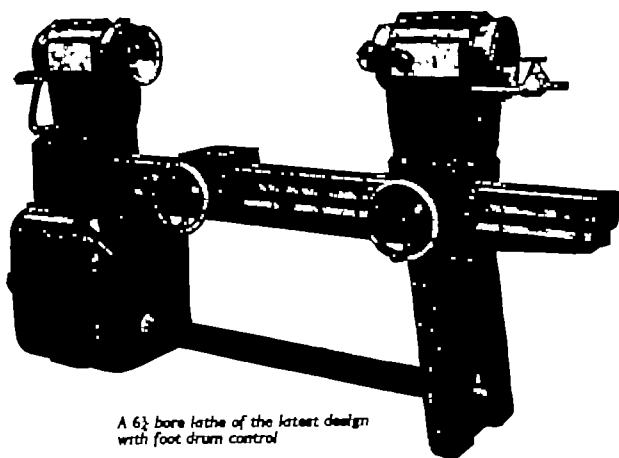
Chromosome preparations were made by the air-drying method, stained with aceto-orcein and photographed. These were coated with Kodak 'AR-10' stripping film, exposed for 4-6 days, processed and re-stained with Giemsa.

Only  $Ph^1$  + ve diploid and polyploid cells were found in the bone marrow, the number of  $Ph^1$  chromosomes corresponding to the ploidy of the cell. The mitotic index rose slightly from 24 to 72 h in culture. In cultures incubated with tritiated thymidine for 3 h there were no labelled mitoses. After 6 h incubation 60 per cent of mitoses were labelled. In both diploid and polyploid cells incorporating tritiated thymidine at the end of S, the Y or Y's were always heavily labelled in comparison to the 4 G group chromosomes, that is ( $Ph^1$  + 3, 21/22 chromosomes). It was impossible to count the grains over this heavily labelled Y while that over any G group chromosome never exceeded 10 grains. The  $Ph^1$  chromosome appeared to be a member of the later labelling G group pair. In tetraploid cells there was some evidence from grain counts of an asynchrony in synthesis between the 2 diploid complements and between the 2 tetraploid complements in 1 octoploid cell.

**Peripheral blood.** Leucocyte cultures were set up by a modification of the technique of Moorhead *et al.*<sup>3</sup>, with and without phytohaemagglutinin. Preliminary experiments indicated that when a single dose of tritiated thymidine was given, both the number of labelled mitoses and the uptake by the chromosomes were low after a 6-h exposure period. This may be due to the fact that chronic granulocytic blood causes a rapid degradation of tritiated thymidine<sup>4</sup>. A total dose of 2  $\mu$ Ci/10 ml. culture was therefore administered in 0.5  $\mu$ Ci aliquots over a period of 1 h. 6 h before collecting, colcemid being added 2 h before. By this method both the number of labelled mitoses and the thymidine uptake were augmented.

The peripheral blood cells incorporating tritiated thymidine at the end of S were classified according to

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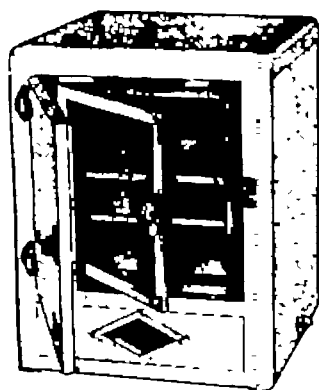
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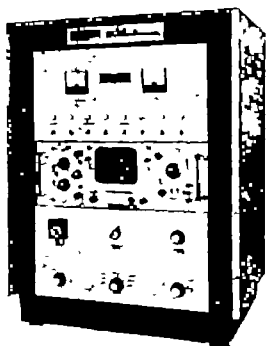
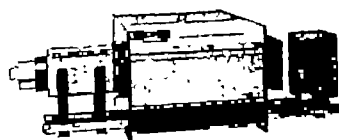
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the labelling pattern of their *G* group chromosomes. Only those cells could be used in which some, rather than all or none, of the *G* group were labelled. Thus, of a total of 60 cells analysed, 34 provided information.

In one cell of (A. C.) the  $Ph^1$  alone of the *G* chromosomes was labelled (that is, indicating the  $Ph^1$  to be a member of the later synthesizing *G* pair), while in two cells 2 intact *G* chromosomes only were labelled (that is, indicating the  $Ph^1$  to be a member of the early synthesizing *G* pair).

In (J. S.) the  $Ph^1$  was a member of the later pair in 8 cells, of the earlier in 4 cells. In (V. C.) the  $Ph^1$  was a member of the later pair in 7 cells, of the earlier in 12 cells. Thus the  $Ph^1$  did not appear to belong constantly to either the earlier or the later synthesizing pair in any patient. In (V. C.) cultures grown with PHA produced a mixed population of  $Ph^1 + ve$  and  $Ph^1 - ve$  cells. The latter, presumably lymphocytes, were more heavily labelled than the  $Ph^1 + ve$  cells with fewer unlabelled chromosomes and the *G* group separated into 2 heavily labelled and 2 lightly or unlabelled chromosomes.

**$Ph^1$  chromosomes.** In order to compare the pattern of synthesis of the  $Ph^1$  chromosome with that of its intact homologue, it is necessary to be able to sort the other 3 *G* group chromosomes into a pair and an odd one on purely morphological criteria. Gilbert *et al.*<sup>1</sup> do this on the principle that chromosome 21 has the larger size and is satellited. In none of the 3 cases presented here was there considered to be a sufficient, morphological, difference between the three intact *G* chromosomes to warrant the selection of a pair among them. Therefore a statistical comparison of the grain count over the  $Ph^1$  chromosome and its homologue was impossible. Furthermore, at the end of *S* period, the grain count over any *G* chromosome is very low. Thus the method of classifying cells by how many of their *G* chromosomes were labelled was adopted. It was assumed that in cells with a late, heavily labelled, sex chromosome those chromosomes that were unlabelled had completed their DNA synthesis before the addition of tritiated thymidine.

The possible significance of very late *S*-period labelling patterns has been pointed out by German<sup>2</sup>.

The results presented here may be interpreted variously. The  $Ph^1$  chromosome could not be consistently classified in any patient as a homologue of either the earlier or the later synthesizing pair of the *G* group. Thus, if it is accepted that in normal cells autosomal pairs synthesize synchronously, either this synchrony has been upset by the chromosomal deletion, and the  $Ph^1$  varies as to what moment it completes its DNA synthesis, or the  $Ph^1$  may not always belong to the same pair. Such an interpretation cannot but be tentative. The possibility of asynchrony in synthesis between the homologues of a normal pair of chromosomes at the end of *S* period cannot be excluded. However, it would seem important to question the general assumption that the  $Ph^1$  chromosome is always derived from the so-called pair 21.

**Length of *G*<sub>2</sub> period.** It was found that an incubation period of 6 h with tritiated thymidine was necessary in all cases to obtain sufficient numbers of mitoses labelled at the end of *S* period. This indicates a longer minimum *G*<sub>2</sub> period for  $Ph^1 + ve$  cells than the 3.5–4.5 h found for normal lymphocytes in this laboratory. Further evidence of this was provided by the mixed population of  $Ph^1 + ve$  and  $Ph^1 - ve$  cells found in (V. C.);  $Ph^1 - ve$  cells appeared to have been labelled earlier in *S* period than the  $Ph^1 + ve$  cells in the same culture. Peripheral blood cultures of (A. C.) (producing only  $Ph^1 + ve$  cells) incubated with tritiated thymidine for 4, 6, 8 h had respectively 3, 60 and 90 per cent labelled mitoses. However, even in cultures collected 12 h after addition of tritiated thymidine, cells late in *S* were found, indicating a very variable length of *G*<sub>2</sub> period.

**Survival of  $Ph^1$ .** Attempts to discover how many times a  $Ph^1 + ve$  cell will divide *in vitro*<sup>3</sup> were unsuccessful.  $Ph^1 + ve$  cells were, however, found in unlabelled

peripheral blood and bone marrow cultures of (A. C.) after 5 days.

The mitotic index of (A. C.)'s bone marrow was highest at 72 h. In peripheral blood cultures from all 3 patients, grown with or without PHA, 72 h was again the time of highest mitotic index of  $Ph^1 + ve$  cells. Thus it seems that the  $Ph^1 + ve$  cell does persist *in vitro* and that, contrary to Tough *et al.*<sup>4</sup>, the mitotic index for this sort of cell is in some cases higher after 72 than after 24 or 48 h in culture.

**Late-labelling X.** Direct cytological evidence for random inactivation of the maternal or paternal X chromosome<sup>5</sup> was obtained from (V. C.). The late-labelling X was either a small, metacentric, size (10–12) chromosome, or a longer, sub-metacentric, size (6–8) chromosome. Of 20  $Ph^1 + ve$  cells with a late-labelling X chromosome, 9 cells could be assigned to the former group and 11 cells to the latter.

These preliminary investigations indicate that: (1) The  $Ph^1$  chromosome does not appear to terminate synthesis consistently as either an early or a late synthesizing *G* chromosome. Possible explanations are that the deletion has affected its pattern of synthesis or that the  $Ph^1$  might vary as to whether it is a chromosome 21 or 22. (2) A minimum time of 6 h incubation with tritiated thymidine was necessary to obtain labelled  $Ph^1 + ve$  cells in both blood and bone marrow cultures. The *G*<sub>2</sub> period of a normal, control, lymphocyte culture is by comparison 3.5–4.5 h. (3) The  $Ph^1 + ve$  cell can persist for up to 5 days in cultures of peripheral blood and bone marrow. In all the 3 cases studied, the highest  $Ph^1 + ve$  cell mitotic index was found at 72 h. (4) Two distinct, morphological, forms of late-labelling chromosome were found in the  $Ph^1 + ve$  cells of one patient. It is suggested that this was due to random inactivation of maternal or paternal X chromosome.

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## Low-alkaloid Mutants of *Lupinus digitatus* Forsk

THE Western Australian blue lupin, which has been variously referred to in the literature as *Lupinus pilosus* L.<sup>1,2</sup>, *L. varius* L.<sup>3</sup> and *L. digitatus* Forsk<sup>4</sup>, occurs as a naturalized exotic in west coastal areas of south-western Australia where the annual rainfall exceeds about 16 in. It grows prolifically on coastal sandy soils overlying limestone, and is also cultivated to some extent in adjacent inland areas on slightly acid sands for soil improvement and sheep feed.

Because of its known adaptation to sandy soils of low fertility, its high seed-yield and the high (30–35 per cent) protein content of its seeds, *L. digitatus* would appear to hold possibilities of development as a grain legume for cropping purposes. However, this has been precluded by a number of wild characteristics of the species, including late maturity, hard seededness, shedding of the seeds as soon as they are ripe, and the presence of alkaloids in the seeds and other parts of the plants. The alkaloids, besides being somewhat toxic, render the seeds unpalatable to all classes of stock except possibly sheep.

Naturally occurring low-alkaloid ('sweet') mutants, which are fully palatable and apparently non-toxic<sup>8</sup>, have been selected in *L. angustifolius* L. and *L. luteus* L. by von Sengbusch<sup>8</sup>, who found five mutant plants among a field population of some 1.5 million. Subsequent searches yielded similar mutants in several other lupin species. There appeared to be no reason, therefore, why homologous mutants could not be isolated from field populations of *L. digitatus*.

A search in 1961 among some 220,000 plants, using Dragendorff's reagent paper for spot testing, failed to disclose any low-alkaloid mutants. It was then decided to try artificial mutation methods. Dry scarified seeds of an early-maturing strain of *L. digitatus*, itself an X-ray-induced mutant<sup>9</sup>, were soaked in 0.3–0.4 per cent ethyl methanesulphonate (EMS) at 20° C for 12 h, and sown in the field in May 1962. The progenies of surviving plants were grown the following year and tested for alkaloid content.

Out of 440 progenies, 11 contained mutants which, on the basis of Dragendorff reaction and taste, could be classified as sweet. These have been confirmed in the *M*<sub>1</sub> generation, although strictly quantitative estimates of alkaloid content have yet to be made. Of the 11 types, 7 appear to be associated with reduced vigour or fertility disturbances or both. However, 4 appear, from *M*<sub>1</sub> and *M*<sub>2</sub> observations, to be normally vigorous, and to have normal or nearly normal fertility. Genetic studies of all the more promising mutants are in progress, together with further selection within lines for vigour and fertility.

The success of artificial mutagenesis in producing low-alkaloid mutants in *L. digitatus*, as in producing low-coumarin mutants in *Melilotus albus*<sup>8</sup>, further attests the usefulness of this method in practical breeding. We would like to direct particular attention to its place in the domestication of wild or semi-wild species. In these species there is seldom much natural genetic variation available to the breeder, except as a result of extensive plant exploration. At the same time the kinds of characteristics he seeks, such as reduced seed shedding or freedom from toxic factors, are precisely those which would be selected against in the natural environment. On the other hand, they are usually due to genetically recessive 'loss' factors, and as such should be fairly readily induced artificially by mutagenic agents.

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## VIROLOGY

### Transport of S-Antigen of Sendai Virus as revealed by means of Antibody labelled with an Organic Mercury Compound

It has been shown that replication of RNA<sup>1</sup> and synthesis of S-antigen of Sendai virus occurs in nucleolar structures while synthesis of V-antigen occurs in the perinuclear zone of cytoplasm<sup>2</sup>. In later stages of infection both components, ribonucleoprotein (RNA + S-antigen) and haemagglutinin (V-antigen), accumulate in the peripheral zone of cytoplasm where mature virions are formed. A modification of the immunofluorescence

method<sup>3</sup> has revealed the details of synthesis of protein components of Sendai virus, but failed to reveal the details of transport of S-antigen through the territory of nucleus and cytoplasm because it was insufficiently sensitive. Therefore a more sensitive method of detection of S-antigen of Sendai virus was used, namely, antibody labelled with an organic mercury compound, para-aminophenylmercurylacetate (pAPMA)<sup>4</sup>.

Normal non-infected cells treated with virus-specific pAPMA labelled antibody showed only a feeble non-specific contrast due to osmium fixation (Fig. 1), being a good background for a sharp specific contrast due to pAPMA labelled antibody bound with viral S-antigen.

S-antigen appeared in nucleoli 3 h after infection of the cells with the virus and accumulated in considerable amount by 6 h. Optically dense masses appeared as granules, threads and less-rectangular formations, the latter depending on the plane of the ultra-thin section. However, their diameter did not exceed 200 Å, which corresponds to the diameter of the helix of nucleocapsid of Sendai virus<sup>5</sup>. By 9–12 h after the beginning of infection nucleoli became empty and masses of the antigen were revealed in nucleus, in nuclear membrane and in cytoplasm (Fig. 2). It is characteristic that no antigen was inside the mitochondria and cytoplasmic vacuoles; this shows that transport of the S-antigen from nucleoli to cytoplasm occurs through the cytoplasmic reticulum. Release of mature virions from the cell can be demonstrated by means of application of whole pAPMA labelled serum (Fig. 3) which contains both S- and V-antibody.

Thus the use of antibody labelled with pAPMA provides the means not only of establishing topography of synthesis of protein components of Sendai virus but also of tracing

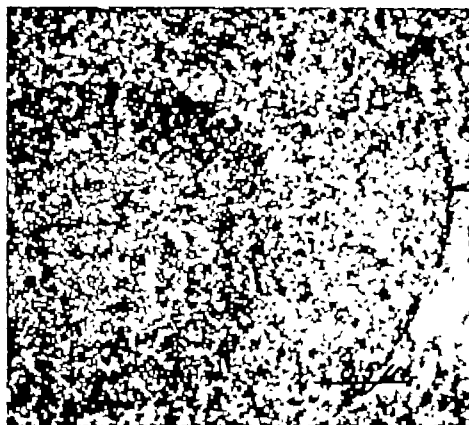


Fig. 1. Non-infected monkey kidney cells treated with Sendai virus S-antibody labelled with pAPMA. A feeble contrast of cellular structures due to osmium fixation ( $\times 18,000$ ).

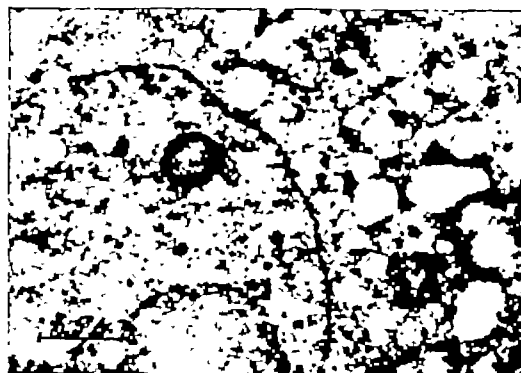


Fig. 2. The same cells 9 h after infection. Amorphous areas of an empty nucleolus, masses of S-antigen in nucleus, in nuclear membrane and in cytoplasm ( $\times 18,000$ ).

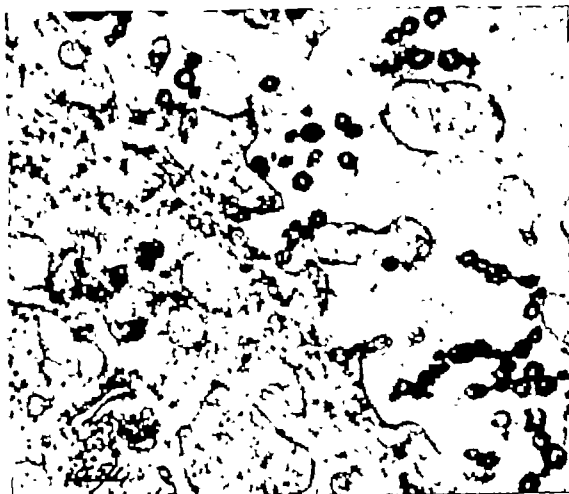


Fig. 3. The same cells 12 h after infection. Mature virions contrasted with the PAPMA labelled whole serum which contains both S- and V-antibody ( $\times 23,000$ )

transport of them from the place of synthesis to place of assembly of mature virions.

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### Antibiotic Carry-over in Cell Cultures of Herpes Simplex Virus

Schneerson and Shore<sup>1</sup> reported that herpes simplex virus grown in cell culture was capable of inhibiting the growth of certain strains of bacteria. In a preliminary investigation in this laboratory, attempts to confirm the findings of Schneerson and Shore were successful. This investigation was then expanded to include testing the virus suspensions collected from different cell lines against a wide range of Gram-positive and Gram-negative organisms representing 15 species. In the course of the assays it was noted that the activity of the virus suspension against test organisms produced a sensitivity pattern similar to that of streptomycin or penicillin. It was noted, however, that this antibacterial activity of the virus culture was lost following serial passage through cells cultivated in antibiotic-free medium. In an attempt to explain the loss of antibacterial activity, the hypothesis was formulated that the antibacterial activity might result from antibiotics carried over from the original inoculum. The purpose of this report is to present the results arising from the testing of this hypothesis.

Two strains of herpes simplex virus were kindly supplied by Dr. Malcolm S. Artenstein, Department of Virus Diseases, Walter Reed Army Institute of Research. Strain Hawkins *EWVK*<sub>1</sub>, isolated in rabbit kidney cell culture from a patient's eye, was in its first cell culture passage. It was ascertained that the medium for cell culture routinely contained antibiotics. Strain Taylor was aseptically obtained from a herpetic lip lesion and suspended in an antibiotic-free solution.

The two strains of *Staphylococcus aureus* and the two strains of *Klebsiella pneumoniae* were laboratory stock

strains of confirmed identity and antibiotic sensitivity. Tube cultures of monkey kidney cells, HeLa cells, Eagle's minimum essential medium and the penicillin and streptomycin mixture were purchased from Microbiological Associates, Inc., Bethesda, Maryland. Monkey kidney medium 'B' (Melnick) and calf serum were purchased from Baltimore Biological Laboratory.

Fluid was decanted from cultures of monkey kidney or HeLa cells and the cell sheet was washed three times with Eagle's minimum essential medium to eliminate any residual antibiotics previously present in the cultures. One-tenth ml. of virus suspension or Eagle's minimum essential medium was inoculated into each tube. After adsorption for 30 min, 1 ml. of antibiotic-free medium was added to each tube, and the tubes were incubated at 37° C for 72 or 96 h until distinct cytopathogenicity was evident in cell cultures infected with virus. The cultures were then frozen at -40° C and later thawed in a 37° C water bath to free the contents as testing material. For comparison, a solution of a penicillin and streptomycin mixture was diluted to a concentration of 150 and 15 units per ml., respectively, and distributed into several test-tubes. Prior to use, half the tubes were stored in the refrigerator and the remaining half were treated as if virus suspensions or cell culture fluids were being prepared.

The disk sensitivity test, utilizing paper disks impregnated with the virus suspension, was conducted as described by Schneerson and Shore<sup>1</sup>. The tube dilution test was performed by routine procedures. The results were recorded after incubation at 37° C for 18 h.

Paper disks impregnated with the herpes virus suspensions were used for sensitivity tests against the four susceptible organisms. Results presented in Fig. 1 (4-8) show that virus suspensions of Taylor strain, regardless of the method of preparation, failed to demonstrate any antibacterial activity. Antibacterial activity was demonstrated in *EWVK*<sub>1</sub> strain virus suspension in its second passage, but not in suspensions of subsequent passages (Fig. 1, 1-3).

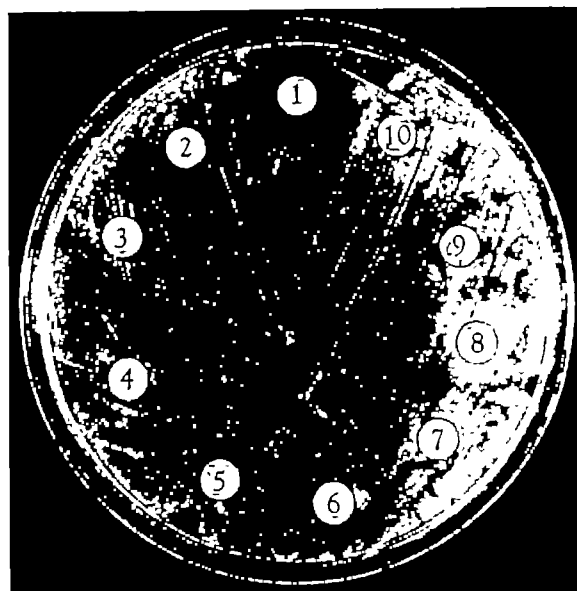


Fig. 1. Sensitivity test against *Staphylococcus aureus* by paper disks impregnated with various viral suspensions: (1) *EWVK*<sub>1</sub> — second passage of herpes virus *EWVK*<sub>1</sub> in monkey kidney cell culture; (2) *EWVK*<sub>1</sub> — third passage of *EWVK*<sub>1</sub> in monkey kidney cell culture; (3) *EWVK*<sub>1</sub> — third passage of *EWVK*<sub>1</sub> in HeLa cell culture; (4) Taylor strain of herpes virus — first propagation in antibiotic-free HeLa cell culture; (5) Taylor strain — first propagation in antibiotic-free monkey kidney cell culture; (6) Taylor strain — first propagation in antibiotic-free monkey kidney cell culture incubated for 96 h; (7) Taylor strain — second passage, monkey kidney cell culture to monkey kidney cell culture; (8) Taylor strain — second passage, HeLa cell culture to HeLa cell culture; (9) monkey kidney cell culture control; (10) HeLa cell culture control.

Table 1. SENSITIVITY OF TEST ORGANISMS TO VARIOUS PREPARATIONS

Organism	Impregnated disks*								
	1	2	3	4	5	6	7	8	9
	Zones of inhibition (mm)								
<i>K. pneumoniae</i> (1)	0	0	19	12	22	14	22	14	16
<i>K. pneumoniae</i> (2)	0	0	19	10	17	10	18	14	16
<i>S. aureus</i> (1)	0	0	23	13	24	15	34	25	17
<i>S. aureus</i> (2)	0	0	25	15	26	14	29	24	19

\* (1) Prepared from Taylor strain propagated in antibiotic-free HeLa cell culture; (2) second passage of (1) in antibiotic-free HeLa cell culture; (3) Taylor strain propagated in HeLa cell culture in medium containing 150 units of penicillin and streptomycin per ml.; (4) second passage of (3) in antibiotic-free HeLa cell culture; (5) HeLa cell culture in medium containing 150 units of penicillin and streptomycin per ml.; (6) HeLa cell culture in antibiotic-free medium after inoculation with 0.1 ml. of (5); (7) solution of penicillin and streptomycin mixture containing 150 units each per ml.; (8) solution of penicillin and streptomycin mixture containing 15 units each per ml.; (9) solution of (8) but incubated, frozen and then thawed.

Since in its primary passage the Taylor strain virus failed to demonstrate any antibacterial activity, it was important to determine whether a result similar to that with *EWK<sub>1</sub>* strain could be obtained if the primary propagation of Taylor virus was prepared in a cell culture treated with a known quantity of antibiotics. One-tenth ml. of an antibiotic-free solution of Taylor's lip lesion fluid was inoculated into tube cultures of HeLa cells. At the end of the adsorption time, 1.0 ml. of medium containing 150 units each of penicillin and streptomycin was added to each tube. After incubation for 72 h at 37° C, the virus suspension collected was used as starting material and was afterwards propagated in antibiotic-free cell culture. For comparison, the same procedure was applied to a few tube cultures, except that they were first inoculated with 0.1 ml. of saline instead of Taylor's lip lesion fluid. Sensitivity tests were carried out with these two suspensions and with a mixture of penicillin and streptomycin in concentrations corresponding to those present in the virus or cell culture suspension. Results are presented in Table 1 and Fig. 2. Whereas the antibacterial activity is absent in Taylor strain virus suspensions prepared from antibiotic-free cell culture (Fig. 1), it is present in suspensions of the second passage propagated in antibiotic-treated cell culture. Similar results were obtained from virus-free cell culture fluid. Furthermore, the size of the

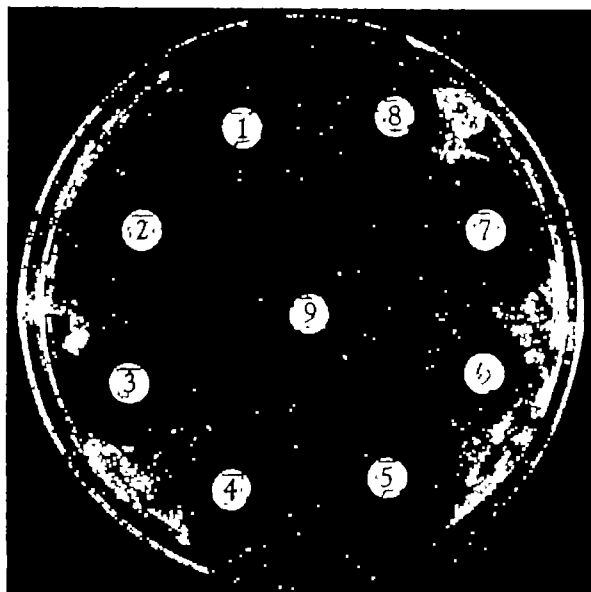


Fig. 2. Sensitivity test against *Klebsiella pneumoniae*. (1) Prepared from Taylor strain propagated in antibiotic-free HeLa cell culture; (2) second passage of (1) in antibiotic-free HeLa cell culture; (3) Taylor strain propagated in HeLa cell culture in medium containing 150 units of penicillin and streptomycin per ml.; (4) second passage of (3) in antibiotic-free HeLa cell culture; (5) HeLa cell culture in medium containing 150 units of penicillin and streptomycin per ml.; (6) HeLa cell culture in antibiotic-free medium after inoculation with 0.1 ml. of (5); (7) solution of penicillin and streptomycin mixture containing 150 units each per ml.; (8) solution of penicillin and streptomycin mixture containing 15 units each per ml.; (9) solution of (8) but incubated, frozen and then thawed.

zone of inhibition decreased proportionally with the decrease of antibiotic concentration in these materials by passage or dilution (Table 1 and Fig. 2).

Since the results strongly suggest a carry-over of antibiotics, a tube dilution test was carried out to detect whether the virus particles or cell debris exert any influence on the potency of the antibiotics. Four antibiotic-containing solutions prepared as described here were assayed against two organisms. Since the quantity of antibiotics present in all preparations was approximately equal, the results shown in Table 2 indicate a decrease in the potency of antibiotics present in the virus suspension and cell culture fluids, as compared with the pure antibiotic solutions. Similar results were also shown in the disk sensitivity test (Table 1). It is speculated that the virus particles or cell debris might adsorb antibiotic molecules and thus render them ineffective. No further investigation was attempted in this respect.

Table 2. POTENCY OF ANTIBIOTICS DERIVED FROM VARIOUS PREPARATIONS  
Effect of antibiotic preparation on test organism

ml. of antibiotic*	<i>S. aureus</i>				<i>K. pneumoniae</i>			
	A†	B	C	D	A	B	C	D
7.5	+	—	—	—	+	—	—	—
3.8	+	—	—	—	+	—	—	—
1.9	+	—	—	—	+	—	—	—
0.95	+	—	—	—	+	—	—	—
0.48	+	—	—	—	+	—	—	—
0.24	+	—	—	—	+	—	—	—
0.12	+	—	—	—	+	—	—	—
0.06	+	—	—	—	+	—	—	—
0.03	+	—	—	—	+	—	—	—
0.00	+	—	—	—	+	—	—	—

\* Penicillin-streptomycin mixture (5,000 units each per ml.) obtained from Microbiological Associates, Bethesda, Maryland. Control No. 51740.

† A, Penicillin-streptomycin mixture present in Taylor strain viral suspension.

B, Penicillin-streptomycin mixture present in HeLa cell culture fluid.

C, Penicillin-streptomycin mixture solution.

D, Penicillin-streptomycin mixture incubated, frozen, and thawed as though viral suspension and cell culture fluid prepared.

† — = no growth of organism, + = growth.

In these experiments it has been demonstrated that: (1) the herpes simplex virus does not exhibit any antibacterial activity if it is isolated in antibiotic-free cell culture (Fig. 1); (2) the antibacterial activity of virus suspensions previously in contact with antibiotics results from antibiotic carry-over rather than from any products of virus-cell interaction. The carry-over mechanism can be visualized as follows: If the virus were isolated from cell culture with medium containing antibiotics of a concentration about 150 units per ml., and if 0.1 ml. of the collected virus suspension were inoculated into an antibiotic-free cell culture, the amount of antibiotics carried over by this process would be diluted to approximately 15 units/ml. The quantity of antibiotics will be further reduced 10-fold by each passage. Such a dilution process readily explains why the antibacterial activity exhibited by the herpes virus suspensions decreased on each passage and finally disappeared (Figs. 1 and 2). In addition, the experimental results further indicate the possible reduction of potency of antibiotics present in the virus suspension or cell culture fluid by some unknown mechanism (Tables 1 and 2).

Prior to Schneerson's report, Gan and Warsa<sup>2</sup> reported the isolation of a bacterial lytic factor from the allantoic fluid of embryonated eggs infected with influenza virus. No further investigations were reported by these authors. Whether any antibiotic can be elaborated from a virus-cell system should be an interesting area of investigation. However, the starting material must be known to be free of antibiotics in order to eliminate any confusion resulting from antibiotic carry-over.

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<sup>1</sup> Schneerson, S. S., and Shore, B., *Nature*, 199, 721 (1963).

<sup>2</sup> Gan, K. H., and Warsa, R., *Amer. J. Hyg.*, 60, 83 (1959).

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

**JUNIOR RESEARCH FELLOW IN PURE MATHEMATICS**—The Registrar, The University, Sheffield (August 4).

**THEORETICAL OFFICER IN THE DEPARTMENT OF ORGANIC AND INORGANIC CHEMISTRY**, to assist the Superintendent of the Laboratory in his duties, which include responsibility for assistant staff, financial and stores administration, buildings and the application of new and existing laboratory techniques and instrumentation—Dr P. Maitland, Secretary of the Faculty Board of Physics and Chemistry, Chemical Laboratory, The University, Lensfield Road, Cambridge (August 4).

**RESEARCH STUDENT** (with a good honours degree in engineering, metallurgy or physics, or expecting to obtain such before 1st October, 1965) for work on surface breakdown friction and wear between unlubricated metal surfaces in sliding contact at speeds up to 250 ft/s.—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (August 6).

**SENIOR LECTURER**; a **LECTURER**; and an **ASSISTANT LECTURER** IN THE DEPARTMENT OF SOCIOLOGY—The Registrar, University of Strathclyde, George Street, Glasgow, G.1 (August 7).

**FELLOW IN THE DEPARTMENT OF STATISTICS OF THE RESEARCH SCHOOL OF SOCIAL SCIENCES**, Institute of Advanced Studies, Australian National University, to engage in some branch of statistical research related to the social sciences, to advise other members of the Research Schools of Sciences and Pacific Studies on sampling and other statistical techniques, and to help in the training of students in such techniques—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 9).

**LECTURER IN COMPUTER SCIENCE** IN THE DEPARTMENT OF MATHEMATICS—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (August 15).

**LECTURER IN THE DEPARTMENT OF MEDICAL PHYSICS**—The Secretary, Royal Free Hospital School of Medicine (University of London), Hunter Street, London, W.C.1 (August 18).

**LECTURERS (2) IN THE DEPARTMENT OF EPIDEMIOLOGY AND PREVENTIVE MEDICINE**—The Secretary of University Court, The University, Glasgow (August 18).

**SENIOR SCIENTIFIC OFFICER/SCIENTIFIC OFFICER** (with an honours degree in dairying, agriculture, science or food science, preferably with postgraduate experience in bacteriology) IN THE AGRICULTURAL BACTERIOLOGY DIVISION OF THE MINISTRY OF AGRICULTURE (successful candidate may be required to undertake teaching duties in the Faculty of Agriculture, Queen's University, Belfast)—The Secretary, Civil Service Commission, Stormont, Belfast, 4, Northern Ireland (August 18).

**AGROLOGIST** (with a degree, preferably with honours, in science or agriculture, and some postgraduate experience in experimental techniques), for work mainly on the control of weeds in sugar beet and other arable crops—The Director, Norfolk Agricultural Station, Sprowston, Norwich, Norfolk (August 14).

**CHAIR OF CONTROL ENGINEERING**—The Deputy Secretary, The University, Southampton (August 14).

**LECTURER IN AGRICULTURAL ZOOLOGY**—The Secretary, The University, Aberdeen (August 14).

**LECTURER IN BIOLOGICAL CHEMISTRY**—The Registrar, The University, Manchester, 13, quoting Ref. 140/65/Na (August 14).

**LECTURER** (with a special interest in radio chemistry) IN THE DEPARTMENT OF CHEMISTRY—The Secretary, The University, Aberdeen (August 14).

**SENIOR LECTURER** (honorary consultant status) IN THE DEPARTMENT OF ORTHOPAEDIC SURGERY—The Secretary, The University, Edinburgh (August 14).

**STUDENT (MAN OR WOMAN) IN THE CHEMISTRY DEPARTMENT FOR RESEARCH IN INFRA-RED SPECTROSCOPY**—The Secretary, Royal Holloway College (University of London), Egham, Surrey (August 14).

**RESEARCH FELLOW IN THE SCHOOL OF MATHEMATICAL AND PHYSICAL SCIENCES** to join a research group working on defects of solids; both point defects and dislocations are being studied in a wide ranging programme, which includes the use of an electron microscope with liquid helium stage facilities—The Assistant Registrar (Establishment), University of Sussex, Slammer House, Slammer, Brighton, Sussex (August 15).

**RESEARCH FELLOW** (preferably with research interests in the field of theoretical solid state, nuclear or elementary particle physics) IN THE DEPARTMENT OF MATHEMATICAL PHYSICS—The Assistant Registrar (Science), University of Birmingham, Birmingham 15 (August 15).

**JUNIOR RESEARCH FELLOW IN BOTANY**—The Registrar, The University, Sheffield (August 31).

**SENIOR LECTURER IN MATHEMATICS**; a **SENIOR LECTURER IN STATISTICS**; a **LECTURER OR ASSISTANT LECTURER IN MATHEMATICS**; a **LECTURER OR ASSISTANT LECTURER IN STATISTICS**; a **LECTURER OR ASSISTANT LECTURER IN NUMERICAL ANALYSIS AND COMPUTING**; and a **LECTURER OR ASSISTANT LECTURER IN OPERATIONAL RESEARCH**—The Registrar, Ref. 114Y/H, Bradford Institute of Technology, Bradford, 7 (August 23).

**LECTURER OR ASSISTANT LECTURER IN PSYCHOLOGY** at the University of Otago, Dunedin, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (August 25).

**RESEARCH AGROLOGIST**; a **SENIOR LECTURER/LECTURER IN AGRICULTURAL BOTANY**; a **RESEARCH BOTANIST/PLANT BREEDER**; a **PLANT INTRODUCTION OFFICER**; and a **PLANT PATHOLOGIST** at AHMADU BELLO UNIVERSITY, NORTHERN NIGERIA—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (August 26).

**SENIOR LECTURER IN SURGERY**—The Registrar, The University, Sheffield (August 28).

**ASSISTANT LECTURER** (mathematician with qualifications in probability theory and statistics) IN THE DEPARTMENT OF MATHEMATICS—The Secretary, University of Lancaster, Bailrigg House, Lancaster (August 31).

**EXPERIMENTAL OFFICER** (interested in the design and construction of equipment for a wide range of psychological experiments) IN THE DEPARTMENT OF PSYCHOLOGY—The Registrar, The University, Manchester, 13, quoting Ref. 130/66 (August 31).

**LECTURER** (graduate, with a good honours degree in sociology, anthropology, or rural sociology) IN SOCIOLOGY IN THE FACULTY OF AGRICULTURAL ECONOMICS, UNIVERSITY OF NEW ENGLAND, ARMIDALE, NEW SOUTH WALES, AUSTRALIA—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 31).

**SENIOR EXPERIMENTAL OFFICER IN CHARGE OF THE ELECTRON MICROSCOPY LABORATORY**—The Deputy Secretary to the Academic Council, University of Belfast, Northern Ireland (August 31).

**SENIOR LABORATORY TECHNICIAN** (with a knowledge of biophysics) IN THE DEPARTMENT OF PHYSIOLOGY—The Assistant Secretary, Royal Veterinary College, Royal College Street, London, N.W.1 (August 31).

**ASSISTANT LECTURER IN BOTANY**—The Secretary, The Queen's University, Belfast, Northern Ireland (September 1).

**LECTURER OR ASSISTANT LECTURER** (graduate in medicine, veterinary science, dentistry or biology) IN THE DEPARTMENT OF HISTOLOGY—The Registrar, The University, Liverpool, quoting Ref. CV/195 (September 1).

**LECTURER IN MATHEMATICS AT THE UNIVERSITY OF NEW SOUTH WALES** (Broken Hill Division)—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 4).

**READER** (with a special interest in either experimental reactor physics, fluid dynamics or heat transfer) IN NUCLEAR ENGINEERING AT QUEEN MARY COLLEGE—The Academic Registrar, University of London, Senate House, London, W.C.1 (September 13).

**KEMPER** (with museum experience, a British university degree or equivalent, and preferably the Diploma of the Museums Association) OF THE DEPARTMENT OF NATURAL HISTORY—The Director, City Museum and Art Gallery, Congreve Street, Birmingham 3 (September 20).

**LECTURER** (with a degree in pure science or agricultural science, and taken zoology to an advanced level) IN AGRICULTURAL ZOOLOGY at Lincoln College (University of Canterbury), New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 25).

**MYCOLOGIST** (with a good honours degree) to work on soil-borne diseases of cereals—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (September 30).

**ASSISTANT LECTURER** (with a degree in physiology, pharmacology or pharmacy) IN THE DEPARTMENT OF OPHTHALMOLOGY—The Academic Registrar, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

**BIOCHEMIST** (national of, and permanently resident in, the United Kingdom or the Republic of Ireland, with an honours degree in biochemistry or Associatehip of the Royal Institute of Chemistry, and at least two years postgraduate experience in medical biochemistry in a hospital laboratory) in Uganda, to take charge of the Biochemistry Division of the Laboratory services located in Kampala and serving all parts of the country and all races—Appointments Officer, Ministry of Overseas Development, Room 404, Strand House, Stag Place, London, S.W.1, quoting Ref. RC/206/183/06.

**CHAIR OF CHEMICAL ENGINEERING**—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 32/G.

**POST-DOCTORAL FELLOW IN SOLID STATE PHYSICS** for work on the transport and optical properties of molecular crystals—The Registrar, The University, Leicester.

**POST-DOCTORAL FELLOW IN THE DEPARTMENT OF PURE AND APPLIED CHEMISTRY**, to assist Prof. M. Gordon in fundamental studies on new reproducible covalently linked gels, rubbers and highly branched liquids—Prof. M. Gordon, University of Strathclyde, George Street, Glasgow, G.1.

**PRINCIPAL SCIENTIFIC OFFICER** (with a good honours degree in a biological subject and considerable experience in ecological research), to assist in the scientific administration of the Council's activities, with particular responsibility in the biological field—The Natural Environment Research Council, Establishments Division, State House, High Holborn, London, W.C.1.

**RESEARCH ASSISTANT** for investigation of the chemistry of carboxylase and other metallo-proteins—The Secretary, Chemical Pathology Department, St. George's Hospital Medical School, London, S.W.1.

**RESEARCH ASSISTANT** (with a Ph.D. or B.Sc. or equivalent in chemistry, mathematics or physics, and interested in the application of computer techniques to molecular theory) IN THEORETICAL CHEMISTRY—The Secretary, Royal Holloway College (University of London), Egham, Surrey.

**RESEARCH FELLOW OR SENIOR RESEARCH FELLOW** (graduate or equivalent, with several years of research experience in ferrous metallurgy, and preferably a knowledge of electron microscopy and fracture mechanics) IN THE DEPARTMENT OF MATERIALS, to participate in a research programme supported by the Science Research Council, into the relationship between metallurgical structure and properties in the weld heat affected zone in low alloy steels, with particular reference to brittle fracture resistance—The Registrar, The College of Aeronautics, Cranfield, Bedford.

**RESEARCH STUDENT IN GEOGRAPHY**—The Registrar, The University, Hull.

**SCIENTIFIC OFFICER OR SENIOR SCIENTIFIC OFFICER** (honours graduate, preferably in chemistry, agricultural chemistry or biochemistry) IN THE CHEMISTRY DEPARTMENT, to take part in a research programme on ruminant nutrition concerned mainly with the relationship of the feeding, digestion and metabolism of the cow to the secretion of fat, protein or lactose in milk—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 65/N/10.

**SCIENTIFIC OFFICER** (with a good honours degree in biochemistry or chemistry, and with a particular interest in lipid metabolism) to investigate the interrelationship between sterol and fatty acid metabolism in animals—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 65/N/11.

**SENIOR ANIMAL TECHNICIAN** (male, 25 years of age or over, and at least an Associate of the Animal Technicians Association, and experience or interest in BFF and germ-free techniques) for interesting work in modern, well equipped laboratory animal houses—The Director, Laboratory Animals Centre, M.R.C. Laboratories, Woodmansterne Road, Camberley, Surrey.

**SENIOR LECTURER** (veterinary graduate with postgraduate qualifications or an honours graduate with a science degree majoring in anatomy and physiology or biochemistry) IN ANIMAL HUSBANDRY (Meat Production) at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

**SENIOR RESEARCH ASSOCIATE IN THE SCHOOL OF CHEMICAL SCIENCES** for work in the preparation and spectroscopic study of disymmetric aromatic molecules or coordination compounds—Prof. S. F. Mason, School of Chemical Sciences, University of East Anglia, Wilberforce Road, Norwich, NOR 77H.

**S.R.C. POSTDOCTORAL FELLOW** (spectroscopist, physicist or physical chemist) for a research project (already partially completed) concerned with the scattering of light in liquids and gases using a laser source—Dr. A. J. Hyde, Department of Pure and Applied Chemistry, University of Strathclyde, George Street, Glasgow, G.1.

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## A SOCIAL SCIENCE RESEARCH COUNCIL

THE Committee on Social Studies was set up with Lord Heyworth as chairman in June 1963 "to review the research at present being done in the field of social studies in Government departments, universities and other institutions and to advise whether changes are needed in the arrangements for supporting and co-ordinating this research". Its principal recommendation is for the establishment of a Social Science Research Council to provide support for research, to keep under review the state of research and the supply of trained research workers, to advise the Government on the needs of social science research, and to give advice on research in the social sciences and its application. Such a Council was recommended in 1961 in a report from the British Academy (*Nature*, 193, 1011; 1962), also in 1962 in a memorandum by Prof. D. V. Glass and Prof. M. Gluckman submitted to the Robbins Committee by the British Association (*Nature*, 196, 704; 1962), and more recently in a paper of the Council of the Tavistock Institute of Human Relations (*Nature*, 205, 729; 1965). This time, however, the recommendation has been accepted by the Government, although details are still under consideration. However, it is rather curious that in a historical survey of the field since 1945 the report now issued by the Committee on Social Studies of the Department of Education and Science\* merely refers to the report of the Clapham Committee in 1946 and its recommendation for an Interdepartmental Committee of Social and Economic Research. The Interdepartmental Committee has not met since 1960 although it is still formally in existence: most of the departments giving evidence to the Heyworth Committee appeared to be unaware of its existence.

In considering the scope of the social sciences, which are regarded as including economics, politics, sociology, demography, social anthropology, social administration and social psychology, the Heyworth Committee draws a distinction between research and fact-finding, the former being regarded as something other than routine fact-finding, or fact-finding for specific administrative purposes. While the distinction is sometimes difficult to draw, it is important to make the attempt if the need for social research is to be assessed accurately and is to be soundly based. The Committee is also wise to emphasize the importance of research in the social sciences to the administrator. Unless an attempt is made to identify long-term problems or penetrate behind everyday decisions, those decisions may be based on wrong or inaccurate data. Whether he knows it or not, anyone engaged on administration in central or local government, in education or in commerce and industry is using methods and techniques to help him deal with his work and solve problems that a social scientist would recognize. It only strengthens the Committee's argument that the use of the computer is allowing decisions to be based on facts to a scale impracticable even a decade ago.

\* Department of Education and Science. *Report of the Committee on Social Studies*. Pp. v+101. (Oxford 1960) (London: H.M.S.O., 1965.) 7s. 6d. net.

Looking next at developments since 1945, the Heyworth Committee estimates that between 1938-39 and 1962-63 the number of university teachers in the social sciences increased from 212 to 1,025, while the number of students obtaining honours degrees in the social sciences also increased five-fold, from 300 to 1,537; since 1959-60 the percentage of full-time students in social science faculties has risen from 10.5 to 11.4 in 1962-63 and 12.1 in 1964-65. In addition, home registrations for external London degrees in sociology have increased from 40 in 1952-53 to 217 in 1963-64 and 460 in 1964-65. Moreover, the Committee formed the impression that a larger proportion of abler students is now entering the social sciences. The rate of growth is not uniform over all the subjects. Thus, while sociology has recently grown explosively, growth in psychology has been largely on the biological side and social psychology has been neglected in British universities compared with its development in the United States, nor is there any centre encouraging the study of law as a social science. Again, while the number of postgraduate State studentships awarded in the humanities and social sciences remained fairly constant at about 250 during the years 1957-61, the number increased in 1962 when 304 were awarded in the humanities, 48 in the social sciences and 95 in related disciplines, compared with 1,607 in science and technology; for 1964 the corresponding figures were, respectively, 626, 184, 190 and 2,453.

The universities are still the main centres for research in the social sciences, and the Committee is satisfied that since 1946 the social sciences have become firmly established in the universities and that a basis has been laid for the future supply of teachers and research workers. Total expenditure by the universities on research in 1962-63 is estimated at £1,575,000 with a further £160,000 from Oxford and Cambridge College funds and endowments, £884,000 from external sources and £300,000 on agricultural economics. Expenditure by research institutes outside the universities, notably by the Tavistock Institute of Human Relations (£170,000), the National Institute of Economic and Social Research (£79,000) and the National Foundation for Educational Research (£35,000), is estimated at £577,000, and for the three Institutes named the expenditure for 1964-65 is put at £220,000, £94,000 and £103,000, respectively. Expenditure by Government research organizations (£780,000) and Executive Departments (£220,000) and overseas (£90,000) at Colleges of Technology (£100,000) and by other bodies and individuals brings the total for 1962-63 to about £5 million, and for 1964-65 the figure is likely to be about £6.5 million.

The Committee attempted to estimate the distribution of this research effort over different fields, but it was only able to do so for some 70 per cent of the total and drew no firm conclusions. Of this 70 per cent, 16 per cent seems to be spent on health and welfare, 14 per cent on industry, 11 per cent on education, 10 per cent on agriculture and



9 per cent each on the general British economy and on developing countries, including race relations, etc. While industry and commerce are taking more graduates in the social sciences and increasing their share of the output, the Committee formed the impression that industry made little use of social scientists in research within its own organization and did not employ them where they would be led to identify problems requiring research and give advice accordingly. Most voluntary associations complained of the difficulty of finding even small sums to finance research and of discovering what research of a similar nature was being undertaken elsewhere. Again, although substantial numbers of social scientists are employed by local government, new town development corporation and other statutory bodies, they are seldom engaged on research. Local Authorities' Associations seem to realize the value of social science research, but this awareness is as yet unaccompanied by a substantial increase in the support or direct contracting of research.

In the Government service the employment of economists and psychologists professionally has been increasing and nearly all departments now employ statisticians. However, the Committee gained the impression that very few sociologists and other kinds of social scientists are employed in departments in a professional or advisory capacity, and that until a few years ago there had been little growth in research in Government departments since the Clapham Committee reported. In June 1964 the National Economic Development Council was employing 20 economists and one sociologist, some of whose work could be described as research, and had arranged for work on contract in university departments. In 1964 the Board of Trade established an economic research unit and research has been strengthened in the Ministry of Housing, the Scottish Home and Health Department and the Ministry of Public Building and Works, although some of this effort is fact-finding rather than research.

This section of the Heyworth Report gives a sidelight on the effect of the premature dissolution of the Overseas Research Council. When in 1962 the Department of Technical Co-operation took over the disbursement of finance for social science and other overseas research the Colonial Social Science Research Council and the Colonial Economic Research Committee were abolished. From 1958-59 to 1962-63 they had contributed £260,000 towards the general running costs of research institutes in colonial territories, £97,000 as grants for specific projects requested by Colonial Governments, £64,000 as grants for projects requested by the Colonial Office or one of the advisory bodies, and £31,000 as grants to individuals for field research in colonial territories. As already noted, it is estimated that in 1962-63 some £90,000 was expended from Government funds in support of research in dependent territories overseas, and in addition to this the Committee estimates that £136,000 went to the developing countries from university research funds and £157,000 from the research institutes.

In the second part of the Report the Committee discusses the need for more research in the light of this situation. Successive chapters deal with the supply and training of research workers, the organization of research and the use of research, leading up to the recommendation for the establishment of a Social Science Research Council and the issues involved in this recommendation. While the Report lists numerous problems suggested to the Committee as needing attention, no comment is made other than the observation that almost all the problems involve contributions from one or more of the social

sciences and often from disciplines outside them. This has a close bearing on the supply of research workers; the Committee considers that the first priority is a further expansion of postgraduate training in the social sciences. It suggests that the 220 awards for postgraduate study in 1964 should be increased to 400 in 1965-66, particularly in sociology and social psychology, with a further increase in 1966-67. It also recommends that at least 20 research fellowships should be available in the social sciences in 1965-66 under the scheme hitherto run by the Department of Scientific and Industrial Research, and not necessarily tenable only in a university but also in a research institute, a Government research station or other research organization.

While it recommends priority for postgraduate studies, it emphasizes also the importance of research assistants, and suggests that married women who are prepared to return to full-time work after bringing up their families should be enabled to enter the research field. Furthermore, it recommends that universities need to examine not only the organization of teaching and research duties in social science departments but also the method of allocating funds for auxiliary staff and facilities for research. It foresees a substantial demand for teachers of the social sciences in the technical colleges, and emphasizes the importance of giving such teachers opportunities to be associated with research. Similarly it directs attention to the importance of utilizing the capacity for training of research institutes outside universities, including the Social Survey of the Central Office of Information and research units in Government departments. However, it urges that expansion of postgraduate training would be carried out most effectively and economically if a few places are built up as research schools.

It is in keeping with the general balance and sanity of the Report that the Committee recognizes the importance of being able to offer reasonable prospects of a career if sufficient able recruits are to be attracted to study the social sciences. The Committee does not consider that many people would be best employed in one institution during their professional career in social science research, but this is not the only reason which it advances in recommending flexible arrangements by the universities as regards teaching duties and research. It is desirable that many who continue to pursue research throughout their career should be able to move from one institution to another. For this reason alone the Committee is on firm ground, with authoritative opinion elsewhere behind it, in wishing to see a range of research posts in universities, units attached to universities, independent research institutions, and Government research establishments, subscribing to a common policy of interchangeable pension rights and seniority grading, and of secondments.

Beyond this, however, if research workers in the social sciences are to have the assurance of a satisfactory career which the evidence submitted to the Committee suggests they often lack, it will be necessary to create more established posts for those qualified in the social science disciplines and trained in the methods of research. Full-time research, however, will often be only a phase, perhaps to be repeated, in the career of men and women who, in other phases, will be university teachers, managers, administrators in the Civil Service or local government, professional economists, sociologists or psychologists, etc. This is another reason for recommending a common policy in pension rights, seniority grading and secondment to encourage and facilitate the necessary mobility and interchange, nor should interchange between the Civil Service

and industry present as much difficulty here as it sometimes does in other spheres. It is also desirable as contributing to meeting the two needs which, the Committee suggests, have to be overcome before we can expect the results of research in the social sciences to be applied effectively.

The first of these is for administrators and managers to be familiar with the scope and value of the social sciences, not only as direct aids to administration but also as disciplines which can limit the uncertainties within which decisions have to be taken and which can evaluate their outcome. Clearly this will be facilitated as interchange grows. However, because problems rarely present themselves to administrators in a fashion which at once shows how they could be clarified by research in the social sciences, there is, secondly, a need for social scientists to work at points where problems first emerge and to help identify and deal with them: this in itself is a reason for encouraging mobility. As the Committee observes, in expressing their research needs and in constructing research units or divisions, user organizations must bring social scientists in a close working relation with administrators.

This is the more important in that in the social sciences, when full allowance is made for the difference in the nature of applied research, there are very few people whose functions correspond to the engineering or development function in the physical sciences, and nowhere are such people trained. This gap needs filling and the Committee remarks that the training given to people to fill this function should include a basic grounding in business economics and also practice in co-operating in research with people trained in other disciplines. It is in connexion with the use of social research that the Committee is anxious to see more social scientists, other than economists, employed in the Government service not only for the research they will do but also because they will help departments to identify the problems to which research in the social studies can contribute, and to track down research already undertaken and apply it to administrative use.

The Committee was asked by the then First Secretary, Mr. R. A. (now Lord) Butler, to comment on the desirability of units of social research in the Departments of Health, Housing and Local Government, Labour, Pensions and National Insurance, and of a unit for demographic research attached to the Central Statistical Office. After pointing out that if the findings of research are to be brought most effectively into the administrative process, professionally qualified staff with experience of research in the discipline concerned must be associated with administrative officers as members of a team. There is, however, need for some central point in the department for the professional social scientists, where day-to-day work is in the various branches. Beyond this, the Committee is content to point out that whether better results would be obtained from a special research unit operating within the department rather than from a programme of research contracts depends on various factors, six of which it specifically indicates, and rather than make specific recommendations it leaves departments to make their own decision in the light of such considerations.

It is at this point that the Committee approaches the question of central organization and interdepartmental action. Its suggestions here are made on the assumption that a Social Science Research Council such as it recommends will, in fact, be established. First it suggests

that the syllabus of the training courses at the Centre for Administrative Studies in the Treasury should include all the social sciences that have a contribution to make to administration. It also recommends that the Treasury should review the conditions of employment of social scientists in the Government service and undertake a central scrutiny of departmental budgets for research for their own purposes: this could usefully be supplemented by consultation with the Social Science Research Council. Better, fuller, more co-ordinated and earlier Government social statistics are needed, especially if their value as a basis for research is to be improved. Here the Central Statistical Office should take the lead, but the link between Government and social scientists outside the Civil Service should be forged through the Social Science Research Council. Dissolution of the Interdepartmental Committee on Social and Economic Research is recommended and also the transfer to the Treasury of the Social Survey, which is at present inefficiently used. The programme of the Survey should be determined by a Committee, under Treasury chairmanship, of representatives of the main user departments and of the Social Science Research Council.

The Committee's observations about the needs of the Government apply in principle to local authorities, statutory bodies, industry and commerce, the judicial system and other users of research: it is against this background that its proposal for a Social Science Research Council should be considered. It rejects the idea of separate research councils for education and the 'built environment'. It considers that the needs of education will best be met by establishment of a Board within the orbit of the Council. Likewise, the needs of planning will be met by establishment of a joint board by the Research Councils concerned with technology, the physical sciences, the arts and the social sciences—presumably after consultation with the bodies which have already constituted a Land Use Research Trust and with specially interested departments of Government.

The Social Science Research Council should consist of an independent chairman and about ten or twelve members, mainly social scientists, but some with experience in industry or 'user organizations', appointed by the Secretary of State for Education and Science, and the Central Government should be represented on the Council and its Committees by assessors. If the Council is brought within the system under which the Council on Scientific Policy advises on the allocation of resources among the Research Councils, the membership of the latter Council should include one or more persons with an up-to-date knowledge of the needs of the social sciences. An expenditure of some £800,000 during the first year is contemplated and £1,000,000 during the second, rising to £2.25 million in the third year, but these figures include some existing expenditure on studentships and research fellowships and on research grants. Existing commitments for research grants are estimated at £170,000 in 1965-66 and £190,000 in 1966-67, but commitments totalling £1 million in the first year and £1.5 million in the second are recommended over varying periods and the figure of £1.5 million is unlikely to be approached until the fourth year.

The recommendations of the Heyworth Committee on finance are subject to review and, in any event, the Government has not yet committed itself on finance beyond accepting the need for some increase in financial support for social science research. It has also not accepted the proposal for a joint board of the various Research

Councils to cover research in the field of urban planning, and it is considering what alternative provision should be made. It is noteworthy that the Committee expresses no opinion as to the ultimate level which expenditure on social science research should reach, although the Council of the Tavistock Institute of Human Relations in 1964 envisaged an expenditure of £25 million by 1980. Its estimate of present expenditure was, however, only £3.5 million a year compared with the £8.5 million in the Heyworth Report.

There are two other points which are important. The Heyworth Committee had reservations as to the value of a register of research in the human sciences such as is at present put out by the Warren Spring Laboratory, but it suggests that the new Research Council should consider whether it should take over this register and extend it to cover the whole field of social sciences. The Committee has no hesitations, however, as to the second point relating to library facilities. Clearly the question of inadequacies in university library facilities for social science must await the report of the Committee considering the needs of university libraries generally. Nevertheless, the Heyworth Committee is satisfied that a strong case exists for providing lending library facilities for the social sciences along the lines of those providing for technology and the natural sciences by the National Lending Library at Boston Spa. It proposes that the Department of Education and Science should arrange for a study to be made of the feasibility of extending these facilities to include the social sciences.

That proposal may merit examination, but it is a less straightforward proposition than might appear at first sight. It requires examination in the light of the library services of the whole country: it should be remembered that in his presidential address to the Library Association last May, Sir Frank Francis insisted that as yet Britain has no library system as such. To create such a system is one of our primary needs, and the dilatoriness over the creation of the new Reference Library for Science and Invention illustrates the absence of any sense of urgency on the part of the Government.

It is one of the merits of the Heyworth Report that it directs attention to the importance of such ancillaries to research. There is no suggestion that, by itself, the creation of a Social Science Research Council—or even a larger supply of men and women trained in the social sciences and techniques of research—will solve our difficulties, nor even that more finance is the first need. The problem is seen as a whole, and if the Government has accepted the recommendation for a Research Council it remains for informed opinion to see that the Research Council is adequately supported and that its work is not hampered by failure to implement the numerous other recommendations on which the effectiveness of the Council largely depends.

## FESTSCHRIFT FOR HAROLD C. UREY

### Isotopic and Cosmic Chemistry

Edited by H. Craig, S. L. Miller and G. J. Wasserburg. Pp. xxv + 553. (Amsterdam: North-Holland Publishing Co., 1964.) 108s.

THE custom of the festschrift has never firmly established itself in the English-speaking countries, although from time to time such a compliment is paid to some outstanding scientist. For the publication to appeal

to a variety of readers, many of whom cannot have had the good fortune to meet the recipient, it is necessary that he should have breadth of interests, a versatile and enquiring mind and, above all, originality in his ideas. In addition, it is helpful if he has taught and inspired a succession of pupils and collaborators, since they may be expected to initiate the production and contribute substantially to its success.

Prof. Harold Urey undoubtedly fulfils these criteria. The career of such a dynamic personality is never dull. "The present volume of contributions cannot hope to match the complete range of topics he has enriched, but almost every paper bears his mark to a greater or lesser degree." It is remarkable to discover that he first graduated in zoology before the First World War. Both the First and the Second World Wars profoundly affected his life and interests. The First led him to chemistry and confirmed his intention to enter academic life; in the Second he played an important part in the Manhattan project and it eventually led to his moving from Columbia to Chicago.

Urey spent a short period at Berkeley at the peak of G. N. Lewis's career, and although the precise effect of this interlude is only a matter of speculation, undoubtedly Urey has remained interested in thermodynamic problems. It is characteristic of both Urey's written and spoken word that he applies a very quantitative analysis even to the most speculative of hypotheses. For several years after this period his principal interest lay in spectroscopy and quantum mechanics. This phase culminated in the collaboration with Ruark in the publication of one of the classics of physical chemistry, *Atoms, Molecules and Quanta*.

Spectroscopy led to an interest in the properties of different stable isotopic species, their separation and abundance. Thus began studies that constitute the central theme of much of his subsequent work and brought him the 1934 Nobel Prize for Chemistry. *Isotopic and Cosmic Chemistry* is extensively concerned with the information that can be gained from measurements of the generally small changes in isotopic composition brought about by natural or laboratory processes.

His earlier interests are represented by two short papers, a note by J. E. Mayer on Loschmidt's paradox and another by A. E. Ruark on classical fields. But the majority of the papers deal with these isotopic effects or with a branch of science that Urey can reasonably regard as his own—cosmochemistry. A part of this subject, the chemical and radiochemical study of meteorites, had indeed been explored for several years by F. A. Paneth, but Urey's work covers a much broader canvas, as can be judged from his book, *The Planets, their Origin and Development*. Urey's insistence on a broad attack—"treating the whole problem"—is another characteristic of his intellectual personality. His interest in this subject, which he has developed rapidly since the Second World War, dates back at least to 1931 when he published a paper in collaboration with Bradley on the origin of the elements, using thermodynamic arguments based on the abundance of the isotopes of the lighter elements.

Among the many interesting contributions may be mentioned three papers dealing with temperature measurements by means of  $^{18}\text{O}/^{16}\text{O}$  ratios. One deals with the application of Urey's original palaeotemperature technique to a series of fossil shells from previously inhabited caves in the Mediterranean area. The other two explore the possibility of extending such temperature measurements to establish the temperature at which oxygen equilibration occurred between different minerals in suitable geological formations—geothermometry. A number of papers treat geochronological measurements; one rather long paper shows how metamorphic processes can invalidate conventional measurements, the data relating especially to  $^{87}\text{Rb}/^{87}\text{Sr}$  measurements.

Seven papers deal with meteorites, their origin, frequency of falls, radiation ages and composition. The longest of these gives a scholarly account of the classification of the chondrites on the basis of their chemical and petrological composition. The abundance of uranium and thorium is discussed in relation to theories of the genesis of the elements, and finally one paper discusses the chemical composition of comets.

All Urey's most speculative interests, the origin of the elements, the planets and life itself are represented. The volume is a worthy tribute to a remarkable man.

A. G. MADDOCK

## GEOLOGICAL AND CHEMICAL EVOLUTION OF EVAPORITES

### Salt Deposits

The Origin, Metamorphism and Deformation of Evaporites. By Prof. Hermann Borchert and Dr. Richard O. Muir. (The University Series in Geology.) Pp. x + 338. (London and Princeton, N.J.: D. Van Nostrand Company, 1964.) 80s.

THIS translation of Hermann Borchert's important book *Ozeane Salzlagernissen* (Geb. Borntraeger, 1959), with a considerable amount of material added by R. O. Muir, the translator, may justly claim to be the first comprehensive work in English on the natural salt deposits, the only previous approach to such a treatment being F. H. Stewart's chapter in the current revision of Clarke's *Data of Geochemistry* (U.S. Geol. Survey Prof. Paper 440-Y. Pp. 52. 1963). It is most valuable to have this summary from one of the leading European authorities, the more so because the deposits of the German Zechstein are considered at some length and these have probably been studied more intensely, and for a longer time, than any in the world.

In the case of the evaporites, as with marine iron ores, the 'principle of uniformity' cannot be applied, in that no extensive marine halite or potash accumulations can be observed in process of formation at the present. The factors controlling development and distribution of salt deposits, past and present, thus have to be inferred from geological evidence, and from the evidence of physical chemistry. After presenting a useful summary of the properties of the 26 important mineral phases found in evaporites, the first five chapters deal with depositional environment, distribution, sedimentation and tectonics, some emphasis being placed on the recurrent rhythmic nature of precipitation. Usiglio's contention that the necessary environment is a basin or series of basins with tectonic bars to control the influx of new sea-water is strongly upheld.

Isothermal evaporation of sea-water is next considered and the paths of crystallization illustrated in terms of the quinary system  $\text{Na}_2\text{O}-\text{K}_2\text{O}-\text{MgO}-\text{CaO}-\text{SO}_4$ . Oceanographic studies show, however, that temperature and concentration gradients exist in all basins, varying with changing current velocities to maintain a dynamic equilibrium. A brave attempt to consider the dynamo-polythermal evaporation of sea-water is therefore made and interesting conclusions are reached about the successive stages in primary facies variation as a basin becomes more isolated from the open sea.

It has long been known that profound mineralogical changes occur in complex salt deposits as they become more deeply buried, arising from the changed temperature-pressure conditions, and from the activity of solutions, especially those being eliminated from deeper levels. Writers in English have hitherto hesitated to describe these changes as metamorphic, since they occur under conditions where silicate minerals in sedimentation are undergoing the changes associated with diagenesis, below the lowest recognized grade of metamorphism. Nevertheless, the authors make a good case for describing the

modifications in the evaporites as metamorphic and show that they can be considered in relation to the phase diagrams for various temperatures in the quinary system mentioned here, rather more effectively than silicates can be deciphered in terms of present knowledge of the physical chemistry of such systems as  $\text{Na}_2\text{O}-\text{K}_2\text{O}-\text{CaO}-\text{Al}_2\text{O}_3-\text{SiO}_2$  at elevated temperatures. Chapters 8-15 deal with metamorphism and its consequences; with normal progressive metamorphism described in terms of melting and the activity of melt liquors; and with retrograde metamorphism described in terms of the effects of water eliminated during dehydration reactions at greater depth and forced upward. The latter type of metamorphism is caused by dilute solutions percolating very slowly into salt deposits; besides waters originating as already noted from the conversion of gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) into anhydrite ( $\text{CaSO}_4$ ) and similar sources, the possibility of activity by hydrothermal solutions of magmatic origin is contemplated, and the well-known solution effects due to meteoric groundwater are also included. Here there are possible grounds for criticism, for changes taking place within the zone of circulating groundwaters do not form part of the silicate petrologist's conception of metamorphism. It must, however, be recognized that it is not easy to avoid this difficulty. The essential distinction between the two types of salt metamorphism proposed is that normal progressive changes are produced by authigenic liquors generated by geothermal melting, and lead to a sequence of mineral associations of which each successive one is in stable equilibrium with a liquid phase that becomes increasingly rich in  $\text{MgCl}_2$ . Retrograde changes, on the other hand, give rise to parageneses stable in brines becoming deficient in  $\text{MgCl}_2$ , but increasingly sulphatic. With the basic conception of salt metamorphism established, it is possible to look at facies variations in the deposit and to attempt to assess their primary or secondary nature; and it is clear that very many have been influenced by post-depositional changes.

A variety of different evaporite districts are described, and there are interesting chapters on the mechanical properties of salts, on salt-tectonics, and on the relationship between evaporites and oil accumulations.

This book is a very important contribution to the literature of the subject, but it is aimed at the advanced student or the specialist, for its authors do not hesitate to enter into controversies the background of which can only be adequately known to those familiar with what has been written during the past two decades about evaporites in several different countries. A less-sophisticated version for the ordinary student of geology or chemistry would also be welcome.

K. C. DUNHAM

## MAGNETISM AND LIFE

### Biological Effects of Magnetic Fields

Edited by Madeleine F. Barnothy. Pp. ix + 324. (New York: Plenum Press, 1964.) 16.00 dollars.

**BIOLOGICAL Effects of Magnetic Fields** is not, as might be supposed, yet another report of a conference attended by workers with a common interest; it is rather a book designed "to provide a theoretical and practical background" for would-be research workers, and also to "bring together in one volume the present-day knowledge in all the active fields of biomagnetic research". It is divided into four main sections: first, "Theoretical Considerations"; secondly, "Effects of Strong Magnetic Fields on Specimens *in vivo*"; thirdly, "Effect of Strong Magnetic Fields on Specimens *in vitro*"; and fourthly, "Effects of Very Weak Magnetic Fields". Finally, there is a fairly comprehensive bibliography relating to the effects of static fields.

On the whole I must confess that I found the book disappointing. Perhaps a failure to achieve some sort of unified

approach should occasion little surprise in a subject which has been worked at rather piecemeal, the interest of one investigator being centred in such a ubiquitous and commonplace phenomenon as geotropism, while the interest of another lies in the significance of biomagnetism for space travel, and so on. But it should surely have been possible, starting from scratch, to have produced a work which was a more coherent and satisfactory introduction to such an interesting bypath (for that is all it is at the moment) of modern investigation than has been done. As it is, most of the articles appear to be restricted accounts of special pieces of work rather than essays, complete in themselves, designed to introduce newcomers into interesting aspects of the broader subject. Perhaps the editor was set the task of attaining two mutually irreconcilable objectives; but it scarcely seems unfair to say that neither has been satisfactorily achieved. This is a pity, for a 'background introduction' and a summary of present-day knowledge and research activity could both have been very useful.

These criticisms do not mean, however, that the book is of little value. To initiated or half-initiated workers it will probably prove very valuable and serve to broaden their horizons. It is rather its lack of any coherent emphasis, treatment and standpoint which is cause for regret, not its failure to include good and interesting material. But that is a common criticism of modern symposia, and no doubt one very difficult to remedy.

On particular articles the following comments may be made from the point of view of a biologist. There is a reasonably adequate introduction to the subject of magnetic fields, and of their possible modes of interaction with biological units, though some clear diagrams would have been a great advantage. The discussion of the characterization of magnetic fields in mathematical terms leaves rather a lot to be desired; it would surely have been better to have made magnetic moment the basis of magnetic definitions rather than pole strength. In this introductory section it would have been a great help had there been a clear résumé of methods of measuring magnetic fields, a requirement which most workers will some time have to meet and about which they may well start off quite ignorant.

The second and third sections, dealing with the effects of strong magnetic fields, contain accounts of interesting but specialized work, such as haematological changes in mice, wound-healing and tissue regeneration in animals, magnetotropism in plants, tissue respiration, increase of bacterial growth and increase of trypsin activity. These articles make it fairly plain that the subject is as yet only in its infancy.

The fourth section, dealing with the effects of very weak magnetic fields, is probably the most interesting of all. The orientational responses of planarians and snails, the reflexes of the water-diviner and the navigational mechanism of birds are lines of investigation that suggest exciting possibilities. Further, the magnetic fields involved might be described as natural ones, and this gives them an added significance.

This is a book which workers in this field will undoubtedly wish to possess. The format leaves something to be desired, and so does the attempt to provide a unified and adequate approach to the topic of biomagnetism; nevertheless it contains much of interest and value.

D. C. SPANNER

## METAL CHELATES

### Chelating Agents and Metal Chelates

Edited by F. P. Dwyer and D. P. Mellor. Pp. xv + 530. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 121s. 6d.

THE publication of *Chelating Agents and Metal Chelates* is overshadowed by the tragic death of one of the authors—Francis P. J. Dwyer. As the inspiration

for its preparation derived mainly from him, the book provides a worthy monument to his memory. The purpose of the book is a good one, and so, in the main, is the execution of this purpose.

The book is a composite work and as a result there is some lack of co-ordination between the different parts but, on the whole, its coverage is satisfactory. It begins with a useful introductory article on "Historical Background and Fundamental Concepts" by D. P. Mellor, followed by a dissertation on the "Nature of the Metal-ligand Bond" by D. P. Craig and R. S. Nyholm. The latter chapter suffers from the lack of an adequate discussion of the ligand field theory. Such discussion seems desirable in view of the frequency with which this theory is referred to in some of the later chapters, particularly that on oxidation-reduction potentials. The introductory chapters are followed by chapters on "Bidentate Ligands" by O. M. Harris and S. E. Livingstone, and "Design and Stereochemistry of Multidentate Ligands" by H. A. Goodwin. The "Metal Chelates of Ethylene-diaminetetraacetic Acid and Related Substances" are the subject of a separate chapter by F. L. Garvan. These chapters contain much useful information on structure and stability of complexes. In a future edition it would be desirable to include a short section on the practical uses of chelating agents in analysis and sequestration.

A chapter on "Optical Phenomena in Metal Chelates", by A. M. Sargeson, describes methods of resolution of optical isomers and brings out the value of conformational analysis and measurement of optical rotatory dispersion in the determination of their structure. Sargeson also co-operates with D. A. Buckingham in an interesting discussion of the factors which determine the oxidation reduction potential of metal complexes. This is a field of considerable importance to those with biological interests and it is unfortunate that the sign convention used here is the opposite of that with which they are familiar. It is a sad commentary on the inability of chemists to agree, that two different sign conventions should be used in different parts of the same book. This appears to confuse even the authors, for on p. 255 the text and Fig. 2 disagree in respect of sign. There is other evidence of carelessness about signs (for example, in Table 4, Chapter 1, and Table 4, Chapter 6) which may confuse some of the students and many of the biologists at whom the book is aimed.

The later chapters dealing with subjects of biological interest are a little disappointing. In the preface, it is stated that the book is primarily intended as a work of reference for senior students and research workers. In this respect it should be very successful. The hope is also expressed that the book will interest biologists and medical scientists and this is less certain of fulfilment. The attempt to bridge the gap between those with biological interests and the co-ordination chemists is laudable, for metals are of great importance in biological processes and our understanding of these processes is often limited by our ignorance of the chemistry of metal complexes. The book does indeed provide a useful basis for further consideration of the biochemical functions of metals, but biologists and biochemists interested in this field will wish that the subject had been developed more effectively in this direction. Dwyer's chapter on "Enzyme-Metal Ion Activation and Catalytic Phenomena with Metal Complexes" deals very scantily with the role of metals in enzyme catalysis, a subject which has for some time been ripe for consideration by experts on the properties of metal complexes. The chapter on "Metal Chelates in Biological Systems", by Dwyer and Shulman, is largely devoted to pharmacological and bacteriostatic effects of synthetic chelates, a field of interest in itself, but one where our knowledge is too empirical to offer much scope for the inorganic chemist except in preparation of suitable chelates. The final chapter on "Physical and Co-ordination Chemistry of the Tetrapyrrole Pigments", by J. E. Falk and J. N. Phillips, provides a good summary of this topic, but again is rather

deficient in its treatment of the naturally occurring complexes—the haemoproteins, chlorophyll and vitamin B<sub>12</sub> and its derivatives.

When the time comes to prepare a new edition it is hoped that more attention will be directed to these natural chelates. In the meantime, those concerned with the writing of the book are to be congratulated on the production of a work that is well worth possessing.

G. LEAF

## A COURSE IN STATISTICAL MECHANICS

### Equilibrium Statistical Mechanics

By Prof. Frank C. Andrews. Pp. xii+206. (New York and London: John Wiley and Sons, Inc., 1964.) 38s.

### Statistical Mechanics and Dynamics

By H. Eyring, D. Henderson, B. Jones Stover and E. M. Eyring. Pp. xiii+508. (London, New York and Sydney, John Wiley and Sons, Ltd., 1964.) 113s.

### Ergodic Theory In Statistical Mechanics

By I. E. Farquhar. (Monographs in Statistical Physics and Thermodynamics, Vol. 7.) Pp. viii+235. (London and New York: Interscience Publishers, a Division of John Wiley and Sons, Ltd., 1964.) 90s.

ONE of the main aims of statistical mechanics is to obtain a more perspicuous insight into the macroscopic behaviour of matter from a knowledge of some of its microscopic properties. Conversely, we are also often able to extend our understanding of its microscopic behaviour by means of judicious comparisons between theory and experiment on the macroscopic scale. From the educational point of view, it is therefore of considerable importance to the professional scientist in many fields. Unfortunately, as Andrews points out in his preface to *Equilibrium Statistical Mechanics*, all too often there is little time in the busy undergraduate curriculum for a unified treatment of the subject, although incursions are frequently made into it from other related disciplines. In this connexion, the three books under review, although published independently, can be regarded as a course in statistical mechanics, of gradually increasing depth and scope of treatment. At a comparatively elementary level, Andrews has set himself the task of providing an introductory account of the basic theory, illustrated by a number of the more important applications, while maintaining a high degree of mathematical simplicity. On the other hand, Eyring *et al.* in *Statistical Mechanics and Dynamics* have produced a much more detailed treatment embracing a wide range of applications, including, for example, such topics as chemical kinetics. *Ergodic Theory in Statistical Mechanics*, by Farquhar, is obviously for the specialist who is interested in a more formal approach to the fundamental ideas of statistical mechanics.

Andrews approaches the problem of developing a logical introduction to the subject by first discussing the elements of probability theory, leading to the concept of the ensemble average. It is commendable that the micro-canonical, canonical and grand canonical ensembles are mentioned at an appropriately early stage in the book, although most of the treatment is in terms of the canonical ensemble. The probability distributions corresponding to the various ensembles are defined and their significance explained in a straightforward manner without recourse to the all too familiar device, involving the rather indiscriminate use of Stirling's theorem and Lagrange's undetermined multipliers, that is so often characteristic of books at this level, in which it is rarely explained with sufficient care to be meaningful (with notable exceptions, of course). Andrews then establishes a link between entropy and probability through the ensemble average

of the energy; the discussion here, although adequate, could profitably have been expanded. He then goes on, in roughly the remaining two-thirds of the book, to a number of illustrative applications, including ideal classical and quantal gases, dense gases and fluctuations. In particular, the relationship between fluctuations and the mechanical view of thermodynamics is explained in order to complete the outline picture of the subject.

While this outline represents quite a satisfactory achievement of the original limited aims, it inevitably lacks completeness in comparison with the more extensive treatment afforded by Eyring's book, to which it would nevertheless form a helpful introduction. Eyring has set a much higher target: that of describing the main basic techniques and including all the applications one could hope to see mentioned in a single volume. We are presented with three methods of approach to the fundamental theory: the technique of compounding a known and an unknown system, the classical approach of Gibbs and the Darwin-Fowler method of steepest descents, with supporting chapters on classical mechanics, thermodynamics and quantum theory, which comprise admirable summaries, in their own right, of their respective contributions to the main theme. It is indeed a luxury to have all this information so neatly assembled. Furthermore, the list of applications is impressive, ranging through specific heats, radiation, electric and magnetic properties, etc., to surface chemistry, with a concluding chapter on relaxation times. Although the treatment of each individual topic is necessarily somewhat compressed, it is still very complete and taken to quite an advanced standard, with many references. It is this completeness which enhances the value of the book, since it extends also to considerations of reaction rates, thus emphasizing, in accordance with the title, the dynamic nature of all physical processes, including equilibrium and the approach to equilibrium. From this point of view, the book is highly to be recommended.

While Eyring tends to regard statistical mechanics as better to be justified *a posteriori*, he does give a short, clear account of the difficulties associated with the ergodic and quasi-ergodic hypotheses. On the other hand, Farquhar quite definitely regards the purely pragmatical view of statistical mechanics as reducing it to the status of an *ad hoc* technique unrelated to the rest of physics. Ergodic theory is concerned with just this problem of examining the precise circumstances in which it is possible to pass from a dynamical to a thermodynamical description of a macroscopic system.

Farquhar's book is the seventh volume of a series of monographs in statistical physics and thermodynamics. It is divided into three sections, dealing in turn with the general principles of the theory and their subsequent application in both classical and quantal statistics. The approach is intended for the physicist rather than the mathematician, and includes an outline of any necessary mathematics, such as Lebesgue measure, as part of the treatment. It is limited also to a consideration of ergodic theory within classical and quantal statistics, and it does not deal with the approach of a system to equilibrium; these restrictions being in accordance with the aims of the series of which the book forms a part. Nevertheless, it constitutes a unique and lucid survey of the problem, as it relates to statistical mechanics, without in any way disguising the various points that still await elucidation. The usefulness of this kind of exercise lies particularly in the stimulation it provides towards a more critical appraisal of the foundations of the subject.

Of these three books, it is more than likely that Eyring's will be found to be the most generally useful, as a recent and most welcome addition to what are usually regarded as the standard texts for the serious student. This judgement does not detract from the contributions made by the works of Andrews and Farquhar in their respective spheres—the latter, especially, warranting the careful attention of the specialist.

F. J. PHARSON



### Bessel Functions

By Andrew Young and Alan Kirk. Part 4: Kelvin Functions. (Royal Society Mathematical Tables, Vol. 10.) Pp. xxvii+97. (Cambridge: At the University Press, 1964. Published for the Royal Society.) 60s. net.

THIS volume continues the series of tables of Bessel functions issued by the Mathematical Tables Committee of the Royal Society and its predecessor, a similar committee of the British Association for the Advancement of Science. The functions tabulated are the real and imaginary parts, and the modulus and phase, of the Bessel functions  $i^n I_n(xe^{i\pi/4})$  and  $i^{-n} K_n(xe^{i\pi/4})$  for real  $x$  and  $n$ . These occur in the solution of the diffusion equation in cylindrical co-ordinates, in the description of eddy currents in cylindrical conductors due to fluctuating magnetic fields, and other problems.

In a mathematical introduction, formulae relating to these functions are listed, the calculation of the tables is described, and a bibliography is provided. In the main tables, the functions are tabulated for  $n = 0(1)10$  and  $x = 0(0.1)10$  to 7 figures, with differences given as aids to interpolation. Basic 15-figure tables for the functions of order 0 and 1 are also given and are believed to be free of errors. There are also 8-figure tables for  $n = 0, 1, 2$  and  $x = 0(0.01)2.5$ , and auxiliary tables for the functions  $i^n x^n I_n(xe^{i\pi/4})$  and  $i^{-n} x^n K_n(xe^{i\pi/4})$ .

The arrangement and printing of these tables are excellent.

A. ERDÉLYI

### Non-Metallic Inclusions In Steel

By Roland Kießling and Nils Lange. Pp. 104. (London: The Iron and Steel Institute, 1964.) 42s.

ALL commercial steels contain non-metallic inclusions which often have important effects on the properties of the steels. Thus, from the earliest days of metallography, these inclusions have been extensively investigated, Swedish metallurgists playing a prominent part in such investigations. The most recent report by Prof. R. Kießling and Mr. N. Lange, of the Swedish Institute for Metal Research, is a fine example of Swedish research.

The investigation of inclusions has been revolutionized by the development of the technique of electron-probe analysis, which enables a quantitative analysis of the inclusions to be carried out *in situ*. The use of this technique, together with optical microscopy of the highest standard, has produced a report on inclusions which will be a standard text for all metallographers who are interested in ferrous alloys.

The authors state that a great many inclusions in steel belong to the system  $\text{MnO-SiO}_2\text{-Al}_2\text{O}_3$ , and the presentation in the report has been based on this system, but inclusions of the general formula:



and a discussion of the different iron oxides are also included. The report consists of a discussion of the various phases present in these systems illustrated by photomicrographs of hybrid inclusions. Each photomicrograph is accompanied by details of the type of steels, its analysis, the electron probe analysis of the various phases in the inclusion and comments. There are also useful introductory remarks and comments through the text.

J. H. RENDALL

### Galvanomagnetic Effects In Semiconductors

By Albert C. Beer. (Supplement 4 to Solid State Physics: Advances in Research and Applications.) Pp. xiv+418. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1963.) 96s. 6d.

THE importance of measurements of the Hall effect and electrical conductivity in determining the carrier concentration and mobility in a simple extrinsic semiconductor is universally recognized. However, the simple

band model and scattering mechanisms that were widely accepted fifteen years ago are now known to be only rarely a good approximation. It is in the more complex realistic situations that the more detailed investigations of the galvanomagnetic effects become particularly fruitful. For example, measurements of the various Hall and magneto-resistance coefficients at low magnetic fields have been used in determining the effective mass tensors in the multi-valley energy bands of several materials. Such measurements are particularly useful when the carrier mobility, even at low temperatures, is too small to allow cyclotron resonance and similar effects to be exploited. The extension of the measurements to high magnetic fields is especially useful for mixed or intrinsic semi-conductors.

Dr. A. C. Beer's book gives a really comprehensive treatment of the galvanomagnetic effects, starting from the simplest extrinsic model and progressing, for example, to multiple bands, non-quadratic energy surfaces and complex scattering processes. It is not a practical book in the sense that it does not describe techniques, but it is nevertheless most suitable for experimentalists since it sets out the theory in an exceptionally plain and straightforward manner. It is a pity that so little attention is paid by Dr. Beer to the thermomagnetic effects, since they can often be measured easily at the same time as the galvanomagnetic effects and they provide useful additional information; they are, moreover, finding practical application in low-temperature energy converters. However, having restricted himself to the galvanomagnetic phenomena, which are, of course, themselves utilized in devices ranging from magnetometers to amplifiers, Dr. Beer has produced an excellent text, well up to the highest standards of the series of which this book forms part. H. J. GOLDSMID

### The Natural Philosopher

Vol. 3. Edited by Daniel E. Garshenson and Daniel A. Greenberg, in association with Dennis Flynn. (A Series of Volumes containing Papers devoted to the History of Physics and the Influence of Physics on Human Thought and Affairs through the Ages.) Pp. 111. (New York and London: Blaisdell Publishing Company, a Division of Ginn and Company, 1964.) 15s.

THIS little manual deals, in four chapters, with a variety of themes of interest in the history and evolution of physics. Its particular merit is that the subjects selected are off the beaten track, and illuminate some unusual recesses of the mind. The first contribution sets Einstein well in the centre of the stage during the period of the wave-particle discussion. We see an aspect of quantum mechanics too often over-simplified, namely the properties of radiation and gases, with special reference to the Bose statistics. This happened in 1924 when Planck's radiation law was derived, not from classical electromagnetic theory as hitherto, but directly from light quanta; in the event, the prelude to Schrödinger's work.

The next paper deals with that somewhat obscure Swiss mathematician Le Sage (1724-1803), who evolved an ingenious theory of gravitation of a strictly mechanical kind, comprising his 'ultramundane corpuscles'. At the time there was no experimental evidence in support of such a system, but about a hundred years later the matter was taken up again, only to be proved untenable by Clerk Maxwell and Poincaré.

Next comes a useful table of references to Faraday's papers on electricity, with explanations and notes.

Finally, the reader is given a very clear account of the Eleatic School of Greek philosophy, and its impact on physics. In effect, this was the fundamental role of logic, even if it contradicted experience. Time was to correct this, without destroying the quest for vigour. We also owe to this school the beginnings of metaphysics, in the recognition of various levels of being, an accomplishment essentially due to Parmenides. F. I. G. RAWLINS



## THE NATURAL ENVIRONMENT RESEARCH COUNCIL

By SIR GRAHAM SUTTON, C.B.E., F.R.S.

Chairman

THE Natural Environment Research Council came into being legally on June 1, 1965. Its formation was the outcome of a long process of consideration. Such a Research Council was first envisaged by the Slater Committee, constituted by the Advisory Council for Scientific Policy, and later, in a somewhat different form, by the Committee on the Organization of Civil Science under Sir Burke Trend. The statutory authority for the creation of the Natural Environment Research Council is contained in the Science and Technology Act, which received the Royal Assent at the end of March 1965.

The new Council is one of four for which the Secretary of State for Education and Science is responsible, the others being the old-established Medical and Agricultural Research Councils and the newly formed Science Research Council. In broad terms, the Natural Environment Research Council is concerned with those sciences, both physical and biological, that relate to man's natural environment. Specifically, to quote its Royal Charter, it is established "to encourage and support research by any person or body in the Earth sciences and ecology and in particular (but without prejudice to the foregoing) in geology, meteorology, seismology, geomagnetism, hydrology, oceanography, forestry, nature conservation, fisheries or marine and freshwater biology". In addition to carrying out research in these subjects by direct control of certain governmental establishments and by support for others, it is also authorized to make grants for post-graduate instruction.

The Council is thus a 'federal' organization, with a number of 'component bodies', that also provides support for certain independent institutions, for example, in marine and freshwater biology. The component bodies, which are directly controlled by the Council, in the past have been within various governmental departments or else had a separate Charter. They are: the Nature Conservancy, the Home and Overseas Geological Surveys (including the Geological Museums), the National Institute of Oceanography and the Hydrology Research Unit. The Council has also taken over the Development Commission's responsibilities for fisheries research as well as policy direction and financial support for research in geophysics (geomagnetism and seismology) carried out at the Observatories of the Meteorological Office. It will also be associated with the general programme of research of the Meteorological Office and, in consultation with the Forestry Commission, will support long-term forestry research in the universities.

The Royal Charter of the Natural Environment Research Council lays down that the Council shall consist of a chairman and not more than fifteen and not less than ten members. The Secretary of State for Education and Science, with the advice of the Royal Society and interested governmental departments, has appointed the following for the initial phase: Sir Graham Sutton (chairman), Lord Howick, Mr. F. C. Bawden, Sir Edward Bullard, Prof. A. R. Clapham, Prof. F. K. Hare, Prof. J. E. Harris, Prof. M. V. Laurie, Prof. M. J. Lighthill, Prof. O. J. Mitcheson, Mr. N. A. F. Rowntree, Prof. S. K. Runcorn, Prof. J. H. Taylor, Prof. V. O. Wynne-Edwards and Dr. O. M. Yonge. The secretary of the Council, who is also its accounting officer, is Mr. R. J. H. Beverton, formerly deputy director of the Ministry of Agriculture, Fisheries and Food's Fisheries Laboratory at Lowestoft. The Headquarters are at State House, High Holborn, London, W.C.1.

At its first meeting on June 30, 1965, the Council set up a number of standing committees. The Charter stipulates that one committee shall be the Nature Conservancy with its present membership. The chairman of this committee is Lord Howick. The other committees are: the Geology and Geophysics Committee (chairman, Prof. Mitcheson), the Oceanography and Fisheries Committee (chairman, Prof. Lighthill, with Prof. Harris as deputy chairman) and the Hydrology Committee (chairman, Mr. Rowntree). The committees, in addition to supervising the work of their respective component bodies, will also be responsible to the Council for recommending research grants, training awards and fellowships in their fields.

The interests of the new Council are thus large and diverse, embracing both physics and biology. The degree of control, however, varies over the field of natural environmental science. The main effort in meteorological research in the United Kingdom comes from the Meteorological Office, which will remain with the Ministry of Defence, as recommended by the Trend Committee. However, as meteorology by its very nature must enter into many parts of environmental science (particularly in hydrology, nature conservation and oceanography) it is clear that the association between the Natural Environment Research Council and the research work of the Meteorological Office must be close. Some aspects of meteorological research, for example those relating to professional activities such as weather forecasting, will continue to be the exclusive concern of the Meteorological Research Committee, but in others, notably climatology and rainfall studies, it will be essential to set up close links. In geophysics there are some difficulties arising as a heritage of the past. Routine measurements of the Earth's magnetic field are made at the Eskdalemuir and Lerwick Observatories of the Meteorological Office and at Hartland Point, which comes under the Royal Greenwich Observatory and is therefore with the Science Research Council. Regular seismological observations will continue at Eskdalemuir under the Meteorological Office, but the programme of research in both these disciplines will be directed by the Natural Environment Research Council. There is a certain amount of administrative untidiness in these arrangements, but this is not expected to have any serious consequences for the research programmes. In other fields the Council should be able to exercise a much-needed integrating function. The formation of one committee to deal with physical oceanography, marine biology and fisheries should be valuable for the development of marine science, and the combination of geology and geophysics, with the amalgamated Home and Overseas Geological Surveys as the component body, illustrates the desire for close union of service and academic interests in this field. In hydrology, there is a clear need for pioneering work to establish a programme of scientific work designed to help the users of water.

The Council recognizes that one of its most important functions will be to provide funds for university research and grants and training awards in the disciplines that come under its control. Hitherto, the administration of this programme was with the Department of Scientific and Industrial Research. Many of the permanent staff that dealt with these matters are now with the Science Research Council; others will be transferred to the Natural Environment Research Council. It is essential that the change-over be as smooth as possible, and it is

a fortunate circumstance that it has proved possible to accommodate the headquarters of both Councils in the same building. For the time being, the machinery of administration will be much the same as hitherto, with the Natural Environment Research Council taking over

responsibility for the parts of the grants and awards programme that lie within its field. The emphasis on continuity should be particularly welcomed by universities and others concerned with this important aspect of the Government's policy for science.

## A PHYSICAL BASIS FOR LIFE DETECTION EXPERIMENTS

By Dr. J. E. LOVELOCK

Bowerchalke, Nr. Salisbury, Wiltshire

THE design of an efficient and unequivocal experiment in extra-terrestrial life detection should take into account: (1) A definition of life stated in terms favourable for its recognition. (2) A description of the past and present environment of the planet to be sampled.

As yet, there is no formal physical statement to describe life from which an exclusive definition for experimental purposes could be drawn. Moreover no comprehensive description is available of the atmospheric as well as the surface physical and chemical environment of any of the planetary bodies.

It is not surprising, in view of the vast expense of space-probe experiments and of the formidable uncertainties already stated here, that the proposed experiments in life detection all ask the cautious geocentric question: "Is there life as we know it?" Most certainly it is difficult to envisage in detail an alien biochemistry; it would seem pointless and very uneconomic to send a space probe to detect a speculative life-form.

It is the object of this article to show that we are not necessarily limited to experiments based on the recognition of a specific life-form, either Earth-like or alien. Also, that it is possible, by accepting a limited phenomenological definition of life, to design simple experiments from the general recognition of life phenomena, including that with which we are familiar. The application of this approach to experiments in life detection is the basis of the discussion which follows.

### Recognition of Life

It is a relatively simple matter to distinguish between living and inorganic matter on Earth by biochemical experiments even though no formal definition of life in biochemical terms exists. Experience suggests, for example, that a system capable of converting water, atmospheric nitrogen and carbon dioxide into protein, using light as a source of energy, is unlikely to be inorganic. This approach for recognition of life by phenomenology is the basis of the experiments in detection of life so far proposed. Its weakness lies not in the lack of a formal definition but in the assumption that all life has a common biochemical ancestry.

It is also possible to distinguish living from inorganic matter by physical experiments. For example, an examination of the motion of a salmon swimming upstream suggests a degree of purpose inconsistent with a random inorganic process. The physical approach to recognition of life is no more rigorous, at this stage, than is the biochemical one; it is, however, universal in application and not subject to the local constraints which may have set the biochemical pattern of life on Earth.

Past discussions of the physical basis of life<sup>1-3</sup> reach an agreed classification as follows: "Life is one member of the class of phenomena which are open or continuous reaction systems able to decrease their entropy at the expense of substances or energy taken in from the environment and subsequently rejected in a degraded form". This classification is broad and includes also phenomena such as flames, vortex motion and many others. Life

differs from the other phenomena so classified in its singularity, persistence, and in the size of the entropy decrease associated with it. Vortices appear spontaneously but soon vanish; the entropy decrease associated with the formation of a vortex is small compared with energy flux. Life does not easily form, but persists indefinitely and vastly modifies its environment. The spontaneous generation of life, according to recent calculations from quantum mechanics<sup>4,5</sup>, is extremely improbable. This is relevant to the present discussion through the implication that wherever life exists its biochemical form will be strongly determined by the initiating event. This in turn could vary with the planetary environment at the time of initiation.

On the basis of the physical phenomenology already mentioned, a planet bearing life is distinguishable from a sterile one as follows: (1) The omnipresence of intense orderliness and of structures and of events utterly improbable on a basis of thermodynamic equilibrium. (2) Extreme departures from an inorganic steady-state equilibrium of chemical potential.

This orderliness and chemical disequilibrium would to a diminished but still recognizable extent be expected to penetrate into the planetary surface and its past history as fossils and as rocks of biological origin.

### Experiments for Detection of Life

The distinguishing features of a life-bearing planet, described here, suggest the following simple experiments in detection of life:

(A) *Search for order.* (1) Order in chemical structures and sequences of structure. A simple gas chromatograph or a combined gas chromatograph-mass spectrometer instrument would seek ordered molecular sequences as well as chemical identities.

(2) Order in molecular weight distributions. Polymers of biological origin have sharply defined molecular weights, polymers of inorganic origin do not. A simple apparatus to seek ordered molecular weight distributions in soil has not yet been proposed but seems worthy of consideration.

(3) Looking and listening for order. A simple microphone is already proposed for other (meteorological) purposes on future planetary probes; this could also listen for ordered sequences of sound the presence of which would be strongly indicative of life. At the present stage of technical development a visual search is probably too complex; it is nevertheless the most rapid and effective method of life recognition in terms of orderliness outside the bounds of random assembly.

(B) *Search for non-equilibrium.* (1) Chemical disequilibrium sought by a differential thermal analysis (DTA) apparatus. Two equal samples of the planetary surface would be heated in a DTA apparatus: one sample in the atmosphere of the planet, the other in an inert gas, such as argon. An exotherm on the differential signal between the two samples would indicate a reaction between the surface and its atmosphere, a condition most unlikely to be encountered where there is chemical equilibrium as in the absence of life. It should be noted

that this method would recognize reoxidizing life on a planet with a reducing atmosphere. This experiment could with advantage and economy be combined with, for example, the gas chromatography mass spectrometry experiment (A1) where it is necessary to heat the sample for vaporization and pyrolysis.

(2) Atmospheric analysis. Search for the presence of compounds in the planet's atmosphere which are incompatible on a long-term basis. For example, oxygen and hydrocarbons co-exist in the Earth's atmosphere.

(3) Physical non-equilibrium. A simplified visual search apparatus programmed to recognize objects in non-random motion. A more complex assembly could recognize objects in metastable equilibrium with the gravitational field of the planet. Much of the plant life on Earth falls into this category.

Experiments A1, B1 and B2 are the most promising for the development of practical instruments. Indeed, the gas chromatography-mass spectrometry combination experiment and the DTA experiment already proposed for planetary probes<sup>7</sup> are, with minor modifications, capable of recognizing the ordered sequences and chemical disequilibrium discussed earlier. Experiment B2, atmospheric analysis, is simple and practical as well as important in the general problem of detection of life. A detailed and accurate knowledge of the composition of the planetary atmosphere can directly indicate the presence of life in terms of chemical disequilibrium; such knowledge also is complementary to the understanding of other life detection experiments and to the planning of subsequent experiments. Even on Earth where life is abundant there are many regions, such as those covered by fresh snow, where a surface sample might be unrewarding in the search for life. The atmospheric composition is largely independent of the site of sampling and provides an averaged value representative of the steady state of chemical potential for the whole planetary surface.

Fig. 1 shows the abundance of hydrocarbons of carbon number between 11 and 33 for abiogenic hydrocarbons of the Fischer-Tropsch process<sup>8</sup> and hydrocarbons of biological

origin, wool wax<sup>9</sup>. Poisson distributions around the predominant hydrocarbon numbers are shown as solid lines. The inorganic hydrocarbons fit closely the expected Poisson distribution for a state of chemical equilibrium. By contrast the biological hydrocarbons show large departures in the distribution of their abundance from this equilibrium state; also, especially for the higher molecular weight alkanes, a two-carbon ordered sequence is well established.

In a similar manner with experiment B1 the disequilibrium associated with life can be demonstrated. A few mg of soil heated in a DTA apparatus in air shows a large exotherm when compared with a similar reference sample heated in argon. The combustion of even a few micrograms of organic matter in these circumstances is capable of generating a detectable signal.

### Detection of Life on Mars

Ordinarily one does not look for fish in a desert, nor for cacti on an ice cap. Should we, therefore, look for microorganisms of Earth-like habits on Mars, or should we rather ask one or more of the general questions discussed here? The answer to this must depend on the history of Mars, past and present.

The following is the only sure information so far available on Mars. It is dry. The atmosphere is thin and contains no more than a trace of oxygen. The flux of solar radiation at the surface, although less than on Earth, is also less filtered and may include an appreciable content of energetic radiation; in particular, short wave-length ultra-violet. The temperature range includes periods above zero centigrade. Finally, but less certain, is the possibility<sup>4</sup> that oxides of nitrogen are present in appreciable amounts.

If these conditions are representative of Mars in the past as well as now, there seems no reason to assume that life, if present at all, can resemble that on Earth. However, it is possible that Mars was once Earth-like (primeval non-living Earth) and has changed physically to its present state; or less likely that the present state of Mars, like that of the Earth, is a consequence of biological change. For success a geocentric biochemical experiment must assume that Mars was once Earth-like and that life is still surviving in a highly adapted form yet still recognizable to the experiment.

If Mars is as it always was or has been changed to its present state by biological action, then life, if there now, would be very different from that we know. Could we conceive of living systems in liquid  $N_2O_4$  as an ionizing solvent? Could they use hard ultra-violet as a source of energy? Is cellular life necessary in a dry environment, or did cell membranes evolve on Earth to offset the overwhelming effects of dilution in the primeval seas? What sort of Martian biochemistry could have generated the present atmosphere?

Answers to these questions are important in the design of experiments to detect particular life-forms; thus, with a growth experiment, or a biochemical experiment, the strength, composition and conditions of incubation of the medium are of vital importance. This information, however, is not needed in the general detection of life. In view of what is not known of conditions on Mars, the physicochemical experiments in life recognition such as experiments A1 and B1 and B2 seem more worth considering for early probe experiments. These simple experiments do not require a prior knowledge of the planetary environment and are not limited to Earth biochemistry.

I thank A. Zlatkis and P. G. Simmonds of the University of Houston for their advice and for conducting and providing me with the results of the differential thermal analysis experiments suggested in the discussions here. I also thank G. Hobby and G. Mamiknian of the Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California, for their advice.

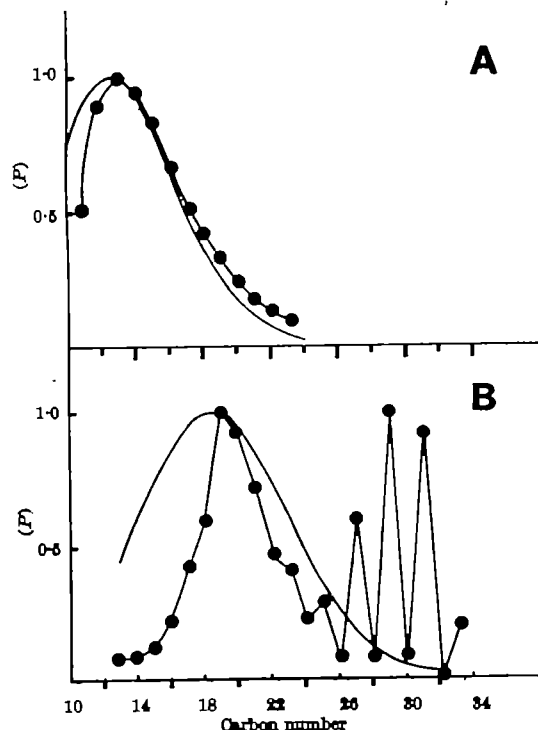


Fig. 1. The abundance of *n*-alkanes from an inorganic source (A), Fischer-Tropsch hydrocarbons, and from a biological source (B), wool wax. The observed abundances (●—●) are compared with normalised Poisson distributions (—) around the predominant alkanes.

This work was supported by a grant from the National Aeronautics and Space Administration (NSG 199-82, J. E. Lovelock).

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## BIRTH-DEATH AND OTHER MODELS FOR MICROBIAL INFECTION

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SHORTLEY and Wilkins<sup>1</sup> have recently analysed certain experimental data derived from microbial infections. They first consider the relation observed between size of dose and the proportion of hosts responding, and draw a conclusion of considerable biological interest, namely, that host resistance is often discontinuously distributed, with the hosts falling into two homogeneous groups. In our opinion these authors lay undue stress on the two-group distribution, which we believe to be supported by very few sets of data. Secondly, Shortley and Wilkins find strong evidence in favour of a simple birth-death model of infection. Our examination of this type of model shows that although its predictions, qualitatively considered, are in remarkable agreement with the data, a detailed analysis encounters serious difficulties.

In most experimental infections a given host is unlikely to respond to inoculation of a single organism, and this finding has been accounted for by two main types of model, originally distinguished by Halvorson<sup>2</sup> in 1935. The first is derived from the pharmacological work of Trevan<sup>3</sup> and postulates an individual effective dose (IED) for each host such that it will certainly respond to a dose equalling or exceeding its IED; the hypothesis is, therefore, deterministic in nature. Inoculated organisms can be thought of as co-operating to 'saturate' the host defences. In the alternative class of model, however, inoculated organisms are assumed not to co-operate but to act independently—hence the designation 'hypothesis of independent action'<sup>4</sup>—and each organism is assumed to have an independent probability,  $p$ , of multiplying sufficiently to produce a response: all hypotheses based on this assumption are, therefore, stochastic. Whenever inoculation of one organism is not invariably followed by a response, the value of  $p$  is clearly less than unity ( $<1$ ). The reasons suggested for this serve to subdivide stochastic hypotheses:

(1) The culture from which inocula are drawn might consist of a mixture of completely virulent and completely avirulent organisms (for which  $p=1$  and 0, respectively) so that the value of  $p$  is the proportion of virulent organisms in the culture. This is unlikely in most systems<sup>5</sup>.

(2) All the organisms in the culture might be fully virulent but each of the sites they reach after inoculation might be either completely favourable or completely lethal to them. This only seems likely when inocula are applied to the external surface of the host, as by ingestion<sup>6</sup> or inhalation<sup>7</sup>, when  $p$  is the probability per inoculated organism of reaching the interior. Both these possibilities presuppose that the outcome is decided once the organisms reach their final sites of deposition, presumably within a short time of inoculation.

(3) In contrast, the birth-death model assumes that the outcome is determined by successive random events which continually operate as long as even one organism remains viable in its host. Thus, in each small interval of time  $dt$  after inoculation, each organism still extant has a probability  $\lambda dt$  of dividing, a probability  $\mu dt$  of dying, and a probability  $1 - (\lambda + \mu)dt$  of doing neither. When  $\mu > \lambda$ , the host must invariably survive if the dose is substantially less than the final number of organisms causing the response ( $C$  in ref. 8), because each clone (that is, each inoculated organism and its progeny) will be extinguished before it can increase to any extent. The model therefore postulates  $\lambda > \mu$  with  $p = 1 - (\mu/\lambda)$  for infections leading to a response. The increasing probability of response with increase in size of dose can here be thought of as reflecting the chance that one or more clones will be 'lucky' enough to increase to  $C$ . Processes of this sort are considered in many branches of science<sup>9</sup> and some of the underlying mathematical arguments are far from novel. For example, equation 14 in Shortley and Wilkins<sup>1</sup>,  $ED_{50} = 0.69 / \{1 - (\mu/\lambda)\}$ , follows immediately from the probability of extinction in a birth-death process derived by Bartlett in 1946 (ref. 10).

Many attempts have been made to establish the model that best describes microbial infection. The deterministic hypothesis of the IED has been objected to<sup>4,11</sup>, because, in systems where the  $ED_{50}$  is relatively small (for example, 10<sup>3</sup>), it has always been hard to see how such a dose could 'saturate' the host. However, viable counts show that, whatever the outcome of inoculation, the number of organisms per host usually increases exponentially at first<sup>12,13</sup>, perhaps for several days, and that only afterwards do the counts on non-responders decline while those for responders continue to rise to  $C$ . Consequently it could be argued that the number of organisms determining the outcome is not that inoculated but the larger number present when the growth curves for responders and non-responders diverge. It may be noted, incidentally, that growth curves on responders certainly do not appear to originate from a proportion  $p$  of the dose, as might occur if infection complied with possibilities 1 or 2 already described here. On the contrary, many growth curves rise from the whole dose, whatever the outcome and whatever the value of  $p$ <sup>12,13</sup>.

Birth-death models are very attractive, since they have far greater predictive power than deterministic models. Given the values of  $p$  and  $C$ , and assuming that the hosts do not differ in resistance, the basic birth-death model developed by Williams<sup>14</sup> predicts many quantitative features of an infection, of which those marked by asterisks below also follow from possibilities 1 and 2. They include

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an exponential relation  $P = 1 - e^{-d}$ , between dose,  $d$ , and proportion of hosts responding,  $P$ ; that  $P$  will be unaffected by splitting a total of  $d$  organisms into several smaller doses  $d_i$ ; a mean response time,  $\bar{T}$ , tending to constancy\* for  $d$  less than the  $LED_{50}$ , with  $\bar{T}$  inversely related to  $\log d$  for values of  $d$  exceeding the  $LED_{50}$ ; the variance of  $\bar{T}$ ; the median and variance of the viable counts for a given dose at different times after inoculation; the probability that a given clone has been extinguished by a given time after inoculation; and the probability for a given dose that  $O$  is largely made up of 1, 2, ... or  $d$  clones\*. The last prediction is often phrased as the probability that a host given a dose of known size will respond owing to the multiplication of 1, 2, ... or  $d$  inoculated organisms. The model can be elaborated to include heterogeneity in host resistance, whose degree may be estimated independently by each type of observation such as viable counts or response times. Moreover, the arguments can be used in reverse to estimate  $p$ ,  $O$ ,  $\lambda$ , and  $\mu$  from epidemiological data like the distribution of incubation periods in epidemics of typhoid and streptococcal sore throat<sup>15</sup>. Some of the predictions have long been used to determine the applicability of stochastic models, two examples being dose-response curves<sup>16</sup> and responses to single clones<sup>17</sup>, first discussed in 1931 and 1934 respectively. The hypothesis of the IED makes only one prediction: that the slope of the dose-quantal response curve is infinite when the hosts are uniform in resistance.

The outcome of previous investigations has tended to favour a stochastic mechanism. The slopes of dose-quantal response curves are almost never greater than predicted, whereas co-operation is consistent with any slope up to infinity, and high values do in fact occur in titrations of toxic chemicals and killed organisms<sup>4,11</sup>. Subdivision of the total dose does not affect per cent response<sup>18</sup>. The mean response time appears to tend to constancy at  $d < LED_{50}$  (refs. 12 and 19), and otherwise to vary inversely with  $\log d$ <sup>12,13</sup>. Most hosts that respond to doses  $< LED_{50}$  of a mixture of distinguishable variants of the same pathogen are expected to contain preponderantly 1 clone, but the results do not usually conform to prediction because the organisms appear to interact at some stage during infection<sup>4,13</sup>. Nevertheless, inoculated organisms evidently behave independently in respect of their ability to form discrete visible foci in internal organs, as shown by the number of foci being proportional to the dose<sup>7</sup> and by each focus containing only 1 clone, although a mixture of distinguishable clones is inoculated<sup>4,11</sup>. A finding inexplicable on stochastic possibilities 1 and 2 is that the course of fatal infections in mice given *Salmonella typhimurium* by intraperitoneal injection is apparently determined by the whole dose,  $d$ , and not by an 'effective fraction',  $pd$ <sup>13</sup>. It is now clear that this is to be expected if the infection conforms to a birth-death process<sup>14</sup>.

The distinction between deterministic and birth-death mechanisms can be made in at least three other ways:

(1) By measuring the value of  $\mu$ . This can be done directly in bacterial infections by obtaining  $\lambda - \mu$  from colony counts and by measuring  $\lambda$  alone by the super-infecting phage technique<sup>20</sup>. The birth-death model requires  $\lambda > \mu > 0$  throughout the infection, whereas  $\mu$  might be zero on a deterministic model when the viable count increases. Such measurements would also test a particularly suspect prediction of the birth-death model which follows from  $p = 1 - (\mu/\lambda)$ : that large changes in  $LED_{50}$  can result from minute changes in  $\lambda$  or  $\mu$ .

(2) By determining the growth curves for non-responders given doses differing in size. The birth-death model predicts that the smaller the dose, the sooner will the median viable count fall, whereas on a deterministic model postulating that the decisive event occurred some time after inoculation, the curves might be identical whatever the dose. Mice have been given 0.03 or 0.000075  $LD_{50}$  of *Salmonella paratyphi* B by intraperitoneal injection

( $1LD_{50} = 10^7$  organisms), but there was no appreciable difference between the two sets of colony counts.

(3) By testing all the data from an experiment for internal consistency. For example, it would be inconsistent on any model to postulate small differences in host resistance to account for a dose-mortality curve when viable counts showed the differences to be gross. We have analysed the data of Meynell and Meynell<sup>12</sup> in the light of the birth-death model<sup>14</sup>, and find considerable discrepancies between observation and prediction, as follows.

(a) The dose-mortality curves flatten out too much at small doses. This is explicable either by a proportion of highly susceptible mice (suggested by Shortley and Wilkins<sup>1</sup>) or by an important cause of mortality independent of the bacterial infection.

(b) The estimates of  $\lambda$  obtained from  $p = 1 - (\mu/\lambda)$  and from the net bacterial growth rate  $\lambda - \mu$  are ridiculously high, as mentioned by Shortley and Wilkins<sup>1</sup>, and correspond to doubling times between 8 and 0.02 sec.

(c) At doses not leading to a response the viable counts show a more consistent upward trend with time than predicted.

(d) The observed variance of viable counts is too high at high doses but too low at low doses.

(e) The logarithmic variance of viable count does not increase with time as would be expected if the variability of  $\lambda - \mu$  was important.

These discrepancies can only be explained by radical changes in the model. The most striking anomaly is (b). Shortley and Wilkins<sup>1</sup> appear to favour an explanation resembling stochastic possibilities 1 and 2 by suggesting that most of the dose is rapidly killed, and the survivors are subject to a birth-death process. However, many viable counts<sup>12</sup> provide no support for this explanation.

Further difficulties arise in analysing epidemics, where the dose cannot be measured. The basic birth-death model<sup>14</sup> does not then permit the separate estimation of all the parameters, but this becomes possible by relaxing the assumption that  $O$  is effectively infinite. The resulting estimates of  $\lambda$  are suspiciously small, with estimated doubling times of the order of 100 times longer than the *in vitro* values<sup>12</sup>. This is in striking contrast to the opposite effect from experimental infections, noted in (b) above.

The experimental evidence considered in detail is therefore by no means wholly in favour of any form of stochastic mechanism, despite the fact that the overall picture provided by the basic birth-death model corresponds remarkably well to what is found in practice. The strongest observation in support of some form of stochastic mechanism is the relatively small slope of dose-quantal response curves in infections as compared to those observed in response to toxic agents<sup>11</sup>. Even this could be accounted for on a deterministic model by arguing that host variation in response to infection would necessarily be larger than to a toxin because the outcome of inoculation is undecided as long as any organisms remain viable in the host, often a period of weeks, so offering greater scope for fluctuation in host resistance which might, moreover, depend on many host functions, each fluctuating independently.

Whichever model is considered, the distribution of host resistance, if allowed for, is usually assumed to be unimodal, whereas Shortley and Wilkins<sup>1</sup> conclude from their examination of reported dose-quantal response curves in terms of a stochastic mechanism that resistance is often distributed into two almost homogeneous groups. However, it should be noted that the compound exponential curve then predicted by all stochastic models is very insensitive to variability in  $p$  (ref. 23) and does not enable the precise form of variability to be easily determined. In an earlier examination of the two-point distribution, Armitage<sup>24</sup> said "the purpose of this discussion is merely to show that some sets of data can be fitted by a two-point distribution and that a reasonably good fit can be made fairly quickly". With a two-point distribution, there are



three parameters (the two values of  $p$ , and the proportion of hosts of each type) to be adjusted at will, so a good fit is likely to be obtained to almost any set of rather imprecise data like those obtained from infectivity titrations<sup>11</sup>. It is dangerous to assert too dogmatically that a two-point distribution of  $p$  is implied by observed curves unless a variety of other distributions has been examined and found wanting. In our view, Shortley and Wilkins<sup>1</sup> do not provide adequate support for their extensive fitting of two-point distributions to dose-response data, and, indeed, they show that one set approximates well to an integrated log-normal distribution with large variance (their Fig. 8), such as would be consistent with a very heterogeneous population of hosts the resistances of which were distributed unimodally.

A bimodal, or indeed any irregular distribution of host resistances, would also reveal itself in the distributions of individual response times to a given dose, provided the two groups of hosts did not differ so much that the doses produced responses in only the more susceptible group. Such distributions are known in mice infected by pneumococci<sup>12,13</sup> or tubercle bacilli<sup>14</sup> and possibly in infection by tumour viruses<sup>15,17</sup>, but some examples arise from trivial causes like non-specific death following inoculation<sup>16</sup>, inadequate chemotherapy<sup>18</sup>, cross-infection<sup>19</sup>, and errors of calculation<sup>6</sup>. Convincing examples are difficult to find, and, in most infections, the logarithms of individual response times appear to be unimodally distributed, often approximately normally<sup>20,21,22,23</sup> with a variance independent of dose<sup>24,25</sup>. This suggests that not only is host resistance unimodally distributed, but also that the response times are not influenced by partial immunity acquired during the infection. One would suppose that the anti-microbial mechanisms responsible for acquired immunity begin to act more efficiently at some time after inoculation. Those members of a dose-group which responded before this time would, therefore, be in their initial state, whereas the course of infection in those which responded later would be modified, for example, if the net microbial growth fell so that each response took longer to occur than would otherwise have been the case (see Fig. 1c in ref. 8). In other words, the hosts, though homogeneous in resistance initially, would become heterogeneous during the experiment. In the few systems in which this appears to occur<sup>26,27</sup> the distributions of individual log response times have greater variance and skewness at small doses than at large doses, which implies that the longer response times associated with small doses give anti-microbial mechanisms a greater chance to take effect. The fact that

the majority of observed distributions are not of this kind and, indeed, appear to be the same for all doses might mean that antimicrobial mechanisms entirely fail to develop in any individual that responds. The other extreme is that these mechanisms develop in each individual but are either completely ineffectual, so that the infection progresses undisturbed, or completely effective and prevent the response. That is, the mechanisms underlying acquired immunity may generally exert an all-or-none effect on the infection which provokes them.

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## SPECTRAL DATA FROM THE COSMIC X-RAY SOURCES IN SCORPIUS AND NEAR THE GALACTIC CENTRE

By R. GIACCONI, H. GURSKY and J. R. WATERS

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**D**URING two rocket flights conducted from White Sands Missile Range on August 28, 1964 (flight I), and October 28, 1964 (flight II), we have obtained information regarding the spectral composition of the X-ray source in Scorpius (ScoX-1) and those along the Galactic equator in the vicinity of the Galactic centre. The results were obtained from two separate detectors and extend over the spectral region 1–25 keV. We find that the radiation from ScoX-1 extends from 15 keV to at least 2.5 keV, and that the spectral distribution is not consistent with black-body radiation. The radiation from the region along the Galactic centre extends to about 25 keV. The latter region apparently contains several distinct X-ray sources as reported by Giacconi *et al.*<sup>1</sup>, Bowyer *et al.*<sup>2</sup> and Fisher *et al.*<sup>3</sup>.

Since the discovery of cosmic X-ray sources by Giacconi *et al.*<sup>4</sup> in 1962, only gross information regarding the spectral composition of the radiation has been reported. In the same paper an effective wave-length near 3 Å was reported based on atmospheric attenuation and the difference in counting rate in two separate Geiger counters. Giacconi *et al.*<sup>5</sup> afterwards reported that the spectrum was consistent with a black-body temperature of about 10<sup>7</sup>°K. Bowyer *et al.*<sup>6</sup> attempted to measure the spectral composition of radiation from the Crab Nebula between 1 and 10 keV by making observations in two independent Geiger counters. Later, they published<sup>7</sup> a critical evaluation of their experiment which showed that one of the counters did not function as expected. In the same paper they presented results on the atmospheric attenuation

of X-radiation from the Scorpius source which was consistent with a black-body temperature of  $2-3 \times 10^6$  °K. In another experiment X-radiation from the Crab has been observed between 20 and 60 keV by Clark<sup>7</sup> using balloon-borne instrumentation.

Discussions of the source mechanism for the generation of the observed X-rays has centred on three possibilities: (1) black-body radiation from a neutron star; (2) radiation from an optically thin hot plasma; (3) synchrotron radiation from high-energy electrons in a weak magnetic field. Specific literature citations appear in refs. 5 and 7 cited here and in a review article by Giacconi and Gursky<sup>8</sup>.

We are reporting here spectral data obtained by placing absorbers in front of the Geiger counters during flight I and by performing a pulse height analysis of the output of a sodium iodide (NaI) scintillation counter during flight II. The several sources along the Galactic equator could not be resolved with these counters and all contribute to the spectral data from that region. Other results from these rocket flights have been reported by Giacconi *et al.*<sup>9</sup>, which demonstrated the separation between ScoX-1 and the sources near the Galactic centre, and by Oda *et al.*<sup>10</sup>, which presented the results of the measurement of the angular diameter of ScoX-1.

The Geiger counter detector used during flight I of this experiment was a bank of twelve individual argon-filled counters with beryllium windows of 0.0 mg/cm<sup>2</sup> thickness and a total sensitive area of about 70 cm<sup>2</sup>. The efficiency, calculated as the product of the absorption in the gas filling and the transmission of the window, lies above 10 per cent between 17 keV and 1.2 keV and peaks at between 3 and 4 keV. This computed efficiency was checked experimentally by exposing counters to the beam from a tungsten target, windowless X-ray tube operated from 1.8 to 10 kV. The observed counting rates were in agreement over the entire range of voltage with those predicted using thick target yield curves and the computed counter efficiency.

The Geiger counters were equipped with rectangular collimators which limited the field of view to 15° full width at half maximum (FWHM) in the direction of rotation of the rocket and 20° FWHM in the direction of the rocket long axis. The counter bank was mounted so that its axis of maximum sensitivity made an angle of 80° with respect to the long axis. Two filters, F1 of 7.04 mg/cm<sup>2</sup> of beryllium and F2 of 1.72 mg/cm<sup>2</sup> of mylar, were placed in front of the detector and were sequentially removed during the flight to allow measurements for approximately equal time-intervals of the X-ray fluxes with F1 and F2 in place, F1 alone in place, and no filter.

The motion of the rocket during this flight (flight I) consisted of a rapid rotation with a period of 0.55 sec about the long axis plus a slow precession of the rotation axis with a period of 83 sec along a cone of a small opening angle. The X-ray source regions were observed during each rotation but, because of the low counting rates, data from successive rotations were added in order to obtain sufficient statistical precision.

During each rotation the detector swept out a band on the celestial sphere the width of which, 20°, equalled that of the collimator. The precession results in an approximately sinusoidal variation of the angle of closest approach of a given celestial object to the centre of this band. During each precession cycle the centre of the band came within 2° of ScoX-1 and moved along the Galactic equator between  $l_{II} = 2^\circ$  to  $l_{II} = 27^\circ$ .

Three full precession cycles were recorded during the rocket flight, and since a filter change occurred almost in phase with the precession we have essentially a complete precession cycle of data for each of the three filter conditions. The data from the precession cycle with no filter in place have been the subject of a previous paper<sup>6</sup>. The counting rates with background subtracted observed

Table 1. COUNTING RATE OBSERVED IN THE TWO-SOURCE REGIONS WITH AND WITHOUT FILTERS

Filter condition	Source region	Counting rate measured c.p.m.	Attenuation relative to no filter
No filter	ScoX-1	620 ± 20	1
F1	ScoX-1	440 ± 18	0.71 ± 0.04
F1 + F2	ScoX-1	350 ± 17	0.56 ± 0.03
No filter	Gal cen	350 ± 18	1
F1	Gal cen	240 ± 18	0.70 ± 0.06
F1 + F2	Gal cen	210 ± 15	0.62 ± 0.05
Typical background rate		30 c.p.m.	

with the three filter conditions when the detector crossed ScoX-1 and crossed the region along the Galactic equator are listed in Table 1. The counting rates represent an average over the same fraction of the precession cycle for the three cases. Listed also is the attenuation, defined as the ratio of the counting rates for the two filter combinations compared with no filter.

The significance of the attenuation factor can be partially understood from the following arguments. Photons at 2.5 keV will give the observed attenuation of about 0.7 per filter. In order to obtain the same attenuation factor in the case of a distribution of energies, the number of lower energy photons present, which are absorbed more strongly, must be compensated for by high-energy photons which are absorbed less strongly. Thus, there must be comparable numbers of photons above and below 2.5 keV. The magnitude of the attenuation depends on the true spectral distribution and can be calculated from the relation:

$$\text{Attenuation} = \int \varphi(\lambda) e^{-\mu(\lambda)x} \epsilon(\lambda) d\lambda / \int \varphi(\lambda) \epsilon(\lambda) d\lambda \quad (1)$$

where  $\varphi(\lambda)$  is an assumed spectrum in terms of number of photons per unit wave-length interval,  $\epsilon(\lambda)$  is the counter efficiency,  $\mu(\lambda)$  is the linear absorption coefficient of the filter and  $x$  is the thickness of the filter. We performed this calculation for three assumed spectra, namely, (1) a power law spectrum of the form:

$$\varphi(\lambda) = A\lambda^{-(\alpha+1)} \quad (2)$$

Equation (2) is equivalent to a distribution of the form  $\nu^{-\alpha}$  ( $\nu$  = frequency) when the spectrum is expressed in terms of power per unit frequency interval.

(2) An exponential spectrum of the form:

$$\varphi(\lambda) = (A/\lambda) \exp(-hc/\lambda kT) \quad (3)$$

Except for the weak energy dependence of the Gaunt factor this spectral distribution describes free-free emission by electrons having a Maxwellian distribution of energy.

(3) A thermal spectrum of the form:

$$\varphi(\lambda) = A\lambda^{-4} [\exp(hc/\lambda kT) - 1] \quad (4)$$

The quantity  $A$  that appears in these three distribution laws is a constant that can be determined from the relation:

$$A = N / \int \varphi(\lambda) \epsilon(\lambda) d\lambda \quad (5)$$

where  $N$  is the observed counting rate.

For each of the distribution laws and for each filter condition, equation (1) was evaluated by numerical integration for a series of values of  $\alpha$  in the case of the power law spectrum and of  $T$  in the case of exponential and thermal spectra. The observed attenuations for ScoX-1 are obtained with  $\alpha = -1.1 \pm 0.3$  for a power law spectrum, with  $T = (3.8 \pm 1.8) \times 10^7$  °K for an exponential spectrum, and  $T = (9.1 \pm 0.9) \times 10^6$  °K for a black-body spectrum. The listed uncertainties result from the statistical fluctuations expected from the total accumulated counts. Within the precision of the measurement, the spectrum from the two-source regions is the same over the interval of wave-lengths to which the Geiger counter is sensitive. Furthermore, it is not possible to decide between the three spectral types from these data alone.

Additional spectral results come from the observation during flight II of the same two-source regions by an NaI(Tl) crystal with area of 38.5 cm<sup>2</sup> and a thickness of 1 mm covered by 6.9 mg/cm<sup>2</sup> of aluminium. The crystal was

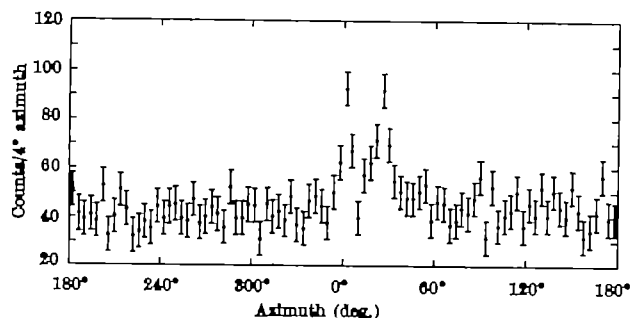


Fig. 1. Azimuthal distribution of counts observed in NaI detector

viewed by a 7188 OBS photomultiplier and the combination had a measured resolution of 48 per cent FWHM at 22 keV. The detector field of view was limited to  $5^\circ \times 25^\circ$  FWHM and the detector was mounted with its axis at  $60^\circ$  from the long axis of the rocket. During the flight this detector scanned substantially the same source regions in the sky as did the Geiger counters in flight I, and the rocket motion was comparable with that of flight I.

The results from this detector consisted of those photomultiplier pulses above a threshold equivalent to about 8 keV. These were stretched to 1 ms with their pulse height preserved and telemetered in real time from the rocket. Pulses from successive spins of the rocket were summed as a function of both pulse height and rocket azimuth. The azimuthal distribution of all telemetered pulses is shown in Fig. 1. Sources are apparent at azimuths of  $2^\circ$  and  $26^\circ$  which correspond respectively to traversal of the regions containing ScoX-1 and the Galactic equator. We have assumed that the same X-ray sources are responsible for the radiation detected in the NaI and Geiger counter detection systems.

The differential pulse height distributions of the data within the source regions are shown with background subtracted in Fig. 2, as well as the background spectrum itself. The data below 10 keV show the cut-off resulting from the electronic threshold. Above 10 keV the distribution of pulse heights observed in the two-source region is markedly different from the background distribution. The distribution observed from ScoX-1 is consistent with a cut-off in the spectrum at about 15 keV, whereas the radiation from the Galactic equator region extends to higher energies and there is evidence for a possible peak at 20 keV from the same region. The observation of this peak must be regarded as only tentative because of the low statistical precision of the results; however, this feature cannot be an instrumental effect since it does not appear in either of the other two spectra.

The ambiguity in the choice of spectral distribution law that arises from the Geiger counter data alone can be partially resolved by considering the NaI data as well. The counting rates observed for the two-source regions by the two detectors are listed in Table 2. The ratio of counting rates for ScoX-1 is  $(14 \pm 3):1$ , which can be obtained for either a power law or exponential spectral distribution with the parameters determined from the Geiger counter data. To fit a black-body distribution, however, requires a temperature of  $(17 \pm 1) \times 10^6$  °K which cannot be reconciled with the Geiger counter data.

Table 2. COUNTING RATES AND ABSOLUTE FLUXES OBSERVED FOR THE TWO-SOURCE REGIONS

Numbers listed for 1–10 keV are derived from Geiger counters and those listed for >8 keV are derived from the NaI detector. The listed errors are based on the count statistics and in the case of the integrated power and flux in the 1–10 keV range include the effect of the uncertainty of the spectral index

	Counting rate counts/cm <sup>2</sup> -sec	Integrated power ergs/cm <sup>2</sup> -sec	Integrated flux photons/cm <sup>2</sup> -sec
ScoX-1			
1–10 keV	$16.8 \pm 0.6$	$(1.61 \pm 0.4) \times 10^{-7}$	$32 \pm 6$
>8 keV	$1.2 \pm 0.3$	$(8.3 \pm 0.8) \times 10^{-8}$	$18 \pm 4$
Galactic equator			
1–10 keV	$4.3 \pm 0.2$	$(0.4 \pm 0.1) \times 10^{-7}$	$8 \pm 2$
>8 keV	$0.67 \pm 0.15$	$(1.9 \pm 0.4) \times 10^{-8}$	$0.8 \pm 0.2$

The black-body temperature consistent with the Geiger counter data predicts a ratio of about 200:1. This analysis applied to the ratio of counting rates observed in the Galactic counter region yields the same general results; namely, that the ratio can be fitted with either an exponential or power law spectrum but that a black-body spectrum yields an insignificant flux in the NaI detector compared with what is observed. It is possible that a series of black-body sources gives rise to the observed radiation which, however, implies that one of the sources has a temperature of several times  $10^7$  °K.

In the case of ScoX-1, if one wishes to fit a thermal spectrum of the order of  $10^7$  °K as is consistent with the Geiger counter data alone plus a high-energy tail to account for the NaI results, the power in the tail must be the order of 25 per cent of that in the thermal portion of the spectrum and to fit a lower temperature requires an even more substantial non-thermal tail. Thus, if a neutron star is postulated as the X-ray source, a large fraction of the observed flux cannot arise from the black-body radiation from that object.

More generally the absence of a substantial thermal contribution to the spectrum indicates that the observed X-rays are being generated in regions that are optically thin. Whether the X-rays are generated in a hot plasma or by the synchrotron process cannot be determined on the basis of these data except for noting that, in a hot plasma line, emission and edge discontinuities can arise, either of which could account for the peak at 20 keV for which there is some evidence from the NaI detector. The synchrotron process cannot give rise to such features.

Table 2 lists the counting rate, the integrated power and photon flux observed in the two-source regions by the two detectors. For ScoX-1 the counting rates are corrected for the shadowing of the collimators and result from the fact that the detector axis comes only within  $2^\circ$  of the source. No such correction was made in the case of the sources near the Galactic centre. In the 1–10-keV region the integrated power and photon flux were obtained by assuming a power law distribution with the spectral index obtained from the filter attenuation of the Geiger counter results. The uncertainty in the spectral index yields the uncertainties in the integral quantity. In the

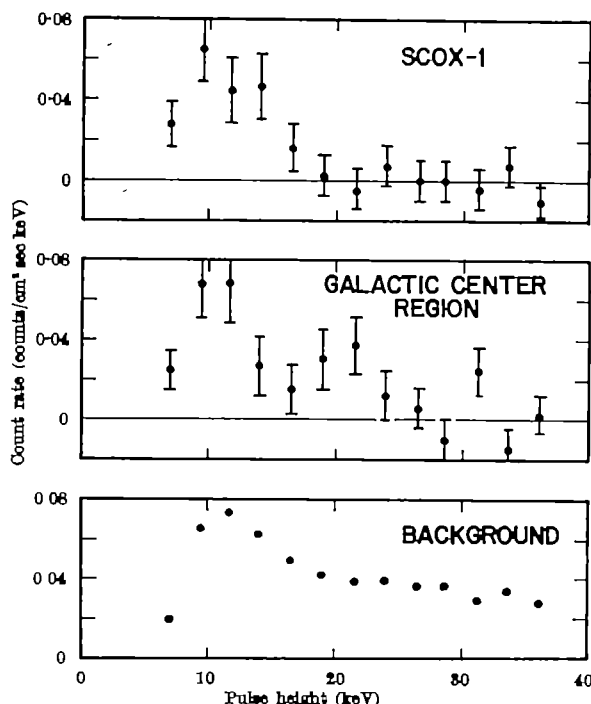


Fig. 2. Pulse height distribution of counting rates observed in NaI detector

> 8 keV region the integrated power and photon fluxes were obtained by summing over the NaI pulse-height distribution.

In summary, the results presented here indicate the following:

- (1) The ratio of the counting rate in the Geiger counters to that in the NaI detector is not consistent with a single thermal spectrum.
- (2) The NaI detector does not detect significant radiation from ScoX-1 beyond about 15 keV, whereas radiation is observed from the Galactic centre region to beyond 20 keV. The minimum detectable flux density at 20 keV (defined as equivalent to 1σ above background) is about  $3 \times 10^{-17}$  ergs/cm<sup>2</sup>-sec (c/s). Clark<sup>1</sup> measures a flux of  $(2.4 \pm 0.6) \times 10^{-17}$  ergs/cm<sup>2</sup>-sec (c/s) over the range of 20–40 keV from the Crab Nebula. The power from ScoX-1 at these high energies is thus considerably below what is emanating from the Crab even though at lower energies (1–10 keV) ScoX-1 is the brighter object as reported by Friedman<sup>2</sup>.
- (3) For both source regions the attenuation of the counting rate in the Geiger counters by the filters indicates

that the X-ray flux below 2.5 keV is comparable with that above 2.5 keV. This result does not agree with that reported by Fisher<sup>3</sup> that little or no radiation is present below 4 keV from these sources.

The work reported here was sponsored by the U.S. National Aeronautics and Space Administration, Office of Space Sciences, under contract NASw-898.

- <sup>1</sup> Clark, G., Garmire, G., Oda, M., Wada, M., Giacconi, R., Gursky, H., and Waters, J., *Nature* (this issue, p. 584). Reported also by Giacconi, R., et al., at Soc. Conf. Relativistic Astrophysics, Austin, Texas (December 1964).
- <sup>2</sup> Bowyer, S., Byram, E. T., Chubb, T. A., and Friedman, H., *Science*, **147**, 204 (1965). Reported also by Friedman, H., at Soc. Conf. Relativistic Astrophysics, Austin, Texas (December 1964).
- <sup>3</sup> Fisher, P. C., Johnson, H. M., Jordan, W. O., Meyerott, A. J., and Acton, L. W. (submitted to *Astrophys. J.*).
- <sup>4</sup> Giacconi, R., Gursky, H., Paolini, F. B., and Rossi, B., *Phys. Rev. Letters*, **9**, 430 (1962).
- <sup>5</sup> Giacconi, R., Gursky, H., Paolini, F. B., and Rossi, B., *Proc. Fifth Intern. Space Sci. Symp.* (to be published).
- <sup>6</sup> Bowyer, S., Byram, E. T., Chubb, T. A., and Friedman, H., *Science*, **146**, 912 (1964).
- <sup>7</sup> Clark, G., *Phys. Rev. Letters*, **14**, 91 (1965).
- <sup>8</sup> Giacconi, R., and Gursky, H., *Space Sci. Rev.*, **4**, 181 (1965).
- <sup>9</sup> Giacconi, R., Gursky, H., Waters, J., Clark, G., and Rossi, B., *Nature*, **204**, 981 (1964).
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## RECENT PROGRESS IN DETERMINING THE ZONAL HARMONICS OF THE EARTH'S GRAVITATIONAL POTENTIAL

By D. G. KING-HELE

Royal Aircraft Establishment, Farnborough

DURING the past year significantly better values have been found for the coefficients of the zonal harmonics in the Earth's gravitational potential. Several authors have given improved sets of values, obtained from analysis of satellite orbits, and the different sets of values agree well, probably because orbits are now available which have a wide variety of inclinations to the equator.

The coefficients  $J_n$  of the zonal harmonics are defined as follows. The gravitational potential  $U$  at an exterior point distant  $r$  from the Earth's centre, and having geocentric latitude  $\varphi$ , is written in a series of spherical harmonics as:

$$U = \frac{GM}{r} \left\{ 1 - \sum_{n=2}^{\infty} J_n \left( \frac{R}{r} \right)^n P_n(\sin \varphi) \right\} \quad (1)$$

where  $G$  is the gravitational constant,  $M$  the mass of the Earth, and  $R$  the Earth's equatorial radius.  $P_n(\sin \varphi)$  is the Legendre polynomial of degree  $n$  and argument  $\sin \varphi$ , and the  $J_n$  are constant coefficients. Equation (1) does not take into account the small variation of  $U$  with longitude, and represents an average over all longitudes.  $GM$  is often taken as 398,802 km<sup>3</sup>/sec<sup>2</sup> and  $R$  as 6,378.163 km, but the last figure in each of these constants is slightly uncertain.

Values of the coefficients of the even zonal harmonics,  $J_2, J_4, \dots, J_{12}$ , may be obtained by analysing the rates of rotation of the orbital planes of  $m$  or more satellites in appreciably different orbits, if it is assumed that  $J_{2m+1}, J_{4m+1}, \dots$  are zero. Similarly, values of  $J_1, J_3, \dots, J_{11}$  may be found by analysing the amplitudes of the long-period oscillations in eccentricity (and other orbital elements) for  $m$  or more satellites in appreciably different orbits.

Recent sets of values of the even and odd coefficients are listed in Tables 1 and 2. It should be emphasized

that the number of coefficients evaluated is a matter for judgement and personal preference. The author who evaluates only a small number of coefficients has to meet the objection that he would obtain a better fit by using an extra coefficient; the author who evaluates a large number of coefficients has to meet the objection that he has fitted a polynomial of too high a degree and that the solution gives a potential which oscillates in an unrealistic manner. So a set with a large number of coefficients is not likely to be any more accurate than a set with a smaller number of coefficients. In fact it can be shown that the perturbations to close satellite orbits caused by the different sets of coefficients in Table 1 (or Table 2) are almost identical for satellites with orbital inclinations between 25° and 90°. Some divergence occurs at inclinations below 25° and there is an urgent need for a satellite in a stable orbit at an inclination between 10° and 20° in order to improve further the values of the coefficients.

Table 2. RECENT SETS OF VALUES OF THE ODD  $J_n$

Authors	$10^4 J_1$	$10^4 J_3$	$10^4 J_5$	$10^4 J_7$	$10^4 J_{11}$
Guler and Newton <sup>1</sup>	-2.69	-0.01	-0.63	0.21	—
King-Hele, Cook and Scott <sup>2</sup>	-2.56	-0.16	-0.44	0.12	—
Kozai <sup>3</sup>	-2.56	-0.18	-0.38	0.04	0.30

A few comments should be made on Tables 1 and 2. The standard deviations in the values, as derived by the authors, are not given, because the error due to neglecting coefficients of degree higher than those evaluated is probably greater than the errors given by most of the authors. A better impression of the likely errors is obtained by comparing the different sets of values: this suggests the errors in most of the coefficients may be of order 0.1. It should be emphasized, however, that the values of the complete potential, not the individual coefficients, are of prime importance, and that the different sets of coefficients give rise to closely similar potentials, despite the differences in individual coefficients.

It is only by chance that the three values of  $J_1$  in Table 1 are so close, and  $J_2$  may well take a slightly different value when near-equatorial orbits can be analysed; even so, the value seems likely to remain between 1,082.6 and

Table 1. RECENT SETS OF VALUES OF THE EVEN  $J_n$

Authors	$10^4 J_2$	$10^4 J_4$	$10^4 J_6$	$10^4 J_8$	$10^4 J_{10}$	$10^4 J_{12}$
King-Hele and Cook <sup>1</sup>	1,082.64	-1.52	0.57	0.44	—	—
Kozai <sup>2</sup>	1,082.63	-1.63	0.59	-0.15	-0.16	-0.29
Smith <sup>3</sup>	1,082.64	-1.70	0.73	-0.46	-0.17	-0.22
						0.19

$1,082.7 \times 10^{-4}$ . If  $10^4 J_2 = 1,082.64$ , the value for the Earth's flattening, using the conventional formula<sup>1</sup>, is  $1/298.26$ .

The coefficient  $J_2$ , which expresses the Earth's tendency towards a pear shape, has a significantly greater numerical value than seemed probable two years ago, being near  $-2.6 \times 10^{-4}$  rather than  $-2.4 \times 10^{-4}$ . With the second set of values for the odd harmonics in Table 2, the sea-level surface at the north pole is 39 m farther from the equator than sea-level at the south pole.

There has also been important progress recently in the evaluation of the tesseral harmonics, which indicate the variation of  $U$  with longitude; but this topic is outside the scope of the present article.

<sup>1</sup> King-Hale, D. G., and Cook, G. H., *Geophys. J.* (in the press).

<sup>2</sup> Kozai, Y., *Publ. Astro. Soc. Japan*, 18, 264 (1964).

<sup>3</sup> Smith, D. H., *Planet. Space Sci.* (to be published).

<sup>4</sup> Guler, W. H., and Newton, R. R., *Johns Hopkins Univ. App. Phys. Lab. Rep.* TG 654 (1964).

<sup>5</sup> King-Hale, D. G., Cook, G. H., and Scott, D. W., *Planet. Space Sci.* (to be published).

<sup>6</sup> Jeffreys, H., *The Earth*, fourth ed., 186 (Camb. Univ. Press, 1960).

## NEWS and VIEWS

### Social Studies In Britain

IN the House of Commons on June 2, Mr. A. Croeland, Secretary of State for Education and Science, announced the publication of the Report of the Heyworth Committee on Social Studies (see p. 559 of this issue of *Nature*). He said that the Government accepted in principle the Committee's main recommendation that a Social Science Research Council should be appointed by the Secretary of State for Education and Science. It also accepted that there was considerable scope for stronger support and better co-ordination of research in this field, and it believed that the Committee was right in concluding that this could best be achieved with the help of such a Research Council, while maintaining support through the University Grants Committee and, where appropriate, through Government departments. The terms of reference of the new Council, its scope and membership, and the arrangements for budgetary control, would require further consideration, but the Government, while accepting that further provisions should be made for research in urban planning, did not believe that the joint board of the various research councils, proposed by the Committee, would be satisfactory. The Government was, in fact, considering what provision could be made for this purpose, and in a written answer on June 14, Mr. Croeland added that the main reason for not accepting this recommendation was that the Research Councils concerned could not provide the professional skills needed, in addition to scientific advice, in judging needs and proposals for research in this field. Another reason was that under such an arrangement research in urban planning would depend primarily on support from three Research Councils, none of which would have more than peripheral interest in this essentially inter-disciplinary field of work. Mr. Croeland also said that the Government accepted that some increase was desirable in financial support for research in social sciences, but it proposed to await the advice of the Council itself. The Government recognized the important part which social scientists could play in formulating and executing policy and intended to ensure that Government departments were so organized and staffed that information and advice from social scientists were available on an adequate scale. Mr. Croeland stated that, at this stage, the Government could not be expected to comment in detail on other recommendations of the Committee.

### National Survey of Staff employed on Information Work

SIR JAMES COOK writes: "One of the functions of the Office for Scientific and Technical Information (recently set up within the Department of Education and Science) is to consider what additional facilities should be provided within Great Britain to train staff for information departments and special libraries, which are growing in number and size as a consequence of the increasing volume of scientific and technical information. As a first step, the Office is conducting a national survey in order to obtain certain facts, estimates and views on the employment of such staff. This is being done by means of two question-

naires—a short one for management and a longer one for individuals employed on work of this sort. So as to preserve complete anonymity the names of organizations and individuals are not being asked for. The distribution of the questionnaires is being carried out chiefly by Government departments, research associations and other grant-aided bodies. I should be grateful, though, for the hospitality of your columns in order to give general publicity to this survey. Anyone concerned with information work, who does not receive a copy of the relevant questionnaire by July 31, may obtain it, either by writing to the Office for Scientific and Technical Information, or by 'phoning Chancery 1262, Ext. 417'.

### Meteorology at the University of Reading

A DEGREE course in meteorology is to be instituted in the University of Reading from October, 1965. Students accepted for the course will read meteorology, physics and mathematics for three years. The course in meteorology will be concerned with the physics and dynamics of the atmosphere with special attention to problems of weather and climate. Students will attend lectures and laboratory demonstration experiments, will carry out synoptic weather analysis and undertake field observations and experiments.

A particularly favourable circumstance is the presence at Bracknell, only eleven miles away, of the headquarters of the Meteorological Office. This is not only the national centre of weather services but also the largest meteorological research centre in Britain. The authorities of the Meteorological Office have shown great interest in the planning of meteorology in the university and, by visits to Bracknell and in other ways, students will be familiarized with the work of the Office. It is intended to institute also a one-year postgraduate course in meteorology which will lead to the degree of M.Sc. It is expected that entry to this course will be restricted to graduates who have followed the B.Sc. course or have comparable qualifications.

Prof. R. C. Sutcliffe, C.B., O.B.E., F.R.S.

DR. R. C. SUTCLIFFE, director of research in the Meteorological Office, has been appointed the first professor of meteorology. He was educated at Whitcliffe Mount Grammar School, Cleckheaton, the University of Leeds (1922-26) and the University College, Bangor (1926-27). He saw service with the Royal Air Force from 1939 until 1946 on meteorological duties and was demobilized as Group Captain. He has taken a leading position in national and international societies and committees for meteorology and related sciences. He is at present, for example, chairman of one Sub-Committee of the Royal Society and member of several others, a past-president of the Royal Meteorological Society and a member of various Government Committees such as the National Oceanographical Council. He is also closely connected with the World Meteorological Organization, being chairman of its Scientific Advisory Committee, and is

vice-president of the International Association of Meteorology and Atmospheric Physics, and a member of the NATO Advisory Group on Meteorology. He has published numerous scientific articles, etc. His main research interests are in mathematical and dynamical meteorology, its application to weather forecasting, and the detailed investigation of the evolution and development of weather systems.

Prof. W. V. Mayneord, F.R.S.

PROF. W. V. MAYNEORD has been awarded the Gold Medal *Pro Meritis Protectione Contra Radiationem* of the Royal Swedish Academy of Science. The Medal will be presented to Prof. Mayneord at the eleventh International Congress of Radiology, which will be held in Rome in September 1965. Until his retirement in September 1964, Prof. Mayneord was professor of physics as applied to medicine in the University of London, and director of the Department of Physics in the Institute of Cancer Research, Royal Cancer Hospital, London. He was a member of the International Commission on Radiological Protection during 1950-59; from 1950 until 1956 he was chairman of the Commission's committee on protection against high-energy radiations (see *Nature*, 204, 22; 1964).

Central Veterinary Laboratory, Weybridge :

Dr. A. B. Paterson

DR. A. B. PATERSON has been promoted deputy director of the Central Veterinary Laboratory, Weybridge, following the death of Mr. J. L. McGirr. Dr. Paterson graduated from Glasgow Veterinary College in 1941. He joined the staff of the Biochemistry Department at the Central Veterinary Laboratory in 1944 after undertaking postgraduate training in chemistry during the tenure of an Agricultural Research Council grant. After work in the field of metabolic diseases of cattle, he took charge of tuberculin production and research, being promoted senior research officer, Grade 2, in 1950. Dr. Paterson was awarded a Ph.D. degree by the University of London in 1954, and became a Fellow of the Royal Institute of Chemistry in 1958. In 1959 he became senior research officer (Grade 1) in charge of the Virology Department.

Psychiatry in the University of Manchester :

Prof. E. W. Anderson

PROF. E. W. ANDERSON, who has been professor of psychiatry in the University of Manchester since 1949, has been appointed a medical visitor in the office of the Lord Chancellor and will relinquish the chair of psychiatry on September 30.

Prof. W. I. N. Kessel

DR. W. I. N. KESSEL has been appointed to succeed Prof. Anderson, from a date to be arranged. Dr. Kessel is at present assistant director of the Medical Research Council Unit for Research on the Epidemiology of Psychiatric Illness and honorary senior lecturer in the Department of Psychological Medicine of the University of Edinburgh. Dr. Kessel was educated at Highgate School and Trinity College, Cambridge, where he was awarded a B.A. degree in 1945 and M.B., B.Chir. in 1949. In 1958 he obtained the diploma in psychological medicine of the University of London with distinction and was awarded an M.D. (Cambridge) in 1963. After qualification he was for one year a house surgeon in University College Hospital, London, and then held successively posts as house physician in the West Middlesex and St. Mary Abbots Hospitals and medical registrar in St. Stephens Hospital, Chelsea. In 1953 he was appointed to the staff of the Maudsley Hospital where for the next five years he held successively the posts of senior house officer, registrar and senior registrar, for the most part in the Professorial Unit. From 1960 he was for one year honorary senior registrar in the Psychiatric Department of University

College Hospital and since 1961 has held his present appointments in Edinburgh, where he has assumed responsibility for the psychiatric care of patients admitted to the emergency ward at the Royal Infirmary. Dr. Kessel's major research interests are in suicide and attempted suicide, the identification and estimation of psychiatric morbidity in general practice, and studies on the epidemiology of psychosomatic medicine. He is the joint author of a book, *Alcoholism*, and in 1965 he delivered the Milroy Lectures to the Royal College of Physicians.

Electrical and Control Engineering in Battersea College of Advanced Technology : Prof. D. R. Chick

DR. D. R. CHICK has been appointed to a chair in, and the headship of, the Department of Electrical and Control Engineering in Battersea College of Advanced Technology (University of Surrey at Guildford designate). Dr. Chick took his first degree in electrical engineering in 1937 and served in various posts dealing with military radio and radar to the end of the Second World War, in particular with the transmitters at Bawdsey and Christchurch, and earned a joint inventor's award for his work on searchlight radar control. He joined the new research laboratory of Associated Electrical Industries, Ltd., at Aldermaston, as a section leader responsible for developing the company's early interests in nuclear engineering, and later as a group leader he co-ordinated the thermonuclear and reactor engineering. With the late Dr. Petrie he developed a successful radio-frequency travelling-wave accelerator for protons to boost the energy of particles leaving the high-voltage electrostatic generator which his Section had built, and he was awarded an M.Eng. by the University of London for this contribution, also an M.Sc. in physics by the University of Reading for his work on static machines. His engineering contributions to the thermonuclear work culminated in the successful operation of the *Sceptre* series of toroidal accelerators, and he gave overall guidance to the design, construction and operation of the first British research reactor, the 5-MW *Martin* at Aldermaston. For his many original engineering papers he was awarded a D.Eng. degree by the University of London. His work has kept him in close touch with research in many universities in the United Kingdom and overseas. He has spent the past two years with the Vickers research organization.

Geography in the University of Edinburgh :

Prof. J. T. Coppock

DR. J. T. COPPOCK, reader in geography in University College, London, has been appointed to the newly instituted Ogilvie chair of geography. This chair is additional to the chair at present held by Prof. J. Wreford Watson. Dr. Coppock was born and went to school in Wales. After service during the Second World War, mainly in the Middle East, he went up to Queen's College, Cambridge, where he was a Foundation Scholar and obtained first-class honours in both Parts of the Geographical Tripos. After a further year at Cambridge as departmental demonstrator in geography, he was appointed in 1950 assistant lecturer in the Department of Geography in University College, London. Promoted to lecturer in 1952, he was accorded his present status of reader in 1964. During the academic session 1963-64 he was visiting senior lecturer in the Department of Geography at the University of Ibadan, Nigeria. He was awarded the degree of M.A. by the University of Cambridge in 1953 and the degree of Ph.D. by the University of London in 1960. Dr. Coppock's principal research interests have lain in the fields of economic and historical geography with particular reference to the geography of agriculture and the problems of rural land-use and land-use competition. In collaboration with R. H. Best he has published a study of land-use changes in Great Britain, and he has recently been appointed a member of the England Committee of



the Nature Conservancy. He has also a general interest in the application of mechanical and electronic aids in data processing and map making and has published an agricultural atlas of England and Wales with the aid of the University of London computer and in collaboration with the Department of Numerical Automation. Dr. Coppock will take up his appointment on January 1, 1966.

#### U.K. Petroleum Statistics, 1963 and 1964

THE United Kingdom *Petroleum Industry Statistics* for the years 1963 and 1964 have recently been published by the Petroleum Information Bureau on behalf of the U.K. Petroleum Industry Advisory Committee (U.K. *Petroleum Industry Statistics: Consumption and Refinery Production, 1963 and 1964*. Pp. 8. London. Petroleum Information Bureau, 1965). Some interesting and perhaps significant contrasts are revealed. In 1963 total consumption of all products (excluding deliveries for bunkers for ships engaged in foreign trade) was 55,692,382 tons; in 1964, 61,199,662 tons. This consumption was apportioned as between: aviation fuels, 2,238,000 and 2,302,931 tons (1963 and 1964 respectively); motor spirit, 9,044,378 and 10,011,922 tons; industrial spirits, 200,429 and 229,880 tons; white spirit, 139,955 and 142,654 tons; kerosene, 1,673,261 and 1,468,218 tons (burning oil) and 183,962 and 147,869 tons (vaporizing oil); derv fuel, 3,305,230 and 3,636,469 tons; gas, Diesel and fuel oils, 5,453,366 and 6,001,368 tons (gas/Diesel oil) and 22,704,005 tons and 24,804,792 tons (fuel oil); lubricating oils and greases, 1,003,482 and 1,081,440 tons; paraffin wax and scale, 52,438 and 58,797 tons; propane, 99,193 and 147,576 tons; butane, 371,364 and 621,456 tons; methane, nil for 1963, 76,917 tons for 1964; bitumen, 1,314,429 and 1,491,291 tons; chemical feedstock, 2,706,351 and 3,126,828 tons; light distillate feedstock, 900,330 and 1,347,312 tons; refinery gases, 426,619 and 483,046 tons. Thus deliveries into consumption of these products, with the exception of kerosene, showed marked increases in 1964 over the preceding year. The figures for propane, butane, methane, chemical feedstock and light distillate feedstock for 1964 are significant of the rapidly expanding petrochemicals and gas industry.

#### Czechoslovak Society of Arts and Sciences in the United States

THE Czechoslovak Society of Arts and Sciences in the United States is a unique organization (*Nature*, 198, 944; 1963). Its 800 members, of whom one-third are teachers at various universities in the United States, are mostly emigrants from Czechoslovakia who have kept their interest in their home-land alive and burning. The Society held two congresses in the United States: one in Washington, D.C., in 1962, and one in New York in 1964. A recent book, *The Czechoslovak Contribution to World Culture* (edit. by Miloslav Rechcigl, jun. Pp. 682. The Hague: Mouton and Co., 1965. Gld. 58.00), is based mainly on the papers presented at the first congress, but seventeen of the fifty-seven contributions were not presented. The subjects range far and wide over the spectrum of Czechoslovak culture, national and international, past and present. The book is divided into ten parts: literature and literary criticism, linguistics, music and fine arts, history, political science and philosophy, sociology, economics, law, science and technology, and Czechs and Slovaks abroad. The volume is concluded with a bibliography on Czechoslovak arts and sciences containing 1,318 references. Short biographies of the fifty-four authors and an index are also included. The individual contributions vary so much in scope and subject-matter that any reader can find an article or two touching directly on his field of interest. The book is neither an encyclopaedia nor an ordered treatise on Czechoslovakia. To a reader interested in all aspects of Slavistics it can be highly recommended; to a student of Czechoslovakia it is

invaluable. It is expected that a similar volume, based on the 1964 Congress of the Society, will be published in due course.

#### Tumour Research

VOLUME VII, *Fascicles 3 and 4* of the *Archivio Italiano di Patologia e Clinica dei Tumori*, is a beautifully printed and attractively produced volume containing 15 articles on various aspects of the study of tumours. The subjects dealt with include methods of producing experimental cerebral tumours resembling human brain tumours, and the morphological, biochemical and immunological properties of these experimentally produced tumours, the difficulties of transplanting them and the promising future of this field of research. Two articles discuss the anti-tumoral activity of a series of biguanide derivatives tested on Ehrlich ascites tumour. Two articles discuss the effects of urethane on the cells of this tumour. Another article describes the morphological, biochemical and immunological properties of Ehrlich ascites tumour cells which have been submitted to several cycles of freezing and thawing. In a paper on the reliability of a technique for the early serodiagnosis of malignant neoplasia, which is based on identifying a typical phospholipid (malignolipin) in the serum, the authors conclude that Kosaki's technique obtains only a mixture of inorganic ions and pirates from the solvent, but no malignolipin. An article on the treatment of patients with malignant tumours with 2,3,5-triethyleniminobenzoquinone by different methods describes palliative effects on one group of patients with no serious side-effects. A second group showed no deaths in the first year, although deaths were recorded among the controls. The volume ends with an illustrated description of 5 cases of histologically benign tumours of the stomach (haemangio-endotheliomas, lipomas and adeno-papillomas and choristomas (aberrant pancreas)), and the author discusses the great difficulties in differentiating on clinical and radiological data between benign blastomas, choristomas and malignant tumours. All the articles give copious references to the relevant literature.

#### Transplantation Research

THE size of the *Proceedings* of the sixth International Transplantation Conference (five times as thick as the first) is an indication of the attraction this subject has for research, but the progress made so far in overcoming the basic problems of tissue incompatibility seems scarcely to justify its present-day popularity with experimental surgeons (*Annals of the New York Academy of Sciences*, 120, 1-806; 1964). Perhaps the most intensive effort has been directed towards finding means of typing human tissues for histocompatibility, as by this it would become possible to pick from a panel of prospective donors that one whose tissue would be most likely to survive in the recipient. Probably the most valuable test in the long run will be an entirely *in vitro* reaction such as that of Bain *et al.*, in which the blood lymphocytes of prospective donor and recipient are allowed to react together. A clinical section (Volume 2) deals largely with attempts to transplant a great many organs in man and animals. While it includes many interesting descriptions of surgical technique and of the pathology of the grafted organs during the rejection which almost invariably occurred, the value of the information obtained sometimes seems scarcely to justify the experiment—especially where human patients were involved. The ethics of such experiments are considered in a thoughtful survey of human kidney transplantation by Murray *et al.* Even in the few "well-tolerated" human kidney grafts described by Hamburger *et al.*, there were recurrent crises which required treatment with immunosuppressive drugs. It thus appears that the presence of the grafted organ alone may not be sufficient to maintain the state of tolerance,



so that in addition to inducing tolerance before grafting we are faced with the problem of maintaining tolerance in the long term. It may be that the continued presence of donor lymphoid cells, intermingled with those of the host, is necessary for tolerance to be permanent. It is this, no doubt, which prompts the considerable attention which is directed to the part played by lymphoid tissue chimerism in the maintenance of the tolerant state. Unfortunately, no firm conclusions can be reported. As a whole, these *Proceedings* are now rather more specialized than previously, and contain less material of interest to the general immunologist, but they remain the best assemblage of advances in transplantation research.

### Food Preservation

THE report of the C.S.I.R.O. Division of Food Preservation for the years 1962-64 records work carried out under the following headings: physics, food chemistry, plant physiology, microbiology, meat research, fish, fresh fruit and vegetables, frozen fruit and vegetables, dried foods, canning, packaging and irradiation (Pp. vi+77. Sydney: Commonwealth Scientific and Industrial Research Organization, 1964). Microbiological work has included investigations on the relation between chemical composition and spore resistance. The heat resistance of spores from the eighteen organisms studied varied by a factor of more than 700; the principal metal constituent in nearly all species was calcium. The calcium content increased significantly with heat resistance while the magnesium content decreased. Diamino pimelic acid also increased with heat resistance and, on a spore unit basis, this acid and dipicolinic acid hexoamine were highly correlated among themselves and with calcium content and spore weight. Studies on responses of meat-spoilage organisms to changes in environment showed that species of *Pseudomonas*, the main spoilage organisms on fresh meat stored in air, are inhibited in certain other atmospheres. Investigations to apply this to carcass preservation are being continued. Meat research was devoted largely to various aspects of meat quality and to a study of the complex biochemical events occurring in post-mortem muscle. Meat from emaciated animals, in addition to having a higher pH, produces more hydrogen sulphide on heating, with resultant staining of cans, and it was suggested that the addition of lactic acid to the meat before canning would reduce such staining. The effects of the removal of muscle from the bones and of proteolytic enzymes on tenderness were also investigated. A pea sheller developed by the Division gave higher recovery of peas than did commercial viners. The rate of damage was reduced, the quality of peas being comparable with that obtained by hand shelling.

### Palynological Society of India

THE Palynological Society of India was founded by an internationally representative section of palynologists, and was inaugurated on January 5 at Calcutta, during the Combined 51-52 Session of the Indian Science Congress. The Society has undertaken the publication of the *Palynological Bulletin* and the *Journal of Palynology* (both of which are annual publications). The membership of the Society is open to all scientists of all nationalities interested in the study of pollen and spores. There are two types of membership, namely: (1) Life Membership, and (2) Ordinary Membership (apart from Institution Membership), in each of which there are two categories A and B. Institution members and members of category A (Life/Ordinary) are entitled to free copies of both the *Journal of Palynology* and the *Palynological Bulletin*, while those in category B (Life/Ordinary) are only entitled to the *Palynological Bulletin*. The membership fees are as follows: *Life Membership*, Category (A). Rs. 100; 24 dollars; £8 (consolidated). Category (B). Rs. 30; 8 dollars; £3 (consolidated). *Ordinary*

*Membership*. Category (A). Rs. 10; 2.4 dollars; 14s. per annum. Category (B). Rs. 3; 1 dollar; 8s. per annum. *Institution Membership*, Rs. 15; 4 dollars; £1 per annum. *Admission fee*, Rs. 2; 48 cents; 8s. for all categories of membership. Further information and membership forms can be obtained from Dr. P. K. K. Nair, general secretary-treasurer, Palynological Society of India, National Botanic Gardens, Lucknow.

### Testing for Seed Health

PROVISION of disease-free seed in agriculture and horticulture is an important factor in raising productivity of food crops. The International Seed Testing Association, Wageningen (Holland), is working towards the accumulation of sufficient knowledge to be able to specify methods for testing seed health which would command world-wide acceptance. A most timely and welcome contribution to this end, from the Plant Pathology Division, Ministry of Agriculture, The Queen's University, Belfast, has recently been published by the Association (*Seed-Borne Fungi: Descriptions of 77 Fungus Species. Handbook of Seed Health Testing*, Series 4 (1). By J. P. Malone and A. E. Muskett. Pp. ii+179-384. Wageningen: The International Seed Testing Association, 1964. 2.05 dollars). The essential feature of the Ulster method is the plating out of seeds on malt extract agar, with or without previous surface sterilization. It is then necessary to be able to distinguish the truly parasitic fungi from those causing little or no damage, so the *Handbook* gives sufficiently detailed descriptions of 77 species or species groups to provide almost forensic standards for recognition. There are, moreover, 284 excellent photomicrographs and photographs of cultures to assist in diagnosis, all of them the sort of practical views encountered in day-to-day examinations. It should be noted that an effective examination for seed contaminants should declare the greatest risk of disease from the sample, but this does not mean that such an amount will necessarily develop, for disease occurrence needs favourable conditions of environment and host reaction in addition to the presence of a parasite. The problem is complicated by the fact that certain seed contaminants may be symbionts beneficial to the plant (for example, an endophytic fungus on *Lolium* spp.) or they may diminish the activity of more active parasites (for example, *Chaetomium* spp. have been held responsible for the low incidence of disease caused by *Helminthosporium victoriae* on oats in Brazil). On the other hand, such potential food grain contaminants as *Aspergillus fumigatus* and *Claviceps* spp. are dangerous to man and livestock, and many fungi deteriorate seeds stored for food. It is therefore essential to be able to assess seed contamination, or more positively seed health, and the detailed information in the *Handbook* should have ready and wide acceptance.

### University News:

#### Liverpool

DR. J. R. ANDERSON has been appointed to the George Holt chair of pathology. Dr. E. Parry has been appointed senior lecturer in anatomy. The following lecturers have also been appointed: Dr. M. J. Roberts (applied mathematics); A. H. Cookson (electrical engineering and electronics).

#### Manchester

DR. P. G. MURPHY has been appointed to the newly established additional chair of physics. The following lecturers have been appointed: Dr. B. J. G. Johnson and Dr. E. Hutton (chemistry); Dr. F. M. Cole (pathology); N. E. Long and Dr. G. K. Garbett (social anthropology); Dr. R. A. James (astronomy); K. S. Williams (mathematics); R. W. Morgan and R. Morton (mathematical statistics); Dr. J. A. D. Aekroyd (mechanics of fluids); Dr. D. G. Lees (metallurgy); Dr. J. G. Baker and Dr. M. A. H. McCausland (physics); Dr. L. M. Cook (zoology); Dr. J. R. Rintoul (anatomy).

## Newcastle upon Tyne

DR. A. JEFFREY has been appointed to the chair of engineering mathematics from September 1. Dr. E. S. Page, director of the computer laboratory, has been appointed to the chair of computing and data processing.

## Sheffield

DR. P. C. KENDALL has been appointed to the second chair of applied mathematics. The following lecturers have been appointed: Dr. G. C. Bye (ceramics with refractories technology); Dr. D. H. Lewis (botany); T. Shanin (sociology); Dr. D. A. Spears (geology). The title of reader has been conferred on: Dr. J. McKenna (organic chemistry); Dr. B. Stevens (photochemistry); Dr. H. J. V. Tyrrell and Dr. P. A. H. Wyatt (physical chemistry).

## Sussex

THE following appointments have been made: *Professorships*, Dr. J. R. Postgate (microbiology); Dr. D. F. Brewer and Dr. M. W. Thompson (physics). *Senior Lectureships*, N. G. Meadows (electrical engineering); Dr. A. W. Simpson (materials science). *Lectureships*, Dr. R. M. Lynden-Bell (chemistry); D. T. Streeter and Dr. J. D. Thomas (biological sciences); Dr. B. V. Jayawant, P. Lindon and Dr. G. Williams (electrical engineering); Dr. R. D. Doherty and Dr. M. E. Ertl (materials science); Dr. J. R. Ellis (mathematics); Dr. D. Bailein and Dr. E. A. Sanderson (theoretical physics); Dr. J. Byrne (experimental physics); M. S. Halliday (psychology); Dr. J. F. Boissvain (sociology); Dr. R. L. Harris (social anthropology).

## Possible Anti-matter Content of the Tunguska Meteor of 1908

PROF. W. F. LIBBY has written to the Editor stating that it has been brought to his attention that Dr. Lincoln LaPaz, of the University of New Mexico, had published earlier results on several aspects of the Tunguska meteorite, referred to in the article by Prof. C. Cowan, C. R. Atluri and Prof. W. F. Libby entitled "Possible Anti-matter Content of the Tunguska Meteor of 1908", which was published on p. 861 of the May 29, 1965, issue of *Nature*. The references which should have been mentioned were as follows: "Meteorite Craters and the Hypothesis of the Existence of Contraterrene Meteorites", *Contr. Soc. Res. Meteorites*, 2, No. 4 (1941); "Contraterrene Meteorites", *Contr. Soc. Res. Meteorites in Popular Astron.*, 49, No. 5 (May 1941); "The Energy of the Podkamennaya Tunguska, Siberia, Meteoritic Fall ( $\pm 1019,609$ )", *Contr. Meteorit. Soc.*, 4, No. 2 (1948).

## Food Preference and Sensitivity of Taste for Bitter Compounds

DR. E. V. GLANVILLE, Dr. A. R. Kaplan and Mrs. F. Griffin have written to the Editor stating that in the article which appeared under the above title by Drs. E. V. Glanville and A. R. Kaplan (*Nature*, 205, 851; 1965), in the fourth from the last paragraph it was stated that taste "... sensitivity to PROP has been shown to be correlated with taste sensitivity to a wide variety of other compounds, including those testing bitter, salty, sour and sweet (Fischer, R., and Griffin, F., 1964)". The discrepancy between this statement and the content of the quoted reference can easily be corrected if the word 'quinine' is substituted for 'PROP'. Taste thresholds for quinine follow a Gaussian distribution, whereas those for PROP, that is, 6-n-propylthiouracil, follow a bimodal distribution. Dr. Glanville, Dr. Kaplan and Mrs. F. Griffin also state that they have shown (*Nature*, 200, 343 (1963), seventh paragraph from the end, as well as *Recent Advances in Biological Psychiatry*, 7, 183 (Plenum Press, New York, 1965)), that subjects with low taste thresholds for quinine are also sensitive tasters of a wide variety of

chemically unrelated compounds—with the exception of hydrochloric acid, the taste threshold distribution of which follows a Gaussian curve.

## Announcements

THE sixteenth international Astronautical Congress will be held in Athens during September 12–18. Further information can be obtained from the Secretary, International Astronautical Federation, 250 Rue Saint-Jacques, Paris 5.

A SYMPOSIUM on "The Ecology of Soil Bacteria" will be held in the University of Liverpool during September 6–12. Further information can be obtained from Dr. T. R. G. Gray, the Hartley Botanical Laboratories, the University, Liverpool 3.

THE one hundred and second meeting of the British Pharmaceutical Conference will be held in Cardiff during September 6–10. Further information can be obtained from the Secretary, British Pharmaceutical Conference, 17 Bloomsbury Square, London, W.C.1.

A CONFERENCE on "Internal Friction in Solids", arranged by the Materials and Testing Group of the Institute of Physics and the Physical Society, will be held in the University of Manchester during September 7–9. Further information can be obtained from Dr. G. M. Leak, Department of Metallurgy, the University, Manchester 13.

A SYMPOSIUM on "Some Biological Systems at the Molecular Level", arranged by the Commission on Molecular Biophysics of the International Organization for Pure and Applied Biophysics, will be held in Naples during September 8–11. Further information can be obtained from Prof. R. C. Williams, Virus Laboratory, University of California, Berkeley, California 94720.

A CONFERENCE on "Fluid Logic and Amplification", organized by the British Hydromechanics Research Association and the Production and Industrial Administration Department of the College of Aeronautics, will be held at Cranfield during September 9–10. Further information can be obtained from the Information Office, British Hydromechanics Research Association, Cranfield, Bedford.

THE first international conference of the International Organization for Medical Physics will be held at Harrogate during September 8–10. The programme will include discussions on: physics in medicine and surgery; physics applied to physiology; physics in radiology. Further information can be obtained from the Secretary, United Kingdom National Committee for Medical Physics, 45–47 Little Britain, London, E.C.1.

A SYMPOSIUM on "Electromagnetic Distance Measurements" will be held at Oxford, under the aegis of the International Association of Geodesy at the invitation of the Royal Society, during September 5–11. The programme will include sessions on: microwave distance measurement systems; electro-optical distance measurement systems; application of masers and lasers to distance measurements; electromagnetic wave propagation questions; airborne and satellite-borne systems; electromagnetic measurements of short distances. Further information can be obtained from the Secretary, Electromagnetic Distance Measurements Symposium, Ordnance Survey, Chesham, Surrey.

CORRECTION. In the biographical note dealing with the appointment of Mr. W. J. Charnley as head of Weapons Department at the Royal Aircraft Establishment (*Nature*, 206, 1097; 1965), it is incorrectly stated that Mr. Charnley graduated at the University of London in 1940. Mr. Charnley in fact graduated at the University of Liverpool in 1942. Furthermore, he was awarded an M.Eng. in 1944 and not 1954 as stated.

## PLANT PHENOLICS INTO PHYTOCHEMISTRY

By DR. E. C. BATE-SMITH, C.B.E.

Low Temperature Research Station, Cambridge

## Origin and History of the Plant Phenolics Group

TWELVE years ago a meeting was held at Long Ashton Research Station, near Bristol, which proved to be the starting point of a highly rewarding adventure. This meeting on plant tannins was organized jointly by Long Ashton and the Low Temperature Research Station at Cambridge, and the invited guests were from other food research laboratories, from leather and forest product research laboratories, and from university departments of botany and chemistry, including those of the University of Bristol. It was here that 'phenolics' were christened and the foundation laid, although unwittingly, for the formation, five years later, of the Plant Phenolics Group.

It is interesting to recall why the Group met such an immediate and enthusiastic response. So far as plant physiology was concerned, the function of the phenolic constituents was unidentified and apparently unimportant. Lignification was an event taken more or less for granted, of concern only to the plant anatomist and the paper industry, tannins had no evident function and, together with many of the simple phenolic constituents, could most conveniently be regarded as waste products of organisms unprovided with excretory apparatus. The phenolases might perhaps be concerned with respiration, but could not be shown to play any such part in normal physiological processes. Only the anthocyanins of flowers and fruits could be allocated a role in fertilization and seed dispersal. Since there was no one in the world of botany to take an interest in the phenolic constituents of plants, the chemists set out to look after the field themselves.

A start had been made in 1947 in the application of paper chromatography to the anthocyanins and flavones of flower petals; but it was Dr. A. E. Bradfield, working for the Indian Tea Association in the laboratories of J. Lyons and Co., Ltd., and later at East Malling Research Station, who really set the wheels in motion. Making skilful use of partition chromatography, he separated a number of fractions from the tea tannins and showed them to be the 8-galloyl esters of catechin and galocatechin, then, using paper chromatography for the first time as an analytical tool in phenolic chemistry, he and I established the configuration of the diastereoisomers of the galocatechins and their galloyl esters. Although, unfortunately, he died before it came into being, it was his authority as a chemist which underpinned the phenolics group in its formative period. His work for the Indian Tea Association and his support of the Group were vigorously carried on by his successor, Dr. E. A. H. Roberts, who later became the second chairman of the Group.

The Plant Phenolics Group was broadly based to cater for the botanist and the chemist, for the university scientist and for the technologist in industry. The subject for discussion at the inaugural meeting at Cambridge, in June 1957, "The Oxidation of Plant Phenolics", was chosen to attract as numerous and representative an attendance as possible. The same objective governed the selection of subjects for discussion at subsequent annual general meetings, the *Proceedings* of which have nearly all been published.

In addition to these annual general meetings, two meetings a year were held on more narrowly based topics. The last of these, foreshadowing the widening of the field which had already been decided on, was a symposium on "The Comparative Biochemistry of the Leguminosae", a subject still largely phenolic in its content, but including also alkaloids, unusual amino-acids, seed proteins and carbohydrates.

## Evolution of a Phytochemical Group

The Plant Phenolics Group had no difficulty in finding attractive subject-matter for three meetings a year, even though the field is a restricted one. The question of an extension of the Group's activities had, however, always been in the minds of successive committees. It had been hoped that other limited subject-areas of phytochemistry might be similarly developed, so that in due course a 'super-group' might be formed co-ordinating the separate activities of a number of such associations. However, many members of the Group were interested in other kinds of plant constituents such as terpenes, alkaloids, carotenoids and amino-acids, and since there was no indication that the group would be trespassing on other people's ground if these were absorbed into its activities, a move was made two years ago to test the feelings of the members about expanding the Group to include the whole range of secondary plant constituents. Finally, at the annual general meeting in April, 1964, the Plant Phenolics Group voted itself into a new phase of existence as the Phytochemical Group.

There are many consequences of this expansion of activity. First, of course, the membership can be expected to increase and, secondly, the programme will inevitably become to some extent sectionalized. But one thing above all else the present Committee wishes to preserve is the comfortable informality of the Group's proceedings. This desire for informality is behind the choice of title; a group can be as formal or informal as it pleases.

There is also a deliberate intention in the choice of 'Phytochemical' as the descriptive element in the title, both a broader and a narrower intention than would be implied, for example, by 'Plant Chemical' or 'Plant Biochemical'. Perhaps the nearest one can get to a definition is that there is at all times an effort to understand the significance of the chemical constituents, especially the secondary constituents, in the life of the plant, and the effect they may have on the lives of man and animals. These objectives give point to the work of the chemist in isolating and characterizing new natural products, and fortify the plant physiologist with knowledge of the identity and possible function of these plant constituents.

There is one reason why the formation of a Phytochemical Group is especially timely, and that is the rapid growth of chemical taxonomy, or biochemical systematics. While much of the initiative for this outburst of activity has come from the chemical side botanists are equally prominent. One international conference has already been held, and its *Proceedings* have been published<sup>1</sup>. An outcome of this conference has been the proposed formation of a Chemical Taxonomy Commission of the Organic Chemistry Section of International Union of Pure and Applied Chemistry, and the creation of a formal link between this and a recently formed committee of the International Association of Plant Taxonomists.

This comes at a time when there is much searching—and some burning—of heart among plant taxonomists themselves, and indeed among classifiers and systematizers of knowledge generally. It can be foreseen that chemical characters will be needed, although reluctantly admitted, along with anatomical, cytological and palynological characters, as adjuncts to the characters of classical taxonomy, which seem to be reaching the limits of their unaided application to problems of classification. All this interest will promote activity among the members of the Phytochemical Group and will be strongly reflected in its programme. Indeed, the subject of the first annual general meeting of the new Group, which was held in

Cambridge in April, 1965, was a three-day symposium on "Comparative Phytochemistry", a report of which is given in the following article.

Much of the knowledge of the chemistry of plants has been gained by the search for pharmacologically active substances, so that the applications of phytochemistry in the pharmaceutical field can also be expected to be prominently featured in the Group's programme. But there is a field of at least equal interest and importance in the occurrence of physiologically active compounds in actual or potential foods and feeding-stuffs. Outstanding examples of these are the oestrogens in leguminous crop plants and the recent discovery of aflatoxin in spoiled groundnuts. Besides these harmful constituents there is a growing need for chemical knowledge—and, in gas chromatography, the means of obtaining it—of the nature of aroma substances in foods. It is to these constituents that so much of the attractiveness, or unattractiveness, of food is due. Both in the breeding and growing of the fresh foods, and in the preservation and enhancement of fresh quality the demand is for an understanding of the nature of the aroma substances. Just as our knowledge of the pigments has advanced during the past few years so our knowledge of the 'aromatics' can be expected to grow during the coming years, and these aroma substances cover

a far wider range than the comparatively few kinds of substances to which the plant pigments are confined. The usefulness of this knowledge can be expected to extend, and is, indeed, already extending, to the palatability of animal feeding-stuffs, now that intensive feeding methods are beginning to assume so much importance in stock raising and dairy farming.

While some regard must be given to sectional interests in the activities of the Group, its objectives will be much better promoted by emphasizing the more general aspects of phytochemistry. With the increasing complexity and specialization both of academic chemistry and the various branches of industry there is a tendency for departmentation and segregation to increase. It will be one of the great advantages of meetings of the Group that departmental barriers can be broken down in the discussion of such topics as biosynthesis and chemical taxonomy, subjects which not only embrace the whole range of natural products, but which also possess an exciting and rewarding intellectual content. These are satisfying activities for the increasing number of those for whom chemistry for chemistry's sake and botany for botany's sake are not enough.

<sup>1</sup> *Chemical Taxonomy*, edit. by Swain, T. (Academic Press, 1964).

## COMPARATIVE PHYTOCHEMISTRY

By DR. J. B. HARBORNE

John Innes Institute, Bayfordbury, Hertford

**A**N international symposium on comparative aspects of plant chemistry, organized by the Phytochemical Group with the financial assistance of the Scientific Affairs Division of NATO, was held in Cambridge during March 30–April 1. The main theme of the meeting was the taxonomic implication of the natural distribution of the various groups of plant constituents, but their biogenesis, evolution and general biochemistry were also discussed. The taxonomist's view of phytochemistry was given by Prof. V. H. Heywood (Liverpool) in the introductory lecture. He dealt with the problems of incorporating chemical data into classification and showed why it was impossible to recommend the phytochemist to use one rather than another of the various plant classifications that are available. He pointed out that a number of systems will always be needed to fulfil different objectives.

In a second introductory lecture, Prof. C. R. Mentzer (Paris) expounded his well-known biogenetic classification of plant constituents, in which substances are placed together according to their biogenetic origin rather than on the basis of chemical complexity as in Beilstein.

The first main session, under the chairmanship of Prof. L. Fowden, was devoted to the distribution of nitrogenous compounds. Dr. E. A. Bell (London) described the distribution patterns of non-protein amino-acids he had obtained using paper electrophoresis of legume seed extracts. These patterns, he said, could be used to divide each of the genera *Lathyrus* and *Vicia* into several distinctive biochemical groups. He pointed out that comparisons of biosynthetic pathways were probably more valuable with unusual amino-acids than considerations of the distribution of single compounds. Prof. R. Hegnauer (Leiden), in considering the taxonomic significance of the comparative chemistry of the alkaloids, found many examples of parallelism and diversification. One of the conspicuous successes of chemical taxonomy has been the discovery that betacyanins and betaxanthins are restricted to one order of plants, the Centrosepermae, and the final paper in this session, given by Dr. T. J. Mabry (Texas), dealt with these purple and yellow alkaloidal pigments.

After describing the chemistry of these substances, Dr. Mabry considered the various arrangements that have been proposed for the betacyanin-containing families and showed that his results definitely discounted the Hutchinson system in this context.

The second session was devoted to phenolic constituents and included a consideration of hydroxyquinones (Dr. O. Mathis, Strasbourg), dihydrochalcones (A. H. Williams, Bristol), glycoflavones (Prof. H. Wagner, Munich) and flavonoid pigments (Dr. J. B. Harborne, John Innes Institute). It was clear from these papers that although phenolics are among the most promising of taxonomic markers, they have not as yet made much contribution to plant systematics. For example, the dianthrone hypericin, as Dr. Mathis found, is characteristic of the genus *Hypericum*, but it only occurs in about half of the 300 species examined. One difficulty in evaluating the results of surveys is the occurrence of 'chemical races' within a single species. A dramatic example of this phenomenon was uncovered by A. H. Williams, who found that some samples of the dihydrochalcone-containing *Smilax glycyphylla* either lacked phenols altogether or contained the xanthone, mangiferin, instead. Glycoflavones are more widely occurring than dihydrochalcones but little is known of their detailed distribution at the generic level. One of the main difficulties with glycoflavones is distinguishing them from the more common flavone O-glycosides, and Prof. Wagner dealt mainly with this aspect of their phytochemistry in his lecture. Because of their importance as petal pigments, the flavonoids are very interesting from an evolutionary point of view, and Dr. Harborne, therefore, sought correlations between flavonoid structures and evolutionary advancement. He also described some recent surveys in the Gesneriaceae and Plumbaginaceae, in which novel correlations have been found between anthocyanin distribution and pollen or leaf morphology.

The third session, under the chairmanship of Prof. H. Erdtman (Stockholm), dealt with hydrocarbons and their derivations. Dr. G. Eglinton (Glasgow) said that the alkane distributions so far studied indicated a reasonable

species constancy, but that the chemotaxonomic use of alkane patterns had so far met with only moderate success. The acetylenic compounds also present taxonomic difficulties, occurring as they do in such disparate groups as the fungi Polyporaceae and the highly advanced Compositae. Dr. J. D. Bu'Lock (Manchester) pointed out that some of the anomalous aspects of acetylene distribution within the Compositae could be explained by considering biosynthetic pathways, so that more knowledge of biosynthesis is vital in this series. Dr. G. Weissman (Hamburg), in considering the occurrence of terpenoids in plants, also found that biogenetic relationships were of prime importance in understanding distribution patterns. In the final lecture of this session, Prof. T. W. Goodwin (Aberystwyth) mentioned that carotenoids were of considerable systematic importance in lower plants, but were of little significance in the angiosperms since all green leaves contain the same set of major carotenoids. Fruit carotenoids do, however, show interesting variations as they can be divided into four categories, but the interest here is mainly their biosynthetic origin rather than their systematic distribution.

The first paper in the fourth and final session of the symposium was given by Dr. E. Percival (London). In surveying the vast and complex field of polysaccharide

chemistry, she pointed especially to similarities and differences in structure between algal and higher plant acidic polysaccharides. She concluded that generic and species differences will only emerge when the fine details of these macromolecules have been elucidated. Prof. A. J. Kjaer (Copenhagen) also had a large area to cover, since he dealt with sulphur-containing amino-acids and alkaloids, thiols, simple sulphides and polysulphides. His main theme, however, was the distribution of the isothiocyanate-producing glucosides, which show a number of interesting correlations with taxonomy. In a joint paper, Dr. T. Swain (Cambridge) dealt with the chemistry and Dr. E. C. Bate-Smith (Cambridge) the distribution of a class of substances including aucubin and asperuloside, now known collectively as iridoids. It was pleasing to find that their distribution in families in the order Tubiflorae fits in particularly well with the recently published twelfth edition of Engler's *Syllabus of Plant Families*. Finally, Dr. Ruijgrok (Leiden) outlined the distribution of the lactone ranunculin in the Ranunculaceae and correlated its occurrence with those of cyanogenetic compounds, which are also present in this family. This talk concluded a very successful meeting, in which both chemists and taxonomists contributed to discussions of the many papers. It is planned to publish the proceedings in full later this year.

## HOT-LABORATORY WORKING METHODS

AN international symposium on working methods in high-activity hot laboratories, organized by the European Nuclear Energy Agency in collaboration with Euratom, was held at the French Centre d'Etudes Nucléaires in Grenoble during June 14-18. The symposium provided a forum for discussion, by some 130 experts from hot laboratories in the nuclear research centres of 15 European and American countries of OECD and Euratom, of specialized methods and equipment for handling radioactive materials with activities greater than 1,000 curies.

The main purpose of hot laboratories, of course, is to permit experimental work on these highly radioactive materials without endangering the personnel carrying out the work. Although in the past such experiments have usually been related to research and development programmes, there is now a growing amount of purely routine work—such as regular analysis of irradiated fuel samples to determine burn-up—for which comparatively simple standardized equipment could often replace the complex and unnecessarily flexible apparatus of the pure research laboratory.

Equipment for both purposes was discussed at Grenoble and it became clear, even for complex research purposes, that laboratory experts preferred simple and economical rather than highly sophisticated equipment provided only that it would do its job in a safe and efficient manner.

Throughout the symposium there was repeated emphasis on the need for designers of hot-laboratory facilities to work more closely with the users, so avoiding the need to make an installation unnecessarily flexible through lack of foreknowledge of its purpose. Improved liaison was also often possible between the hot-laboratory operator and his "client"—the reactor operator or research engineer—so that the one could know as accurately as possible what the other wanted him to do. The earlier this liaison took place, the less complicated the hot-laboratory equipment might well be.

On the other hand, a too-rigid limitation of use could lead to considerable expense when, as was often the case, advancing technology required modifications to what had become standard test procedures. Economics and flexibility were in fact often in opposition, and an efficient compromise was not always easy to find.

Although the symposium was concerned with hot laboratories for all purposes, most of the papers presented dealt with installations for work on irradiated fuels, and several stressed the problems to be expected from the advancement of fast reactors. Monsieur André Valentin (Commissariat à l'Energie Atomique, Fontenay-aux-Roses, France), in summing up the situation, mentioned that such reactors would undoubtedly operate to very high fuel burn-ups—for example, it was generally thought that plutonium carbide fuels would not be interesting unless taken to burn-ups greater than 50,000 MW days/tonne. This was likely to lead to considerable and important developments in hot-laboratory methods.

Mr. K. R. Ferguson (Argonne National Laboratory), in reviewing present techniques in the United States, stressed the value of experience in building up these techniques. Such experience enabled consistently reliable results to be obtained from comparatively simple apparatus having the highest standards of safety and protection, while less-experienced operators would feel impelled to use more complex equipment which was certainly less economical and could also be less reliable.

A discussion of various types of 'enclosure' directed attention to the advantages of incorporating all or most of the remote handling operations of a particular process within a single complex of interconnected containments. By thus reducing the frequency of transfer across the containment barrier, both containment reliability and operating efficiency could be greatly increased. Such cell complexes are to be used at the Fuel Recycle Pilot Plant at the Hanford Laboratories, the Oak Ridge Transuranium Laboratory, the Oak Ridge Thorium-uranium Recycle Facility, and the Alpha-Gamma Metallurgical Hot Cell at Argonne.

Concerning the use of 'boxes' within hot cells, it was generally agreed that these were necessary for handling materials above a certain activity (for example, above a total alpha count of  $10^{11}$ /min was suggested) to avoid excessive time loss in decontamination and to provide added assurance of safety. Boxes were also needed to avoid cross-contamination between different materials handled simultaneously in the same laboratory.

There was some discussion on methods and equipment for both individual and general dosimetry, and here again

it appeared that there was scope for closer liaison between the two specializations concerned, namely the radio-chemical technology of the hot laboratory and the physico-biological technology of radio-protection. There was at least one suggestion that this matter might be the subject of a future symposium.

It was perhaps not surprising that on most matters considered at Grenoble, the conclusions of the experts from the various countries represented were similar. Indeed in many fields it was clear that standard methods were beginning to be adopted (particularly for non-destructive

testing) and that this was leading to the adoption of standardized items of equipment in all laboratories. Participants generally welcomed this trend which doubtless owes its origins to previous international discussions which, if on a less ambitious scale, were none the less the forerunners of the Grenoble Symposium.

The *Proceedings* of the Symposium, including all papers and discussions as well as a directory of high-activity hot laboratories in operation in OECD countries, will be published by ENEA towards the end of September.

## MENDEL MEMORIAL BUILDING, HYNČICE

ON June 13, the birthplace of Gregor Mendel at Hynčice, Czechoslovakia, was officially opened to the public and a memorial plaque unveiled to celebrate the occasion.

The house, built by Mendel's father on the site of a former wooden building, has been reconstructed, thanks to the efforts of the Regional Department for the Preservation of Historical Monuments in Ostrava, the District National Council, and the District Museum in Nový Jičín. On the ground floor, the Moravian Museum of Brno has restored the former kitchen and the room where Mendel was born.

The arrangement of the memorial building was, to a great extent, determined by the rooms available. This necessitated dividing them into two parts, representing, on one hand, Mendel's life and, on the other, his scientific activities.

In the first part, Mendel's birth certificate is displayed in the room where he was born in 1822. Other exhibits there record his genealogy, family background, and details of the schools where he obtained his basic education. There are also photographs and documents of the period, testifying to Mendel's entry into the Monastery of Old Brno, his teaching activities at the Brno Secondary School, and finally his election as Abbot in 1868. The final exhibits in this section include the obituary notice issued by the Monastery on the day of his death, and the photograph of the Monastery vault in the Central Cemetery in Brno, where he gained his final resting place.

The second part, which is aimed at acquainting the visitor with Mendel's work and its significance, particularly stresses his interest in natural science. It was this interest which led him, after his theological studies had been completed, to study at the University of Vienna.

Later he returned to Brno where for many years he performed his experiments on the heredity of peas. It was these, now classical experiments, which led to the formulation of his theory of dominant and recessive characters and its publication in 1865. His theory, however, was not recognized until it was publicized by de Vries, Correns and Tschermak at the beginning of the present century. The final documents exhibited in this section indicate the great use which scientific research workers throughout the world have made of Mendel's work.

The official opening of Mendel's birthplace aroused considerable interest among the general public, and at the ceremony Dr. V. Orel, head of the Gregor Mendel Department of Genetics in the Moravian Museum in Brno, gave an inaugural address in which he briefly outlined Mendel's life and explained the significance of his work. He also recalled Mendel's close ties with his family and his native village.

The opening of this memorial building gave Hynčice the chance to inaugurate the official celebrations of the centenary of the publication of Mendel's classic paper, and in this way of paying honour to its most famous son.

LUDMILA MARVANOVÁ

## POSITIONS OF THREE COSMIC X-RAY SOURCES IN SCORPIO AND SAGITTARIUS

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AND

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WE have determined the positions of three cosmic X-ray sources in the constellations of Scorpio and Sagittarius within uncertainty areas of 1.2–3 square degrees. Positions for these sources were reported earlier by us at the Austin Conference on Relativistic Astrophysics on the basis of a preliminary analysis of the same data<sup>1</sup>. One of these sources is ScoX-1, which was first detected by Giacconi *et al.* in 1962 (ref. 2). (Our designations specify the constellation and the order of discovery, for example, ScoX-2 for second X-ray source in Scorpio.)

The other two, SgrX-1 and ScoX-2, lie near the galactic plane in the complex of sources the existence of which was reported in a previous publication<sup>2</sup>. We have also located a probable fourth source to within 1° of a segment of a great circle that is almost parallel to and about 4° below the galactic equator. The Kepler supernova remnant SN1604 was scanned and no evidence of an X-ray source at its position was found.

We obtained the data with a rocket that was launched from White Sands, New Mexico, on October 26, 1964, at a sidereal time of 20h 20m, and reached an apogee of 224 km. Above the atmosphere the rocket spun around

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its long axis with an almost constant angular velocity of approximately 808 deg/sec. The spin axis precessed with an angular velocity of 5.51 deg/sec around a circular cone the apex angle and spatial orientation of which did not vary by more than  $0.3^\circ$  during the 260 sec when useful X-ray data were obtained.

The effects of the rocket motion on the observations are most easily visualized in the rocket frame of reference which we define to be a co-ordinate system fixed in the rocket with its  $z$ -axis parallel to the spin axis. During each spin any given point on the celestial sphere moved through  $360^\circ$  of azimuth in the rocket frame at an almost constant angle of elevation with respect to the rocket equator. In addition, the precession caused the elevation angle of an object to vary periodically with an amplitude equal to one-half of the apex angle of the precession cone. Thus an object was observed repeatedly in successive spins whenever it moved within the elevation range of a given detector's field of view.

In describing our analysis, we shall refer to the precession frame of reference which we define to be a co-ordinate system the  $z$ -axis of which is parallel to the axis of the precession cone, and the  $x$ -axis of which is in the direction of the vector product  $\hat{s}_c \times \hat{s}_p$ , where  $\hat{s}_c$  and  $\hat{s}_p$  are unit vectors parallel to the  $z$ -axes of the celestial and precession co-ordinate systems, respectively. We define the spin azimuth of a direction to be its azimuthal angle measured around the spin axis from the direction of  $\hat{s}_p \times \hat{s}_c$ , where  $\hat{s}_c$  is a unit vector in the direction of the spin axis. We define the bearing of a direction to be the sum of its spin azimuth and the precession azimuth of the spin axis. During one complete precession cycle the spin azimuth of a given direction fixed in space decreases non-uniformly by  $360^\circ$ . In the same period the precession azimuth of the spin axis increases uniformly by  $360^\circ$ . Therefore, the bearing of a fixed direction varies about a mean value which, as one can easily see, differs by  $90^\circ$  from the azimuth of the fixed direction in the precession frame. The amplitude of this variation is determined by the angle of the precession cone and the elevation of the direction with respect to the precession equator.

The rocket carried a variety of instruments, among which were several X-ray detectors and two star sensors. The data consisted essentially of the occurrence times of pulses from these detectors and sensors. Four of the X-ray detectors were banks of Geiger tubes, each having sensitive areas of 70 cm<sup>2</sup>, beryllium windows 8 mg cm<sup>-2</sup> thick, and argon fillings giving a gas thickness of 5.4 mg cm<sup>-2</sup>. Three of the banks had slit collimators that gave rectangular fields of view with full widths of  $3.0^\circ$  in the narrow dimension, and  $30^\circ$  (detectors GH0 and GH20) or  $40^\circ$  (detector GV10) in the wide dimension. The narrow dimensions of detectors GH0 and GH20 were perpendicular to the rocket equator, while that of detector GV10 was parallel. The fourth bank, detector GMC0, had a modulation collimator<sup>4</sup> the response function of which, in the direction perpendicular to the rocket equator, was a saw-tooth function with maxima at rocket elevation angles given by the formula  $1.4^\circ \pm \arctan(0.0417n)$ , where  $n$  is an integer. In the direction parallel to the rocket equator the response function of detector GMC0 was triangular with a base width of  $15^\circ$ . The star sensors, SS0 and SS30, had effective fields of view of  $1.0^\circ \times 5.2^\circ$ , with their long dimensions perpendicular to the rocket equator. In our detector designations the number following the letter code specifies the nominal elevation in degrees of the centre of its field of view above the rocket equator. We determined the actual orientations of the detectors and star sensors in the rocket frame of reference to accuracies of  $\pm 0.3^\circ$  in elevation and azimuth by optical measurements on the ground before and after the flight.

We solved the problem of determining the orientation of the rocket in the celestial frame of reference at any

given instant in the following way. We first made use of the fact that ScoX-1 was within the elevation range of the modulation collimator throughout the precession cycle. We could therefore determine the amplitude of its variation in elevation from the observed modulation caused by the motion of ScoX-1 back and forth over the saw-tooth response function. This amplitude, which is equal to the full apex angle of the precession cone, was found to be  $5.1 \pm 0.1^\circ$ . (This angle could also be determined from the star sensor data, though not with as high accuracy as from the modulation collimator data.) From the relative times of the signals from the star sensor SS0, we identified seven stars between the second and fourth magnitude as the sources of the signals. We then determined the orientation of the precession cone axis and the phase of the precession motion which minimized the mean square difference between the predicted and observed times when these seven stars entered and left the elevation range of the star sensor during the precession cycle. We found that the precession axis lay in the direction with right ascension  $302.2^\circ \pm 0.5^\circ$  and declination  $38.2^\circ \pm 0.3^\circ$ . From these data we could calculate the celestial orientation of the spin axis at any given instant. Analysis of the nearly periodic pulses from SS0 produced by various stars repeatedly crossing the field of view showed that the spin frequency increased approximately linearly with time by about 0.2 per cent during the useful period of the flight. Thus the bearing of the axis of any given detector, which for constant precession and spin frequencies would be a linear function of time, could be expressed by a quadratic function of time. The average deviation between the computed and the actual bearing was less than  $\pm 0.5^\circ$  over the useful observation period.

To determine the relative bearings of the X-ray sources and reference stars, we plotted the numbers of counts per bearing interval from each of the detectors as a function of the bearing of the detector's axis, reduced modulo  $360^\circ$ . The data accumulated over four precession periods are shown in Fig. 1. The main peak of the distribution for detector GV10 in Fig. 1a is due to ScoX-1. The difference between its average bearing and that of the star  $\beta$ -Cetus, observed by the star sensor SS0, is  $239.8^\circ \pm 0.3^\circ$ . A segment of the great circle through the precession axis direction with this azimuth relative to  $\beta$ -Cetus is marked A in Fig. 3. We conclude that ScoX-1 lies within  $0.3^\circ$  of this segment.

The second, broader peak in Fig. 1a is due to the complex of sources near the galactic centre. From the fact that the ratio of the amplitude to the area of the peak is less than one-half times that of the ScoX-1 peak we conclude that it contains more than two sources. Arcs B and C in Fig. 3 are loci of positions the average bearings of which are equal to the values marked B and C in Fig. 1a. They are segments of great circles drawn through the precession axis, and they bound the region within which these sources lie. The Kepler's supernova remnant SN1604 lies  $2^\circ$  outside this region, and the radio source SgrA at the galactic centre is just barely within it. The separate peak, the average bearing of which is marked D in Fig. 1a, is  $3.5^\circ$  above the background. It is probably caused by a separate source that lies somewhere along the great circle segment marked D in Fig. 3.

The bearing distribution for the GMC0 detector is shown in Fig. 1b. It has separate peaks due to ScoX-1 and the galactic centre complex, but it has a low angular resolution and does not improve the bearing determinations already made with the GV10 data.

Figs. 1c and 1d show the bearing distributions for the two detectors GH0 and GH20 the fields of view of which were narrow slits parallel to the rocket equator. Both show the presence of sources which are a part of the complex between circles B and C, and which lie within the two 8-degree wide elevation bands scanned by the detectors during the precession cycle. These bands are bounded by the small circles marked GH0 and GH20 in



Table 1. POSITIONS (EPOCH 1950.0) OF THREE X-RAY SOURCES  
The preliminary values of the position co-ordinates given earlier in ref. 1 are shown in parentheses

Source	Right ascension	Declination
ScoX-1	16h 18m or 16h 19m	-15.6° -14.0
ScoX-2	16h 50m	-30.6° (-41°)
SgrX-1	17h 44m (17h 57m)	-23.2° (-24°)

Fig. 3. It is apparent from the absence of a peak at the bearing of ScoX-1 in either distribution that ScoX-1 does not lie within either of these bands.

To obtain more precise information about the elevation of a source with respect to the precession equator we examined the precession variation in the rates of counts in an appropriate bearing interval round the average bearing of the source. At any fixed bearing the precession elevation of a detector axis varied with an amplitude equal to one-half the apex angle of the precession cone. If a source lay in the region scanned, this variation in elevation caused a variation in the X-ray counting rates that could be fitted to the known response curve of the detector by a proper choice of the elevation of the source with respect to the precession equator. Fig. 2a shows the results for the GV10 detector. The abscissa is the relative elevation which we define to be the precession elevation of the detector axis at the centre of the indicated bearing interval minus its fixed rocket elevation. The fact that the counting rate was a maximum at the maximum relative elevation of the spin axis shows that ScoX-1 is above the maximum precession elevation of the GMCO axis, which limit is indicated by the dashed line that crosses the bearing circle A. On the other hand, ScoX-1 cannot lie above the band bounded by the GH20 lines because the observed elevation variation is too small. Therefore, ScoX-1 must lie between the dashed line and the lower boundary of the GH20 band.

To refine the elevation determination of ScoX-1 we used the GMCO data the elevation variation of which is

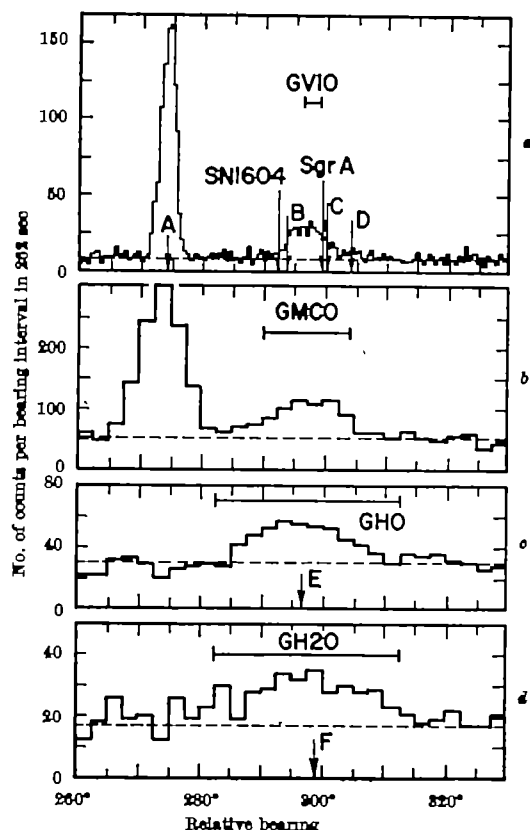


Fig. 1. Bearing distributions of the counts from the four detectors in the vicinity of ScoX-1 and the galactic centre complex. The full widths of the fields of view are indicated under the label of each detector. The background levels are indicated by the dashed lines

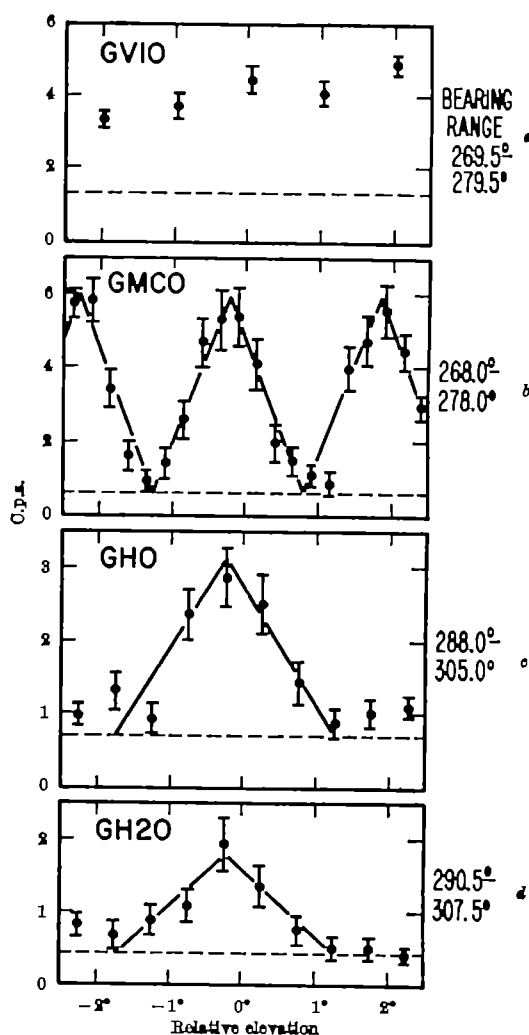


Fig. 2. Variation of the counting rates with the relative elevation of the detector axis at the midpoint of the indicated bearing interval. The background levels are indicated by the dashed lines

shown in Fig. 2b. From the completeness of the modulation, as well as that of the modulation observed in a previous experiment with a similar but higher resolution collimator, it was possible to conclude that the angular diameter of ScoX-1 is less than 7 arc min (ref. 5). One sees here that ScoX-1 lies on one of the maxima of the response function when the precession elevation of the GMCO axis is  $-2.3^\circ$ ,  $-0.2^\circ$  or  $+1.8^\circ$ . The solid lines crossing the bearing circle A show the positions of the two maxima which fall between the limits previously determined. We are not able to choose between the two intersections on the basis of the data from this experiment. The boxes around each of the two intersections are  $0.6^\circ$  wide by  $1.0^\circ$  long, and they indicate the estimated errors in the bearing and elevation determinations, respectively.

Figs. 2c and 2d show the elevation variation of the counts from the sources observed by the GH0 and GH20 detectors. In both cases the sources appear to lie about  $0.3^\circ$  below the midpoint of the elevation scan as indicated by the line crossing the bearing circles D and E. We determined these latter bearing circles using only the data from the parts of the precession cycle when the sources gave the highest counting rates. As before, the boxes around each intersection indicate the estimated errors in the position determinations.

The two alternative positions we find for ScoX-1 are both within the uncertainty circle of  $2^\circ$  radius around the position published by the NRL group<sup>4</sup>. The lower of the two is within  $0.5^\circ$  of the position given by Fisher *et al.*<sup>1</sup>.

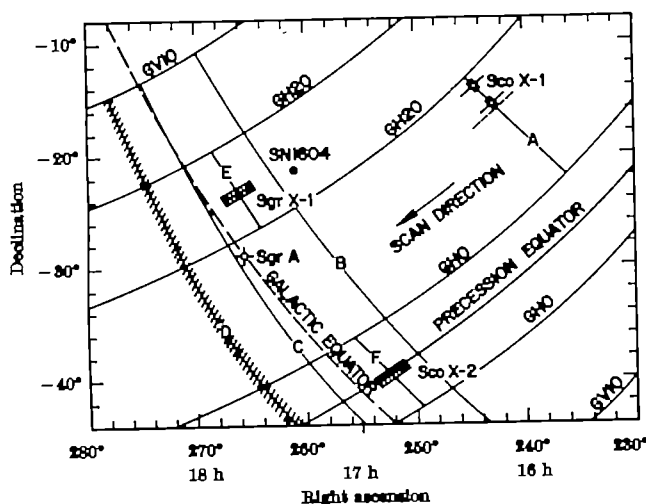


Fig. 2. Mercator projection of the celestial sphere in the region of the galactic centre. The cross-hatched areas show the locations of the sources as determined in this experiment. The small circles labelled GV10, GH0, etc., are the extreme boundaries of the regions scanned by the corresponding detectors.

*Sgr X-1* is  $5^\circ$  away from the Kepler supernova remnant *SN1604*. The absence of a significant peak at the average bearing of *SN1604* in the *GV10* data in Fig. 1a shows that any X-ray emission from this remnant must be several times smaller than that of the nearby sources. Therefore, our observations do not support the identifica-

tion of an X-ray source with *SN1604*, as suggested by the *NRL* group<sup>6</sup> on the basis of data obtained with an instrument of lesser angular resolution.

*Sco X-2* lies more than  $5^\circ$  away from the position of the nearest source reported by the *NRL* group<sup>6</sup>.

Two of the circular arcs along which Fisher *et al.*<sup>7</sup> have located sources pass within  $1^\circ$  of our locations for *Sgr X-1* and *Sco X-2*, respectively.

Finally, we note that there is a striking lack of symmetry with respect to the galactic centre in the distribution of the general X-ray emission from this region. The centre line of the emission region between lines *B* and *C* in Fig. 3 passes the galactic centre at a galactic latitude of  $b_{\text{ll}} = +2.5$ . It is also apparent that the radio centre of the galaxy, *Sgr A*, is at most a weak X-ray source compared with the other sources nearby.

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## A HIGH-SENSITIVITY SURVEY OF M31 AND SURROUNDINGS AT 1,415 MEGACYCLES PER SEC

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MAPS of the Andromeda and Perseus regions made at 600 and 1,415 Mc/s with the Ohio State University 260-ft. radio telescope have recently been published<sup>1,2</sup>. The telescope data output for these maps was recorded in analogue form (pen and paper chart) and all data were processed manually. The observations were completed in January 1964. By September 1964 a new stepped-amplitude twin-feed horn<sup>3</sup> and digital output had been installed for the 1,415 Mc/s receiver and a new survey of the *M31* region lasting four months was begun at this frequency. Using a liquid-nitrogen refrigerated parametric amplifier<sup>4</sup> and repeated scans a sensitivity (root mean square noise temperature) of  $0.01^\circ$  K was achieved with all data reduction done on the University's IBM 7094 computer. With this sensitivity and method of data processing, *M31* is resolved into a number of sources, one of which is close to the optical nucleus. Much detail is also brought out in surrounding areas that was not apparent on the earlier map. The region surveyed extends between right ascensions of 23h 30m to 02h 00m and north declinations of  $39^\circ 10'$  to  $43^\circ 10'$ . The antenna beam-widths at half power are  $13'$  in right ascension by  $38'$  in declination.

The results of the new observations are illustrated by the 17 profiles in Fig. 1 as plotted by the computer. The profiles are at declination intervals of  $15'$ . Each profile as plotted is the average of no less than four drift scans taken on different days except for one profile which is the average of three scans. A total of 72 scans is involved. A digital integration time of 10 sidereal sec was used so that more than 60,000 basic data points are involved in the 17 profiles. With additional scans at the central

declination ( $41^\circ 10'$ ) a profile involving the average of eleven scans was also made. This profile shows little difference as compared to the four-scan central profile in Fig. 1 even with respect to small details, indicating that with four scans the results are close to the resolution limit of the telescope. This means that a further increase in the telescope sensitivity would reveal no more significant detail at this frequency although the same results could be obtained with fewer scans.

The galaxy *M31* is evident as the extended object near the centre of Fig. 1 and appears to be resolved into as many as ten distinct radio features. A number of other prominent objects appear elsewhere in the figure as peaks on several adjacent profiles. (A point source should appear on three or four adjacent profiles and should trace the antenna pattern in right ascension with a time between half-power points of 53 sec.) Two sources in the third Cambridge list (3C13 and 3C54) are apparent in Fig. 1 although their centres are beyond the region surveyed. About twenty Ohio list *A* (O*A*) sources tabulated in the earlier survey are evident. A like number of additional sources are also apparent. These have been given new O*A* numbers and are listed in Table 1. The new sources are: O*A* 5, 16-1, 17, 23, 25, 27, 29, 35-1, 35-2, 35-3, 35-4, 35-5, 35-6, 35-7, 35-8, 35-9, 38-1, 39, 43, 53, and 61. Source O*A* 53 is an extended object which surrounds O*A* 52 and 54. (Sources are listed in Table 1 only for the right ascensions of Fig. 3.)

Fig. 2 is a contour map of the central region of Fig. 1 showing *M31* and its immediate surroundings. The contour interval is  $0.025^\circ$  K antenna temperature referred to as an arbitrary zero-level. The contours were drawn

manually on a map printed by the computer with each contour step indicated by a single digit. The contours outlining the emission from the galaxy show marked asymmetry. In both directions from the nucleus along the major axis the emission conforms to the axis but beyond swings away into narrow north and south spurs. The ten radio features associated with M31 are designated O436 and O435.1 through O435.9. The source O435.3 is less than 3' from the optical nucleus. Taking the distance of M31 as 2 million light years and assuming that this source is in the plane of the galaxy, it would be less than 2 kiloparsecs from the nucleus. Although the sources O436 and O435.6 may be background objects, their location in the north and south spurs gives some probability to their being associated with the galaxy. There is no indication of emission from NGC205 and none is apparent from M32, but the closeness of O435.3 makes this somewhat inconclusive. The fact that several sources, such as O435.4, O435.7, O435.8, rise only one contour-interval above the surroundings gives them only a low probability of reality. Sources O433, 35.2, 35.5, 36, 37, 38 and 39 appear at or close to peaks in MacLeod's map<sup>4</sup>, while O435.6, O437 and 38 are in the list of Scott, Ryle and Hewish<sup>5</sup>. Besides the much greater detail in Fig. 2 as compared with the earlier map<sup>1</sup>, the most noticeable difference is the absence of the spurs preceding and following M31 along its minor axis. The spurs may be real features but do not appear in Fig. 2 because the computer programme for removing long-term drift tended to discriminate against weak objects extended in an east-west direction.

Fig. 3 is a partial contour map of a larger part of the region of Fig. 1, in which a few selected contours are shown to indicate the locations of the principal sources with their OA or 3C designations. Of particular interest are the two clusters of sources about 30 min preceding and following M31 and the cluster to the south. The preceding cluster consists of O414, 16, 16.1, 17 and 18, and the following cluster of O442, 45, 46, 50, 52 and 54. The cluster to the south consists of O425, 26, 28, 29, 3013, O436, 37, 38 and 38.1. In the discussion of the earlier map the question was raised as to the possible relation of the preceding and following clusters with M31 (ref. 1). To these might also be added the cluster to the south. Thus, might some of the sources in these clusters be material ejected from the nucleus of M31 at some earlier epoch? There is an apparent north-south elongation of some of the sources in the preceding and following clusters and a marked beam broadening (east-west elongation) of some of the sources in the south cluster. The preceding and following clusters are at

Table 1. SOURCES ON OHIO STATE UNIVERSITY 1,415 Mc/s MAP

Source	Position* (1950 0)			+ Dec.		Flux density†
	h	m	s	deg	min	
O4 5†	23	40	23	40	56	0.4
O4 8†	23	51	30	36	54	0.6
O4 14	23	58	21	40	39	1.0
O4 16†	23	58	40	41	30	0.7
O4 16.1	00	02	02	41	23	0.5
O4 17	00	06	24	39	43	0.6
O4 18†	00	10	54	40	31	1.4
O4 23	00	15	40	42	28	0.3
O4 24†	00	19	01			0.8
O4 25	00	22	04	30	49	0.4
O4 26	00	22	39			
O4 27	00	22	52	42	17	0.5
O4 28	00	27	08	39	50	0.3
O4 29	00	29	58	39	40	0.4
3C 13	00	31	45			
O4 33‡	00	35	48	41	17	0.5
O4 35.1	00	37	38	40	19	
O4 35.2‡	00	38	30	40	45	
O4 35.3	00	39	48	40	50	
O4 35.4	00	40	57	41	06	
O4 35.5‡	00	42	08	41	20	
O4 35.6‡	00	41	55	42	27	0.5
O4 35.7	00	42	26	40	55	
O4 35.8	00	43	38	41	43	
O4 35.9	00	44	06	42	10	0.4
O4 36‡	00	39	41	39	56	0.8
O4 37.1‡	00	45	47	40	06	0.7
O4 37.1	00	48	52	42	28	0.4
O4 38.1‡	00	51	41	40	25	0.7
O4 38.1	00	53	47	39	25	0.6
O4 39‡	00	53	22	41	18	0.4
O4 43	01	00	44	42	17	0.6
O4 43	01	02	00	39	45	0.8
O4 45†	01	03	47	42	08	0.8
O4 46	01	06	52	39	43	0.4
O4 50†	01	08	21	41	35	1.1
O4 52	01	10	38	40	08	0.7
O4 53	01	12	15	40	15	
O4 54†	01	13	40	40	07	0.7
O4 59†	01	20	40	40	21	0.7
O4 61	01	28	29	39	41	0.4

\* Probable error for OA sources:  $\pm 5$  s in R.A.,  $\pm 5'$  in dec.† In flux units of  $10^{-26}$  W m<sup>-2</sup> (c/s)<sup>-1</sup>, probable error  $\pm 50$  per cent.

‡ In list of Scott, Ryle and Hewish (ref. 6) (178 Mc/s).

§ On map of MacLeod (ref. 5) (610 Mc/s).

opposite ends of an axis through M31. Circles centred on the nucleus of M31 and drawn through the members of these clusters, as shown by the dashed lines in Fig. 3, have a relatively uniform spacing. We offer the conjecture that some of the sources in these clusters may be associated with the wave crests of the shock-front of material ejected from M31 in a prior epoch and further that some of the sources in the south cluster may be at a comparable distance but in a direction from M31 more nearly along our line of sight. On the other hand, the cluster associations may be entirely a coincidence and the sources unrelated to M31. Further investigation is needed to decide between these possibilities.

Scott, Ryle and Hewish<sup>5</sup> list thirteen sources in the area of Fig. 3. Of these, twelve correspond to O45, 8, 16, 18, 24, 35.6, 37, 38, 45, 50, 54 and 59. Omitting one source which is complex, the systematic difference in

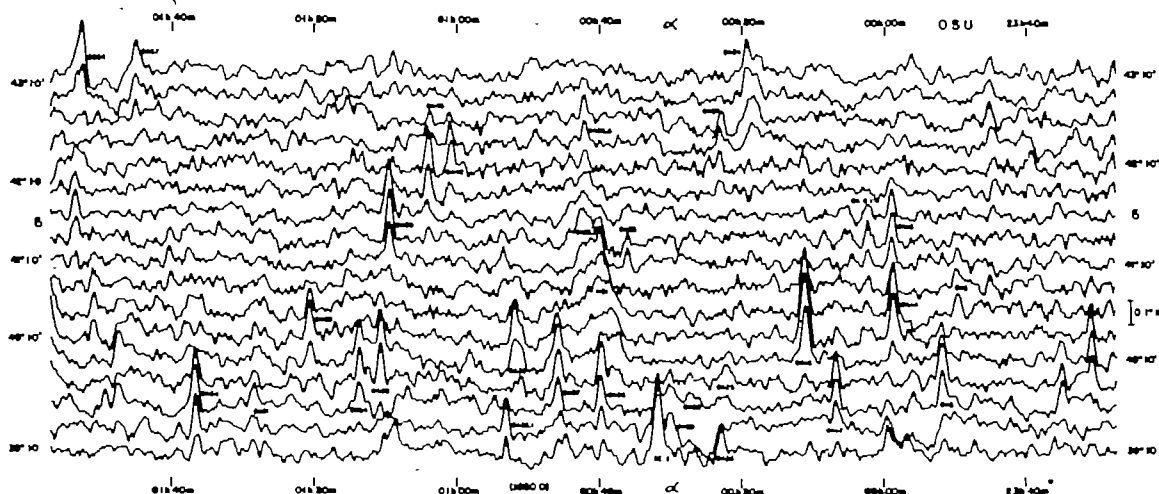


Fig. 1. Oblique view of M31 region as shown by high-sensitivity profiles made with Ohio State University 260-ft. radio telescope at 1,415 Mc/s. The sensitivity is 0.01° K with the strongest sources having an antenna temperature of less than 0.4° K. The centres of 3013 and 54 are beyond the declination limits of the survey but appear owing to their proximity. The same is true for the sources O4 24, 26 and 57.

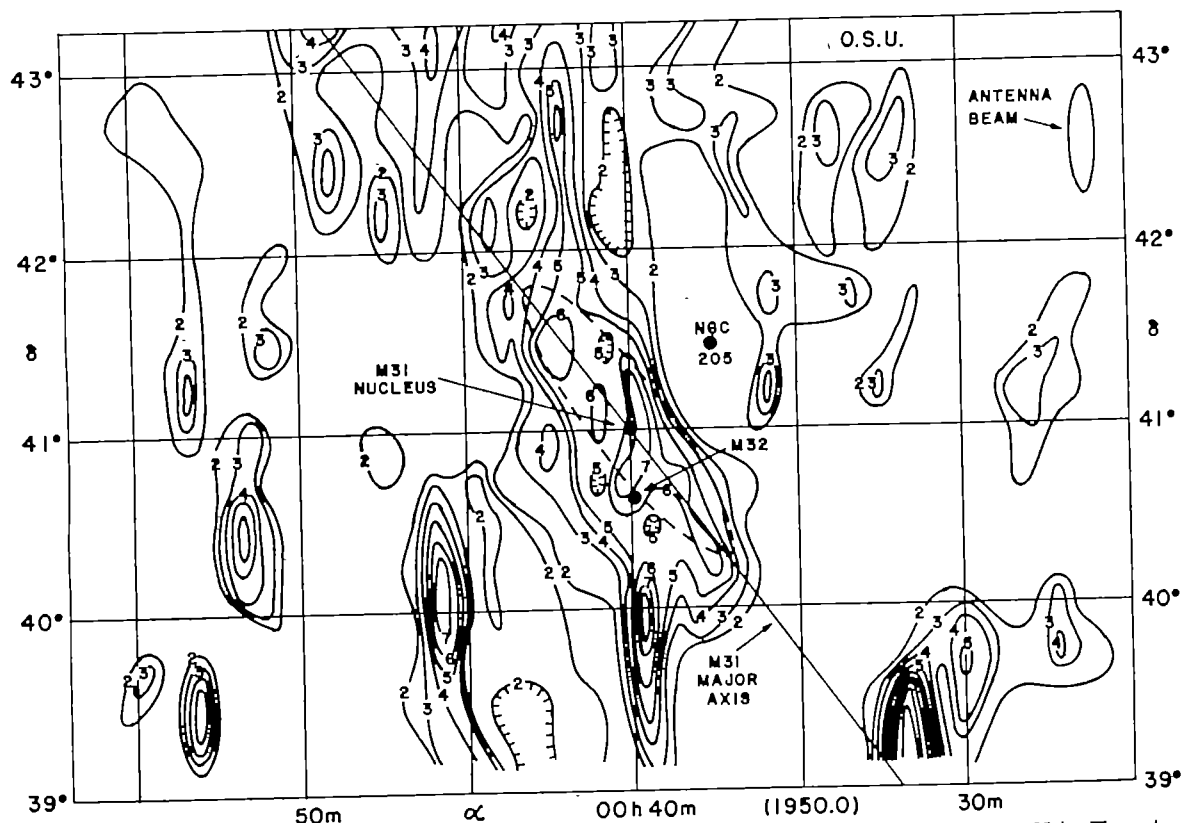


Fig. 2. Contour map of M31 and immediate surroundings made with the Ohio State University 250-ft. radio telescope at 1,415 Mc/s. The contour interval is 0.025° K antenna temperature. The dashed ellipse indicates the extent of the Andromeda galaxy (M31) on the usual photograph

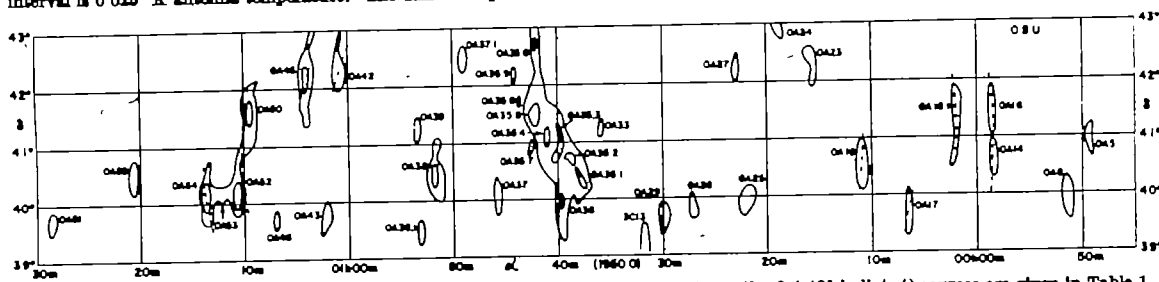


Fig. 3. Partial contour map of M31 region showing principal radio sources. Data on the OA (Ohio list A) sources are given in Table 1

their positions and ours amounts to less than 1 sec of time in right ascension and less than 3 min of arc in declination with standard deviations of 5 sec of time in right ascension and 3 min of arc in declination.

A more complete discussion of the techniques and results of our survey with more accurate position and flux data will be published in due course.

During the observations the telescope receiver and antenna were operated by S. R. O'Donnell, R. Townsend, G. Mikesell and W. Truss. R. O. Fisher, of the University's Computer Center, was responsible for much of the computer programme, and O. P. Haught assisted in its

execution. S. Y. Meng also assisted in the work. This work was supported in part by the U.S. National Science Foundation, the U.S. Air Force Cambridge Research Laboratories, and the Merston and Development Funds of the Ohio State University.

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## RE-SURVEY OF HURRICANE EFFECTS ON THE BRITISH HONDURAS REEFS AND CAYS

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CONSIDERABLE attention has been directed in recent years to the morphological and vegetational effects of catastrophic storms on the coastal features of high islands, such as Mauritius<sup>1,2</sup> and Guam<sup>3</sup>, and of atolls, particularly

Jaluit Atoll, Marshall Islands<sup>4,5</sup>, and Ulithi Atoll, Caroline Islands<sup>6</sup>. Most of these surveys were carried out shortly after the occurrence of major storms, and recorded immediate effects by comparison with pre-storm conditions.

On October 30–31, 1961, Hurricane *Hattie* crossed the atoll and barrier reefs of the British Honduras coast, causing major damage to reefs and islands shortly after a complete survey of island physiography and vegetation<sup>1</sup>. This made possible the detailed analysis of hurricane-induced changes during an expedition three months after the storm<sup>2,3</sup>. While the immediate effects of major storms is thus well documented, less is known about the long-term stability of hurricane-induced changes, and hence of the significance of such storms in the development of the surface features of coral reefs and islands. At Jaluit Atoll, the storm effects were re-surveyed three years after Typhoon *Ophelia*, when Blumenstock, Fosberg and Johnson<sup>4</sup> reported considerable changes in topography and vegetation during that period. During March–April 1965 a small expedition revisited the British Honduras reefs to collect data on reef and island changes in the 3½-year period since the immediate post-hurricane survey, in order to assess the long-term role of tropical storms in the evolution of reefs and reef islands. This report briefly notes the major conclusions of this re-survey, which may be compared with those of Blumenstock, Fosberg and Johnson at Jaluit Atoll<sup>4</sup>.

#### Nature of Immediate Hurricane Effects

The British Honduras coast is fringed by a 180-mile long barrier reef and coastal lagoon with three atolls (Turneffe Islands, Lighthouse Reef and Glover's Reef) to seaward. The reef flats carry numerous small islands or cays, which in topography and sediment composition are strikingly zoned in response to exposure to prevailing winds and waves. In their natural state the cays carry a littoral thicket of *Cordia*, *Bursera* and *Thrinax*, now largely replaced by coconut plantations. Hurricane *Hattie* crossed the northern part of Turneffe Islands and Lighthouse Reef and the northern barrier reef, accompanied by winds gusting to more than 200 m.p.h. and by a storm surge 45 miles wide and up to 15 ft. above normal high water.

Damage to the reef itself was considerable but superficial: over a narrow zone of the barrier reef more than 90 per cent of the reef corals disappeared, destroying the groove-spur system on the reef front; damage decreased to north and south of this zone, but was greater to the north where winds were mainly easterly. Damage to islands was also concentrated near the storm track. In a central zone many small reef islands disappeared, and most others were overtopped by the storm surge and underwent surface sand-stripping and marginal shoreline erosion. Most of the pre-hurricane vegetation was destroyed in this zone, which extended farther to the north of the storm track than to the south. Outside the high surge area, topographic change was limited to marginal beach erosion with some deposition inland, and the exposure of semi-lithified cay sediments: this zone extended for some 40 miles north and south of the storm track. Outside this area, wind effects were dominant, resulting in the aligned felling of coconuts and other trees, and the defoliation of mangroves, particularly on Turneffe Islands and in the barrier reef lagoon. In the area near the storm track, many nearshore plants disappeared with the erosion of their substrate; and dominants such as *Tournefortia*, *Suriana* and *Borrichia* were no longer to be seen in a 40-mile wide zone north and south of the storm track immediately after the hurricane.

In the southern part of the barrier reef lagoon, particularly on densely vegetated cays off Placencia, and also on larger cays near the storm track which had not been cleared for coconuts, fresh shingle and coral rubble were piled against the vegetation hedge on the windward sides of the islands, above high-water level, and in these cases the main hurricane effects were aggradational rather than erosional. This suggested that the recent clearance of littoral thicket for coconut plantation, especially since 1850, may have had a major influence on the nature of hurricane effects, and may account for the increasing

numbers of cays totally destroyed during such major storms<sup>11</sup>.

#### Geomorphic Changes since 1962

During the 1965 re-survey, islands were revisited, mapped, and photographed from land and air over the whole area damaged by the 1961 hurricane. By contrast with the post-storm changes at Jaluit Atoll, geomorphic adjustments in British Honduras have been minor. Reef recovery, in particular, has been surprisingly small. In the area suffering greatest reef damage in 1961, the only corals still living in any quantity are those which survived the storm itself, particularly *Montastrea annularis*, and in places *Siderastrea siderea*, *Diploria*, and massive *Acropora palmata*. Recolonization has been limited to a few scattered *A. palmata*, all less than 30 cm tall, *Millepora* sp., *Agaricia agaricites*, and infrequent non-frame-building corals such as *Manicina*, *Bumelia*, *Myosiphylia* and *Porites* (*P. astreoides*, *P. furcata*, *P. porites*). Formerly extensive corals, such as *Acropora cervicornis*, are still rare or absent in the most severely damaged areas, and wide areas of flat-lying rubble and dead coral heads are thickly blanketed by non-calcareous algae, chiefly *Padina* with some *Halimeda*. Larger algae, such as *Turbinaria turbinata* on beach rock habitats, are much less numerous than before the hurricane. Sponges and gorgonians, while not numerous in damaged areas, have made a more rapid recovery than the corals.

Why reef recovery is delayed is not clear. The substrate of mobile debris is probably unfavourable to coral colonization, and non-calcareous algae are more rapid colonizers. In these circumstances the estimate made by Stephenson<sup>12</sup> at Low Isles, Great Barrier Reef, of 10–20 yr. being required for reef recovery is probably minimal following such major devastation; it seems likely that the reefs studied in 1959–61 had only recently reached a climax state following the major hurricane of 1931.

Some of the dead reef debris has been moved on to reef crests and island shores to form rubble ridges since the 1962 survey, but otherwise there has been little shoal-water topographic change since the hurricane.

Hurricane effects above high-water level on reef islands appear relatively permanent. Of the seven islands which disappeared in 1961, none has reappeared. In the intertidal and nearshore areas minor changes have occurred in beach profiles, with filling or cutting depending on exposure, but these changes are generally within the range of seasonal fluctuation. In the area of most intense reef damage slight erosion of seaward shores has occurred, consequent on increased exposure. Above and away from beaches there has been no topographic change, except at Northern Cay, Lighthouse Reef, where wind action has built small dunes on a hurricane-deposited sand sheet. In cases where large vegetated islands were reduced to shifting sandbars by the storm, as at Goff's and Sergeant's Cays, continuous minor changes of form and location are taking place, typical of those of unvegetated islands. On islands where rubble and shingle bars and ridges were built during the hurricane, as at Scipio and Colson Cays, these remain essentially intact and are now being vegetated; at Jaluit, by contrast, such bars were largely eroded on the seaward sides of islands in the three years following the typhoon<sup>13</sup>. Gravel and sand sheets on islands and channels cut through island surfaces (except where the latter have been filled by man) remain essentially unaltered.

The most interesting geomorphic changes since the hurricane were found where areas of incipient lithification of island sediments were exposed at and above high-tide level by beach erosion and retreat. This lithification was of two main types: cay sandstone and beachrock. At Big Cay Bokel, Deadman Cays, Grand Bogue Point and Harry Jones, Turneffe Islands, platforms of slightly bonded sand and shingle were uncovered, filled with coconut roots which could be traced upwards into the overlying un-bonded sands. Bonding was so slight that the rock could

be easily crumbled in the fingers. The highest of these exposures reached 60 cm above mean sea-level, and the lowest only slightly above high water level (tidal range about 20 cm), and their height correlated directly with the height of the land behind the beach. By 1965, these exposures had been converted, mainly by case-hardening, into a tough limestone of reef fragments in a carbonate sand matrix.

In all the cases of case-hardening noted, the rock was subject to spray-wetting, and where this was not the case, as at Cay Chapel, the cementation remained weak and the rock friable. The sandy examples are similar to the cay sandstones formerly noted at Harry Jones, Turneffe, and Northern Cay, Lighthouse Reef, and the conglomerates to rubble platforms at Half Moon Cay, Lighthouse Reef. In these cases the existence of lithified platforms above present sea-level had suggested the possibility of recent relative sea-level shifts, but the new data indicate that such high-standing platforms can be produced with stable sea-levels, by the lithification of sub-island sediments exposed by storms. This may have implications in the Pacific, where similar, though larger, topographic features have been used as indicators of sea-level changes<sup>19</sup>. Weakly bonded intertidal beachrock exposed on many islands by the storm (for example, Sandbore Cay, South Water Cay) had been strongly though superficially lithified by 1965.

#### Vegetation Changes since 1962

The major reef island changes in the period 1962-65 have been vegetational. In the zone of major damage, vegetation recovery, as at Jaluit, has depended largely on whether the original soil cover was preserved during the storm (mainly in the interiors of large islands), or whether it was either stripped by surface erosion or buried by fresh sand or rubble deposits (on the margins of larger islands, and across the whole surface of smaller islands near the storm centre). In the case of soil preservation, recovery of herbaceous vegetation and grasses has been rapid, and the ground cover of *Wedelia*, *Canavalia*, *Ipomoea* and *Stachytarpheta* may reach 100 per cent. Where soil stripping occurred the vegetation cover is generally less than 25 per cent, and consists of discrete patches of *Sesuvium*, *Sporobolus*, *Ipomoea* and *Euphorbia*. In cases of soil burial, vegetation colonization depends on the calibre and thickness of the hurricane deposits. Thick nearshore sand sheets may be so densely covered with *Ipomoea* and *Canavalia* that the physiographic effects of the hurricane are largely hidden. Deposits of shingle and rubble especially where thin and overlying a deeply eroded cay surface, have a very patchy cover of *Euphorbia* and *Sesuvium*. Where whole islands were reduced to unvegetated sandbores by the storm, and in 1962 carried a pioneer strand flora dominated by *Portulaca oleracea*, the more stable areas are now covered with carpets of *Ipomoea*, *Canavalia*, *Euphorbia* and grasses.

The dominant nearshore shrubs destroyed by the hurricane in the zone of major damage have made a remarkable recovery. The stripped south shore of Half Moon Cay now has a 50 per cent cover of *Tournefortia gnaphalodes* in patches up to 10 metres in diameter and 1.5 m tall. *Tournefortia* is the most widespread of the nearshore colonizers, but elsewhere *Suriana maritima*, *Sophora tomentosa*, *Borrichia arborescens* and *Conocarpus erecta* reach heights of 1-3 m, regenerating from seed rather than stumps.

Tree recovery has been less spectacular. Coconut trees which retained their crowns during the storm are now fruiting, and trees planted in 1962 now have heights of 1-3 m. Fallen trunks and exposed root nets are rapidly rotting away. *Thrinax* has made a good recovery even where blanketed with shingle in nearshore areas. Of the littoral thicket dominants, *Cordia sebestena* has been most resilient, flowering freely in 1965 from the most shattered stumps; *Coccoloba uvifera*, while less widespread, has also

regenerated from stumps in most cases; but *Bursera simaruba* appears to have been killed in all cases of severe damage.

While vegetation on the sand cays is rapidly reverting to normal, that on mangrove islands has suffered permanent damage. *Rhizophora*, *Avicennia*, *Laguncularia* and *Conocarpus* were defoliated during Hurricane Hattie within a zone extending 20-25 miles north and south of the storm track: the 1965 survey showed that most of this defoliation resulted in death. Particularly in the barrier reef lagoon, mangrove islands are grey and dead over a 50-mile wide zone, the boundaries of which are sharply delimited and pass from mainly dead to mainly living mangrove within 5-10 miles. Within the mortality zone there are small patches of living *Rhizophora* in areas sheltered from extreme winds and waves, but otherwise vegetation growth is limited to fresh colonization by *Batis maritima* and *Cyperus* spp. on higher land. Similar mangrove mortality following major storms has been noted elsewhere (in Guam<sup>14</sup> and Florida<sup>15</sup>) but its precise cause is unknown. As a result of this massive mortality, the formerly abundant *Rhizophora* seedlings are now rare in shoal water areas throughout the central area, and it may be many years before the dead mangrove is replaced by new growth.

#### Economic and Other Adjustments

Following Hurricanes Janet (1955) and Hattie (1961) economic investment on the cays has practically ceased. The failure of coconut rehabilitation projects after Janet has led the British Honduras Government to decline to sponsor further projects after Hattie, and private concerns have made no large plantings. Clearing on one large island for tourist development has ceased, and with the decline of a settled population many of the cays are reverting from intermittently cleared coconut plantation to a coconut-dominated thicket. The change in nature and increasing density of vegetation will undoubtedly increase island resistance to catastrophic damage during later hurricanes<sup>11</sup>. Two destroyed lighthouses have been replaced by automatic lights, and the Government has built small jetties at larger cay and coastal settlements. Few of the cays abandoned after the storm, however, have been reoccupied, and in the central area especially, soil-stripping and the lack of vegetation growth mean that permanent reoccupation may be long delayed. Only at St. George's Cay, a holiday centre near Belize, and at Big Cay Bokel, a tourist centre on Turneffe, has any post-hurricane development taken place, but in exposed locations and lacking vegetational protection further destruction seems inevitable during later hurricanes.

Bird life on the cays has returned to normal since 1962. The nesting of *Sula sula sula*, the pink-footed booby, delayed several weeks in 1962, occurred normally in February-March 1965. Large numbers of pelicans (*Pelecanus occidentalis*), ospreys (*Pandion haliaetus*) and man-of-war birds (*Fregata magnificens*) were seen throughout the damaged area in 1965.

#### Conclusions

The conclusions of the 1965 resurvey reinforce those tentatively made immediately after Hurricane Hattie. In areas of major reef damage, recovery will probably take 25-30 years, or more if the colonization of unfavourable substrates is long delayed. On islands the critical nature of pre-hurricane vegetation is emphasized: with dense littoral thicket aggradation may occur during storms, whereas with coconuts erosion is more probable. Whichever occurs, the geomorphic changes during the storm at and above sea-level appear to be permanent, at least until modified by later hurricane action. Vegetation growth on favourable substrates has been rapid, and with the abandonment of settlements on the cays there may be a reversion to littoral thicket vegetation in place of coconut plantations, with decreased susceptibility to future hurri-

cane damage. Where soils were destroyed, vegetation colonization has been slower, and its future development will probably depend on the rate of soil formation, about which little is known on reef islands. Useful comparisons can be made between these data and the results of the Jaluit Atoll re-survey, and continued observation of reef and island recovery on the British Honduras coast is likely to yield more information on the long-term significance of major storms in the building of coral reefs and islands.

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## A POSSIBLE QUANTITATIVE MECHANISM FOR CARCINOGENESIS BY ULTRA-VIOLET RADIATION

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BY assuming that cells contain two chemical agents, one advancing and one inhibiting growth, it is possible to construct a simple model which quantitatively accounts for the growth-rate of cancers induced by ultra-violet radiation. This article suggests and outlines a mechanism based on departures of these chemical agents from equilibrium in the cell. It may be regarded as an alternative point of view to Blum's theory<sup>1,2</sup>.

The experimental results for volumetric growth of ultra-violet-induced tumours are described by Blum<sup>3</sup> by the relation:

$$\ln \frac{V_i}{V_0} = \frac{SU_i^2}{(i - \alpha)} \quad \text{For } i > \alpha \quad (1)$$

The volume is  $V_i$  at some initial time  $t = t_0$  and  $t_i$  is the time elapsing from  $t = t_0$  to the time when the volume of the tumour is  $V_i$ . This is usually called the development time. The amount of ultra-violet energy per dose is  $U$ ,  $i$  is the interval between doses,  $S$  and  $\alpha$  are constants. This formula will be derived by considering the growth to be directly related to the influence ratio,  $\gamma$ , of two chemical substances,  $A$  and  $I$ , which respectively advance and inhibit cellular growth:

$$\gamma = \frac{A}{(I - I_0)} \quad (2)$$

Evidence has been presented by Szent-Györgyi *et al.*<sup>4,5</sup> showing that certain tissue cells contain two substances which promote and retard the growth of ascites tumours in mice. However, the interpretation of experimental data on tumour growth rates is not straightforward, as pointed out by Blum<sup>3</sup>, since these rates are coupled to the general metabolism of the animal and will change with age, feeding conditions, etc. In the case of these experiments it is not clear but that regression of tumours may be due to non-selective starvation of local tissue in the cancerous region. Only further research can determine the scope of reversible effects and the seriousness of these objections. For the present, however, we shall neglect such objections in the model which follows.

We suppose the total amount of inhibiting agent in the cell to be governed by radiation decomposition, thermal decomposition, chemical decomposition, and regeneration. In the normal cell the last three processes are in a steady state condition and maintain  $I$  constant. When irradiation occurs, the balance is upset by anomalous decomposition. When ultra-violet light is the carcinogenic agent, the rate of tumour growth is increased each time the dosage

is repeated, indicating that a cumulative process is at work. Thus we might expect the total amount of  $I$  present to be inversely proportional to the accumulated ultra-violet radiation, which is itself proportional to the time over which the dosage has been administered. Differentiating this relationship gives a formula for the rate of change of  $I$  in the cell:

$$\frac{dI}{dt} \sim - \frac{(i - \alpha)}{U i^2} \quad \text{for } i > \alpha \quad (3)$$

We propose a natural generalization of this formula. During a reaction, the change in concentration of a chemical species is usually proportional to the amount of that species present. Our interpretation of  $I$  as a compound in the cell then suggests that we write the decay rate of the inhibitor in a cell in the more general form:

$$\frac{dI}{dt} = - \frac{C(i - \alpha)}{U i^2} I \quad \text{for } i > \alpha; C = \text{constant} \quad (4)$$

This is a hypothesis which can be tested in which  $\alpha$  represents a threshold effect. If  $\alpha$  is a function of physiological conditions—such as previous dosage—and therefore of time, it may be possible to provide apparent immunity by using intervals of less than  $\alpha$  between doses, assuming  $\alpha(t)$  to be a monotonic function. It should be borne in mind that  $\alpha$  may be a function of energy per dose as well as of total dosage. We might, for the present, interpret this threshold effect as a regeneration mechanism for 'normal' semicontinuous dosage. If the intervals between doses are so short that the destruction of the inhibitor becomes a 'normal' condition, the (as yet unspecified) mechanism for maintaining a steady state concentration of the inhibitor will be modified to a new equilibrium-level as the cell adjusts. The value of  $\alpha$  found experimentally is about 0.5, although setting  $\alpha = 0$  and thereby assuming no threshold effect still gives a fair description of experimental data. In this case the accumulated energy would just be  $U i / i$ .

Integrating equation (4) between the limits  $I_0$ ,  $I$ , and  $t_0$ ,  $t$ , gives:

$$I = I_0 \exp \left( \frac{C(i - \alpha)}{U i} \right) \quad (5)$$

where:

$$I_0 = I' \exp \left( - \frac{C(i - \alpha)}{U i_0} \right) \quad (6)$$

Evidently  $I'$  is the amount of inhibitor present at time  $t = 0$ . Equations (5) and (6) are not meaningful for this



model if  $t_0 = 0$  since then  $I = 0$  independent of time. Possibly this indicates a discontinuity such as an initial ionization by an ultra-violet photon which triggers the growth of a tumour. For  $t_0$  finite,  $I_0$  is less than  $I'_0$  and as  $t_0 \rightarrow 0$ ,  $I_0 \rightarrow 0$ . We shall next suggest an interpretation of  $I_0$ .

Since the induction of a tumour requires at least several months, we may assume the rate of decrease of the inhibiting agent to be small and during most of the growth period, for  $t/t_0 \gg 1$ :

$$\frac{O(i - \alpha)}{Ut} \ll 1 \quad (7)$$

This is the criterion for a first-order approximation. Expanding the exponential in equation (5) and ignoring second-order terms in  $O(i - \alpha)/Ut$  we find:

$$(I - I_0) = I_0 \frac{O(i - \alpha)}{Ut} \quad (8)$$

This first-order solution to equation (4) for the decrease in  $I$  could have been obtained directly by assuming that the ultra-violet light perturbs the amount of the inhibitor present in the cell in the manner characteristic of an equilibrium system, that is,  $I = I_0 + (\delta I) + (\delta I)^2 + (\delta I)^3 + \dots$ . Later, however, we will want to return to equations (5) and (6).

It appears that  $I_0$ , which is always less than  $I'_0$  (the amount of inhibitor present initially in equilibrium), is the appropriate fiducial point for the measurement of  $I$  for given values of  $O$ ,  $i$ ,  $\alpha$ ,  $U$  and  $t_0$ . Then  $(I - I_0)$  is the effective amount of inhibitor present. Equation (8), which can in principle be tested experimentally, shows that the effective amount,  $I - I_0$ , of the inhibiting agent present after irradiation is begun is inversely proportional to the total dosage of ultra-violet light received, modified by the threshold constant. As the irradiation continues, the amount of  $I$  present in the cells decreases from  $I'_0$  toward  $I_0$ . It is interesting to examine the dependence of  $(I - I_0)$  on the interval between doses and the intensity per dose. If we define a parameter,  $\lambda$ , equal to the ratio between these quantities:

$$\lambda = \frac{(i - \alpha)}{U} \quad (9)$$

and use equation (6), we may write equation (8) as:

$$(I - I_0) = I'_0 \frac{O}{i} \lambda \exp\left(-\lambda \frac{t}{t_0}\right) \quad (10)$$

Consider equation (10) as a function of  $\lambda$ . If we plot  $(I - I_0)$  on the ordinate against  $\lambda$  on the abscissa, the curve will rise to reach a maximum at  $\lambda_0 = t_0/O$ , after which it will fall. On the rising portion of the curve, an increase in the interval between doses, or a decrease in the energy per dose, will result in an increase in  $(I - I_0)$  and therefore an increase in the effective amount of the inhibiting agent. It is not unreasonable to associate this with a quantitative expression of a recuperation effect. For  $\lambda > \lambda_0$ , either an increase in  $(i - \alpha)$  or a decrease in  $U$  decreases the effective amount of inhibitor. Small doses at long intervals may appear as single irreversible events to the cells and a general recuperation mechanism may not become effective in such circumstances. The dependence of  $\lambda_0$  on  $O$  is consistent with this interpretation. For as  $O$  becomes smaller, the rate of decrease of  $I$ , that is,  $-dI/dt \sim O$ , also becomes smaller. Hence, as the destructive power of the dose is reduced,  $\lambda_0$  increases and there is a larger range of parameters over which recuperation is possible. On the other hand, as  $t_0 \rightarrow 0$ ,  $\lambda_0 \rightarrow 0$ , there is no recuperation, and the model breaks down. This may again be due to the irreversibility of the singularity associated with the initial triggering event.

Let us now suppose that the growth rate is directly proportional to the influence ratio,  $\gamma$ , for cell proliferation. This may work as a chemical control which permits 'cancer templates' or possibly virus particles to replicate more rapidly as the growth ratio increases and thus

somehow convey information of the extraordinary conditions induced by the ultra-violet radiation to neighbouring cells. This modification of information transfer is consistent with the growth of transplanted malignant cells. Here it should be possible to introduce a more detailed mechanism for information transfer and feedback control between cells into this chemical equilibrium model when more experimental work becomes available.

According to Szent-Györgyi<sup>1</sup>, the chemical agent tending to advance growth is the more stable of the two compounds. For simplicity, therefore, we imagine it to be unaffected by ultra-violet radiation and thus maintained at a constant value in the tissue cells. Since the rate of cell proliferation is also proportional to the volume of the tumour, we have:

$$\frac{dV}{dt} = \beta \gamma V = \frac{\beta A U t}{O(i - \alpha) I_0} V \quad (11)$$

where  $\beta$  is a constant. Integrating this between  $V_0$  and  $V_1$ , and  $t_0$  and  $t_1$ , the tumour volume is found to be given, to high accuracy, by the experimental relation (1) for  $t_1 \gg t_0$ , where:

$$S = \frac{\beta A}{2 O I_0} \quad (12)$$

This model also qualitatively accounts for the observed break in the relationship between tumour development time and dose<sup>2</sup>, which suggests that there is an upper limit to the rapidity of tumour growth, as expected from general metabolic considerations. As  $t$  becomes very large in equation (5), we see that  $I$  goes to  $I_0$ , which is given by equation (6). However,  $I$  approaches  $I_0$  asymptotically and for large  $t$ ,  $dI/dt$  is very small. Hence  $\gamma$  is approximately constant. From equation (11) it immediately follows that for the maximum growth rate:

$$\frac{1}{V} \frac{dV}{dt} \approx \text{constant} \quad (13)$$

which is also observed<sup>3</sup>.

As a third comparison of this model with experimental results, we consider tumour growth and development time after irradiation ceases. Equation (4) then becomes:

$$\frac{dI}{dt} = -\frac{EI}{t} \quad (14)$$

where  $E$  is a constant equal to the left-hand side of equation (7) with  $t$  replaced by  $t_0$ , the time at which the dosage is stopped. The amount of inhibitor present at this time is  $I_0$ , which is inversely proportional to  $t_0$ . If this time is less than the development time, the tumour has a volume  $V_0$  which is approximately equal to  $V_1$  since the growth curve is exponential with a long time constant. Applying the same procedure used to derive equation (1) then yields:

$$\ln \frac{V}{V_0} = \frac{\beta A t_0}{I_0(E + 1)} \left[ \left( \frac{t}{t_0} \right)^{E+1} - 1 \right] \quad (15)$$

In the case that  $E \ll 1$ , consistent with equation (7), comparison of equation (15) with equation (1) shows that:

$$t_0 \sim \sqrt{t_1 t_0 - t_0^2} \quad (16)$$

which approximately fits the small amount of data available<sup>4,5</sup>.

This equilibrium formulation has the additional advantage of providing a natural basis for investigation of the reversibility of tumour growth in terms of a stable or unstable equilibrium. At present, however, the experimental data do not appear sufficiently accurate to justify a detailed reversibility analysis.

I thank Prof. H. F. Blum for his advice. This work was begun at the Bartol Research Foundation of the Franklin Institute.

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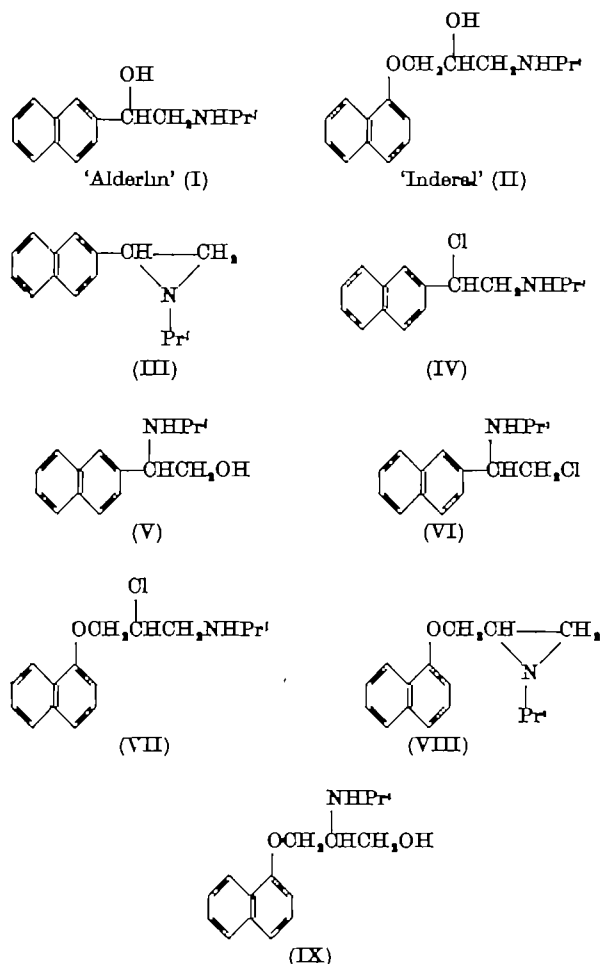
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## CARCINOGENICITY OF 'ALDERLIN' (PRONETHALOL) IN MICE\*

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'ALDERLIN' (I)<sup>1</sup> was shown by Black and Stephenson<sup>2</sup> in 1962 to block specifically cardiac and other  $\beta$ -adrenergic receptors in laboratory animals. In 1964, Alcock and Bond<sup>3</sup> described the production in mice of thymic tumours and other sarcomata associated with the administration of 'Alderlin', which had not appeared to be carcinogenic to rats, guinea-pigs or dogs. 'Inderal' (II)<sup>4</sup> (propranolol) has recently been shown<sup>5</sup> to be about ten times more potent than 'Alderlin' as a  $\beta$ -adrenergic receptor blocking agent and non-carcinogenic to mice<sup>6</sup>, rats, guinea-pigs or dogs<sup>7</sup>.



The major metabolites of 'Alderlin' have been identified<sup>8,7</sup> for the mouse and the rat, and their similarity suggests that it is unlikely that they can be concerned in the carcinogenicity of 'Alderlin'. In particular there was no evidence to suggest that the ethyleneimine (III), a member of a class of compounds known to be carcinogenic<sup>8</sup>, was a minor or transient metabolite of 'Alderlin' in the mouse; nor was there evidence of a sulphate or phosphate ester of 'Alderlin' which might be a precursor of the ethyleneimine (III). Nevertheless some biological

\* The terms 'Alderlin' and 'Inderal' are registered trade marks of Imperial Chemical Industries, Ltd.

and chemical observations led us to test the idea that the ethyleneimine (III) derived from 'Alderlin' might be the actual carcinogen.

The  $\beta$ -chloroethylamine (IV) related to 'Alderlin' was originally prepared<sup>9</sup> as a possible means for providing a slow release of 'Alderlin' *in vivo*. It was known<sup>10</sup> that such  $\beta$ -aryl- $\beta$ -chloroethylamines could be hydrolysed *in vitro* to the parent ethanolamine via an ethyleneimine. The  $\beta$ -chloroethylamine (IV) was as active as 'Alderlin' as a  $\beta$ -adrenergic receptor blocking agent. The onset of  $\beta$ -receptor blockade was rapid and the compound did not show any significantly prolonged activity. The observed biological activity is presumed to be due to hydrolysis *in vivo* to 'Alderlin' via the ethyleneimine (III), and hydrolysis must be rapid because of the speed of onset of  $\beta$ -receptor blockade. This explanation is supported by the fact that, whereas the position isomer (V)<sup>11</sup> of 'Alderlin' is virtually inactive as a  $\beta$ -receptor blocking agent, the  $\beta$ -chloroethylamine (VI)<sup>9</sup> related to it is as active as 'Alderlin'. Hydrolysis *in vivo* to 'Alderlin' via the common ethyleneimine (III) could explain the observed biological activity of (VI). Somewhat surprisingly the  $\beta$ -chloroethylamine (VII) related to 'Inderal' was devoid of  $\beta$ -receptor blocking activity, which suggested that it was not hydrolysed to 'Inderal' *in vivo*. It may, however, have been hydrolysed *in vivo* to the position isomer (IX) of 'Inderal', which is not active as a  $\beta$ -receptor blocking agent. An examination of the hydrolysis of the  $\beta$ -chloroethylamines (IV), (VI) and (VII) and the ethyleneimines (III) and (VIII) has confirmed our interpretation of the biological observations.

The hydrochlorides of (IV), and (VI), were separately heated at 100° C with 1 equivalent of 0.04 N sodium hydroxide for 1 h and then the solutions were cooled and rapidly extracted with ether after liberation of the organic base from its salt by the addition of a further equivalent of sodium hydroxide. Pure 'Alderlin' containing no trace of the position isomer (V) (checked by thin-layer chromatography) was obtained<sup>12</sup> in both cases, thus substantiating the hypothesis that the  $\beta$ -chloroethylamines (IV) and (VI) are converted to 'Alderlin' *in vivo* via the common ethyleneimine (III). Similar conditions applied to the hydrochloride of (VII) gave the ethyleneimine (VIII) as an oil (characterized as the picrate), and not 'Inderal' or its position isomer (IX). The initially elusive ethyleneimine (III) related to 'Alderlin' was obtained by treatment of the hydrochloride of (IV) with 5 per cent sodium bicarbonate and isolation of the free base (IV), which on standing dismutated to a readily separable equimolecular mixture of the hydrochloride of (IV) and the ethyleneimine (III) (an oil, characterized as the picrate). Pure 'Alderlin' was obtained when a solution of the ethyleneimine (III) in 1.1 equivalents of 0.1 N sulphuric acid was heated at 100° C for 0.25 h, cooled, and then rapidly extracted with ether after liberation of the organic base from its salt by the addition of 1.1 equivalents of sodium hydroxide. Under similar conditions the ethyleneimine (VIII) was recovered virtually unchanged. This ethyleneimine was, however, hydrolysed to the extent of 10–15 per cent when the heating period was prolonged to 1 h. The product of the hydrolysis was the position isomer (IX) of 'Inderal'. Pure 'Alderlin' hydrochloride was obtained<sup>13</sup> by heating the hydrochloride of (IV) at 100° C for 2 h with water or

N hydrochloric acid and then evaporating the solution to dryness. Under similar conditions the hydrochloride of (VII) was recovered unchanged. Thus the interpretations put forward to explain the results of the  $\beta$ -receptor blockade experiments were substantiated.

The conversion of the  $\beta$ -chloroethylamine (IV) to 'Alderlin' *in vivo* via the ethyleneimine (III) suggested a means of testing the ethyleneimine hypothesis proposed by Rose and Howe<sup>3</sup> to account for the carcinogenicity of 'Alderlin'. Administration to mice of the  $\beta$ -chloroethylamine (IV), which should produce an equivalent amount of the ethyleneimine (III) *in vivo*, ought to lead to the development of significantly more thymic tumours than would be expected from 'Alderlin' if the ethyleneimine (III) were but a minor metabolite. After the latent period the tumours might also be expected to appear on average at an earlier date in the case of (IV). The ethyleneimine (III) itself was not administered to mice because we did not have a more convenient method of presenting it than as the hydrochloride of (IV).

In an experiment performed by colleagues in these laboratories, groups of 25 male and 25 female mice were given either 'Alderlin' hydrochloride or the hydrochloride of (IV) in the diet at each of the dosage levels shown in Tables 1 and 2. The number of days between the beginning of the experiment and the day on which the thymic tumour was first observed was recorded, and the tumours were afterwards confirmed on post-mortem examination. The experiment with 'Alderlin' terminated on day 454 and that with the  $\beta$ -chloroethylamine (IV) on day 265.

Table 1	
% 'Alderlin' in diet	Day on which thymic tumour was observed
0.2%	♂ 223 ♀ 171, 257
0.1%	♂ 115, 320, 450 ♀ 141, 240, 358, 454
0.05%	♂ None ♀ 172, 220, 428
Control	♂ 228 ♀ None

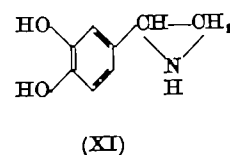
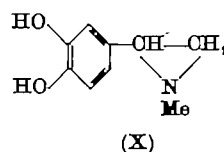
Table 2	
% (IV) in diet	Day on which thymic tumour was observed
0.2% reduced to 0.15% after 2 months	♂ 78, 92, 92, 100, 101, 103, 104, 113, 117, 120, 130, 141, 146, 149, 151, 167, 183, 190, 210, 253 ♀ 100, 156, 187, 231
Control	♂ None ♀ 158, 142, 153, 173

Unfortunately, the  $\beta$ -chloroethylamine (IV) was selectively toxic to female mice. In the first 90 days seventeen of them died, the majority of which showed severe kidney damage. The test is being repeated using groups of 50 male and 50 female mice, and at a dose-level of 0.1 per cent in the diet. It is hoped that this dose will prove less toxic to the females so that we can determine whether or not the apparently low incidence of tumours is entirely due to the early mortality of the females. Nevertheless the striking increase in the incidence of thymic tumours in the males dosed with the  $\beta$ -chloroethylamine (IV) as compared with the incidence in those dosed with 'Alderlin' strongly suggests that the ethyleneimine (III) could be the carcinogenic agent formed from 'Alderlin' in mice.

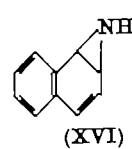
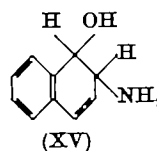
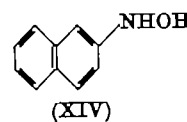
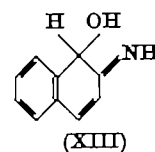
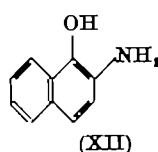
Rose has shown<sup>12</sup> for a series of aromatic nitrogen mustards that a certain level of chemical reactivity is necessary if a compound is to be an effective tumour growth inhibitor. If in a similar way chemical reactivity is necessary for tumour initiation then our chemical evidence suggests that the ethyleneimine (VIII) related to 'Alderlin' would probably be non-carcinogenic.

The hypothesis that the  $\beta$ -hydroxyethylamine side-chain of 'Alderlin' might be converted to an ethyleneimine *in vivo* suggests further lines of work. It would be interest-

ing to know whether or not the ethyleneimines (X) and (XI) related to adrenaline and noradrenaline are carcinogenic.



These ethyleneimines might arise as abnormal metabolic products if the normal metabolism of adrenaline and noradrenaline is impaired and might be responsible for the incidence of 'spontaneous' tumours in animals and man. In connexion with the carcinogenicity of aromatic amines, the *ortho*-hydroxylation hypothesis proposed by Clayton<sup>14</sup> and Walpole *et al.*<sup>15</sup>, and the *N*-hydroxylation hypothesis proposed by Miller *et al.*<sup>16</sup>, might be examined from a new point of view. For example, in the case of 2-naphthylamine, 1-hydroxy-2-naphthylamine (XII) and *N*-hydroxy-2-naphthylamine (XIV) are known metabolites which are carcinogenic<sup>17</sup>. The former presumably arises from the latter *in vivo* by an arylhydroxylamine rearrangement involving the *ortho*-quinolimine (XIII). Miller and Miller<sup>18</sup> have demonstrated that such a re-arrangement occurs *in vivo* in the case of *N*-hydroxy-2-acetylaminofluorene. Reduction *in vivo* of the *ortho*-quinolimine (XIII) or 1-hydroxy-2-naphthylamine (XII) could give the  $\beta$ -hydroxyethylamine (XV) which might be metabolized further to the ethyleneimine (XVI).



I thank my colleagues, Mr. P. J. Taylor, who is at present examining the kinetics of the formation and solvolysis of the ethyleneimines (III) and (VIII), and Dr. J. W. Black, Dr. R. G. Shanks, Dr. P. Lemon, Mr. P. A. Melvin, Miss S. J. Alcock and Miss M. J. Tucker for the biological results.

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## ROLE OF THE CARRIER IN DEVELOPMENT OF DELAYED SENSITIVITY TO THE AZOPHENYL-ARSONATE GROUP

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**G**UINEA-PIGS immunized with hapten-protein conjugates produce antibody which is hapten-specific, but exhibit a delayed hypersensitivity which is directed largely toward the protein carrier<sup>1</sup>. When, however, arsanilic acid was used as a hapten and polytyrosine as the carrier, a delayed hypersensitivity resulted which was hapten-specific<sup>2</sup>. Since this represented the only clear-cut case of hapten-directed delayed sensitivity, it became of interest to assess the relative importance of the hapten and the carrier in its production.

The present report concerns the function of the carrier and is a study of the immune response produced by injection of guinea-pigs with the arsanilic acid hapten attached to a variety of molecules which may be roughly divided into the following categories: (1) non-antigenic homopolymers of amino-acids; (2) antigenic co-polymers of amino-acids; (3) proteins of varying antigenicity; (4) proteins which are non-antigenic by virtue of derivation from serum of the same species.

Conjugates were prepared by adding the appropriate amounts of diazotized arsanilic acid to ice-cold solutions of carrier maintained at pH 8-9. Coupling was allowed to proceed overnight to ensure complete reaction, and the resulting coloured materials were purified for the most part by dialysis against running water. All proteins were conjugated with 2 mg of arsanilic acid to 10 mg of protein. Synthetic polypeptides were conjugated with slightly smaller amounts to provide one azophenyl-arsonate group (ars) per 3 tyrosine or lysine residues. Guinea-pig serum albumin (GSA) and insulin were conjugated with excess arsanilic acid for use as skin test antigens.

White male guinea-pigs weighing 400 g were immunized by injection in the four foot-pads of 100 µg of conjugate in 0.1 ml. of complete Freund's adjuvant<sup>3</sup>. Two weeks later they were shaved and depilated and tested for hapten-specific delayed hypersensitivity by intradermal injections of 1 µg N of ars-GSA and ars-insulin. Reactivity to the carrier was assessed by simultaneous injections of 20 µg of the immunizing conjugate and carrier alone, except where these gave non-specific toxic reactions. All skin tests were measured after 24 h to determine the extent of induration and erythema. Table 1 lists the results of immunization with conjugates of arsanilic acid and several synthetic polypeptides. The homopolymers of either tyrosine, histidine, or lysine (purchased from the New England Nuclear Corp., Boston) produced almost universal hapten specific delayed hypersensitivity when this was assessed by reactions to ars-GSA. Reactions to ars-insulin were, however, somewhat greater in ars-polytyrosine immunized animals and less frequent in ars-polyhistidine or ars-polylysine immunized animals. This might in some way reflect contributions by adjacent amino-acids to the reactivity of the azophenyl-arsonate group, since insulin is especially rich in tyrosine and poor in lysine. It is of some interest that the polymers of both L- or D-lysine appeared to be equally effective as carriers for the production of hapten-specific delayed sensitivity.

Despite the effectiveness of polylysine as a carrier, the copolymer of glutamic acid and lysine was ineffective in 8 animals tested. Only one animal, in fact, produced any reaction to the immunizing antigen itself. The addition of tyrosine to the polymer resulted in a carrier

that was universally effective in producing hapten-specific delayed sensitivity. Again, there was a suggestion of some contribution by the tyrosine group to the azophenyl-arsonate reactivity in the markedly greater reactions produced with ars-insulin. The copolymer of glutamic acid, alanine and tyrosine was also an effective carrier for production of delayed sensitivity with all animals showing reactivity to ars-insulin. In confirmation of studies by Benacerraf *et al.*<sup>4</sup>, the polymer synthesized from the D-isomers of glutamic acid, alanine and tyrosine was completely ineffective, even in a ten-fold greater immunizing dose.

The very much larger delayed reactions obtained on skin testing with the immunizing antigen in the case of the polymers of glutamic acid, lysine and tyrosine and glutamic acid, alanine and tyrosine suggested that a good measure of carrier specific reactivity was also present. In other instances, carrier specific reactivity could not be assessed because of the tendency of the conjugates to give non-specific reactions.

The results of immunization with conjugates prepared from a variety of proteins is shown in Table 2. Taken as a whole, a wide range of effectiveness is seen, ranging from total inability to produce hapten-specific delayed sensitivity to almost complete effectiveness. When the various protein carriers are considered in terms of their generally known potencies as antigens in a variety of animal species, some suggestion as to the relation between this property and the ability to produce hapten-specific delayed sensitivity emerges. Thus, bovine ribonuclease (RNase), insulin and serum albumin (BSA) were totally ineffective in the production of hapten-specific delayed sensitivity. Only one animal immunized with ars-BSA showed any reactivity with the heterologous skin test conjugates. While hapten-specific reactivity was lacking, large delayed reactions were seen in all cases after tests

Table 1. RESULTS OF IMMUNIZATION WITH CONJUGATES OF ARSANILIC ACID AND SYNTHETIC AMINO-ACID POLYMERS

Immunizing antigen (100 µg)	No. of reactions/No. tested and average diameter of delayed reactions produced with:		Immunizing antigen (20 µg)
	Ars-GSA (1 µg N)	Ars-insulin (1 µg N)	
Ars-polytyrosine	16/17 (13)*	5/7 (16)	
Ars-polyhistidine	14/17 (13)	2/7 (14)	
Ars-poly-L-lysine	12/13 (13)	2/4 (12)	
Ars-poly-D-lysine	9/10 (11)	7/10 (11)	
Ars-glutamic-lysine	0/8	0/8	1/8 (23)
Ars-glutamic-lysine-tyrosine	8/8 (13)	8/8 (18)	7/8 (25)
Ars-L-glutamic-alanine-tyrosine	3/5 (12)	5/5 (13)	5/5 (24)
Ars-D-glutamic-alanine-tyrosine	0/6	0/6	
Ars-D-glutamic-alanine-tyrosine (1 mg)	0/6	0/6	

\* Min of induration and erythema.

Table 2. RESULTS OF IMMUNIZATION WITH CONJUGATES OF ARSANILIC ACID AND PROTEINS OF VARYING ANTIGENICITY

Immunizing antigen (100 µg)	No. of reactions/No. tested and average diameter of delayed reactions produced with:		Carrier protein (20 µg)
	Ars-GSA (1 µg N)	Ars-insulin (1 µg N)	
Ars-gelatin	8/10 (9)*	4/10 (11)	10/10 (13)
Ars-claritidin	5/7 (12)	7/7 (14)	7/7 (17)
Ars-insulin	0/7	—	7/7 (16)
Ars-BSA	1/10 (13)	1/10 (12)	10/10 (19)
Ars-oxidized BSA	5/6 (7)	5/6 (6)	6/6 (13)
Ars-RNase	0/7	0/7	7/7 (20)
Ars-oxidized RNase	3/6 (7)	3/6 (9)	6/6 (21)
Ars-H haemoglobin	4/8 (7)	3/6 (7)	6/6 (20)
Ars-pepsin	4/5 (10)	4/5 (10)	5/5 (17)
Ars-GGA	—	3/15 (13)	11/11 (17)
Ars-GGG	8/10 (12)	8/10 (15)	10/10 (17)

\* Min of induration and erythema.

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with the immunizing antigen, as well as the carrier protein alone, thereby demonstrating the large measure of carrier specificity following immunization with these conjugates.

On the other hand, gelatine, haemoglobin and pepsin are ordinarily considered to be poor antigens for antibody production<sup>4-6</sup>. In the form of conjugates with arsanilic acid, all were found to produce some modest degree of hapten-specific delayed hypersensitivity as evidenced by skin reactions to ars-GSA and ars-insulin. Since inherent lack of antigenicity of the carrier protein was felt to be of importance in its ability to produce hapten-directed sensitization, an attempt was made to alter drastically this antigenicity in two cases (BSA and RNase) by oxidation of all disulphide bonds<sup>7</sup>. When arsanilic acid conjugates of oxidized BSA and RNase were used for immunization, small but definite hapten-specific delayed sensitivity was produced in many of the animals. Once again a large degree of carrier specificity could be elicited by tests with the immunizing conjugate and the carrier alone.

Advantage was taken of the fact that guinea-pig serum proteins would not be antigenic in the guinea-pig, and conjugates of two such proteins, albumin (GSA) and  $\gamma$ -globulin (GGG), were used as examples of poor or non-antigenic carriers. When immunization with ars-GSA and ars-GGG was carried out, excellent hapten-specific delayed sensitivity was produced in most of the animals receiving the globulin conjugate but only in an occasional animal receiving the albumin conjugate. In all cases, the immunizing antigen produced marked reactions, but the carrier alone produced none.

While the positive results obtained with ars-GGG seem to be striking confirmation of the contention that poorly antigenic carriers are required, the lack of hapten sensitization obtained with ars-GSA appears to be completely opposed to this hypothesis. One possible way to reconcile these apparently opposing data lies in the recognition that antigenicity of the carrier *per se* need not be qualitatively or quantitatively identical in the conjugate. In other words, immunization with a conjugate cannot be considered merely as sensitization to the native carrier plus sensitization to the hapten. It is well known, for example, that the process of conjugation can denature proteins and can either add or delete antigenic determinants<sup>8</sup>. Thus, it might be that the rather heavy conjugation with arsanilic acid in the present instance so denatured GSA that it became a relatively antigenic material, while GGG was not similarly affected.

These results do not yet allow an exact description of the qualities a carrier must possess to direct sensitization to an appropriate hapten but do suggest that good antigenicity in respect to antibody production militates

against the production of hapten-directed reactivity. This was shown by the ineffectiveness of conjugates of RNase, BSA and insulin to sensitize to hapten. When the antigenicity of such proteins is decreased by oxidation, some increase in ability to sensitize is then seen. The results obtained with the relatively poorly antigenic polymers of amino-acids seem in general to fit this pattern. On the other hand, a poorly antigenic carrier such as gelatine was only occasionally effective, although so few aromatic groups are present in this molecule that it could not be ascertained whether sufficient conjugation did in fact occur. Elastoidin, a tyrosine-rich protein from collagen<sup>9</sup>, was much superior to gelatine, which is poor in tyrosine. This enhancement of carrier effectiveness for directing specificity toward the hapten by the addition of tyrosine groups was similar to that observed with the glutamic-lysine and glutamic-lysine-tyrosine carriers (Table 1).

The failure to obtain sensitization with the polymer of the D-isomers of glutamic acid, alanine and tyrosine, while the polymer of D-lysine was effective, suggests caution in the interpretation of these results in terms of inherent digestibility or recognition of natural and unnatural amino-acid polymers<sup>3</sup>.

While the azophenyl-arsenate group is probably not unique in being able alone to sustain a delayed reaction, the commonly used haptens, such as *p*-aminobenzoic acid, sulphanilic acid and *p*-aminophenylacetamide, do not apparently contain the necessary complementarity or binding potential to produce hapten-specific reactions<sup>10</sup>. The work recorded here demonstrates that the production of hapten-specific delayed hypersensitivity requires not only a particular type of hapten able to express completely this reaction, but that in addition sensitization can be accomplished only by conjugation of this hapten to special kinds of carriers.

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## CORRELATION BETWEEN CODING-TRIPLETS AND AMINO-ACIDS

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A TABLE containing 62 nucleotide sequences of RNA codons was recently published by Trupin *et al.*<sup>1</sup> and by Nirenberg *et al.*<sup>2</sup>, who also noted that "amino-acids which are structurally or metabolically related (such as synthesized *in vivo* from a common precursor) often have similar RNA codons". It seemed of interest to explore: (a) what correlations exist between a coding triplet and the structure of the amino-acid coded for; (b) whether the properties of an amino-acid coded by a given triplet on one DNA-strand will in some respects be complementary to those of an amino-acid coded by the complementary triplet (as given by Watson-Crick pairing) on the second DNA-strand. The following correlations, shown schematically in Fig. 1, were found.

The columns headed U and C, that is, those containing all amino-acids the codon of which has uridilic acid or cytidilic acid in second place, enclose the hydrophobic amino-acids, the molecule frequently ends in a methyl group (CH<sub>3</sub>). Those with branched methyl groups are in the U column; OH-groups are seen in the C column. The acid and basic amino-acids have A or G as the second base and are found in the third and fourth columns. No amino-acids; the molecule frequently ends in a methyl. The only exception is serine with AGU and AGC, but this placement seems uncertain since the incorporation in the relevant experiment is scarcely above background.

Simple changes in the structure of an amino-acid are accompanied by a change of one base only in the codon.

		← 2 <sup>nd</sup> →					
1 <sup>st</sup> ↓		U	C	A	G		3 <sup>rd</sup> ↓
		PHE PHE <chem>c1ccccc1</chem>	SER SER <chem>OC(C)C</chem>	TYR TYR <chem>c1ccc(cc1)C(C)C(=O)O</chem>	CYS CYS <chem>SCC</chem>		U C
U		LEU LEU	SER SER		TRYP TRYP <chem>c1ccc2c(c1)c(c[nH]2)C(C)C(=O)O</chem>		A G
		LEU LEU <chem>CC(C)C</chem>	PRO PRO <chem>C1CCN1C(=O)O</chem>	HIS HIS <chem>c1ccc(cc1)C(C)C(=O)O</chem>	ARG ARG <chem>NC(=O)C(CCN)C(=O)O</chem>		U C
C		LEU LEU	PRO PRO	GLUN GLUN <chem>NC(=O)CC(C)C(=O)O</chem>	ARG ARG		A G
		ILEU ILEU <chem>CC(C)C(C)C</chem>	THR THR <chem>CC(C)C(C)C(=O)O</chem>	ASPN ASPN <chem>CC(C)C(C)C(=O)O</chem>	SER SER		U C
A		MET MET <chem>CSCC</chem>	THR THR	LYS LYS <chem>CCCCN</chem>	ARG ARG		A G
		VAL VAL <chem>CC(C)C</chem>	ALA ALA <chem>CC(C)C(=O)O</chem>	ASP ASP <chem>CC(C)C(=O)O</chem>	GLY GLY <chem>C(C)C(=O)O</chem>		U C
G		VAL VAL	ALA ALA	GLU GLU <chem>CCC(C)C(=O)O</chem>	GLY GLY		A G
		BRANCHING	CH <sub>3</sub> FREQUENT	OH	ACID NO	BASIC CH <sub>3</sub>	

Fig. 1. Correlation of codons and amino-acids. The first base in a triplet is noted downwards in the column on the left, the second base horizontally on top, the third base in the sequence U (uridine), C (cytosine), A (adenine), G (guanine) for each row in the narrow column on the right. For example, the codon for tyr, in the third column, top row, is either UAU or UAA. Abbreviations: phe, phenylalanine; leu, leucine; ser, serine; tyr, tyrosine; cys, cysteine; tryp, tryptophan; pro, proline; his, histidine; glun, glutamine; arg, arginine; ileu, isoleucine; met, methionine; thr, threonine; aspn, asparagine; lys, lysine; val, valine; ala, alanine; asp, aspartic acid; glu, glutamic acid; gly, glycine. Smaller letters to the right denote from which compound an amino-acid is derived: PB, propionic acid; OA, oxopropionic acid; Im, imino acid; VA, valeric acid; IVA, isovaleric acid; BU, butyric acid; SU, succinic acid; GL, glutamic acid; AC, acetic acid. The arrows indicate stepwise changes (see text). B =  $\text{H}_2\text{N}-\text{CH}-\text{COOH}$ , the group common to all amino-acids except pro.

Table 1. CORRELATION OF A STRUCTURAL DIFFERENCE OF AN AMINO-ACID WITH CHANGE OF CODON

aa <sub>1</sub>	change	aa <sub>2</sub>	Codon changes from	to
gly	CH <sub>2</sub>	ala	GGU	GCU
ala	O	ser	GCU	UCU
ala	CH <sub>2</sub> -C-H	val	GCU	GUU
ala	OOO	asp	GCU	GAU
asp	CH <sub>2</sub>	glu	GAU	GAA
glu	-OH	glun	GAA	CAA
asp	+NH <sub>2</sub>	aspm	GAU	AAU
asp	-OH	leu	GUU	CUU
val	CH <sub>2</sub>	ileu	GUU	AUU
val	OH	tyr	UCU	UAU
ser	benzene	phe	UCU	UUU
ser	-OH	cys	UCU	UGU
ser	+benzene	tryp	UUG	UGG
ser	-OH	his	UAU	CAU
ser	+8H	thr	UCU	ACU
ser	-OH			
ser	+indole			
tyr	-benzene OH			
ser	+imidazole			
ser	CH <sub>2</sub>			

For example, gly changes to ala by conversion of the terminal hydrogen to a methyl group, the codon from GGU to GCU; the difference between ala and ser, replacement of one hydrogen in the terminal CH<sub>2</sub> group by hydroxyl (OH), is accompanied by a change from GCU to UCU. Altogether fifteen such changes of one amino-acid into another by means of addition of one group connected with a corresponding one-step change in the codon are shown in Fig. 1 and Table 1. Conversely, transformations involving a more complicated rearrangement of an amino-acid molecule usually involve more than one step in the change of the codon, for example, val to met. These conversions are not necessarily steps in the actual synthesis of amino-acids or in the evolution of these compounds. They are additions or rearrangements of single components of amino-acids.

The amino-acids derived from propionic acid, namely, ala, ser, phe, tyr, cys, tryp and his, are situated in such a manner that conversions by a single step are possible; similarly glu and glun derived from glutaric acid, asp and aspm derived from succinic acid. Leucine and ileu derived from caproic acid are in adjacent squares, while the conversion of lys to ileu in two stages also involves changing two bases in the codon. No easy conversion of met into thr, both derived from butyric acid, seems possible.

The third base in a triplet seems to influence the coding only by being either a purine or a pyrimidine (previously suggested for the second base by Eek<sup>3</sup>) or it does not do so at all, especially when C is the second base of the triplet.

The correlations noted here might be explained either by the evolution of the coding system or by assuming that a structural relationship exists between codons and amino-acids. On balance the latter explanation seems to be more likely, since during evolution a random distribution of triplets and amino-acids would be expected if the two are not connected structurally; the probability of achieving by chance the well-ordered and logical arrangement reported here would be negligible.

The enzyme which attaches an amino-acid to its appropriate tRNA must recognize both; the tRNA in turn recognizes the codon on the mRNA. If as shown in this article a close structural relationship between codon and amino-acid exists, it has to be assumed that the two sites on the enzyme are also structurally related.

The second question posed in this article, namely, the possible complementarity of amino-acids, can now be considered in more detail. If thr is found in a given position in a protein chain, the appropriate codon on the mRNA might be ACA, formed from TGT on the DNA. The complementary triplet on the second strand of DNA would be ACA, which in turn would form UGU which codes for cys. In this way pairs of amino-acids complementing each other can be derived. Since the strands of DNA are anti-parallel the second triplet might have to be read backwards but this is not certain<sup>4</sup>. It is obvious that amino-acids with U or C as the second base will complement with those with A or G respectively in the same place. The marked differences between the respective amino-acids appearing in these columns will usually lead to pairing of members of different groups. For example, no acid or basic amino-acid can complement with another acid or basic amino-acid, and amino-acids containing a CH<sub>2</sub> group cannot complement with each other. The properties of pairs of complementary amino-acids are at present being explored by means of model-building.

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## HISTONES IN DEVELOPMENT, GROWTH AND CANCER

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UNICELLULAR organisms such as bacteria, protozoa and algae, when placed in a favourable environment containing all the required nutrients, grow at a maximum rate characteristic of the species. So long as adequate supplies of nutrients are maintained and waste products removed, as in a chemostat, this maximum growth will continue. In a simple medium containing salts and a carbon source, the unicellular organisms synthesize all the necessary proteins which in turn catalyse reactions producing all the required precursors for RNA, DNA and other macromolecules, as well as other products used as energy sources or for other special purposes.

A cancer cell is, in terms of its growth and multiplication, similar to the unicellular organisms. It continues to grow in the host or in tissue culture at a rate characteristic of the type of tumour, and probably limited largely by eventual failure of the nutrient supply. The unicellular organisms are in a real sense cancerous (consider, for example, a bacterial infection that overwhelms the body's defences) and conversely the cancer cell is behaving like a unicellular organism in a favourable environment.

The unicellular organism appears to lack any mechanism for growth control. The cancer cell lacks a functional mechanism for growth control.

Since it has been suggested that nuclear DNA-associated histones are the biological repressors which regulate gene action in cells from higher animals<sup>1-3</sup>, it is of considerable interest to ask the following questions:

(1) Do unicellular organisms such as bacteria, protozoa and algae contain DNA-associated histones which are used for regulating gene expression?

(2) Is the possible loss of growth control in a cancer cell a general failure of the histone regulating mechanism, or perhaps a limited failure of this mechanism, particularly with respect to metabolic pathways important to cell division?

It is obvious that these questions cannot be answered unequivocally as yet. The ability to ask the right questions, however, is an important part of scientific endeavour, and there are a number of interesting considerations and some data pertinent to these questions which will be discussed here. These considerations, in turn, may lead to some useful experimental approaches to further our understanding of growth.



With respect to the first question, there is a striking paucity of information concerning histones in unicellular organisms. Only a few scattered reports of histone-like materials in various bacteria, protozoa or algae are found. Most, or all, of these investigations have been carried out by histochemical techniques, and in the few occasions reported it is possible to raise serious doubts as to whether the supposed histone: (1) has a typical histone amino-acid pattern; (2) is associated with DNA in a functional way. In one investigation in which DNA nucleoprotein was isolated from *E. coli* and the amino-acid composition of the protein determined<sup>8</sup>, it proved to be completely different from the typical amino-acid pattern of histones and, in fact, had high proportions of dicarboxylic acids and low proportions of basic amino-acids—just the reverse of histone composition. This, of course, does not refute the possibility that the more acidic protein of the deoxyribonucleoprotein of *E. coli* may function in the same way as histones do in cells of higher animals. Recently Beck and Walker, using a fluorescent immunochemical technique, were unable to demonstrate histones in a number of different trypanosome nuclei<sup>9</sup>. Also, Butler and Godson found no evidence for histone-like protein in nuclear material from *Bacillus megaterium*<sup>10</sup>. In the absence of any good positive evidence for histones in unicellular organisms, the hypothesis that they lack this type of cellular control material is, for the moment, intact.

It is of interest to observe that the Jacob and Monod formulation of the operon system as applied to bacteria<sup>11</sup> does not specifically require histones for its functioning; although it appears quite possible that specific proteins may act as repressors in some cases.

At this point, it may be well to consider that a basic difference between the unicellular organism and the cell of a higher animal is that the latter can undergo differentiation, so that from a single cell type a diverse variety of cells with greatly different functions and properties may be formed. This would certainly require a mechanism for allowing expression of certain gene functions and not others, and for this reason the histone-regulator hypothesis is attractive. Since the unicellular organism does not differentiate in the ordinary sense, it has no need for such a mechanism. (It is true that certain bacteria undergo sporulation which might be considered to be a primitive type of differentiation. This is a process induced, generally, by unfavourable, rather than favourable, growth conditions.) In minimal medium, its entire complement of enzyme systems is presumably functioning at maximum capacity. In enriched medium, it uses (probably in common with cells of higher animals) a system of feedback controls to 'turn off' the synthesis of unneeded proteins.

Thus, addition of a single amino-acid to a minimal medium will prevent the production of all the enzymes involved in the synthesis of that amino-acid. The process is envisaged by Jacob and Monod as a combination of amino-acid with a regulator substance (which may be a protein), which in turn combines with the operon, a regulator gene, inactivating it. So long as the operon is inactive, no gene in the related group produces messenger RNA, and hence no enzyme involved in the synthesis of the particular amino-acid is produced. This is a simple on-off mechanism and if, as suggested, the regulator substance is protein, it need not be a histone. There have been suggestions and there is some evidence that the regulator substance in unicellular organisms may perhaps be RNA<sup>12</sup>, or a more simple molecule, such as an amino-acid or nucleotide, which might act directly at the gene-level on the operon. It has been postulated recently that small peptides may function in this capacity<sup>13</sup>. In unicellular organisms there is no need for an orderly, progressive sequence of gene expression or repression which is required during the development and differentiation of higher animal cells. There is no need for the unicellular organism to stop proliferating at a specific time as is the case for almost all higher animal cells.

It is of special interest to note that many types of tumour cells show a striking lack of differentiation. If histones control differentiation, this is especially significant to the thesis proposed here.

The cancer cell may be visualized, then, as a cell in which the histone control of orderly protein synthesis is faulty. Certain proteins, particularly those involved in or needed for cell division, may be synthesized at a continuing rate, which may be slow or rapid depending on the rate of cell proliferation. Other proteins which would normally be synthesized by the mature cell are not produced at all.

Since we are not certain whether histones control the production of messenger RNA or the sequence of its production, or if they do, how this is accomplished, one can only speculate on how this control may be lost in the tumour cell. Allfrey *et al.* have suggested that acetylation and/or methylation of histones may be the method by which they are activated or inhibited in the performance of their regulatory functions. They reported that acetylated histones lost their ability to repress the activity of RNA polymerase<sup>4</sup>. It is possible to visualize a defective acetylating system, which normally might be highly selective in its action. No striking differences in the overall composition or amount of histones from tumours or homologous or related normal tissues have been detected<sup>14,15</sup> (with one exception mentioned in ref. 7). Although some metabolic differences have been noted<sup>16</sup>, it is possible that differences in the degree of acetylation of different histone fractions from normal and tumour tissues may exist, but thus far this has not been examined. Briefly, the mechanism of the formation of a tumour cell might involve the following steps:

(1) Interference by some chemical, physical or biological agent with the enzyme system or systems responsible for acetylation or other chemical modification of histones resulting in an interference with their proper functioning.

(2) This process might then lead to a fairly random pattern of release of some histone repressions and subsequent synthesis of some enzymes. It might also entail addition of suppressing histones to some genes normally active, the net result being a reversion of the cell to a less differentiated type. There is an example in which the synthesis of the embryonic type enzyme in a tumour, as distinct from the different adult type, occurs and this observation offers support for this concept<sup>17</sup>.

(3) In a few cases the pattern of random changes resulting might be such as to favour continued growth and cell division. It would be expected that all degrees of growth efficiency would result, leading to formation of some tumours capable of proliferating rapidly and others only slowly.

One interesting consequence of this hypothesis is that no gene mutations are required except possibly one—in the gene or gene system related to histone control. Thus a failure to find genetically altered proteins in tumour cells would in general be supporting evidence for a carcinogen mechanism involving histone control. Thus far no clear-cut case has been presented for the production of a protein from tumour cells which has an altered amino-acid sequence, although in some tumours it has been reported that certain enzymes have somewhat altered properties, for example, the *Km* of hepatoma aspartate aminotransferase has been reported to be different from that of the normal liver enzyme<sup>18</sup>. This could well be due to other changes in the cell and not related to a change in the amino-acid pattern of the protein.

(4) If changes in the properties and control activity of histones occur, they would be, presumably, permanent and it would appear to be a difficult, if not impossible, task to selectively reverse them. On the other hand, these changes lead to fairly well defined alterations in metabolic patterns in the tumour, which can be explored for possible rate-limiting reactions or alternative pathways not present or important in normal tissues. Thus, the present-day

research approach which embodies obtaining a further understanding of tumour metabolism as an effective method of devising tumour chemotherapy would not be seriously affected.

Since tumour-causing viruses apparently bring about very selective changes in the cell which then lead to the cancerous state, an examination of viral mechanism of action at the histone- and enzyme-level would seem to be one of the most promising approaches to explore. Particularly important would be the determination of the relationships, if any, between the virus and cell histones, or the acetylation or other chemical modification of cell histones which might alter histone action.

Undoubtedly many other possibly fruitful experimental approaches will be suggested to the investigator and this, it is hoped, will justify the speculation presented here.

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## A NEW RED-CELL AUTOANTIBODY IN NZB MICE

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MICE of the 'New Zealand Black' (NZB) strain develop autoimmune haemolytic anaemia with red-cell autoantibodies<sup>1</sup>; direct antiglobulin tests are positive in some mice from the age of 16 weeks, and in nearly all mice by 36 weeks<sup>2</sup>. It has been reported that male NZB mice give positive direct antiglobulin tests on average some weeks earlier than females, and that males develop a more severe anaemia<sup>3</sup>. At about the time when red-cell autoantibodies appear, germinal centres and plasma cell proliferation may be seen in the thymus. These thymic changes are found in a majority of the mice and, according to Burnet, may in females precede the appearance of the red-cell autoantibodies, while in males the reverse may be the case<sup>3,4</sup>. Blood from NZB mice has sometimes yielded positive lupus erythematosus cell tests, and Norins and Holmes have reported serum antinuclear factors in about 40 per cent of adult mice<sup>4</sup>.

During investigation of the occurrence of autoantibodies in this and other mouse strains, we have noted in the NZB the appearance of another red-cell autoantibody distinct from that already described.

A colony of about 300 NZB mice has been bred at Taplow from stock obtained from the Laboratory Animals Centre, Carshalton. These originated from Bialohowsky strain stock and were imported from New Zealand at F63. Strict inbreeding has been maintained throughout, and intra-strain skin grafting in the Taplow colony is uniformly successful.

Rabbit antiserum prepared against whole-mouse serum, and absorbed with washed mouse cells, was used for direct antiglobulin tests as described by Holmes, Gorrie and Burnet<sup>5</sup>. Goat antiserum against mouse globulin was conjugated with fluorescein isothiocyanate and absorbed with powdered rabbit liver. This conjugate was used as previously described<sup>6</sup> to carry out indirect immunofluorescent tests for antibodies in mouse sera, which were usually diluted 1:2 in buffered saline before use.

**The new autoantibody.** As a rule, cryostat sections of unfixed calf thyroid tissue were used as a source of nuclear antigens in the immunofluorescent test for antinuclear factor. On one occasion when sections of human thyroid from

a Group O infant were used as well, however, it was noticed that serum from a 36-week-old male NZB mouse gave fluorescent staining of capillaries in the human thyroid tissue, as shown in Fig. 1a. Red cells present in the larger blood vessels were also positively stained (Figs. 1b and 2). Similar staining of vascular contents in human tissue sections was afterwards obtained with sera from many of the older NZB mice. It appeared that the fluorescence seen in the capillaries and other blood vessels was due to staining of the red cells disintegrated by freezing and thawing in the preparation of cryostat sections, rather than to staining of a vascular endothelial component. Similar staining of capillary and vascular contents was obtained with sections of human stomach. Brief immersion of the air-dried cryostat sections in 95 per cent ethanol destroyed their ability to give this staining with positive sera.

Absorption of the anti-mouse-globulin conjugate with mouse  $\gamma$ -globulin (obtained by DEAE column fractionation of mouse serum<sup>7</sup>) inhibited staining by positive sera, although not inhibiting the ability of the conjugate to stain the glomerular component in mouse kidney sections which is related to mouse  $\beta$ -globulin<sup>8</sup>.

Sera giving positive staining of human tissue sections gave similar staining of intravascular red cells in cryostat sections of tissues (kidney, lung, liver, salivary gland, stomach) from NZB mice, as well as from mice of other strains. Furthermore, positive sera showed auto-immune reactivity, staining red cells present in sections of tissues of the mice from which they were taken. In cryostat sections of mouse fetuses taken near term, positive sera stained not only red cells present in the vascular spaces but also nucleated cells morphologically resembling normoblasts. Sera from 111 mice of 12 other strains taken at 6-9 months of age gave no staining of the type described.

**Staining of blood smears.** As well as staining red cells present in cryostat sections of human and mouse tissues, positive sera gave bright staining by indirect immunofluorescence of the red cells in unfixed smears of human or mouse blood prepared and treated as described by Johnson and Holborow<sup>9</sup> (Fig. 3a). Such sera also stained

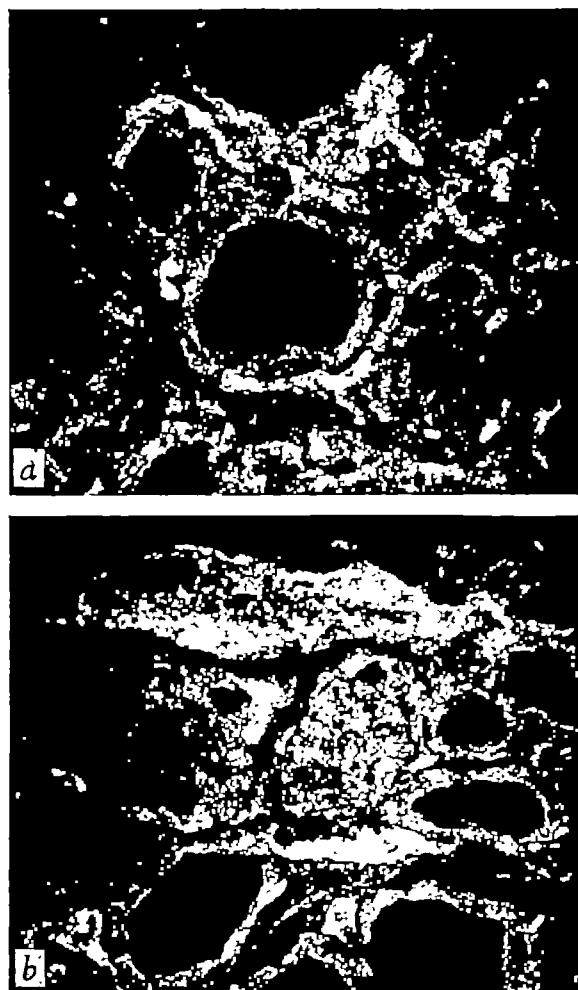


Fig. 1. Unfixed cryostat section of human infant thyroid (group O) treated with serum from a 9-month-old NZB male, and then with anti-mouse globulin conjugate. Ultra-violet microscopy ( $\times c. 206$ ). *a*, Immunofluorescent staining of capillary contents, *b*, immunofluorescent staining of contents of larger blood vessel.

mouse red cells in smears taken from the donors of the sera, that is, gave an auto-immune reaction. It was noticeable that in positively stained smears of NZB blood, the erythrocytes stained brightly but the many reticulocytes often present stained only faintly or not at all. NZB sera negative against human tissue gave no staining of human red-cell smears, and no staining, or very weak staining, of mouse red-cell smears. This latter mouse specific serum antibody may be that detectable in some NZB sera by indirect antiglobulin test<sup>14</sup>.

Complement-fixing activity was tested for by an immunofluorescent method, in which uptake of added guinea-pig complement in the reaction of red-cell smears with antibody was demonstrated with a rabbit anti-guinea-pig complement antiserum and a goat anti-rabbit serum conjugate. In this way, sera reactive with human erythrocytes in sections or smears as judged by the immunofluorescent antiglobulin test were shown to fix complement with mouse red cell smears.

**Absorption of mouse sera with red cells.** Staining of both human and mouse tissues and human and mouse blood smears could be prevented, partially or completely, by absorbing positive sera with packed washed human group O red cells or mouse red cells. Repeated absorptions at 37° C were necessary; three such absorptions with equal volumes of packed mouse cells removed staining activity of positive sera against both human and mouse tissue and red cells; three absorptions with packed human cells removed most of the staining activity against human

material, and reduced, but did not completely prevent, staining of mouse cells.

However, when the cells used for absorption (even of an excess of serum) were washed and tested with anti-mouse globulin serum, no agglutination was seen. Similarly, smears made from mouse or human red cells used to absorb positive sera showed no immunofluorescence after treatment with anti-mouse globulin conjugate. These findings were consistent with the observation noted below that in some NZB mice the new antibody was detected in the absence of a positive direct antiglobulin test.

**Absorption with red cell stromata.** The foregoing results suggest that the new antibody reacts with antigen present



Fig. 2. Control section of thyroid treated with serum from a 3-month-old NZB mouse. No immunofluorescence of capillaries, or blood vessel contents (top centre) ( $\times 240$ ).

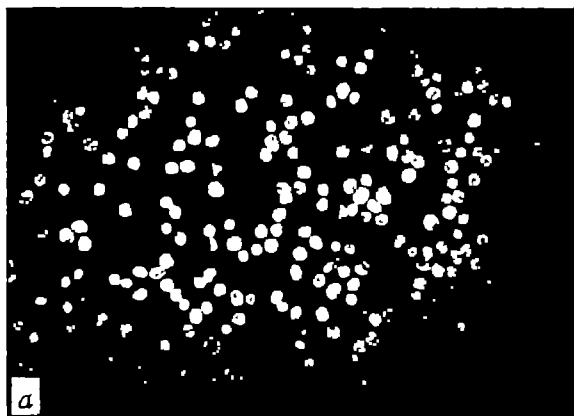


Fig. 3. *a*, Smear of NZB mouse blood treated with NZB serum positive against human red cells; indirect immunofluorescence; *b*, as in (*a*), but treated with positive serum absorbed with human red cell stromata ( $\times c. 185$ ).

in both mouse and human red cells, but not readily available or poorly represented on the surface of the intact erythrocyte; this antigen is evidently rendered accessible in cryostat sections or in blood smears by the effects of freezing and/or drying on red-cell integrity.

Human or mouse red-cell stromata were therefore prepared by the method of Milgrom and Layrisse<sup>11</sup> and used to absorb positive sera, in the proportion of 1 mg of dried stromata powder to 0.1 ml. serum. One such absorption (37° C for 0.5 h) with mouse stromata removed activity against human as well as mouse tissues and smears; a similar absorption with human stromata (Fig. 3b) removed all activity against human tissue or human blood smears, although not invariably all activity against mouse. When the stromata (either human or mouse) used for absorption were eluted with saline at 56° C the eluate obtained stained both human thyroid sections and mouse red-cell smears by the indirect anti-globulin immunofluorescent test.

Absorption with human red-cell stromata also removed the ability of positive sera to fix complement with mouse red-cell smears, as judged by the indirect immunofluorescent method used. By this method, however, eluates from human stromata could not be shown to possess complement-binding activity with red cells present in human thyroid sections.

Results of absorption tests are summarized in Table 1. Age incidence of antibodies. The age incidence of positive direct antiglobulin tests and of the anti-human

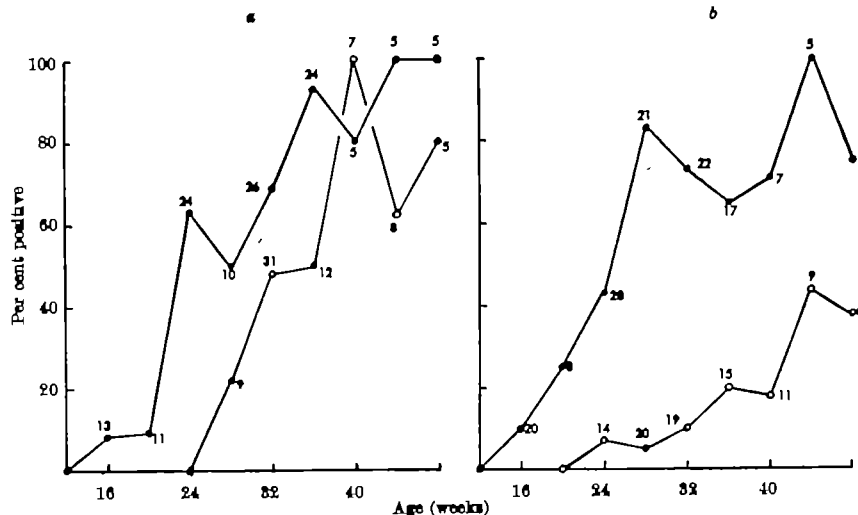


Fig. 5. Age frequency as in Fig. 4 in (a) male NZB mice (left), (b) female NZB mice (right). In Figs. 4 and 5 numbers of mice tested at each age are shown.

cross-reacting antibody are shown in Fig. 4. The age-frequency curve of the latter roughly follows that of the direct antiglobulin tests, but with a time-lag of 10 of more weeks. Thus, as might be expected, in individual mice the autoantibody cross-reactive with human red cells was usually found in the presence of a positive antiglobulin test. However, among 120 NZB mice in which development of positive direct antiglobulin tests had been observed, we encountered 5 in which this test afterwards became negative again. Three of these 5 later acquired the new antibody although their anti-globulin tests remained negative during the course of the study. In 4 other NZB mice the direct antiglobulin test never became positive, but nevertheless the appearance of the new antibody was observed.

Figs. 5a and b show the age incidence of the antibodies by sexes. It will be seen that in males the new antibody has a higher incidence than in females. Tests so far performed indicate an incidence of more than 50 per cent in males over 24 weeks, and of about 20 per cent in females over this age. Numbers of mice older than 10–12 months of age so far tested are small, however. The rate at which mice in this series acquire positive direct antiglobulin reactions is somewhat irregular, and does not show the clear difference between the sexes reported by Holmes and Burnet<sup>3</sup>.

Antinuclear factor (ANF). Fig. 4 shows also the incidence of ANF (Fig. 5) in NZB mice. There is little relation between the antinuclear and the anti-red cell antibodies with regard to age incidence; up to 48 weeks of age the incidence of antinuclear factors does not exceed 20 per cent, and is approximately equal in the two sexes. These

Table 1. EFFECTS OF ABSORPTION (a) WITH INTACT RED CELLS OR (b) WITH RED-CELL STROMATA ON IMMUNOFLOUORESCENT REACTIVITY OF POSITIVE NZB SERUM

Tissue	Unabsorbed serum		Serum absorbed, ×3 with Human mouse RBCs		Serum absorbed once with Human mouse stromata	
	AMG anti-O	anti-O	AMG	anti-O	AMG anti-O	anti-O
Human thyroid	+	±	Neg.	Neg.	Neg.	Neg.
Human blood smear	+	±	Neg.	Neg.	Neg.	Neg.
Mouse blood smear	+	+	Neg.	+/-Neg.	Neg.	Neg.

AMG—tested with fluorescent anti-mouse globulin.  
Anti-O—tested for uptake of guinea-pig complement with rabbit-anti-guinea-pig and goat-anti-rabbit fluorescent conjugate

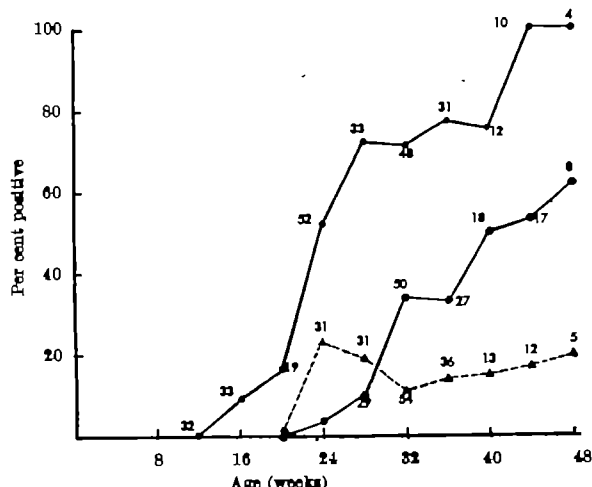


Fig. 4. Age-frequency of positive direct antiglobulin test (closed circles); positive anti-human red cell antibody (open circles); and ANF (dotted line) in NZB mice



Fig. 6. Human thyroid section as in Figs. 1 and 2, treated with ANF-positive NZB mouse serum and anti-mouse globulin conjugate (×180)

results do not suggest that development of antinuclear autoantibodies is closely related to the progress of disease in these mice. It may be mentioned here that in a strain of O57 brown mice we have found a higher incidence of ANF (about 50 per cent) without gross evidence of accompanying disease. Antinuclear antibodies have been reported in mouse strains A/J, O57 BL/6 (ref. 12), A/He and O57 BL/6J (ref. 13), and we are investigating their incidence in some other strains.

### Discussion

These results show that as well as the mouse-specific autoantibodies against red cells already known, NZB mice may also develop a different autoantibody, reactive with antigens present in both human and mouse red cells. It appears that the antigenic sites with which this second antibody reacts are not abundantly present on the surface of intact erythrocytes, but are readily accessible in dried red cell smears or in red cell stromata. Tests for cross-reactivity of this antibody with the red cells of other species are being undertaken. That this antibody indeed differs from that responsible for the positive antiglobulin tests of NZB mouse red cells is further attested: (a) by its occasional occurrence in mice with negative antiglobulin tests; (b) by its complement-fixing activity, which has proved to be demonstrable not only by immunofluorescence, but also by conventional complement-fixation tests. The spontaneous appearance of this second autoantibody in a considerable proportion of NZB mice more than 24 weeks old suggests that its production may be related to the thymic changes which have already begun to take place by this age, and this relationship needs closer study. One possibility to be considered in this connexion is viral infection, for it is known that this can give rise to anti-red cell antibodies<sup>14</sup>, and that the effects of thymic deficiency may be averted in mice by germ-free rearing<sup>15</sup>. The effect of rearing NZB mice in a germ-free environment is at present being examined. The question also arises whether the higher incidence of the new anti-

body in males than in females is causally related to the greater severity of the anaemia<sup>7</sup> in males, as already noted<sup>8</sup>. Like Holmes *et al.*<sup>8</sup>, we have succeeded in transferring the direct antiglobulin reaction to young NZB mice by injecting intraperitoneally washed spleen cells from older mice already antiglobulin positive. We have, in addition, shown that when the spleen cells are obtained from donors positive for the new antibody described here, the ability to produce this new antibody is also transferred, so that after a few days the sera of the young recipient mice begin to give positive immunofluorescent tests with human thyroid sections.

In experiments now in progress we are investigating whether the haematological effects of transferring spleen cells from older NZB mice to young mice of this strain differ according to whether the cell donors are positive or negative for the new antibody. The effects of immunization with human red cell stromata on young NZB mice are also under investigation. We thank Miss N. Bell and Miss J. Holliday for assistance.

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## SOIL HUMIC ACID AS A HYDROXYPOLYSTYRENE: A BIOCHEMICAL HYPOTHESIS

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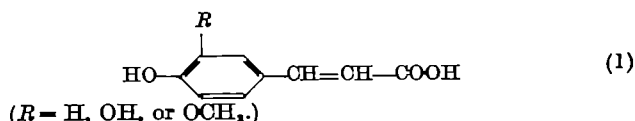
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THE stable humic acid of soil has an unknown chemical structure; it is considered to be a complex phenolic polymer formed from unknown biological precursors<sup>1</sup>. This article presents the hypothesis that hydroxystyrenes formed under natural conditions may play an important part in forming soil humic acid. This concept followed on the finding that hydroxystyrenes are produced during bacterial decarboxylation of certain phenolic acids of plant origin (4-hydroxycinnamic acids)<sup>2</sup> and the realization that soil humic acid in many of its described properties resembles oxidized polystyrenes.

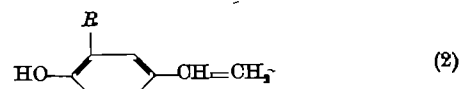
The hypothesis is suggested on the basis of the following information: 4-hydroxycinnamic acids and their derivatives are abundant in fallen, decaying vegetation; conversion of the acids to hydroxystyrenes involves simply a known enzymatic decarboxylation; styrenes and hydroxystyrenes thus formed would spontaneously polymerize and oxidize under natural conditions<sup>3</sup>; and oxidative polymerization of such hydroxystyrenes would give rise to polymers which, it appears, could account for many of the properties characteristic of humic acid.

The enzyme responsible for decarboxylation of 4-hydroxycinnamic acids, forming hydroxystyrenes, was found in widely occurring, free-living species of *Aerobacter*<sup>4</sup>. It readily and specifically attacks the hydroxy-

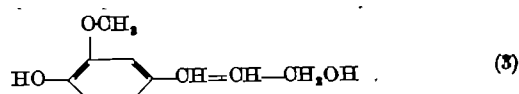
cinnamic acids (structure 1), *p*-coumaric, caffeic and ferulic acids, common plant constituents.



The product hydroxystyrenes, after decarboxylation, have the general structure:



Hydroxystyrenes could also arise through bacterial degradation of other plant products, such as hydroxycoumarins and perhaps flavonoids, which can yield their chemically related hydroxycinnamic acids. Lignin also is closely related to the hydroxycinnamic acids; lignin from conifers is often considered a polymer of coniferyl alcohol:



During plant decay, lignin is reported to undergo depolymerization and demethoxylation and, further, is oxidized to aromatic carboxylic acids<sup>4,5</sup>. Decomposition of lignin to hydroxycinnamic acids<sup>6</sup> and the participation of lignin in forming mature soil humic acid<sup>6</sup> have been documented.

The proposed hypothesis is that humic acid forms through the following reactions in soil: liberation and/or synthesis of hydroxycinnamic acids from the decay of plant litter (the presence of *p*-coumaric acid and related phenolic acids in soils has been reported<sup>7</sup>); decarboxylation of the hydroxycinnamic acids by common micro-organisms, producing hydroxystyrenes; and oxidation, polymerization, and copolymerization of the resultant hydroxystyrenes to humic acid.

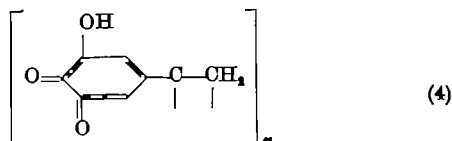
Two main questions would have to be answered to verify that the proposed processes contribute in significant amount to the formation of soil humic acid. (1) To what extent do decarboxylation of hydroxycinnamic acids and polymerization of their hydroxystyrene products take place in soil? There are no data bearing on this. (2) To what extent would the properties of such hydroxypolystyrenes, oxidized to an unknown degree, resemble those of humic acid? This question can be examined. As a first approach toward evaluating the hydroxypolystyrene hypothesis, I will enumerate several properties of humic acid preparations that are similar to and consistent with the expected properties of the complex polystyrenes described.

Soil humic acid has been repeatedly reported as a (1) stable<sup>8</sup> amorphous aromatic polymer of varying degrees of molecular condensation<sup>1</sup>. It is (2) water and alcohol insoluble<sup>1</sup>, to a degree depending, as with other polymers, on molecular weight<sup>9</sup>. Humic acid, a major component of the soil humus complex, gives to soils a (3) friable character (comparable in this respect to the 'soil conditioner' effect of synthetic vinyl polymer additives<sup>10</sup>).

Humic acid is (4) soluble in alkali<sup>11</sup>. Such solubility would be consistent with the properties of either a hydroxypolystyrene (which would form phenolate salts in alkali solutions) or a carboxylic acid, or a mixture of these.

Humic acid contains a (5) great concentration of stable free radicals. These have been interpreted as arising from oxidized phenolic compounds, such as quinones and quinhydrone<sup>12</sup>. Hydroxystyrenes oxidized at the quinone-level would, likewise, display free radical behaviour.

The (6) molecular composition of soil humic acid is similar to that of an oxidized polystyrene. Composition data on humic acid preparations fall within a recognized range, but with some variation dependent on the soil of origin. Gillam<sup>13</sup>, analysing nitrogen-free preparations of humic acid, found the average C—H—O proportions 61—4—35 per cent. A hydroxypolystyrene quinone of the structure:



(derived from oxidative decarboxylation of, for example, *p*-coumaric acid) has the C—H—O proportions 64—4—32 per cent. The undecarboxylated parent (*p*-coumaric acid) oxidized similarly would have the proportions 56—3.1—41 per cent. Analyses of humic acid indicate about 0.5 mole carboxyl group per mole of aromatic monomer when a formula weight of 200 is assumed<sup>1</sup>. A 50—50 copolymerized mixture of styrene and carboxylic acid at the foregoing oxidation-level would have the C—H—O proportions 60—3.6—37 per cent. Methylation in some degree (see structure 1) would bring the figures still closer to the ratios found by Gillam. A small proportion of methylated 3-hydroxy groups and other variations of structure, such as hydroxylated side-chains and oxidative ring cleavage products, are evidenced by humic acid

analyses<sup>14</sup>. Some of the variability in composition among soil humic acids may be accounted for by these variations. Of course, composition data, particularly when variable, can only be suggestive; such data cannot of themselves prove the presence of any particular chemical structure.

Of particular interest, however, is the observation that during ageing of soils changes occur in chemical composition: more mature, highly aromatic, highly condensed soils have more C relative to O+H and more O relative to H<sup>1</sup>. These changes are consistent with continuing oxidation and decarboxylation of copolymerized hydroxycinnamic acids to oxidized hydroxystyrenes in a polystyrene copolymer. The (7) ease of decarboxylation of unknown structural components of humic substances has been repeatedly confirmed and attributed to constituent carboxylic acids<sup>15</sup>. The analytical figures of Schwartz and Asfeld<sup>14</sup> on heat decarboxylation of extracts of lignite soil agree well with this picture.

The presence of carboxylic acids in a humic acid copolymer would also account for its (8) base exchange properties. The molecules could be interconnected and copolymerized by cross-linkages between the side-chains, as in polystyrene, thus leaving the carboxyl groups free.

Another type of copolymerization, or perhaps entrapment within a polystyrene matrix, could take place with aromatic amino-acids derived from protein degradation of organisms in the soil. The (9) nitrogen content of humic acid preparations varies widely (< 0.5 to > 6 per cent) among soils<sup>1,11,15</sup>. The variation, and the ease with which nitrogen content is often reduced by simple extractions, has led many to conclude that the nitrogen compounds in humic acid are not derived from primary humic acid precursors, are hence variable in composition and dependent on the chance proximity of nitrogenous biological products<sup>1,15</sup>.

It appears, then, that many of the important characteristics of soil humic acid may be reconciled in terms of a complex, variable polymer with oxidized hydroxypolystyrene as a major structural feature. The decarboxylation-polymerization reactions that have been described almost certainly take place in vegetable compost to some extent. Both plant hydroxycinnamic acids and ubiquitous *Aerobacter* species (and possibly other species) which decarboxylate them are bound to occur in juxtaposition in many (or most) soils<sup>16</sup>. Continuing decarboxylation, polymerization and oxidation could proceed for a long period of time. Subsidiary reactions of demethoxylation and hydroxylation, reaction with nitrogen-containing residues from amino-acids, etc., would be assumed to also occur in the soil at various rates, some slowly, in a complex manner, over many years. At what rate the described decarboxylations and polymerizations actually do proceed in soil; whether the rates of hydroxystyrene monomer formation and its polymerization, copolymerization, and oxidation could, or do, account significantly for the formation of humic acid in soils: these are questions which require investigation.

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## MITOSIS IN HYBRID CELLS DERIVED FROM MOUSE AND MAN

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IN a previous article some characteristics of heterokaryons produced by fusing together HeLa and Ehrlich ascites cells were described<sup>1</sup>. In cultures containing such composite cells it was observed that some of the cells rounded up in mitosis were very much larger than those normally seen in cultures of HeLa or Ehrlich ascites cells. Preliminary cytological investigation of these large mitotic cells revealed that many of them contained a mixture of metacentric and acrocentric chromosomes. Since published reports indicated that there may be one or at the most two metacentric chromosomes in tetraploid Ehrlich ascites cells<sup>2</sup> and relatively few acrocentric chromosomes in HeLa cells<sup>3</sup>, it seemed likely that metaphase figures containing large numbers of both were derived from hybrid cells which originally contained both human and murine nuclei. This observation has now been confirmed by a more detailed examination: hybrids produced by the fusion of HeLa and Ehrlich ascites cells are indeed capable of undergoing mitosis, and, at metaphase, they contain chromosomes characteristic of both mouse and man.

The HeLa cells were obtained from a stock suspension culture and the Ehrlich ascites cells from the peritoneal cavity of Swiss mice. A suspension containing  $2 \times 10^7$  cells of each type was treated with 8,000 haemagglutinating units of ultra-violet-irradiated Sendai virus, as previously described<sup>1</sup>. The treated cell suspension was then seeded into four 20-oz. medical flat bottles, each containing approximately 40 ml. of culture medium. The cultures were maintained at 37° C and samples were taken at 24, 48 and 72 h for cytological investigation.

'Colcemid' (CIBA), at a final concentration of approximately  $10^{-5}$  M, was added to the culture, and 1.5 h later the bottle was shaken to free the cells in mitosis from the glass. The freed cells were sedimented by centrifugation for 3 min at 200g, resuspended in warm hypotonic sodium citrate solution (1 per cent wt./vol.) and left to stand at room temperature for 10 min. The cells were again sedimented and then fixed by several changes of a mixture of 3 parts ethyl alcohol and 1 part glacial acetic acid. Preparations were made by air drying<sup>4</sup>, either from the fixative or from 45 per cent acetic acid. Similar preparations were obtained from Ehrlich ascites cells taken directly from carrier mice.

The chromosome count in 37 out of a sample of 40 Ehrlich ascites cells fell within the range 73–80 with a weak mode (9 cells) at 76. The counts in the remaining three cells were 62 (possibly a broken cell), 151 and 154. Thirty-four of the cells examined contained a long metacentric marker chromosome like that described by previous workers. Many cells also contained up to three additional marker chromosomes. Two of these were very similar, perhaps identical, long acrocentric chromosomes with prominent sub-terminal (distal) secondary constrictions. The third was a long acrocentric chromosome with a closely proximal secondary constriction (Fig. 1). Otherwise, the chromosomes were typical of the mouse, that is, they were all acrocentric with minute (or undetected) short arms. The range in length between the longest and shortest (about 4:1) was, however, clearly greater than in the normal somatic chromosomes of the mouse (about 2.5:1).

Most of the mitotic cells in the mixed culture were immediately recognizable as HeLa cells by their chromosomes. Counts made in 53 of them revealed a sharp mode at 57 (36 cells), an 'inner' range of 54–59 and an extreme range of 48–82. The mode is much lower than any reported by previous workers<sup>5</sup>. The chromosome set as a whole was clearly human in character. Only three decisive marker chromosomes were identified, although detailed examination would almost certainly reveal more in the D, E, F and G groups. One of these marker chromosomes had an arm ratio of approximately 4:1, as in the B group, but was as long as, or even longer than, the presumptive No. 1 and No. 2 chromosomes. The other two markers correspond to the C group in length, but have arm ratios of 5:1 or 6:1, one being somewhat longer than the other and having a relatively longer short arm. A cell with the modal number is illustrated in Fig. 2 and the markers are indicated. This cell contains 13 acrocentric



Fig. 1. Ehrlich ascites cell ('tetraploid' line) at metaphase. 77 chromosomes. Arrows indicate the three marker chromosomes described in the text.



Fig. 2. HeLa cell at metaphase. 57 chromosomes. Arrows indicate the three marker chromosomes described in the text.



chromosomes, of which nine and two, respectively, are not obviously distinct from the normal *D* and *G* groups and two are intermediate in length between them. These are the only chromosomes of the HeLa karyotype that could be confused with any of the Ehrlich ascites chromosomes (other than the metacentric marker already mentioned).

Mitotic cells were present at all three times of gathering, but the best preparations were obtained from the 72-h material and these alone were used for detailed examination. Hybrid cells in mitosis (Figs. 3 and 4) were easily identified in these preparations by their large number of chromosomes and the high proportion of acrocentrics among them. An unselected sample of 28 cells was examined, and counts ranging from 111 to approximately 412 chromosomes were recorded. Twenty-three of the counts are believed to be substantially correct and are set out in Table 1 in comparison with the expected counts from various combinations of Ehrlich ascites and HeLa nuclei, minimum, modal and maximum values being calculated from the modes and 'inner' ranges already given. On this basis eight of the cells fall in the 1 Ehrlich/1 HeLa class, five in the 1 Ehrlich/2 HeLa class, two in the 1 Ehrlich/3 HeLa class and two in the 2 Ehrlich/2 HeLa class. The remaining six cells could be the products of fusion between cells with numbers of chromosomes lying outside the 'inner' range or, more probably, artefactual counts due to breakage of the rather fragile cells and loss of chromosomes during cytological processing. Of the five cells excluded from Table 1, one had 112

Table 1. COUNTS OF THE CHROMOSOMES IN 28 HELa/EHRlich ASCITES HYBRID CELLS

Nuclear combination	Expected count			Actual counts
	Min.	Mode	Max.	
1E+1H	125	133	139	111, 116, 118, 120, 122, 129, 131, 132, 133, 134, 137, 153, 171, 172
1E+2H	177	190	198	173, 181, 185, 189, 193
2E+1H	198	211	219	
1E+3H	239	247	257	230, 241
2E+2H	250	266	278	271, 275 -
3E+1H	271	287	299	

chromosomes but was quite clearly broken, three were estimated to contain 150, 300 and 300 chromosomes, respectively, and one was the cell with 412 chromosomes already mentioned (Fig. 4). Two of the 28 cells exhibited large numbers of structural changes in the chromosomes, particularly 'double minutes', but other changes, at both the chromatid and chromosome levels, were also identified (Fig. 4).

A detailed analysis was made of one of the cells (Fig. 3). Of a total of 181 chromosomes, 72 (including one marker) were identified as of Ehrlich ascites origin, 100 (including two markers) as from HeLa, and nine could not be assigned to either. These are very close to the numbers expected from the fusion of one modal Ehrlich cell and two modal HeLa cells.

These hybrid mitotic figures could have arisen in four ways: (1) by chance association of HeLa and Ehrlich cells which have dried out together to give a single group of chromosomes; (2) by coalescence of HeLa and Ehrlich cells simultaneously in mitosis; (3) by mitosis in synkaryons (heterokaryons in which the individual nuclei have fused together to form a single nucleus); (4) by synchronous mitosis in the nuclei of multinucleate cells. The first possibility can be ruled out on the grounds of the evenness of distribution of the chromosomes in many spreads, the thorough mixing of HeLa and Ehrlich chromosomes in most spreads and the rarity of free Ehrlich ascites cells in mitosis. This line of Ehrlich ascites cells does not grow *in vitro*, and only one example of an Ehrlich ascites cell in mitosis was found in the mixed culture. This last consideration also eliminates the second possibility, which would in any event require the very improbable juxtaposition of several single cells simultaneously in mitosis to account for mitotic figures containing several chromosomal sets.

We now have evidence that hybrid mitotic figures can be produced by both the third and the fourth mechanisms. Mitotic prophase has been seen in synkaryons of various sizes, and there is thus no doubt that at least some of these cells can undergo mitosis. The evenness of distribution and thorough mixing of the chromosomes in most of the spreads made from the 72-h culture strongly suggest that most of the hybrid mitotic figures seen at this time arose in this way. In earlier experiments mitosis was not seen in the nuclei of multinucleate cells, but in the present experiments prophase has been seen in such nuclei in preparations made 24 h and 48 h after cell fusion. In most cases only some of the nuclei in the multinucleate cells were in prophase, while others remained in interphase, a situation apparently similar to that described by Stubblefield<sup>8</sup> in multinucleate cells induced by 'Colcemid'. In view of the fact that only a small percentage of HeLa-Ehrlich heterokaryons show synchrony of DNA synthesis in their nuclei<sup>1</sup>, the occurrence of asynchronous nuclear mitoses is not surprising. A small number of multinucleate cells have, however, been seen in which the individual nuclei enter prophase together. Hybrid mitotic figures may therefore also be produced by synchronous metaphase in multinucleate cells. Fig. 5 illustrates the two mechanisms by which hybrid mitotic figures may be produced. Two synkaryons are shown in prophase, and, in the same field, a dikaryon with both nuclei in synchronous prophase. If post-mitotic reconstitution in these multinucleate cells gives rise to cells with a single large nucleus or cells with fewer nuclei than



Fig. 3. Hybrid cell at metaphase. 181 chromosomes. Arrows indicate 2 HeLa and 1 Ehrlich ascites marker chromosomes.



Fig. 4. Hybrid cell at metaphase. Approximately 412 chromosomes (centromeres). Note large number of structural changes.

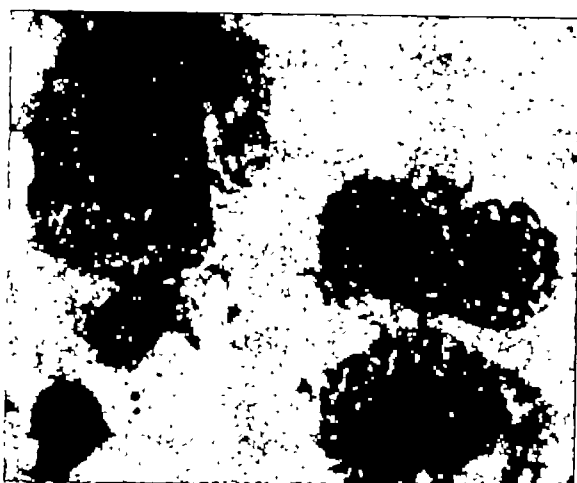


Fig. 5. Two synkaryons in prophase and a dikaryon with both nuclei in synchronous prophase

were present originally, the progressive nuclear fusion which is characteristic of these hybrid cultures might be produced by this mechanism rather than by direct coalescence of interphase nuclei, as previously suggested<sup>1</sup>.

While it is thus clear that hybrid cells produced by fusing together HeLa and Ehrlich ascites cells are capable of undergoing mitosis, the question remains whether these cells can multiply continuously to give rise to a strain of mixed parentage. Populations of heterokaryons made with high doses of virus and consequently containing relatively large numbers of nuclei per cell show no net growth: the number of hybrid cells remains more or less stationary for about 5 days and then gradually falls<sup>1</sup>. It is therefore clear that most of these multinucleate cells do not give rise to viable progeny. This observation is borne out by the fact that giant mitotic figures containing several chromosomal sets, like that shown in Fig. 4, disappear from the cultures within 5 or 6 days. In cultures examined 10 days after cell fusion the only hybrid mitoses

seen were those in which the number of chromosomes approximated to 2 chromosomal sets. Table 1 shows that even at 72 h hybrids containing only one chromosomal set from each parent cell were already the commonest type of hybrid undergoing mitosis. This type of hybrid thus resembles the spontaneous hybrids discovered by Baraki *et al.*<sup>2</sup> in mixed cultures of cell lines from the same species: in both cases the hybrid cells appear to contain one set of chromosomes from each parent cell. It is not unlikely that the hybrids discovered by Baraki *et al.* also arise through the action of viruses, especially in the light of recent experiments which indicate that para-influenza viruses may be carried by cell lines in a latent form<sup>3</sup>.

Further examination of the reproductive capacity of HeLa-Ehrlich hybrids is frustrated by the fact that these hybrids are invariably overgrown in the cultures of HeLa cells. Several attempts have been made to find cultural conditions which would select for the hybrids, but so far without success. It is, however, of interest to note that a rapidly growing strain of rat-mouse hybrid cell has recently been isolated by the use of selective media<sup>4</sup>. The fact that the parent cells are derived from different animal species is not, therefore, in itself a barrier to continued reproduction of the hybrid progeny. In the present case it may be the inability of the Ehrlich ascites cell to maintain itself *in vitro* which is responsible for the poor growth of the HeLa-Ehrlich hybrid relative to the HeLa cell. The technique of cell fusion permits the exploration of a wide range of other combinations<sup>5</sup>.

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## NUCLEIC ACID SYNTHESIS AND THE MITOTIC CYCLE IN MAMMALIAN CELLS TREATED WITH NITROGEN MUSTARD IN CULTURE

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A DIFFERENTIAL inhibition of DNA and RNA synthesis by nitrogen mustard (methyl-bis[ $\beta$ -chloroethyl]amine, or HN2) has been observed in various types of animal cells<sup>1,2</sup>. In guinea-pig kidney cells adapted to *in vitro* growth (strain ROP), a dose of 2  $\mu$ g/ml. of HN2, which reduces survival to negligible values (0.002 per cent), inhibits cell division severely, but allows the cells to grow in size for many days: the RNA content of such undividing giant cells may attain a seven-fold increase, whereas their DNA content scarcely doubles<sup>3</sup>. Data are also available which seem to indicate that RNA synthesis still remains DNA-dependent after HN2 treatment<sup>4,5</sup>.

The increased amount of DNA per cell, which was also observed after treatment with HN2 by other authors<sup>3,4,6</sup>, has been interpreted to mean that the cells synthesise DNA up to the pre-mitotic level, and then DNA synthesis ceases. Gelfant<sup>7</sup> suggested that the inhibitory effect of nitrogen mustard is felt in the G2 phase of the cell cycle: HN2 would thus inhibit cell division after DNA synthesis has occurred, but it would not prevent the synthesis of DNA in those cells which

have not as yet reached the G2 period. The hypothesis has also been advanced that the blockage of cells in a pre-mitotic phase of the cycle could be due to some change induced in a protein of the nucleus, perhaps of the chromosomes, which must occur in the G2 period in order for cells to enter mitosis<sup>8</sup>.

On the other hand, the cross-linking action of difunctional alkylating agents (such as HN2) on nucleic acids<sup>9</sup> suggested to us a mechanism based on a direct action of the agent on the DNA molecule, which could also explain the relative insensitivity of RNA synthesis, as compared with DNA synthesis<sup>10</sup>. It may be envisaged that the degree of cross-linking between the complementary deoxy-ribose chains produced by the particular HN2 concentration used may still allow a partial unfolding and separation of the twin polynucleotide chains, but not the complete partition of the newly formed double strands in two daughter chromatids. Therefore, alkylated chains may still serve as templates for a single round of DNA synthesis, but no division could follow, and subsequent DNA synthetic cycles would thus be impeded. A scheme of this sort would therefore explain why DNA synthesis

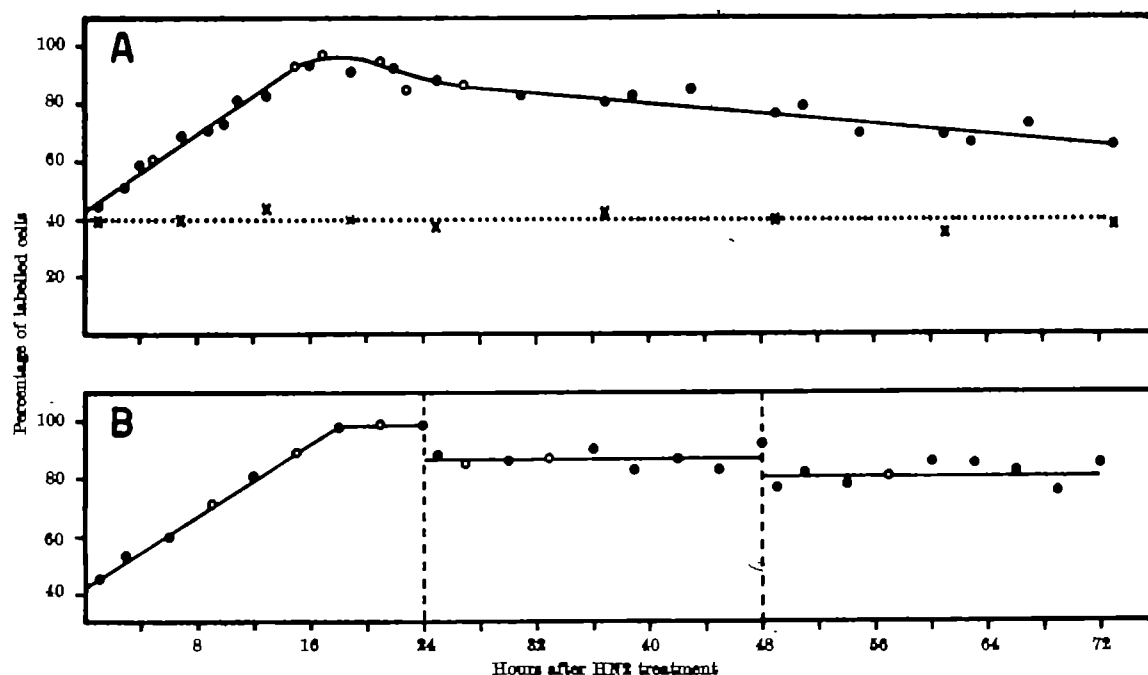


Fig. 1. *A*, Fraction of labelled *AUP* cells in untreated (broken line) and in HN2-treated (unbroken line) cultures, incubated for 1 h with 1.7  $\mu$ Ci/ml. of tritiated thymidine (specific activity: 1.9 c./mM) at different times after treatment. At each point 500 cells were scored. Data from two separate experiments. *B*, Fraction of labelled *AUP* cells in cultures treated with HN2, incubated up to 24 h with 0.5  $\mu$ Ci/ml. of tritiated thymidine. Some of the cultures were incubated during the first, some during the second, some during the third day after treatment. At each point 500 cells were scored. Data from two separate experiments.

stops only after the cell has doubled its normal DNA content. On the contrary, DNA-dependent RNA synthesis, which does not seem to require transition of primer DNA to the single-stranded form<sup>11</sup>, would not be affected by any degree of cross-linking of DNA molecules.

If this were so, cells would be stopped in a phase the nearer the end of the *S* period, the more related are the two processes: DNA duplication and partition of the new molecules into daughter chromatids. Moreover, the effects of DNA alkylation on the progress of cells along the cycle would differ according to the position of cells at the time of treatment. We have therefore undertaken an investigation of the action of nitrogen mustard (2  $\mu$ g/ml.) on human amnion cells grown *in vitro* (strain *AUP*), the results of which are described in detail elsewhere<sup>12</sup>. The first point we observed, in agreement with the findings of other authors<sup>13</sup>, is that cells which were in *G2* at the time of treatment are not prevented by HN2 from entering mitosis, and are able to complete cell division. In fact, all mitotic phases drop to zero values only after a time which corresponds to the length of *G2* (about 3 h) in the particular strain used. About 20 h after treatment, mitosis is resumed, but we observed only prophase and abnormal metaphases (showing chromosome fragmentation); not one anaphase or telophase was observed until the end of the fourth day (more than 4,000 cells were scored per point). Moreover, after continuous incubation with tritiated thymidine, labelled mitoses (abnormal metaphases) appear in cultures treated with HN2 only after the 20th h, whereas, by treating the cells after 3 h of tritiated thymidine incubation, the frequency of labelled metaphases rises as in control cultures. On the other hand, the percentage of labelled cells, after continuous incubation with the radioactive precursor, rises in HN2 as in untreated cultures.

These results seem to confirm the hypothesis that *G2* cells are not blocked by HN2 during the first mitotic cycle, but are prevented from entering mitosis during the second, and show at the same time that all the cells are able to pass at least once throughout *S*, and are blocked afterwards. If, as suggested, the inhibitory effect of the mustard is felt in *G2*, the block would there-

fore be placed just at the beginning of this phase. In these experiments, however, the DNA content per cell can reach much more than the double per cell level, even if a differential inhibition of DNA and RNA synthesis is still induced by HN2 (Table 1). Moreover, the percentage of labelled cells after a 'pulse' of tritiated thymidine given at different times after treatment with HN2 may reach 90–100 per cent values between the 16th and the 24th h, and still at the end of the second day the percentage of cells in *S* is almost double that of controls (Fig. 1*A*).

The cells must therefore be blocked in *S*, where they are able to synthesize DNA for several cycles of replication, up to levels 4–5 times greater than normal. Cells which eventually escape the block are definitively stopped in metaphase, where the alkylation of DNA results in chromosome fragmentation. That these cells cannot complete a new cycle is confirmed by the fact that no cell enters from *G1* into *S* after the first day of treatment (Fig. 1*B*).

These results are not in disagreement with those previously reported, which seemed to indicate a blockage of DNA synthesis at the pre-mitotic level<sup>2,3</sup>. In fact, the cells treated with HN2 had up to twice the mean normal amount of DNA found in an asynchronous control population; as the last included cells which had already doubled DNA (those in *G2* and in the first phases of mitosis) the double cellular level found in treated cultures really exceeded the normal pre-mitotic level. Only Caspersen *et al.*<sup>4</sup>, by using quantitative cytochemical methods on single mouse fibroblasts grown *in vitro*, have shown that DNA synthesis was arrested in all the cells when they had attained the pre-mitotic content, within a 48-h period after treatment with HN2. But it cannot be excluded that the synthesis would have continued later on: the same conclusion in fact could have been reached from our data also (Table 1), if we had limited the observations to the second day after treatment. It must be noted that strain *AUP* cells are much less sensitive to HN2 than *ROP* cells we have previously used<sup>12</sup>; survival at the same dose of 2  $\mu$ g/ml., measured by colony counts, is increased in the former by a factor of 60. HeLa cells, which show

Table 1. THE AVERAGE CONTENT OF DNA AND RNA PER CELL (STRAIN AUP), AFTER TREATMENT WITH HN2 (2 µg/ml)\*

Days after treatment:	0†	1	2	3	4	5
DNA A	1.0	1.3	1.6	1.8	2.6	3.8
B	1.0	1.4	1.8	2.6	3.6	5.2
RNA A	1.0	1.5	2.2	3.4	3.8	5.6
B	1.0	1.8	2.8	4.0	7.0	10.1

\* From cultures of known cellular density, nucleic acids were extracted with perchloric acid and determined spectrophotometrically, following a method described elsewhere<sup>12</sup>; three cultures per point were used. Data from two separate experiments (A, B) are reported.

† The unit at zero time refers to the average of three determinations made on exponentially growing cultures, just before treatment.

an intermediate degree of sensitivity to HN2, are able to synthesize DNA for 5 days, up to a level three times greater than the mean control value<sup>14</sup>. The different action on DNA synthesis could therefore be related to a different inactivation of the enzymes or other features of the biosynthetic pathway, which seem also to be involved in the process of inhibition of DNA synthesis<sup>15</sup>. Recently the different sensitivity of cells to alkylating agents has been shown to depend on the activity of enzymes which repair damaged DNA<sup>16</sup>.

In summary, whatever their position in the cycle at the time of treatment, all the cells are able to proceed and are stopped only when they reach S. The blockage of cells in this phase could still be due, as suggested before, to the induction of cross-linkages between the complementary deoxyriboside chains, the partition of which would be impeded. Clearly the two events, DNA duplication and partition of the new molecules into daughter chromatids, must be much closely related, as suggested also by the model for chromatid duplication and organization proposed by Taylor<sup>17</sup>.

Perhaps the most significant of our results, independently of the molecular mechanism which it determines, is the possibility for cells to undergo several cycles of DNA replication without cell division (and therefore without partition of the synthesized DNA). As a matter of fact, there is considerable evidence that each segment of the chromosomal DNA replicates only once during each cell cycle. Taylor's detailed model seems to explain this known feature and to provide the necessary control

mechanism for the single DNA reproduction in the cell cycle. However, as Taylor has suggested<sup>18</sup>, the control mechanism could be prevented by breakage of DNA between the normal sites for replication. The fixation of the deoxyriboside chains, which is known to follow if some of the cross-links induced by difunctional alkylating agents split off<sup>9</sup>, could therefore open new primer sites on the DNA molecule and permit several cycles of replication during the same cell cycle. This mechanism could also explain why DNA which is alkylated *in vitro* by HN2 serves as a better primer than the non-alkylated DNA<sup>19</sup>. The possibility that a fraction of the DNA molecule may replicate more than once before the completion of normal duplication of the rest of the molecule has recently been shown for the chromosome of *E. coli*<sup>12</sup>. It seems that the experimental conditions which cause such a defective replication result also, in this case, in the induction of new primer sites on the DNA molecule.

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## SHERPA PHYSIOLOGY

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THE physiological investigation of the Himalayan high-altitude residents (Sherpa) which was sponsored by the Indian Council of Medical Research and carried out on the Himalayan School House Expedition, 1964, led by Sir Edmund Hillary (sponsored by *World Book Encyclopedia*, Chicago), recently concluded its programme after a period of three months in the field. The physiology team was organized and led by Dr. S. Lahiri of the Department of Physiology, Presidency College, Calcutta. Two other members of the team were Dr. J. S. Milledge, Christian Medical College, Vellore, Southern India, and Mr. A. K. Bhattacharyya, Presidency College, Calcutta. Mr. Bhattacharyya was, however, unable to continue his participation in the altitude programme.

The expedition set out from Katmandu in the middle of September 1964, and reached the base camp, Lukla, 9,500 ft. (2,900 m), after 14 days' trekking. The physiology camp was established on October 5, 1964, at 16,000 ft. (4,880 m) within a day's walking distance from Lukla.

Three lowlanders and four Sherpa residents occupied the camp and served as subjects of the physiological programme. There were visitors, both lowlanders and Sherpas, from time to time, who also served as subjects for some of our experiments. The camp was taken down and the members returned to the base camp on December 6, 1964, and flew out to Kathmandu from there by December 11.

We had a weekly cycle of weather change, 3-4 days' fine weather with clear skies followed by mist, snowfall and short storms. Afternoon mist was almost a regular feature. The barometric pressure in early October was 425 mm mercury which gradually decreased to 418 in December as winter set in. The maximum daily variation was 3 mm mercury, a fall being recorded in the evening.

A large dome tent was used as the main laboratory. Morning laboratory tent temperature immediately after sun-rise was below freezing, often down to minus 10° C. During the working hours the inside temperature fluctu-

ated between 7° and 24° C. Exercise experiments were carried out mostly on a bicycle ergometer outside the tent under a shade and a few climbing experiments on a mountain slope.

The main objective of this investigation was to investigate the physiology of the Sherpa high-altitude residents, and compare the results with those of the lowlanders acclimatized to the same altitude. This was the first systematic attempt to examine the Sherpa physiology which not only recorded new data on an isolated group of people but also advanced knowledge about some aspects of human adaptation to altitude. The following principal physiological investigations were carried out:

(1) *Respiratory regulation.* The ventilatory response to carbon dioxide and hypoxia were examined at rest with respect to alveolar carbon dioxide pressure and oxygen pressure using a technique worked out by Lloyd and Cunningham and their associates in the Laboratory of Physiology, University of Oxford.

(2) *Muscular work.* Oxygen consumption, pulmonary ventilation, alveolar gas tension and arterial blood pH were measured under resting condition and during muscular work at 475, 900 and 1,285 kg/min on the bicycle ergometer. In some experiments the effects of breathing oxygen at higher and lower pressures were also recorded. The relationship between ventilatory function and work was also investigated in a few climbing experiments.

(3) *Heart rate.* Heart rates were systematically recorded in all the experiments mentioned in section (2).

(4) *Maximum oxygen consumption and recovery.* Oxygen consumption, ventilation and heart-rates were measured during and after work at highest work rates the subjects could manage for about 3 min. The recovery was followed up to 15 min on stopping work. Blood was taken for lactate estimation at 3rd, 10th and 15th min on stopping work.

(5) *Acid base balance.* Arterial blood and cerebro-spinal fluid pH, arterial oxygen content and capacity, and carbon dioxide content of arterial blood and cerebro-spinal fluid were measured at rest. Tonometry of the cerebro-spinal fluid and oxygenated and reduced blood at different carbon dioxide pressures were performed at 38° C and pH carbon dioxide pressure and contents were determined.

(6) *Arterial oxygen saturation.* This parameter which gives a measure of diffusion limitation was measured at rest and exercise breathing air and oxygen.

(7) *Urinary steroids and Na/K ratio.* The urinary 17-ketosteroids and ketogenic steroids and Na/K ratio were measured in the lowlanders and the residents before and after the administration of adrenocorticotrophin gel at altitude to determine the response of the adrenal cortex. These experiments were also performed in the lowlanders at sea-level.

(8) *Electrocardiogram.* Complete electrocardiograms of lowlanders and Sherpas were taken at 9,500 ft. (2,900 m) and 16,000 ft. (4,880 m). Sea-level records of the lowlanders were also made.

(9) Blood-haemoglobin values of the Sherpa residents were determined to compare their values with those of the published data of the Andean residents of similar altitude. Haemoglobin of the lowlanders was also determined.

(10) *Alveolar and mixed venous gas pressures.* Alveolar oxygen pressure and carbon dioxide pressure and mixed venous carbon dioxide were determined in the lowlanders and residents at different altitudes. In these experiments, a few lowlanders examined had residence at altitude for a period up to four years.

## Results

*Respiratory regulation.* The high-altitude residents breathe less than the recently acclimatized lowlanders both at rest and in different grades of exercise, but breathe more than the average sea-level values. The ventilation carbon

dioxide response line was lower (shifted to the left) at altitude but there was no marked difference between the two groups. The carbon dioxide sensitivity also was not markedly different in the two groups, but the Sherpas showed a markedly reduced sensitivity to hypoxia. For example, the effect on Sherpas of reducing the alveolar oxygen pressure from 200 to 40 mm mercury at constant carbon dioxide pressure was to increase the ventilation by only 10 per cent of that found in lowlanders.

*Alveolar and blood gas tensions.* Alveolar and mixed venous carbon dioxide pressures were higher in the residents. Along with this, arterial blood and cerebro-spinal fluid pH were also lower in the Sherpa residents compared with the lowlanders at altitude, which seems to tie up in a cause and effect relationship. The calculated arterial and cerebro-spinal fluid carbon dioxide pressure was higher in Sherpa residents.

Tonometry experiments showed that there was no significant difference in the buffering power of cerebro-spinal fluid and of blood between the two groups to account for the difference in pulmonary ventilation.

*Blood pH.* Arterial blood pH in the acclimatized European members turned more alkaline in exercise but significantly acidic in the Indian member studied. Acidic change may be related to the lactate accumulation in blood of the latter subject. This pH value, if anything, changed to acidic side in the Sherpa residents. A corresponding change in the alveolar gas pressures was also noted, that is, carbon dioxide pressure remained almost unchanged in the Sherpa subjects but decreased in the European subjects. Thus the Sherpa subjects were able to maintain higher arterial oxygen pressure at a given saturation of oxygen, because of the Bohr effect on the oxygen dissociation curve.

*Oxygen breathing.* Breathing a gas mixture enriched with oxygen to sea-level pressures during exercise greatly diminished pulmonary ventilation in the lowlanders but usually produced no definite effect on the resident's ventilation. Thus in the lowlanders oxygen breathing increased not only alveolar oxygen pressure but also alveolar carbon dioxide pressure and arterial hydrogen-ion concentration, while in Sherpas oxygen breathing resulted in little change in carbon dioxide pressure or pH.

*Arterial oxygen saturation.* Resting arterial blood oxygen saturation was similar in both groups, which was about 80 per cent. Moderately heavy exercise while lowering this saturation increased the alveolar oxygen pressure due to hyperventilation. Thus alveolar-arterial oxygen gradient became large, showing a limitation of oxygen diffusing capacity of the lung at altitude. Oxygen breathing raised the saturation to normal values both at rest and in exercise. The experiment on arterial oxygen in exercise could be performed in a lowlander subject only. But capillary blood drawn from a warm hand for pH measurements on other subjects including Sherpas showed visibly a difference: blood on oxygen inhalation was bright red.

*Heart rate.* Resting heart rates were lower in the Sherpas and the normal maximum heart rate of 190-200 was achieved in two subjects at the work rate of 1,285 kg/min. The maximum heart rate in the lowlanders was lower, between 140-160, at 900 kg/min. However, oxygen breathing at sea-level pressure raised the maximum in the lowlanders. In Sherpa residents oxygen lowered the rate at all levels examined. Breathing low oxygen mixtures at altitude depressed the rate: very severely in the lowlander, less so in the Sherpas. There was no appreciable difference between the two groups in the rate of recovery after stopping work.

*Oxygen debt.* Oxygen consumption at rest and during work on the bicycle ergometer including the maximum was similar in both groups but the oxygen debt appeared to be smaller in the Sherpa subjects. Resting blood lactate was similar but its level after maximum and during recovery period was lower in the Sherpas.

Lactate concentration in the cerebro-spinal fluid was similar in both groups, about 25 mg/100 ml. Although the cation ( $\text{Na}^+$  and  $\text{K}^+$ ) concentration was similar and did not change from the sea-level values,  $\text{K}^+$  concentration both in cerebro-spinal fluid and plasma was in the higher range in the Sherpa subjects.

**Haemoglobin.** In general, haemoglobin concentration in the residents was lower than the lowlanders exposed to the same altitude.

**Electrocardiograph.** All electrocardiographic records were within the normal limits. Normal axis in the lowlanders was vertical or showed often a deviation to the right. A few in Sherpa group showed horizontal axis.

These observations show that the altitude residents respond less to the hypoxic stimulation of altitude than

the well-acclimatized lowlanders. They select a different combination of cardiovascular and respiratory function from the acclimatized lowlanders to achieve the same physiological end. It seems that acclimatization, as the term is usually understood, consists of a number of short-term adjustments of a lowlander, enabling him to survive and perform more efficiently under conditions of low oxygen tension at altitude. But in the high-altitude residents more fundamental long-term changes have come about perhaps at cellular level which result in more efficient working of the organism and a certain reversal of the short-term adjustments when the first crisis is overcome. Whether these fundamental changes are genetic in origin or acquired over many years or generations of high-altitude residence is one of the unsolved problems of altitude physiology.

## BIOLOGICAL PROPERTIES AND RADIOSENSITIVITY OF TUMOURS : DETERMINATION OF THE CELL-CYCLE AND TIME OF SYNTHESIS OF DEOXYRIBONUCLEIC ACID USING TRITIATED THYMIDINE AND AUTORADIOGRAPHY

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AS part of a series of investigations on biological properties and radiosensitivity of tumours, their proliferating cycle has now been examined in this laboratory. Specifically, I have compared the kinetics of two types of tumours with varying growth rates, both indigenous to the same host. The analysis was based on the quantitative autoradiographic estimation of the number of cells incorporating tritiated thymidine as a precursor in DNA synthesis, thus permitting determination of the time the cell spends in the various phases of its cycle. The data were plotted and the curves are shown in Figs. 1 and 2. Inspection reveals a striking difference between the  $T_s$  phase of the two tumour-types. The  $T_s$  phase of the spindle cell tumour is approximately twice as long as that of the epithelial tumour. (There are also some differences between the other phases of the life-cycle of these tumour types but these will not be considered at present.)

The usefulness of carbon-14- and phosphorus-32-labelled precursors and improved autoradiographic techniques for determining the cell cycle and the time of DNA synthesis in growing plants was first recognized by

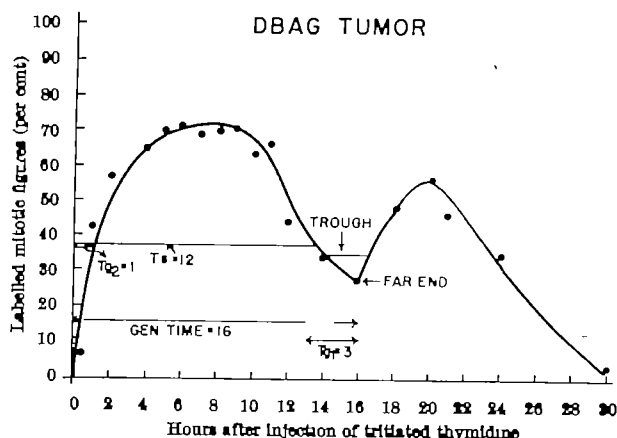


Fig. 1. Curves illustrating the percentage of labelled mitoses in the spindle cell tumour, plotted against time between tritiated thymidine injection and killing of the tumour-bearing mouse. Each symbol represents average counts of several tumour nodules of two or three mice

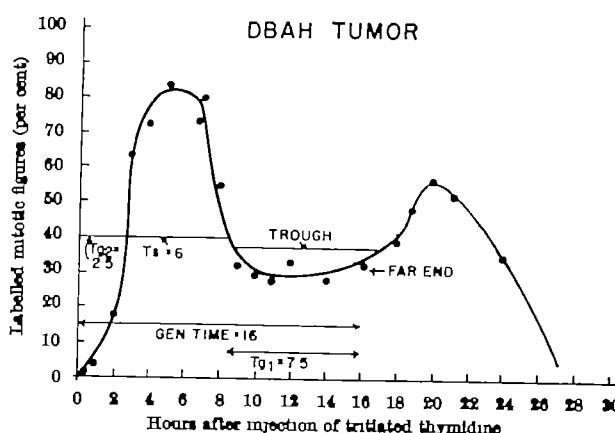


Fig. 2. Curves illustrating the percentage of labelled mitoses in the epithelial cell tumour, plotted against time between tritiated thymidine injection and killing of the tumour-bearing mouse. Each symbol represents average counts of several tumour nodules of two or three mice

Howard and Pelot<sup>1</sup>. This technique was later applied to human bone marrow cells cultivated *in vitro*<sup>2</sup>. Since thymidine is one of the nucleosides of the DNA molecule<sup>3</sup> and is specifically utilized by proliferating cells (mammalian, plant or micro-organisms) for synthesis of DNA, its successful tritiation<sup>4</sup> led to its widespread use in the examination of cellular kinetics and the timing of DNA synthesis. Because it emits  $\beta$ -particles of low energy (0.019 MeV), thereby permitting high resolution of autoradiography, it is a valuable tracer for identification of those cells which are actively engaged in DNA synthesis prior to cell division.

During the past several years, tritiated thymidine and the autoradiographic technique have found wide application not only in kinetic investigations of a variety of normal mammalian cells, but also in investigations of neoplastic cells, particularly those growing in ascites form. (The references pertaining to these investigations are cited in Table 1.) However, I know of only one report in the literature on the use of tritiated thymidine in examinations of the mitotic cycle and DNA synthesis time in a mouse mammary tumour grown in a solid form

Table 1. DNA SYNTHESIS TIMES ( $T_s$ ) OF VARIOUS CELL TYPES

Cell types	Medium	$T_s$ -Phase (h)	Ref. No.
Bone marrow	<i>In vivo</i>	12-15	2
C3H mammary tumour	<i>In vivo</i>	9-12	5
Epithelium forestomach	<i>In vivo</i>	12-5	13
Epidermis	<i>In vivo</i>	20	14
Ehrlich ascites tumour cells	<i>In vivo</i>	17-0 female 11-0 male	15
Spermatogonia	<i>In vivo</i>	12.5-14.5	21
Tibia (rat)	<i>In vivo</i>	8-0	22
Colon	<i>In vivo</i>	11-14	23
Colon	<i>In vivo</i>	6-5	24
Ascites tumour	<i>In vivo</i>	8-5	25
Ascites tumour	<i>In vitro</i>	8-0	26
U-strain fibroblasts	<i>In vitro</i>	9-0	27
Myeloid cells	<i>In vivo</i>	5-0	28
Ascites L-5178Y	<i>In vitro</i>	6-0	29
Chinese hamster cells	<i>In vitro</i>	7-4	30
Rat liver	<i>In vivo</i>	9-0	31
Mammary epithelial tumour	<i>In vivo</i>	6-0	32
Spindle cell tumour	<i>In vivo</i>	12-0	33
Tooth enamel of young rabbits	<i>In vivo</i>	9-5	35

in isologous hosts<sup>5</sup>. I am not aware of any comparative investigations of the kinetics of two morphologically and biologically diverse but genetically isologous tumours, both grown in parent hosts. Thus, we thought that the two types of mouse mammary tumours which have been under investigation in this laboratory for several years would constitute a particularly suitable test system for an examination of the dynamics of cellular proliferation.

These tumours are: a slow-growing epithelial one, designated DBAH, and a rapidly growing spindle cell type, designated DBAG. Both were developed from the mammary gland tissue of the inbred DBA/212 strain of mice and are propagated by serial transplants in isologous hosts. Their history and biological properties have been described<sup>6</sup>. Several of their metabolic and morphological properties examined at cellular and sub-cellular levels, as well as their response to ionizing radiation, are also known<sup>7,8</sup>. Their latent periods are 4-6 days for the spindle cell tumour and 10-12 days for the epithelial tumour. The spindle cell tumour was in its 458th passage and the epithelial tumour in its 38th passage of serial transfer at the time of these examinations.

In my investigations I used the following method: Male mice were implanted with several tiny particles of either epithelial or spindle cell tumour. Each tumour-bearing mouse was injected intraperitoneally with 0.3 c.c. aqueous solution containing 25  $\mu$ c. of tritiated thymidine. (The tritiated thymidine with specific activity of 1.9 c./mmole was obtained from Schwarz Bio-Research, Inc., Orangetown, New York.) Since the mice ranged in weight between 20 and 25 g, each received approximately 1  $\mu$ c./g body-wt. After injection of the labelled precursor, the mice were killed by cervical dislocation at hourly or bi-hourly intervals and the tumour nodules immediately removed. These procedures were carried out during a 30-h period.

Small particles were randomly removed from each tumour with sharp razor blades, fixed for 1 h in 3:1 alcohol-acetic acid and then kept overnight in a 10 per cent neutral formal-saline solution. Autoradiographs were made of deparaffinized sections 2-3  $\mu$  thick and dipped in nuclear photographic emulsion, NTB or NTB<sub>2</sub>, obtained from Eastman Kodak, Rochester, N.Y. After 10-12 days' exposure in a freezer at -20° C, the autoradiographs were developed for 6 min in Kodak D-19 at 16° C and fixed for 10 min in Kodak acid fixer. The usual procedure of washing, staining with Harris haematoxylin and eosin, and dehydrating followed. Labelled mitotic figures were identified by the presence of activated silver grains. The cells were usually heavily labelled and the background was low. In a number of instances mitotic counts were made on Feulgen-stained squash-preparations and compared with those of sections. Generally, fairly good agreement was obtained, indicating that my sections 2-3  $\mu$  thick provided good autoradiographic efficiency. This is in agreement with Kisilevski, Baserga and Vanpotic<sup>9</sup>. The number of labelled and unlabelled mitotic figures in all phases was determined for 100-200 mitoses per mouse.

The percentage of labelled mitotic figures, including all phases, was plotted against time after administration of tritiated thymidine into the tumour-bearing mice. The results are presented in Figs. 1 and 2. Each point constitutes average counts of 3-4 tumour nodules of the same mouse and also averages of two repeated experiments for each time-interval. Confidence intervals of positive mitotic counts among all mitotic counts observed are expressed using the formula:

$$p \pm 2 \frac{\sqrt{p(1-p)}}{n}$$

where  $p$  = percentage of positive mitoses,  $n$  = total counts and resulted in  $p \pm 10$  per cent. (Calculated by Dr. A. Berger, of the Statistics Department of Public Health, New York City.)

The estimation of the time-intervals of the cell-cycle and of DNA synthesis is made according to the method originally used by Quastler and Sherman for mammalian cells<sup>10</sup>, and afterwards by many other investigators. In brief, the phase of the cell-cycle was divided as follows:  $T_{G_1}$  = post-synthetic, pre-mitotic period from the time of injection of the labelled precursor to the earliest time of appearance of labelled mitotic figures. In the case of the spindle cell tumour (Fig. 1),  $T_{G_1}$  phase lasted about 1 h, whereas in the epithelial tumour (Fig. 2),  $T_{G_1}$  was about 2.5 h. The frequency of the labelled mitotic figures rises rapidly after the  $T_{G_1}$  period and reaches a peak of approximately 76 per cent for the spindle cell tumour and about 80 per cent for the epithelial tumour. The time of DNA synthesis ( $T_s$ ) was estimated from the points between the 50 per cent level, that is, where half-labelled mitoses intercept on the ascending and descending portions of the curve. Inspection of the curves shows that the average  $T_s$  for the spindle cell tumour is 12 h and for the epithelial tumour 6 h.

The number of labelled mitoses in the spindle cell tumour decreased (after the peak) to approximately 27 per cent at 16 h after administration of the labelled precursor. At this point a second wave of labelled mitoses started and reached 56 per cent at 20 h. These labelled mitoses could well constitute the daughter cells of the initially labelled mitotic figures which had passed through interphase and had arrived at the next division. Following the 20-h period, a gradual decrease in labelled mitoses occurred in this tumour, reaching 3 per cent labelling 30 h after the administration of the labelled precursor.

Regarding the epithelial tumour, it can be seen in Fig. 2 that a gradual decrease in labelled mitoses occurred after the peak, resulting in 27.6 per cent at 11 h and in a plateau lasting about 4 h. Following this period, a slow rise in labelled mitoses occurred, reaching a second peak of approximately 57.5 per cent at 20 h. Then followed a decline to about 35 per cent at 24 h and about 28 per cent at 30 h after the administration of tritiated thymidine.

The generation time for both tumours was then determined. This is the distance from the beginning of the rising points of the curve at zero to the far portion of the trough (allowed in these cases where  $T_{G_1}$  and mitoses are short compared to  $T_s$  time). For both types of tumours, it was found to be approximately 16 h.

The post-mitotic, pre-synthetic period ( $T_{G_2}$ ) was obtained by subtracting the sum of the  $T_{G_1}$  and  $T_s$  periods from the generation time. According to the results (Figs. 1 and 2),  $T_{G_2}$  lasted 3 h for the spindle cell tumour and 7.5 h for the epithelial tumour.

In the field of cellular kinetics the most reliable data are generally deemed those obtained for  $T_{G_1}$  and  $T_s$  phases. Consequently, these two phases, and particularly the  $T_s$  phase, are emphasized in the work recorded here. A detailed analysis of the literature reveals that  $T_s$  values differ not only between various cell types but also between cells of the same morphological classification. Examples are listed in Table 1. Additional results can be found in a table compiled by Oehlert, Seemayer and Lauff<sup>11</sup>.



There are differences in opinion among investigators as to the constancy of  $T_s$  phase for various cell types. For example, Cameron<sup>13</sup> expressed the opinion that the 6-8 h  $T_s$  period should be regarded as a constant for various cell types. Wolfberg<sup>13</sup> reported a  $T_s$  period of 13.5 h for the stomach epithelium of the mouse. Another report<sup>14</sup> cites a  $T_s$  period of 30 h for mouse ear epithelium. This extremely long  $T_s$  period is assumed to be caused by the 17° C temperature of the mouse ear, which is lower than the 37° C at which the mammalian cells normally metabolize. Baserga<sup>15</sup> reports a  $T_s$  of 17 h for the Ehrlich ascites tumour cells grown in female mice and a  $T_s$  of 11 h for those grown in male mice.

The question arises as to why there exists a wide range in the  $T_s$  period, which is one of the most important phases in the life-cycle of the cell. This question becomes even more significant if one compares the  $T_s$  period of the two types of tumours examined here, both of which are of the same genetic derivation and are propagated in isologous parent hosts. The difference in their  $T_s$  periods could be solely attributed to differences in their inherent cellular composition.

To shed light on this problem, the following explanations are offered:

(1) Some of the metabolic events which are involved in DNA synthesis may be slower in the spindle tumour cells than in the epithelial tumour cells. This assumption is based on results of previous experiments<sup>6,7</sup>, which showed that the oxidative phosphorylation capacity of the spindle cell tumour mitochondria is significantly less efficient than that of the epithelial cell tumour mitochondria. Electron microscope investigations<sup>8</sup> which revealed that the mitochondria of the spindle cell tumour are fewer in number than those of the epithelial tumour and of inferior quality, containing fewer membranes (cristae), attested to these results. Furthermore, there appeared to be fewer and smaller ribonucleoprotein particles (RNP) in the spindle cell tumour than in the epithelial tumour<sup>16</sup>. This would indicate that the epithelial tumour cells, which are richer in the essential cytoplasmic components, are more efficient in the production of the necessary precursors for DNA synthesis. Conversely, the spindle tumour cells produce these precursors less efficiently, thus limiting the rate of DNA synthesis and extending the  $T_s$  period. These results are in line with recent concepts concerning the participation of the cytoplasmic compounds including mitochondria in DNA synthesis<sup>17,18</sup>.

(2) The number of chromosomes may also play a part in the time of synthesis of DNA. It has been found by cytophotometric measurements and by chromosome counts<sup>19</sup> that the spindle tumour cells contain 32 per cent tetraploids, 6 per cent octaploids, 49 per cent diploids and 13 per cent aneuploids, whereas the epithelial tumour cells contain 85.5 per cent diploid, 10 per cent aneuploid and 4.5 per cent hypoploid cells. Thus, in order to replicate two or four times more DNA for tetra- or octa-ploid cells, more time could well be required, particularly in the case of the spindle cell tumour, where the nucleotide pool is inadequate.

Conclusions reached by Defendi and Manson<sup>20</sup> that the number of chromosomes has no bearing on the phase of DNA synthesis do not seem to be borne out by closer scrutiny. These authors tabulated results from the literature on the relationship of chromosome numbers to DNA synthesis ( $T_s$ ), and expressed the opinion that the duration of  $T_s$  is independent of the number of chromosomes. However, their table reveals that in the case of Ehrlich *ELT* tumour containing 92 chromosomes, the  $T_s$  phase ranges from 13-14 h; in the majority of cells containing 42-46 chromosomes, and in two cases of cells with 60 chromosomes, the  $T_s$  period ranges from 6-8 h. It would therefore seem that the question of the influence of chromosome numbers on the  $T_s$  period will have to be further explored.

Based on the previous observations on the structural and functional properties of these two tumour types, the inference is strong that the longer  $T_s$  of the spindle cell tumour, relative to that of the epithelial tumour, is caused by the diminished or impaired synthesis of the necessary nucleotides. The thought is suggested that the clue to the variation in  $T_s$  phases of other cell types may be found in the availability of free nucleotides.

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## A PERMEABILITY FACTOR (TOXIN) FOUND IN CHOLERA STOOLS AND CULTURE FILTRATES AND ITS NEUTRALIZATION BY CONVALESCENT CHOLERA SERA

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THIS article reports the finding of a heat-labile factor in sterile filtrates of both cholera stools and young cultures of *Vibrio cholerae* which evokes induration and a prolonged increase in capillary permeability following intra-

cutaneous injection in guinea-pigs and rabbits; and which is neutralized by convalescent sera from cholera patients.

Although Koch in 1884 considered cholera to be essentially a toxicoosis, caused by a poison excreted by the

causative organism, most workers during the ensuing decades have favoured the view that *V. cholerae* does not produce a soluble exotoxin, but rather contains endotoxins which are liberated on autolysis or disruption of the organisms. Many workers, using a wide variety of extraction procedures, have obtained from cholera vibrios a number of deleterious substances which exhibit many of the properties generally associated with endotoxins. The indicator of toxicity for these materials has, however, been limited largely to death following parenteral injection of animals, and association of these endotoxic substances with the clinical manifestations of cholera has been lacking<sup>1</sup>.

More recently, however, De and Ghose<sup>2</sup> and De *et al.*<sup>3</sup> have shown that certain young culture filtrates of *V. cholerae* contain an antigenic and heat-labile substance which produces dilatation of the isolated gut loop; and Oza and Dutta<sup>4</sup> and Finkelstein *et al.*<sup>5</sup> have shown that similar preparations cause a fatal, dehydrating diarrhoea when introduced into the gut lumen of infant rabbits. Both these effects appeared to be specifically neutralized by antisera prepared against the toxic filtrates. Basu Mallik and Ganguli<sup>6</sup> showed that intracutaneous injection of cholera stool filtrates evoked an immediate increase in permeability of skin capillaries in rabbits by the intravenous dye technique. Similar activity, however, was demonstrated to a lesser extent in non-cholera stools; and specific neutralization of the effect was not reported.

The recognition of these heat-labile, extracellular, toxic factors once again suggests the possibility that a 'true' exotoxin may play a significant part in the pathogenesis of cholera, in spite of long-held views to the contrary.

For the work recorded here, stools from cases admitted to the Pakistan-SEATO Cholera Research Laboratory in Dacca, East Pakistan, in 1964, were centrifuged at 10,000 r.p.m. at 5° C for 30 min and the supernatant fluids were passed through 450-mμ 'Millipore' filters. Formed stools were diluted 2- to 10-fold in sterile saline before centrifugation. All stools were cultured for *V. cholerae* as well as other common bacterial enteric pathogens. Filtrates were diluted in sterile saline and injected intracutaneously in 0.1-ml. volumes in partially randomized sites on the backs of albino guinea-pigs or rabbits. Guinea-pigs were prepared by clipping and depilating with barium sulphide paste, while rabbits were clipped only.

The results of the first 56 stools tested in guinea-pigs are shown in Table 1. Stools from 13 out of 20 bacteriologically confirmed cholera cases produced marked induration and erythema beginning 6-8 h after injection, reaching a peak in 18-24 h, receding somewhat by 48 h, but persisting for 4 or 5 days, in both guinea-pigs and rabbits. No induration was produced by any of the filtrates of stools from 33 patients with acute diarrhoea from which *V. cholerae* was not isolated. Of these 33 negative cases, 16 were suffering from a severe watery diarrhoea, similar clinically to cholera, with dehydration and collapse. By replicate titration in both guinea-pigs and rabbits, it was found that the diameter of induration bore a linear relationship to log dose of filtrate, over a range of 6-15 mm. In order roughly to quantitate the activity in each stool, the amount of filtrate in a 0.1-ml.

volume capable of producing induration of 8 mm in diameter at 18-24 h was established as an arbitrary 'induration dose'. All specimens were tested in at least two animals simultaneously, and active cholera stools were used repeatedly for investigation of the properties described here. Most of the same stools were also tested in duplicate in rabbits, and results were essentially identical in both animals.

From three patients with confirmed cholera, stools were collected in the acute purging stage and again after recovery. In one case, both early and late stools were negative. In the other two, stools collected on the first day were strongly positive (> 100 induration doses/ml.), whereas stools collected 57 and 68 days after onset were negative.

The effect of stool filtrates on skin capillary permeability was investigated by the intravenous injection of pontamine sky blue 6XB, 0.12 ml. of a 5 per cent solution/100 g, at varying times before and after the intracutaneous injection of test materials<sup>7,8</sup>. Filtrates capable of evoking induration also caused an increase in permeability of skin capillaries of at least 24 h duration, and the diameter of the blue lesion bore a linear relationship to log dose of filtrate within the range of 5-12 mm. The time-course of increased permeability was determined by injecting filtrates at various intervals followed by intravenous injection of dye when the skin was bearing lesions of different ages. A final injection of filtrate and of saline was given immediately after the dye to determine the immediate permeability effect as well as the effect of trauma. Readings were made 1 h after the injection of dye. Immediately after injection there was a marked increase in capillary permeability followed by recovery within 1 h. During the next few hours the permeability gradually increased again, reaching the maximum diameter, but not maximum intensity, by 8 h. The bluing became more intense between 8 and 18 h and was maximum at 18-24 h. Between 24 and 48 h intensity faded. Induration developed more gradually during the first 24 h; but the peak was the same as for bluing. Permeability returned to normal by 72-96 h, whereas induration gradually receded over a 4-5-day period. Residual induration was palpable at 6-7 days. The time-course of permeability and induration in rabbit skin was essentially the same as that in guinea-pigs. On the basis of these time-course investigations, 18-h lesions were selected for the study of other properties of the stool permeability factor. In all stool filtrates so far examined, increased capillary permeability at 18 h has paralleled the induration response.

Filtrates of *V. cholerae* cultures grown in 5 per cent Difco 'Bacto-Peptone', pH 7.3, as used by De *et al.*<sup>3</sup> for production of cholera enterotoxin, evoked skin lesions indistinguishable from those produced by the skin-factor found in cholera stools. Living vibrios injected intracutaneously failed to evoke any induration or permeability change. De *et al.*<sup>3</sup> showed that the *in vitro* production of cholera enterotoxin active in the ligated rabbit-gut segment was related to surface-volume ratio of the culture medium. This association appears to hold true also in the case of skin-reactive factor. Shallow cultures with surface/volume ratios of 2.5:1 yielded approximately 30 times as much active material as did tube cultures with a ratio of 0.2:1.

The influence of length of incubation on skin-factor production was investigated by titrating permeability factor content of filtrates of two strains of *V. cholerae* grown in shallow cultures in Erlenmeyer flasks (surface/volume ratio of 2.5) for different periods of time at 37° C. The permeability factor was absent at 2 h, detectable at 4 h, reached a maximum titre at 24-48 h and began to wane at 72 h.

The skin reactions evoked by stool and culture filtrates were compared on the same set of guinea-pigs. Time-course of the permeability factor as well as dosage-response

Table 1. TWENTY-FOUR HOURS' INDURATION IN GUINEA-PIGS FOLLOWING INTRACUTANEOUS INJECTION OF STOOL FILTRATES

Source of stool specimen	Induration doses/ml stool filtrate				Positive Total
	<5	5-10	10-100	100-1,000	
Acute cholera*					
Ogawa	4	—	3	1	
Inaba	3	2	2	5	
Total acute cholera	7	2	5	6	13/20
Convalescent cholera	3	—	—	—	0/3
Non-cholera diarrhoea*					
Non-cholera vibrio, Heiberg II	4	—	—	—	
No pathogens					
Pulseless	16	—	—	—	
With pulse	13	—	—	—	
Total non-cholera diarrhoea	29	—	—	—	0/23

\* Within 48 h of onset.

curves of induration and the permeability factor of both stool and culture filtrates were indistinguishable qualitatively and quantitatively, suggesting that the two materials are identical.

In order to determine whether substances with similar effect on skin were elaborated by other organisms, the following strains were grown simultaneously in shallow cultures of 5 per cent 'Bacto-Peptone', pH 7.3: *Shigella B*; enteropathogenic *E. coli*; *E. coli*, non-typable; non-cholera vibrio, Heiberg Group II; and *V. cholerae* Ogawa. Neither induration nor change in capillary permeability was produced by intracutaneous injection of undiluted filtrates of cultures of the first four organisms, whereas cholera filtrate gave rise to typical lesions at a dilution of 1:40.

Both the induration-evoking capacity and the permeability factor of both stool and culture filtrates were completely destroyed at 56° C for 30 min. Dialysis of both stool and culture filtrates for 24 h at 5° C. against 0.85 per cent sodium chloride caused a 25-50 per cent loss of skin reactivity. Stool filtrates retained full activity after exposure to trypsin, 100 µg/ml. for 15 min at 37° C at pH 7.0.

The neutralizing capacity of convalescent cholera sera was investigated as follows: acute and convalescent sera from cholera cases were inactivated at 56° C for 30 min. Three-fold serial dilutions of serum were mixed with constant amounts of stool filtrate or culture filtrate and the mixtures were incubated at 37° C for 30 min. The mixtures were injected intracutaneously in guinea-pigs, and intravenous dye was given 18 h later. Convalescent sera from confirmed cholera cases were capable of preventing both the induration and permeability factor effects of both stool filtrate and culture filtrate at a higher dilution than was acute phase serum. The permeability factor-neutralization titres of a number of sera against culture filtrates are set out in Table 2. Titres are expressed as the highest serum dilution which prevented the increase in capillary permeability induced by the standard dose of toxic filtrate. Rises in titre were demonstrable in all cases of confirmed cholera so far tested, but not in human beings given commercial cholera vaccine or in rabbits given living vibrios grown on 1 per cent tryptose slants. Substantial agglutinin titre rises were demonstrated, however, in both the cholera cases and in persons and animals vaccinated with whole organisms. Normal persons and some acute phase sera contained low titres of neutralizing substance. The lowest titres were found in normal rabbits and in the acute phase sera of those cholera patients who showed the highest titres during convalescence.

The foregoing findings indicate that skin-reactive substances found in the stools of cholera patients and in culture filtrates are identical. Moreover, their properties are those of a typical bacterial exotoxin. Hence, the term "skin-toxin" would seem applicable. For the pre-

Table 2. TOXIN-NEUTRALIZING TITRES OF HUMAN AND ANIMAL SERA (Toxin from Ogawa B1307)

	Source of sera	Day of disease or vaccination	Neutralization titre	Agglutination titre	
				Ogawa	Inaba
I	Confirmed cholera cases				
	Case No.	Organism			
	977	Inaba	1	< 40	< 40
			10	1,280	1,280
	1,021	Inaba	0	< 40	< 40
			10	160	160
			30	40	80
	978	Inaba	1	< 40	< 40
			12	640	640
	1,188	Inaba	1	< 40	< 40
		10	> 270	320	
944	Ogawa	0	< 40	< 40	
		16	90	160	
890	Ogawa	0	< 40	< 40	
		9	640	1,280	
II	Non-vibrio cholera				
	1,011	No pathogen	0	< 40	< 40
			13	< 40	< 40
			59	—	—
III	Normal human controls (East Pakistan residents)				
	44-I	—	10	< 40	< 40
	37-I	—	10	< 40	< 40
	A-684	—	10	< 40	< 40
IV	Pakistani personnel vaccinated with killed Ogawa + Inaba vaccine				
	1	—	0	< 20	< 20
			28	30	160
	19	—	0	30	40
		28	30	640	
V	Rabbits vaccinated with living <i>V. cholerae</i> Ogawa				
	RJ-6	—	0	< 40	< 40
			36	< 10	1,280
	RJ-7	—	0	< 40	< 40
			36	< 10	10,240

paration of skin-toxin *in vitro*, the medium and growth conditions are the same as those used for the preparation of the enterotoxin of De. Both are heat-labile. These facts suggest the possibility that the skin-toxin and the enterotoxin are also identical.

At present there is no evidence that the skin-toxin plays a part in the morbid sequence of cholera in man. Skin-toxin could, however, exert a deleterious effect on the gut, perhaps by altering permeability of capillaries in the villi, resulting in the passage of exocoel fluid into extravascular spaces and then into the gut lumen.

Evidence indicates that the skin-toxin is antigenic in man, that it reaches antibody-producing elements during the course of clinical cholera, and that the antibody elaborated behaves like an antitoxin. Moreover, antibody against the skin-toxin does not appear to be evoked by vibrio antigens capable of evoking agglutinins.

This work was supported in part by U.S. Public Health Service special research grant 7-F3-A1-9409-02 from the National Institute of Allergy and Infectious Diseases.

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## COMPARISON OF FERRITINS FROM NEOPLASTIC AND NON-NEOPLASTIC HUMAN CELLS

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IN previous work<sup>1</sup> it was shown that two lines of neoplastic human cells grown *in vitro* synthesize distinctive kinds of ferritin. By electrophoresis in polyacrylamide gels, ferritin from HeLa cells was separated into 3 fractions (α, β, and γ) with mobilities that differed from those of corresponding fractions extracted from human liver cells. Only an α-ferritin was obtained from KB

cells and this had the mobility of the α-ferritin of HeLa cells. These mobilities, like those of other ferritins, are independent of the FeOOH micelles that form the core of the ferritin molecule<sup>2-4</sup>.

We now report results obtained with ferritins synthesized *in vitro* by two strains of embryonic human skin cells, and by HEP-2 carcinoma cells.

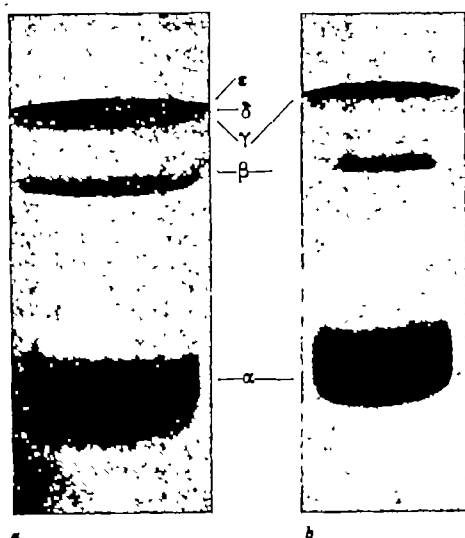


Fig. 1. Electrophoretic separations in 5 per cent polyacrylamide gels at pH 6.6. *a*, SK-1 ferritin, separated into five fractions. *b*, Mixture of SK-1 ferritin and ferritin from human liver. Note single  $\alpha$ ,  $\beta$ , etc., fractions. Minor fractions are obscured in this picture.

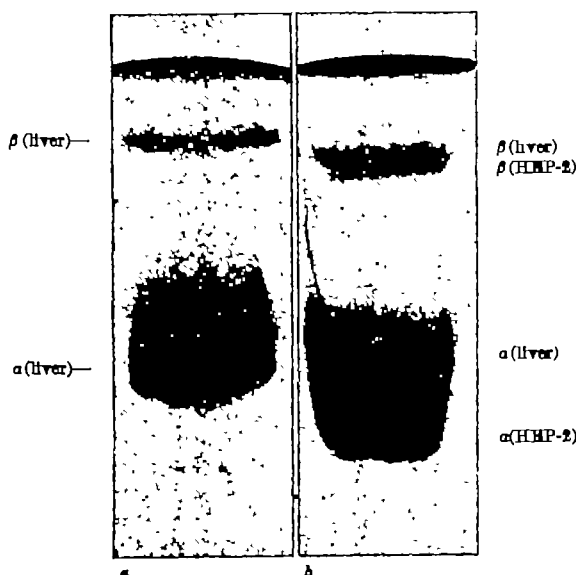


Fig. 2. Electrophoretic separations in 5 per cent polyacrylamide gels at pH 6.6. *a*, Ferritin from human liver. *b*, Mixture of HEP-2 and liver ferritins. Note separate  $\alpha$ - and  $\beta$ -fractions ( $\gamma$ -bands are obscured). HEP-2 fractions have greater mobilities than corresponding fractions from human liver (spleen, kidney).

A diploid strain of embryonic human skin (M-5) was obtained from Microbiological Associates, Bethesda, Md., after 8 serial passages. We have called this strain SK-1. Its modal chromosome number after 20 further passages has remained 46. Cells of another diploid strain of embryonic human skin, SK-2, were donated by Dr. Albert Kettler of the Public Health Research Institute of New York City. HEP-2 cells were supplied by Microbiological Associates and these were heteroploid. Cultures were grown in Eagle's medium containing 0.01 per cent  $\text{FeSO}_4$ , as previously described<sup>1,4</sup>. Extraction and purification of the ferritins, and the electrophoretic and serological analyses were done as already described<sup>1</sup>. Although equivalent results were obtained when electrophoretic separations were carried out at diverse pH values (4.8–8.6), a pH of 6.6 was most satisfactory. After separation in polyacrylamide gels at pH 6.6, the fractions were either stained by the Prussian blue method for ferric iron or with amidoschwartz for protein. In separate experiments, the fractions were eluted, and checked for the presence of ferritin by electron microscopy<sup>1,8</sup>.

Typical findings are shown in Fig. 1, where it can be seen that SK-1 ferritin was resolved into at least three fractions, identical in mobility with the  $\alpha$ -,  $\beta$ - and  $\gamma$ -fractions of human livers or spleens. SK-2 cells yielded three fractions that had mobilities exactly like those of the corresponding three SK-1 fractions. By contrast, HEP-2 cells produced  $\alpha$ -,  $\beta$ - and  $\gamma$ -fractions with mobilities that were greater at (pH 6.6) than those of the corresponding fractions from SK-1 cells, SK-2 cells or from livers and spleens (Fig. 2). Moreover, the mobilities of the HEP-2  $\alpha$ -,  $\beta$ - and  $\gamma$ -fractions were the same as those of the corresponding three fractions from HeLa cells.

It was noted previously that in double diffusion tests HeLa and KB ferritins had the serological specificities of ferritin from human livers and spleens, when tested with rabbit antisera specific for splenic and hepatic ferritin<sup>1</sup>. Similar precipitin tests in gels have revealed no serological differences between SK-1 ferritin, SK-2

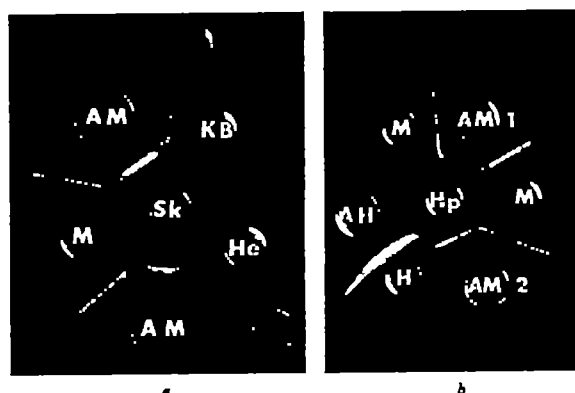


Fig. 3. Results of precipitin tests in agar gels. *a*, Comparison of SK-1 (SK), HeLa (He), and KB (KB) ferritins with ferritin from human liver (M). Rabbit antiserum (AM) to M was used in this test. Note that precipitin bands in front of each well are joined completely to those in front of neighbouring well(s). No serological differences between antigens were revealed by this or by similar tests. *b*, Comparison of HEP-2 ferritin (Hp) with ferritins from human liver (M) and from horse spleen (H). AM-1: rabbit antiserum against M. AM-2: rabbit antiserum against another preparation of human ferritin (from spleen). AH: rabbit antiserum against H. Note that Hp and M gave reactions of identity when tested with AM-1 or AM-2. There is a weak band between H and AM-2. No band was formed between Hp and AH, but this may have been due to insufficient concentration of Hp since—as can be seen—M reacted only weakly with AH in this test.

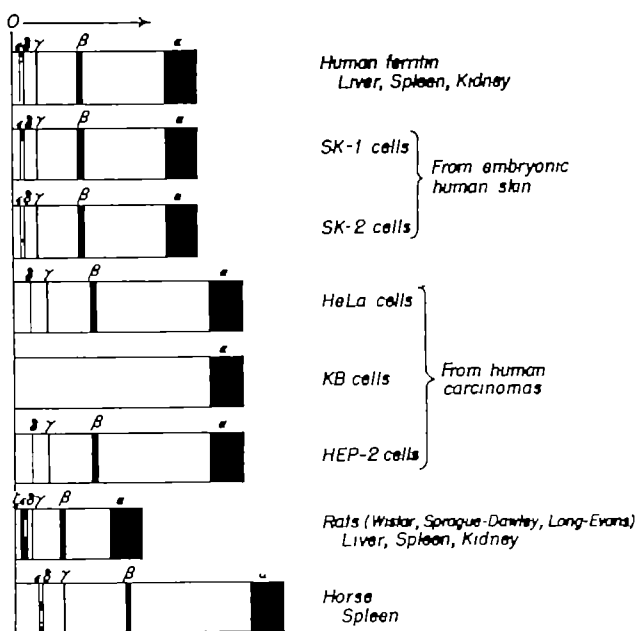


Fig. 4. Electrophoretic mobilities of various ferritins relative to each other. Five per cent polyacrylamide gels at pH 6.6.

ferritin, HEP-2 ferritin and the ferritin from human liver and spleen (Fig. 3a and b). Thus, all these materials gave reactions of identity when tested with rabbit antisera to unfractionated, highly purified human ferritin.

The results obtained with various sorts of ferritin by means of electrophoresis in polyacrylamide gels are summarized in Fig. 4. The evidence reveals a pattern in which neoplastic cells are separated from non-neoplastic cells (or tissues), but tests of ferritins produced by tumours *in vivo* are needed to evaluate the significance of these results in relation to neoplasia. While the synthesis of

distinctive ferritins in neoplastic cells may indicate potentialities that are unrelated to neoplasia, it is obviously important to investigate the possibility that there is a link.

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## EFFECT OF CARRIER-GAS ON GAS-LIQUID PARTITION

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IN gas-liquid chromatography the carrier-gas is normally almost completely insoluble in the stationary phase, but it may affect the solubility of another gas or vapour by altering its fugacity. Different carrier-gases will affect the solubility of a given vapour to different extents, and this has been shown in gas-chromatography experiments utilizing capillary columns, by Desty, Goldup, Luckhurst and Swanton<sup>1</sup>. These authors also derived an equation relating the  $k'$  factor of a gas chromatographic column (the ratio of the liquid phase capacity to the gas phase capacity) to the mean absolute column pressure,  $\bar{p}$ , and the second virial coefficient,  $B_{12}$ , accounting for interactions between unlike molecules of the mixed gases. From a plot of  $\log k'$  against  $\bar{p}$  the activity coefficient at infinite dilution of a solute,  $\gamma_2^\infty$ , may be estimated without the need to correct for carrier-gas/solute interactions. Values of  $B_{12}$  have also been calculated from the gas chromatography experiments and good agreement has been obtained with values calculated from the Beattie and Bridgeman formula<sup>2</sup>.

The solubility of the vapours of chloromethanes in squalane and dimonyl phthalate have been measured by Freeguard and Stock<sup>3</sup> by a static technique involving a McBain-type spring balance. In the present work we have been concerned to measure directly, by a similar static technique, the effect of an inert carrier-gas (for the most part nitrogen) on the solubility of these vapours. From these measurements it should then be possible to obtain values of  $B_{12}$ , and verify directly the equation of Desty *et al.*<sup>1</sup>:

$$\log k' = A + \frac{\bar{p}}{2 \cdot 303 RT} (2B_{12} - v_2^\infty)$$

where  $v_2^\infty$  is the molar volume of the solute at temperature  $T$ .

The static method has the advantage that the contribution of adsorption and solubility of the carrier-gas can be determined easily.

A McBain-type balance similar to that described by Freeguard and Stock<sup>3</sup> was used, but the system was modified so that thorough mixing of the two gases (absorbate and inert gas) was achieved, and so that gases could be admitted to the system without loss of absorbate vapour already present, by back-diffusion.

To ensure thorough mixing of the gases a glass 'loop', surrounded by a water-jacket and maintained at the same temperature as the balance case, was connected to the top and bottom extremities of the balance case. This permitted free circulation of the gases in the system, and an actual flow of gas was promoted by means of a glass paddle, in which was embedded a small magnet, and which was made to revolve at high speed by means of a magnetic stirrer. This device was found to be effective even when the total pressure in the system was only about 2 or 3 cm.

To prevent back-diffusion when adding fresh charges of gas, a doser technique was employed, that is, additional gas was displaced into the system by means of a mercury piston, operating through a series of valves. In practice this procedure was only necessary when quite high pressures of gas were already present in the system.

The inert gas was dried by passing it over an activated molecular sieve (Linde 5A). The involatile liquids used were squalane and dimonyl phthalate coated on 'Celite', the liquid loading being about 30 per cent by weight. An experiment was performed using squalane alone, but equilibrium times were so long (cf. Freeguard and Stock<sup>3</sup>) that other measurements using involatile liquids alone were not attempted.

All the experiments described here were carried out at 30° C using carbon tetrachloride as the absorbate, and the following procedure was adopted to measure the effect of the inert gas on the absorption of this vapour. The solid support and involatile liquid were contained in a light glass bucket and suspended by a fused quartz spring (extension approximately 1 m per g) and degassed to better than 10<sup>-4</sup> mm of mercury. A charge of carbon tetrachloride vapour was then admitted and the increase in weight of the sample noted at equilibrium. Inert gas was then admitted, the gases were thoroughly mixed and allowed to equilibrate and the change in weight of the sample was noted. Further charges of inert gas were added in a similar way. It was ascertained that the uptake of the inert gas was negligible by performing similar experiments in the absence of the absorbate. The change in weight of the sample, after correcting for buoyancy, was therefore due to absorption or desorption of the solute caused by interactions in the gas phase between the solute and the inert gas.

It was at first thought that the presence of the inert gas might so retard the equilibration of the absorbate vapour with its solution in the involatile liquid that it would prove impossible or very difficult to determine when equilibrium had been attained, hence a preliminary experiment was carried out in which a charge of nitrogen was admitted to the previously evacuated balance case until a pressure of about 20 cm was reached. Charges of carbon tetrachloride vapour were then admitted and an isotherm measured in the usual way. The results were presented graphically by plotting the increase in weight of the sample against the apparent pressure of the carbon tetrachloride, that is,  $(P - p_1)$ , where  $P$  was the total pressure in the system and  $p_1$  was the pressure of nitrogen. The curve thus obtained should be identical with that obtained when performing the experiment in the absence of nitrogen, since the term  $(P - p_1)$  takes into account interactions between the carbon tetrachloride and the nitrogen (assuming negligible effect on the fugacity of the nitrogen by the carbon tetrachloride). Good agreement between the curves was found,

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suggesting that equilibrium was established under the experimental conditions.

Some results using squalane as the involatile liquid are presented graphically in Figs. 1 and 2. In Fig. 1 each curve shows the change in weight of the sample for a fixed pressure of carbon tetrachloride but varying pressure of nitrogen. In practice the pressure of carbon tetrachloride did not remain constant throughout each experiment because of the small amount desorbed by the sample on admitting nitrogen. The volume of the system was so large, however, that the increase in pressure due to desorption was considered negligible. In all the experiments desorption of carbon tetrachloride was observed when either nitrogen or carbon dioxide was added to the system, regardless of the initial pressure of the absorbate or the pressure of the inert gas. This is consistent with predictions based on the theory of corresponding states<sup>4</sup>.

In Fig. 2 absorption isotherms of carbon tetrachloride in squalane are shown. Curve 1, showing the largest

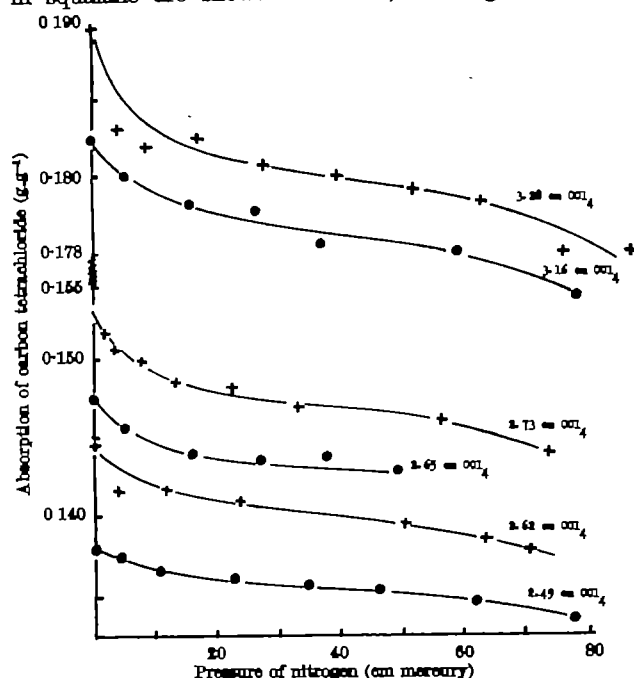


Fig. 1. Variation in absorption of carbon tetrachloride by squalane at 30°C with increasing pressure of nitrogen

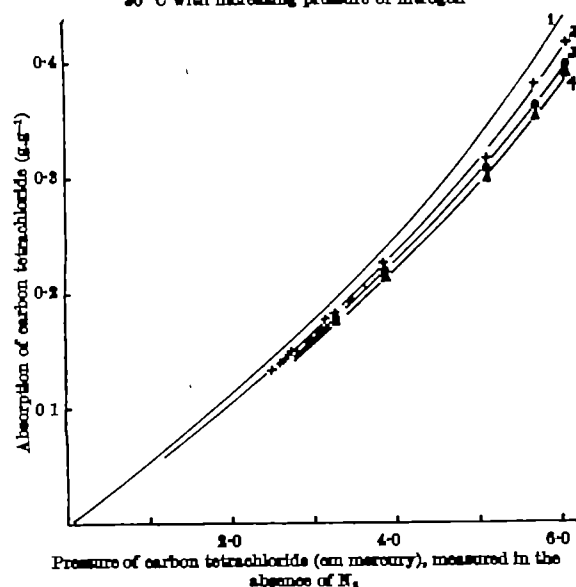


Fig. 2. Absorption isotherms of carbon tetrachloride in squalane at 30°C, effect of nitrogen. Curve 1, pure  $\text{CCl}_4$ ; 2, 10 cm  $\text{N}_2$ ; 3, 30 cm  $\text{N}_2$ ; 4, 60 cm  $\text{N}_2$ .

uptake of vapour, is the isotherm measured in the absence of an inert gas. Curves 2, 3, and 4 are isotherms measured at 10, 30, and 50 cm of nitrogen respectively. They show that the higher the pressure of nitrogen, the greater the depression of the isotherm. Points for curves 2, 3, and 4 were extracted from the curves in Fig. 1, and some additional curves not shown.

It is possible to calculate  $B_{11}$  from the above results in the following way. If the absorption by the involatile liquid is plotted against the fugacity of the absorbate then all points should fall on the same isotherm, regardless of the amount of inert gas present. The isotherms in Fig. 2 show the plot of absorption against pressure, therefore horizontal lines drawn through these curves join points of constant fugacity of carbon tetrachloride vapour. The fugacity,  $p_1^*$ , of the pure vapour at pressure  $p_1$  is given<sup>5</sup> by:

$$\ln p_1^* = \ln p_1 + \frac{B_{11} \cdot p_1}{RT} \quad (1)$$

where  $B_{11}$  is the second virial coefficient<sup>6</sup> for the pure vapour. In the presence of an inert gas, the fugacity of the vapour at pressure  $p_1'$  is given<sup>5</sup> by:

$$\ln p_1'^* = \ln p_1' + \frac{P}{RT} [B_{11} - (1-y)(B_{11} - 2B_{12} + B_{22})] \quad (2)$$

where  $P$  is the total pressure;  $B_{11}$  is the second virial coefficient of the inert gas;  $y$  is the mole fraction of the solute in the gas phase. Hence when the fugacities are equal the following expression for  $B_{11}$  is obtained from equations 1 and 2:

$$B_{11} = \frac{1}{2}(B_{11} + B_{22}) - \left[ RT \ln \frac{p_1'}{p_1} + B_{22}(P - p_1) \right] / 2P(1-y) \quad (3)$$

It is interesting to note that this is the Lewis-Randall approximation with an additional term.

In calculating results for  $B_{11}$  no allowance was made in the pressure for desorption of carbon tetrachloride from the sample, as already mentioned, hence  $p_1'$  was taken as the initial pressure of absorbate before admitting nitrogen, and  $y$  was assumed to be  $p_1'/P$ . In equation 3 the measured quantities are  $P$  and  $p_1'$ ;  $p_1$ , the pressure of pure absorbate which would give the same uptake in the absence of inert gas, was either taken from the appropriate isotherm, or calculated from an empirical equation for the isotherm, known to give a good fit over the narrow range of pressures under consideration. As might be expected, the latter method gave more consistent results.

The value of  $B_{11}$  for the system: carbon tetrachloride/nitrogen at 30°C calculated from the corresponding states theory of Guggenheim and McGlashan<sup>4</sup> is approximately -158. Values of  $B_{11}$  obtained by the above method ranged from about -226 to about -10,000. Values should remain constant regardless of pressure, whereas in the present work  $B_{11}$  usually approached the value given by the corresponding states theory as the pressure of nitrogen increased. These discrepancies may be accounted for in part by the method of calculation; slight variations in the term  $\ln p_1'/p_1$  in equation 3 are greatly magnified and seriously affect the value of  $B_{11}$ . On the other hand, the curves in Figs. 1 and 2 show the expected reduction of the fugacity of carbon tetrachloride by nitrogen and there is a measure of consistency in the curves not apparent in the values of  $B_{11}$ . Similar results were obtained with the systems carbon tetrachloride/nitrogen/dimethyl phthalate and carbon tetrachloride/carbon dioxide/squalane.

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## LETTERS TO THE EDITOR

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## PHYSICS

## Effect of Pressure on the Viscosity of Water

An accurate knowledge of the viscosity of water as a function of pressure is important in the interpretation of the effect of pressure on the electrical conductance of aqueous electrolytes. For this reason we have recently measured the viscosity of water at pressures up to 10,000 kg/cm<sup>2</sup> relative to that at atmospheric pressure over the temperature range 2.2° C–100° C (ref. 1). The only other measurements at pressures greater than 900 kg/cm<sup>2</sup> are those of Bridgman<sup>2</sup>; but these are of limited accuracy because of the experimental difficulties he encountered when he used his viscometer to investigate water.

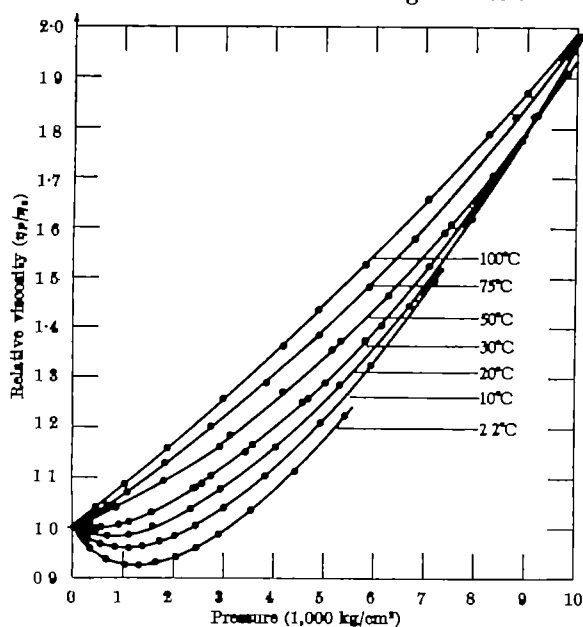


Fig. 1. Relative viscosity of water

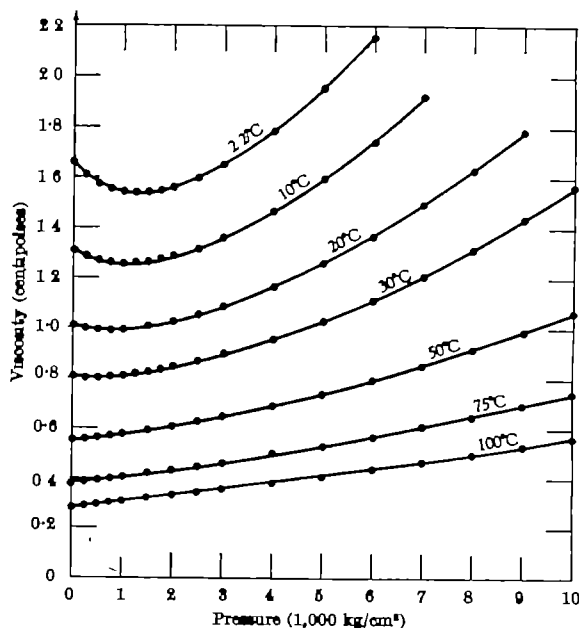


Fig. 2. Absolute viscosity of water

The viscometer used in our investigation is of the falling-body type; it consists of a stainless steel tube containing two sinkers, one of high and the other of low density. Measurements are made by inverting the pressure vessel containing the viscometer and timing the fall of each sinker through the fluid separately by means of an electronic timer actuated by an electrical induction system within the vessel. The technique is novel in that the use of two sinkers permits measurements to be made of the density of the fluid as well as the viscosity.

Comparison of the unsmoothed experimental results for the relative viscosity of water given in Fig. 1 with those obtained by Bridgman shows that the deviations do not conform to a general pattern. At 10° C his results are



lower than ours whereas at the other temperatures they are higher, the discrepancy at 75° C and 10,000 kg/cm<sup>2</sup> being as large as 25 per cent. The agreement with low-pressure data<sup>3-5</sup> is generally good and within the experimental error of our observations, which is estimated to be  $\pm 1$  per cent. Interpolation of our results shows that the temperature at which the initial pressure coefficient is zero is 33.5° C. Values of the absolute viscosity of water given in Fig. 2 have been calculated from our results with the aid of Bingham and Jackson's results for the viscosity of water at atmospheric pressure<sup>7</sup>.

We thank the Council for Scientific and Industrial Research for providing funds to enable us to build the high-pressure viscometer.

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### Calculations with Two Nearly Equal Quantities

A FAMILIAR experimental problem arises when two quantities of about equal magnitude are measured, and a third quantity equal to the difference between the two is to be calculated. The relative error in the calculated result can be intolerably high. An example of such a situation is the measurement of airglow radiations from the upper atmosphere in the presence of Rayleigh scattering from the lower atmosphere, where the strength of the desired signal may be small compared with that of the background. Initial measurements of airglow which we made with a simple photometer resulted in plots of calculated airglow intensity versus time with such large fluctuations that no interpretation was possible.

In these circumstances, information can be extracted with greater reliability by taking ratios rather than differences. Taking the example referred to here, let the intensity of the background be  $B$ , and that of the signal-plus-background be  $S + B$ . If each is measured by counting the total number of photons arriving in a standard time-interval, then the standard deviations,  $E_B$  and  $E_{S+B}$ , are  $\sqrt{B}$  and  $\sqrt{S+B}$ , respectively. We wish to compare the relative errors in  $\bar{S} = (S+B) - B$ , and  $R = (S+B)/B$ , denoted by  $e_S$  and  $e_R$ , respectively. It is easily shown that in the situation being considered:

$$e_S = \sqrt{\frac{S+2B}{S^2}}$$

$$e_R = \sqrt{\frac{S+2B}{B(S+B)}}, \text{ and}$$

$$\frac{e_S}{e_R} = \sqrt{\frac{B(S+B)}{S^2}}$$

It is of interest to note that while the accuracies of both  $S$  and  $R$  can be improved by increasing the counting time, the ratio of these accuracies is independent of counting time.

A plot of  $(e_S/e_R)$  versus  $R$  is shown in Fig. 1. The ratio of errors has the value unity at  $R = 2.6$ , or  $S = 1.6B$ . For  $S < 1.6B$ , the advantage lies with taking the ratio. This is consistent with my finding that plots of  $R$  calculated from my data had considerably less scatter than plots of  $S$  calculated from the same data.

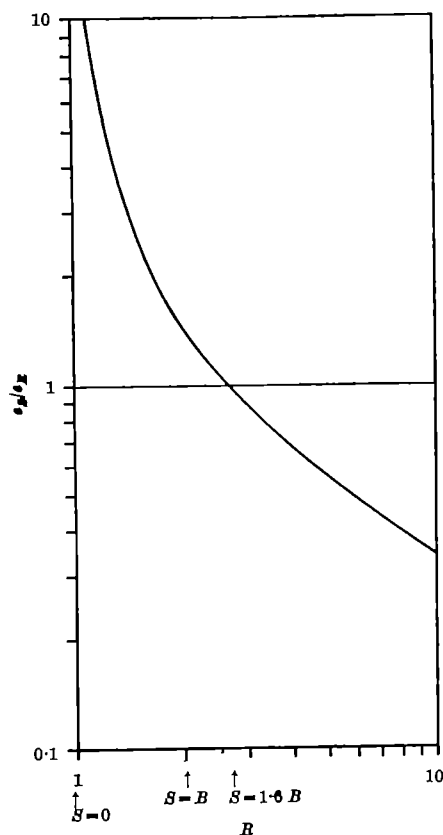


Fig. 1. Relative errors in  $S$  and  $R$ .

This work was supported by a grant from the Lowell Technological Institute Research Foundation. I thank K. H. Olson for his advice.

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## GEOLOGY

### An Interglacial Soil at Teindland, Morayshire

RECENTLY a fairly well preserved podsol was found buried beneath glacial deposits at Teindland, Morayshire (map ref. O.S. 299586). Briefly, the section shows at the top a semi-podsol developed in 6-8 ft. of sandy till and outwash gravel overlying a fossil iron podsol developed in glacio-fluvial outwash. The upper part of the buried soil which originally was the organic and leached layers has been transformed to thin bands of black, dark grey and light grey material. This banding has most likely been produced by solifluxion.

The black bands were sampled in September 1963 and found to contain 1.44 per cent organic carbon. A sample submitted to the National Physical Laboratory (reference NPL-78) for radiocarbon measurement was dated to  $28,140 \pm 480$  and  $-450$  years (B.P.), where the uncertainties quoted are derived from one standard deviation of the activity measurement. The age must be regarded as a minimum, since the nature of the sample precluded alkali pretreatment.

Hence the history of the site may be reconstructed as follows: (1) Glaciation (Riss ?) followed by the deposition of glaciofluvial deposits. (2) Soil formation in the above deposits to produce a well-developed iron podsol. (3) Periglacial conditions which caused disturbance and solifluxion of the surface layers. (4) A second period of glaciation (Würm ?) during which the soil was buried with till and outwash. (5) Holocene pedogenesis in the upper part of the Würm deposits.

The buried soil is more leached and differentiated than the contemporary one formed above it at the present surface. Therefore, it was differentiated either over a longer or over a shorter, more intense period of soil formation than the holocene. If the latter, the buried soil could be of interstadial age. However, it is doubtful if such conditions existed during the Würm interstadials, and a more likely explanation is that it developed as a result of long and continuous leaching during the last interglacial period.

The age of the organic matter merely indicates the time at which the soil was buried. It would seem that it lay outside the limits of Würm glaciation for a considerable time undergoing solifluxion and vegetative change, and that only during the middle to late Würm did the ice advance sufficiently to bury this soil.

I thank Mr. A. C. B. Hutcheon of this Department for the determinations of organic carbon, and the National Physical Laboratory for the radiocarbon measurements and age estimate provided in report C.65 of August 27, 1964.

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### A Possible Main Würm Glaciation in West Pembrokeshire

Two carbon-14 age determinations from marine mollusc fragments in glacial outwash in Pembrokeshire have indicated that the last glaciation of West Wales from the Irish Sea probably occurred within the past 38,000 years.

At Mullock Bridge (grid ref: SM 811080), just north of the mouth of Milford Haven, a large gravel-pit has been cut into a terrace of fluvi-glacial outwash material. The locality lies within the area of so-called 'Older Drift' in West Wales (Fig. 1)<sup>1</sup>. The terrace has a maximum surface altitude of 100 ft. O.D., and in the sides of the pit at least 25 ft. of current-bedded sands are visible; these sands appear to have been deposited deltaically, in the sea or in a pro-glacial lake. The sands are interbedded with, and overlain by, fine shelly gravels, which are in turn overlain by coarse torrential outwash. In parts of the gravel-pit unleached shelly gravels are seen within 3 ft. of the terrace surface. The northern part of this surface has a patchy covering of stony and gravelly till,

beneath which the shelly gravels are severely contorted. It seems, therefore, that the ice-front may have been in the vicinity of Mullock Bridge during the deposition of the gravels, and later advanced for an unknown distance across the outwash terrace. A sample of about 400 g of well-preserved and solid fragments of marine molluscs from shelly gravels beneath the till has been dated at  $37,960 \pm 1,700$  years B.P. (NPL-80).

Eighteen miles to the north a small gravel-pit has been cut into shelly outwash gravels at Tre-llys (grid ref.: SM 898349), near the north Pembrokeshire coast. The gravels, which lie at an altitude of 200 ft. O.D., have a hummocky surface expression, and were considered by Charlesworth<sup>2</sup> to be part of the 'South Wales End-moraine'. As at Mullock Bridge, shelly gravels are irregularly interbedded with sands in the pit; they are generally overlain by about 6 ft. of coarse torrential outwash, but in places shelly gravels occur within 4 ft. of the ground surface. In places the outwash contains severe slump features and contortions, and in view of the interbedding of small till masses it may be proposed that the deposits were laid down in an ice-contact environment during the wastage of an ice-sheet. More than 300 g of marine mollusc fragments from this pit have been dated at  $37,310 \pm 1,515$  years B.P. (NPL-97).

The shell fragments collected at each locality for carbon-14 dating represent a wide range of environments, and therefore constitute a 'mixed' fauna<sup>3</sup>. So far as could be ascertained, no Crag species were included in the collections, but nevertheless the occurrence of the cold indicators *Trophonopsis clathratus* (L) and *Chlamys islandica* (Müller) alongside species found in Pembrokeshire waters to-day may indicate that the molluscs vary greatly in age and provenance. The carbon-14 dates must be considered as mean dates, and the possibility borne in mind that many shells older than about 38,000 years B.P. may be mixed with shells which lived after that date.

There is no evidence that either sample was contaminated. Laboratory pre-treatment included the rejection of apparently weathered shell fragments and the removal by leaching of the outer third of the shells in each test sample. The middle and inner thirds of each test sample were dated separately, with almost identical results.

The widespread occurrence of marine molluscs at Mullock Bridge and Tre-llys indicates that the ice-sheet which incorporated the molluscs and later deposited the shelly outwash and gravelly till probably moved across western Pembrokeshire from the north and north-west. This suggestion is supported by the presence of erratics from the St. David's Peninsula in the gravels at Mullock Bridge. There appears to be little doubt that the ice-sheet was a true Irish Sea ice-sheet. If some of the dated shells lived in the Irish Sea within the past 38,000 years, then it follows that the last glaciation of West Wales occurred even more recently.

This dating seems to contradict several previously published opinions concerning the age relationships of drifts around the Irish Sea. Mitchell<sup>4</sup> and Synge<sup>5</sup> consider that all the glacial drifts of West Wales are the equivalent in age of the Eastern General till of Ireland, which in turn they correlate with the Gipping till of East Anglia. They consider that the maximum extension of the Irish Sea ice during the Last Glaciation is represented by the Midland General-Clynnog Fawr morainic limit, which passes from the Screen Hills of Wexford to the Llyn Peninsula (Fig. 1). Alternatively Charlesworth<sup>2</sup>, while agreeing that most of the till in West Wales is of 'Older Drift' age, has proposed a limit for the ice of the Last Glaciation at the so-called 'South Wales End-moraine' of north Pembrokeshire. On the other hand, the evidence from Mullock Bridge and Tre-llys indicates that the Irish Sea ice of the Last Glaciation probably extended much farther south, and over-rode the whole of western Pembrokeshire. Thus it appears that neither the Midland

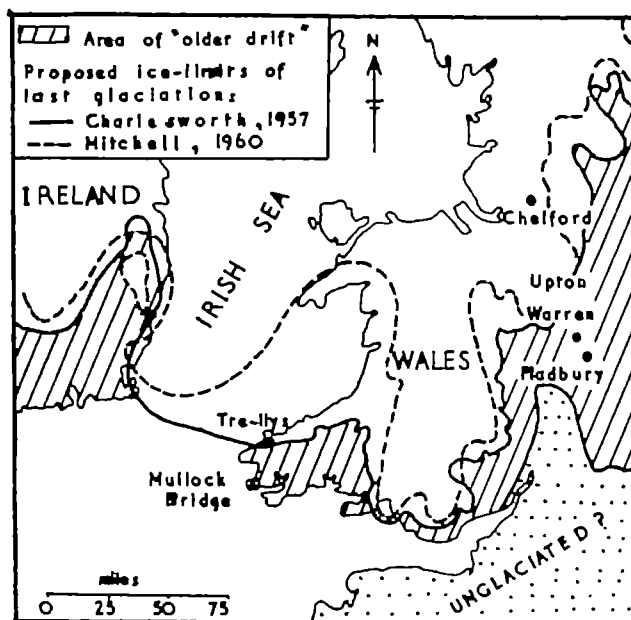


Fig. 1. Current ideas concerning the glaciation of Wales and the southern Irish Sea Basin

General-Clynnog Fawr moraine nor the South Wales End-moraine can be identified as a true terminal feature, unless it represents minor re-advances or retreat stages during deglaciation.

The Mullock Bridge and Tre-llys dates, themselves almost identical, are markedly similar to radiocarbon dates from Upton Warren (41,900 years B.P.) and Fladbury (38,000 years B.P.) in the Midlands. At the latter localities faunal and floral remains record the presence of fluctuating interstadial conditions, and likewise the marine mollusc assemblages in the Pembrokeshire outwash gravels indicate that boreal conditions probably prevailed at about this time. The Pembrokeshire evidence lends support to the suggestion of Coope, Shotton and Strachan<sup>7</sup> that interstadial conditions in Britain around 42,000–38,000 B.P. may be correlated with part of the Middle Würm period of fluctuating sub-arctic and boreal climates proposed for Northern Europe by Gross<sup>8</sup>.

Concerning the absolute dating of the southernmost advance of the Irish Sea ice during the Würm, two recent suggestions have been made. Coope, Shotton and Strachan consider that this advance occurred between 57,000 and 42,000 years ago; this is based on the interpretation of radio-carbon dated beds at Chelford<sup>9</sup>, Upton Warren, and Fladbury, and their relationships with the Severn-Avon terraces and the Midland tills. Similarly, Bowen<sup>10</sup> has proposed that the 'Main Welsh Glaciation' of South Wales was the equivalent of the European Early Würm, followed by a smaller 'Welsh readvance' during the Main Würm. On the other hand, Penny<sup>11</sup> has suggested that the maximum ice advance of the Last Glaciation throughout Britain was the approximate equivalent in time of the European Main Würm glaciation, which was most extensive between 25,000 years B.P. and 17,000 years B.P.<sup>12</sup>

The evidence from Pembrokeshire suggests that there was an extensive glaciation in Western Britain later than 38,000 years B.P., and it therefore appears to provide support for Mr. Penny's suggestion. However, the possibility remains that an even more extensive glaciation could have occurred before that date, perhaps during the Early Würm. Some recent work in north-east Scotland by Dr. E. A. FitzPatrick and D. E. Sugden has a bearing on this problem. Dr. FitzPatrick<sup>13</sup> has suggested on the basis of a recently obtained carbon-14 date from Tiendland, near Elgin, that non-glacial conditions persisted in north-east Scotland from the beginning of the Würm until 28,000 years B.P., and this is supported by traces of a long phase of corrie glaciation in the Cairngorm Mountains prior to the development of the Main Würm ice-sheet<sup>14</sup>. In view of this evidence it is difficult to envisage an Early Würm glaciation which could have extended as far south as the approaches of the Bristol Channel.

In Scotland and in the northern Irish Sea basin<sup>15</sup> there are signs of an extensive glaciation after 28,000 years B.P., which correlates with the European Main Würm maximum. In view of the apparent synchronicity of events in Britain and Northern Europe, it seems reasonable to propose that the Last Glaciation of West Wales was a part of this Main Würm glaciation. Whether or not the foregoing correlations are correct, the carbon-14 dates from Pembrokeshire strongly suggest a widespread glaciation of Western Britain some time after 38,000 years B.P., and the possibility emerges that other landscapes south of the Newer Drift limit may have been glaciated during this glaciation.

It is hoped that the evidence for the points made here will soon be published in a more detailed analysis of the Mullock Bridge exposure. So far, no reliable clue has been obtained as to the absolute age of the Irish Sea till of West Wales and Eastern Ireland; stratigraphic evidence suggests that the till may be no older than the dated shelly outwash, but this may be misleading. Two carbon-14 age determinations on organic material from this till may soon be completed, and perhaps it will then be possible to

suggest a tentative chronology for the Late Pleistocene events of the southern Irish Sea basin.

I thank Dr. Marjorie Sweeting, Miss Gillian Groom and Mr. D. F. W. Baden-Powell for their advice. I also thank the National Physical Laboratory for the radio-carbon dating of the Pembrokeshire samples.

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### Seed-bearing *Ottokaria*-like Fructifications from India

ALTHOUGH Plumstead<sup>1-4</sup> has described a number of male, female or bisexual fructifications obviously attached to fertile leaves of *Glossopteris*, *Gangamopteris* and *Palaeovittaria*, not a single specimen among them shows clearly determinable seeds or sporangia. *Glossopteris*-like fertile leaves, called *Lidgetttonia*<sup>5</sup>, were found closely associated with bodies which appear to be very similar to undoubted seeds and sporangia described by Pant<sup>6</sup> and Pant and Nautiyal<sup>7</sup>. Nevertheless, no one has so far reported any indubitable seeds or sporangia actually borne on attached fructifications of *Glossopteris*. Pant<sup>6</sup> has, however, described *Arborella*-type sporangia attached to certain disks which are associated with *Glossopteris* leaves but, so far, seeds have not been found attached even on isolated fructifications occurring in association with *Glossopteris*. The present seed-bearing *Ottokaria*-like fructifications from the Lower Gondwanas of Bihar are therefore of extraordinary interest.

In all, four fructifications have been found, two of which are illustrated in Figs. 1–5. One of them (Figs. 1, 2) is apparently attached, by means of a slender stalk, to a scale or *Gangamopteris*-like leaf. Some of the veins in the leaf are distinctly anastomosing (Fig. 3) but it shows no midrib, at least in the apical part (the lower part is not seen). The stalk of the head appears to be coming out from its side but it is presumably attached to the bottom of the head which is in contact with the rock. The head bears a number of laterally flattened oval seeds at the periphery and its upper face shows vague rounded scars in the centre. We believe that the disk-like head originally bore seeds all over the face now exposed. During fossilization, when the whole structure was compressed, seeds borne in the centre stood erect (for want of space) and therefore became rounded due to antero-posterior flattening, while seeds near the margin were compressed laterally and appear oval. Some marginal seeds in this specimen are flanked by sterile wing-like structures on either side. The specimen also shows, lying over the stalk of the fructification, a detached seed, exactly like the attached ones but without any flanking sterile tissue. The second specimen, illustrated in Figs. 4 and 5, shows a fructification lying near the apex of a *Glossopteris* leaf but not attached to it. The associated leaf is similar to *Glossopteris fibrosa* in having narrow meshes and fibres between veins. This second fructification apparently shows a few veins in its stalk which run up to the centre of the head and from there branch and spread out on its

surface like the veins of *Ottokaria beugaleensis*<sup>1,10</sup> or *Sourum*. Attached seeds are also seen projecting from the periphery of this specimen. Some sterile tissue is seen by the side of seeds even here. Carbonaceous material of the fructifications is preserved in all our specimens. The seeds are sessile and orthotropous. They are ovoid in shape, having a more tapering micropylar end and a broader rounded base. Their surface shows elongated rectangular cells in longitudinal rows. The external form of these seeds is therefore quite like that of some seeds described by Pant<sup>7</sup> and Pant and Nautiyal<sup>8</sup>. Like the detached seeds of these authors, the attached seeds in our fructifications are either found compressed (Figs. 1 and 2) or mud-filled (Figs. 4 and 5). We regard the foregoing two types of fructifications as representing two views of the same type of structure which had a slender stalk attached on the sterile back of a terminal flat or slightly convex side of a peltate or semipeltate disk. The back of the disk also shows veins radiating round the stalk. On the front (possibly concave) side of the disk were attached a number of seeds. The sterile tissue seen between the seeds may represent their wings, sterile flesh, cupules or interseminal bracts. If these were bract-like structures they could have been of the same nature as the so-called 'male bracts' of *Sourum*<sup>3</sup>. However, as preserved, our fructifications could never be regarded as bivalved and there is no evidence to show that they were bisexual. We believe that, contrary to Plumstead's interpretation, the fructifications described by her may, similarly, not be bivalved. As they appear to us, our multi-ovulate fructifications are rather like the detached microsporangiate disks described by Pant<sup>7</sup>. However, in place of the seeds in the present female disks, the male disks bear numerous *Arborella*-type of sporangia which contained two winged spores of *Striatites* type. Examination of detached seeds like those borne by our disks has shown that they were pollinated by similar *Striatites* type of pollen grains<sup>11</sup>. We are therefore in-

clined to think that *Glossopteris* was a gymnosperm the fertile leaves of which bore separate male and female disk-like fructifications. The general organization of the male (and even female) disks could not have been very different from that of *Potonia*. Possibly, the female disks of *Glossopteris* could be compared with the multi-ovulate cupules of *Calathospermum* in the same manner as we can compare the flat disks of *Dorstenia* with the hypanthodia of *Ficus*. Of course the disks and cupules of the Moraceae are sympatric developments of inflorescences bearing flowers, quite unlike the ovule bearing disks and cupules of *Glossopteridaceae* and *Calathospermum*.

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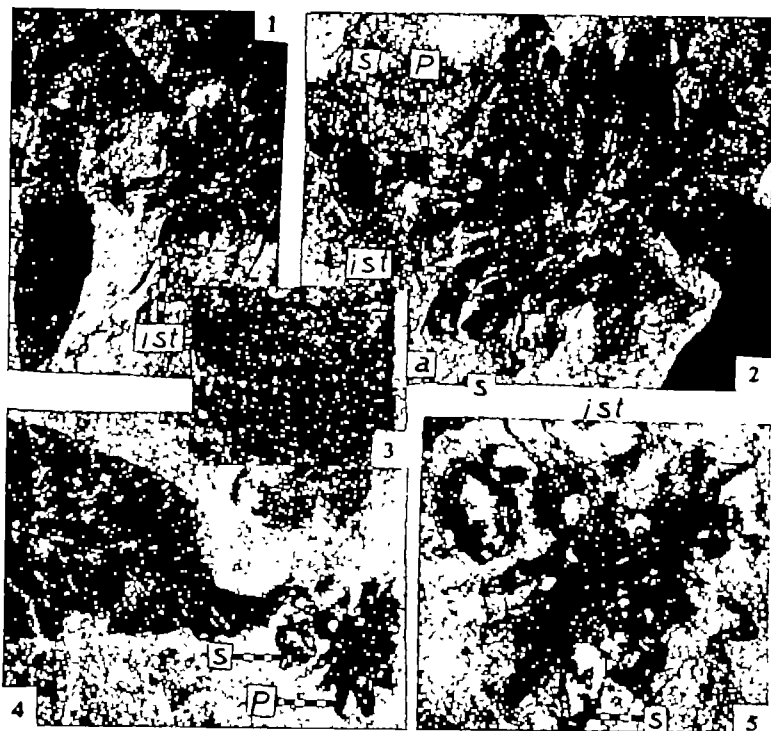
### Age of the New Red Sandstone in South Devonshire

RADIOMETRIC dating of the Exeter Volcanic Series (the Exeter Traps) recently gave ages of  $270 \pm 6$  m.y. and  $281 \pm 11$  m.y. for the Killerton mica-lamprophyre (minette)<sup>1</sup> and the Dunchideock basalt<sup>2</sup> respectively. These and other extruded masses of the Series are interbedded with breccias and sandstones of the New Red Sandstone both north and south of Exeter, and as far west as Hatherleigh; new evidence is thus provided of the age of the strata.

The main intrusive event of the Dartmoor granite is dated at 295 m.y. (ref. 3), the paroxysmal phase of the Variscan Orogeny being regarded as preceding this by only a short time<sup>4</sup>. Smith<sup>5</sup> puts the Permo-Carboniferous boundary at 280 m.y.

Definition of stratigraphic units in the New Red Sandstone in the coast section from Paignton to Seaton is in progress. The succession consists of over 9,000 ft. (2.75 km) of unmetamorphosed red breccias, sandstones and siltstones, lying unconformably on deformed Devonian and Carboniferous rocks. A relief mountainous topography is shown by the unconformity, and the New Red formations overlap progressively northwards. Two sequences can be distinguished in the succession, of which the lower and thinner one is recognized only in the Torquay district. They are separated by a small but clear angular unconformity recording contemporaneous tectonic activity.

The age of the New Red Sandstone has long been a subject of discussion. The only fossils are reptile remains (Triassic) high in the succession<sup>6</sup>, an unrecognizable plant, and some trace fossils; consequently, lithological similarities have been urged as evidence of age. Thus Irving<sup>7</sup> argued that the Budleigh Salterton Pebble Bed formed the base of the Triassic by analogy with the succession in the Midlands, a conclusion accepted with reservation by Ussher<sup>8</sup>. The Permian age of the strata beneath had been postulated by Murchison<sup>9</sup>, and was supported by the presence of the lavas which were compared with similar types in the Permian of Germany<sup>10</sup>.



Figs. 1-5 1, A seed-bearing *Ottokaria*-like head with the seminiferous face exposed. The stalk is apparently attached to a scale or *Glossopteris*-like leaf. A detached seed is seen lying over the slender stalk ( $\times 0.1$ ); Fig. 2, Disk in Fig. 1 more magnified to show wing-like sterile tissue flanking some seeds ( $\times 0.2-7$ ); Fig. 3, Portion of leaf in Fig. 1 more magnified to show vein anastomoses ( $\times 0.8-4$ ); Fig. 4, A detached seed-bearing fructification lying by the side of a *Glossopteris* leaf. The specimen shows the back of the disk and seeds projecting from margin ( $\times 0.1-4$ ); Fig. 5, Fructification in Fig. 4 more magnified to show details ( $\times 0.2-7$ ); one of the vein anastomoses; *ist*, interseminal tissue; *p*, stalk; *s*, seed

# GEOPHYSICS

## Significance of Strontium Isotope Ratios in Theories of Carbonatite Genesis

The radiometric evidence now invites a re-appraisal of the age question, requiring a correlation of the lava horizons with the succession on the coast. South of Exeter, field work indicates that the Dunchideock lava lies at a level equivalent to the lower beds of the upper sequence, as suggested by Ussher<sup>11</sup>. Marker horizons are lacking, however, and such correlation cannot be definite. Although fragments of the Exeter lavas have not been positively identified in the rocks of the coast section, they are common in the Crediton Valley in New Red strata above those associated with the extrusions. The succession of fragment facies established by Hutchins<sup>12</sup> in that area can be traced to the coast section<sup>13,14</sup>, and this also suggests that the lavas were contemporaneous with the lower beds of the upper sequence, probably the basal limestone breccias. Fragments and boulders of dominantly acidic volcanic and dyke rocks are abundant in both lower and upper sequences, but are not closely related to the Exeter lavas. As they occur in sediments that show evidence of derivation from the direction of Dartmoor, they presumably result from volcanicity on the roof of the granite pluton<sup>15</sup>, predating the lowest visible New Red deposits, and probably contemporaneous with the intrusion.

From the available evidence, and accepting the dates for the Exeter lavas and the Permo-Carboniferous boundary, it is probable that: (a) the lower sequence at Torquay and the lower beds (Cadbury Beds<sup>12</sup>) in the Crediton Valley are of Upper Carboniferous (Stephanian) age; (b) minor tectonic movements occurred at or near the end of the Carboniferous Period in the region. Later movements affecting the New Red Sandstone appear to be Cretaceous (probably Aptian) in age, since similar movements affected strata up to Wealden near Weymouth<sup>16</sup>, but not the Upper Greensand of the Haldon Hills. Mid-Tertiary wrench faulting also affected the region<sup>17</sup>.

The youngest rocks involved in the Variscan folding in North Devon are Lower Westphalian (*G<sub>1</sub>*), slightly older in the Exeter region<sup>18</sup>. Assuming that the New Red sedimentation commenced after the main folding, the date for this event can be narrowed down to somewhere between early Westphalian and late Stephanian. Early Stephanian (Asturian) is the earliest date possible for folding in the Radstock coalfield<sup>19</sup>, and is the date favoured by Fitch and Miller<sup>8</sup> for the main event, though Owen<sup>20</sup> prefers mid-Westphalian. The granite intrusion date, 295 m.y., is early Stephanian on the present time scale<sup>21</sup>; this is presumably also the date of the culminating orogenic phase and the pre-New Red volcanicity, though important orogenic movements in the Westphalian are not ruled out.

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RECENTLY, several investigators<sup>1-3</sup> have compared the <sup>87</sup>Sr/<sup>86</sup>Sr ratios of carbonate rocks from carbonatite complexes with those of sedimentary limestones. Powell *et al.*<sup>1</sup> quote a mean value of 0.7035 for carbonatites while limestones are usually greater than 0.7080. All results are normalized to <sup>87</sup>Sr/<sup>86</sup>Sr = 0.1194 and relative to a value of 0.7085 for normalized <sup>87</sup>Sr/<sup>86</sup>Sr on the Eimer and Amend standard SrCO<sub>3</sub>. Hamilton and Deans reported a mean <sup>87</sup>Sr/<sup>86</sup>Sr ratio of 0.7060 for nine carbonatites. Powell *et al.* concluded that the difference in <sup>87</sup>Sr/<sup>86</sup>Sr values demonstrates that carbonatites are not mobilized limestones. For this criterion to be effective, however, two requirements must be fulfilled: (a) there must be a negligible overlap of the strontium isotope ratios of carbonatites and limestones and (b) the <sup>87</sup>Sr/<sup>86</sup>Sr ratios of limestones must not be altered by metamorphism. Powell *et al.*<sup>1</sup> showed that a xenolith of Trenton limestone (Ordovician) from the Mount Royal gabbro (Montreal) has essentially the same <sup>87</sup>Sr/<sup>86</sup>Sr ratio as the Trenton limestone at some distance from the intrusion. From these measurements, they inferred that requirement (b) is generally satisfied. It seems to us, however, that the behaviour of a small xenolith that has merely been recrystallized but not otherwise reacted on by the enclosing magma cannot readily be extrapolated to cover the case of a large mass of limestone that might be melted and intimately incorporated in a magma with extensive reaction.

We have examined calcite separated from Grenville marble and from a number of calcite-silicate rocks that represent extensively metasomatized limestone. The specimens have been collected from well-mapped parts of the Grenville province in Ontario and Quebec and there can be no reasonable doubt that the carbonate is derived from Grenville limestone. The results obtained are shown in Table 1. Isotope ratios are normalized to <sup>87</sup>Sr/<sup>86</sup>Sr = 0.1194. The error at the 95 per cent confidence-level which has been estimated from replicate analyses of a standard solution is ±0.0006 for a single analysis and ±0.0004 for the average of two analyses. An account of the double collection system used with an A.E.I. MS 2 mass spectrometer will be given elsewhere.

While the investigation is not yet complete, there does seem to be sufficient evidence to make the following statements:

- (1) If the <sup>87</sup>Sr/<sup>86</sup>Sr ratios in the limestones described here were not affected by the metamorphism, then there must have been a very considerable spread in the <sup>87</sup>Sr/<sup>86</sup>Sr ratios in the original limestones. This range would in fact overlap to a large extent that shown by carbonatites.
- (2) If our samples are assumed to have originated with some common limestone value for the <sup>87</sup>Sr/<sup>86</sup>Sr ratio, then

Table 1

Sample description	Normalized <sup>87</sup> Sr/ <sup>86</sup> Sr
White crystalline limestone with accessory graphite and phlogopite—recrystallized Grenville marble with no introduction of components. Monmouth Twp., Ontario, Canada.	0.7071 0.7072
Calcite from calcite-phlogopite-diopside rock containing enough phlogopite and diopside to indicate considerable introduction of components from granite magmas. Monmouth Twp., Ontario, Canada.	0.7064 0.7060
Calcite from calcite-garnet-pyroxene skarn formed by metasomatism of Grenville marble by gabbro. Chandos Twp., Ontario, Canada.	0.7056
Calcite from pod of orange calcite-apatite-fluorite-pyroxene rock (calcite crystals 3" across) Grenville metasediments. North of Ottawa, Quebec, Canada.	0.7048 0.7048
Calcite from alkali feldspar-biotite-calcite rock formed at the junction of Grenville marble with a syenite intrusion. Monmouth Twp., Ontario, Canada.*	0.7038 0.7032

\* It is important to emphasize that this rock is a reaction product between Grenville marble and a syenite which does not contain any calcite. There can be no reasonable doubt geologically that the calcite in this rock was derived from Grenville marble.

there is a significant decrease in this ratio with increase in the degree of metamorphism/metasomatism. Whichever is correct it can scarcely be said that strontium isotope measurements offer a clear demonstration that carbonatites are not mobilized limestones.

We do not suggest that carbonatites are either limestone xenoliths or mobilized limestones but we doubt that the studies of strontium isotopic composition prove that they are not.

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## GEOCHEMISTRY

### Estimation of Elapsed Time since a Certain Climatic Change for Lake Vanda

WILSON<sup>1</sup> has considered the estimation of elapsed time since a climatic change for Lake Vanda (77° 35' S., 161° 39' E.), which is 5 miles long, 1 mile wide and occupies the lowest part of the Wright Valley, an ice-free valley in Victoria Land, Antarctica.

The estimation procedure makes use of the equation:

$$C = [M/(Dt)^{\frac{1}{2}}] \exp[-h^2/(4Dt)]$$

where  $C$  is the concentration of calcium chloride at distance  $h$  from bottom after an elapsed time  $t$ ,  $h$  is the distance above bottom,  $D$  is the diffusion coefficient of calcium chloride (0.68 cm<sup>2</sup>/day at 10°—see ref. 2), and  $M$  is the total mass of calcium chloride per unit area.

Measurements were made on the concentration of calcium chloride at a distance  $h$  from bottom. Originally the data were presented by Wilson<sup>1</sup> in the form of a graph, but more recently he has presented them (personal communication) as in Table 1.

Table 1

$h$ (ft.)	$C$ (equiv./L)
0	2.36
5	2.28
10	2.12
15	1.93
25	1.53
35	0.99
45	0.473
50	0.306
55	0.142
60	0.0604
65	0.0303
70	0.0230

$h$  is the distance above bottom in feet, and  $C$  is the concentration of calcium chloride.

It should be noted that Wilson (personal communication) gives these data and points out that in Fig. 2 of ref. 1 there is an error in the concentration scale of about 0.5. This reduces the former good fit for approximately 1,200 years to the smaller value given in this communication.

It should be noted that the equation involved can be written:

$$\log C = -h^2/4Dt + \log[M/(Dt)^{\frac{1}{2}}]$$

where  $\log$  refers to natural logarithm.

For the values  $0 = h_0 < h_1 < \dots < h_n$  we have associated  $C_0, C_1, \dots, C_n$ , respectively. We write:

$$W_i = \log(C_i/C_0) = -h_i^2/4Dt \text{ for } i = 1, 2, \dots, n$$

The value of  $t^*$  which minimizes the sum of squares  $\sum_{i=1}^n (W_i - W_i^*)^2$ , where  $W_i^*$  is computed from the observed values  $C_i^*, C_1^*, \dots, C_n^*$  of  $C_0, C_1, \dots, C_n$ , is:

$$t^* = \frac{-\sum_{i=1}^n h_i^4}{4D \sum_{i=1}^n h_i^2 W_i^*}$$

If we assume that  $W_i = -h_i^2/4Dt + \epsilon_i$ , where  $\epsilon_i$  is normally distributed with mean 0 and variance  $\sigma^2$  for  $i = 1, 2, \dots, n$ , then  $1/t^*$  is normally distributed with mean  $1/t$  and variance  $16D^2\sigma^2 / \sum_{i=1}^n h_i^4$ . An unbiased estimate of  $\sigma^2$  is  $s^2$ , where:

$$(n-1)s^2 = \sum_{i=1}^n W_i^{*2} - \left( \sum_{i=1}^n h_i W_i^* \right)^2 / \sum_{i=1}^n h_i^4$$

It will follow that:

$$(1/t^* - 1/t) \left[ \left( \sum_{i=1}^n h_i^4 \right)^{\frac{1}{2}} / 4Ds \right] = T(n-1)$$

where  $T(n-1)$  is the Student  $t$ -distribution with  $n-1$  degrees of freedom. Therefore, if  $T_\alpha(n-1)$  is such that  $\text{Prob}(T(n-1) > T_\alpha(n-1)) = \alpha/2$ , then a  $(1-\alpha)100$  per cent confidence interval for  $t$  has:

$$\frac{t^* \left( \sum_{i=1}^n h_i^4 \right)^{\frac{1}{2}}}{\left( \sum_{i=1}^n h_i^4 \right)^{\frac{1}{2}} + 4Dt^*sT_\alpha(n-1)} \leq t \leq \frac{t^* \left( \sum_{i=1}^n h_i^4 \right)^{\frac{1}{2}}}{\left( \sum_{i=1}^n h_i^4 \right)^{\frac{1}{2}} - 4Dt^*sT_\alpha(n-1)}$$

when  $0 < 4Dt^*sT_\alpha(n-1) < \left( \sum_{i=1}^n h_i^4 \right)^{\frac{1}{2}}$

The method of estimation used by Wilson<sup>1</sup> was simply trial and error. He observed graphically that the observed points fell between the graph for  $t = 1,000$  and  $t = 1,500$  and that  $t = 1,200$  looked like a good fit. By the described statistical estimation method one obtains  $t^* = 984$  years and 95 per cent confidence interval  $576 \leq t \leq 3,360$ . This is a much more satisfactory method of analysis since it gives an estimate with the optimal properties of least squares as well as a measure of the accuracy of the estimate.

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<sup>1</sup> Wilson, A. T., *Nature*, **201**, 176 (1964).

<sup>2</sup> *Handbook of Chemistry and Physics* (Chemical Rubber Publishing Co., Cleveland, Ohio, 1964).

DR. ROBERTS's treatment of my data gives additional confirmation to my tenet that the chemical gradients found in Lake Vanda can be explained by chemical diffusion and that such chemical composition profiles in Antarctic lakes can provide valuable paleoclimatic information<sup>1</sup>.

I would, however, like to make a few comments on the treatment used by Dr. Roberts. The model I used, and that adopted by Dr. Roberts, was diffusion from a planar source of negligible thickness on the bottom of a rectangular trough of infinite volume. This assumes the Lake has a flat bottom with vertical sides. As my Fig. 2 (ref. 1) and the mathematics of Dr. Roberts show, this is, in fact, a reasonable first approximation. Dr. Roberts is concerned with secondary refinements, and to do this one cannot give all

points equal loading as he has done. The data near the bottom should be loaded more highly than those further from the bottom. This is because the real Lake Vanda does not have perfectly vertical sides so that the cross-sectional area will always become larger with increasing distance from the bottom. This will lead to a lowering of the concentration faster than the rectangular model would predict—this correction, while small near the bottom, becomes more important the further one goes from the bottom. Thus the least squares fit on the basis of a rectangular trough model will always give a minimum age. In my previous communication<sup>1</sup> data were plotted to emphasize the lower region of the Lake and show that in the lower 45 ft. the experimental points lay between 1,000 and 1,500 years. It was then concluded that the true age lay between these or about 1,200 years. It can be seen in my Fig. 2 that the experimental points above 50 ft. from the bottom are better fitted by the 1,000 year age, but this was neglected for the reasons given here.

More bathometric data on Lake Vanda would enable a more accurate date to be obtained. Further corrections would also have to be made for variation of the diffusion coefficient of calcium chloride with temperatures and concentration.

These are only minor objections in that Dr. Roberts's "rectangular trough age" and my estimate are essentially in agreement. In the Antarctic there are no good dating procedures, since there are little or no carbonaceous remains and when these are found they are suspect due to the "carbon-14 age" of the original carbon, be it from glacial melt waters or from the sea.

My original paper<sup>1</sup> was to persuade geologists that Lake Vanda was not 10,000 years old, as was believed by some<sup>2</sup>, or that the presence of upper lake levels meant that it was drying up, as was believed by many<sup>3</sup>. It was also my intention to persuade them that the salt gradients in lakes could yield valuable palaeoclimatic data.

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## OCEANOGRAPHY

### Observations on the Entrapment of Organic Matter within the Particle Structure of Calcareous Sediments

In the course of experiments on the metabolism of intact sediment communities we had to determine the concentration of ninhydrin-positive substances associated with the sediments. In addition, data on the uptake of free amino-acids from solution by these sediments was needed.

The organic compounds associated with natural sediments and their concentration have been well documented in recent literature<sup>1-4</sup>. Most of these analyses have dealt either with compounds in interstitial waters or in the hydrolysates of whole sediments. During the present work it became apparent that, as well as organic compounds associated with the interstitial water of these sediments, there was also a considerable amount bound within the sediment particles. The present investigation was an attempt to find the amount of this ninhydrin-positive organic matter as well as its origin.

The sediments under investigation were aerobic, calcareous sediments from Biscayne Bay, Florida. They had a uniform grain size with 90 per cent of the particles falling in the 0.5–1.0 mm range. Samples were collected by scraping the surface of shallow-water sediments to remove only the well-oxidized surface layers. These were

then freed from interstitial water and macroscopic organisms by washing and decanting repeatedly. Prolonged washing of the sediments on a coarse sintered glass filter removed as many of the microscopic organisms as possible. Sub-samples of these cleaned sediments were used immediately.

Chromatography columns were loosely packed with the sediment and wetted with artificial sea water. They were then washed with various solutions (Table 1). Some of these solutions were acidic and dissolved most of the sediment during the course of the experiment. Final complete dissolution was achieved with concentrated hydrochloric acid. The non-calcareous fraction remaining after this treatment was subjected to further washing. Samples of the solutions obtained after washing were analysed for ninhydrin-positive substances by the method of Moore and Stein<sup>5</sup> with minor modifications to eliminate the precipitate which formed in the presence of sea-water.

Table 1. ILLUSTRATION OF DIFFICULTY EXPERIENCED IN WASHING CALCAREOUS SEDIMENTS FREE OF NINHYDRIN-POSITIVE COMPOUNDS

Wash solution	Wash No.	Extinction at 570 mμ after ninhydrin colour development
Artificial sea-water, pH 8.0	1	1.050
	2	0.250
	3	0.163
	4	0.090
	5	0.068
Artificial sea-water, pH 5.5	6	0.107
	7	0.090
	8	0.152
Acetate buffer, pH 5.0	9	0.975
	10	0.650
	11	0.496
	12	0.460
	13	0.496
	14*	too high to read
Complete dissolution of calcareous sediment	15†	—
Distilled water	16‡	0.000

\* Sediment left overnight in twice its own volume of buffer.

† Dissolved in concentrated hydrochloric acid.

‡ Wash of siliceous sand remaining after dissolution of calcareous sand.

To reduce interference from naturally occurring organic compounds similar columns were washed with artificial sea-water until a stable low level of ninhydrin-positive substances was obtained in the wash water. The columns were then loaded with small volumes of 0.02 M amino-acid solutions. Amino-acid analyses were performed on fractions of the effluent.

Cleaned sediment samples were weighed out into clean dry flasks. A known amount of amino-acid was added in sufficient sea-water solution to exceed the water-holding capacity of the sediment. The flasks were then placed on a shaker for short periods of time to permit equilibration. The fluid was recovered by filtration on sintered glass followed by partial displacement of the liquid held by the sediment with a small volume of sea-water. Amino-acid analyses were performed on the solutions recovered from such batch uptake experiments.

Artificial sea-water was supersaturated with respect to calcium carbonate by lowering the pH to 4.0 and temperature to near freezing. Calcium carbonate was added until solid calcium carbonate persisted. The sea-water was freed of the solid material and made 0.02 M with respect to the amino-acid being tested. The pH and temperature were then raised, causing calcium carbonate to reprecipitate. The solutions were freed of the solid material and analysed for their amino-acid concentration.

Washing sediment columns with sea-water eventually yielded an eluant with a low concentration of ninhydrin-positive substances (see Table 1). However, washing with acidic solutions released ninhydrin-positive substances in proportion to the amount of sediment dissolved. These observations are interpreted to indicate the presence, on the surface of the sediment particles, of a loosely bound organic fraction. In addition there was an organic fraction within the physical structure of the particles comprising the sediments.

The column uptake experiments did not yield clear-cut results but there appeared to be some retention of the



acidic amino-acids. For example, a column of sediment ( $2.5 \times 5.0$  cm, with an estimated void volume of five ml) was washed with artificial sea-water (pH 8.0) until the effluent showed a stable low level of ninhydrin-positive substances. The column was then loaded with 10 ml. of a 0.02-M solution of aspartic acid, followed by elution with artificial sea-water. After passage of 100 ml. of the eluant through this system the effluent still possessed twice the concentration of ninhydrin-positive substances established before the addition of the amino-acid. The 45–50-ml. fraction possessed the highest concentration, indicating some retardation of the amino-acid. Similar results were not obtained with leucine or lysine.

Table 2. PERCENTAGE RECOVERY OF ADDED AMINO-ACIDS IN BATCH UPTAKE EXPERIMENTS

Amino-acid added	Percentage recovery in different experiments using the same sediment (%)
Arginine	94
Lysine	100
Serine	100
Glutamic acid	95
Aspartic acid	96
Proline	60–73
Tryptophan	78–88

Table 2 presents percentage recovery figures from the batch experiments. It would appear that the heterocyclic compounds tryptophan and proline are weakly associated with these sediments. Even they, however, could be eluted with a single wash with artificial sea-water. Artificial calcium carbonate sediments, free from organic matter, were obtained from a local water purification plant. They were sieved to conform in size with the natural sediments under study. With these sediments no uptake of free amino-acids from solution was observed nor did they show the retardation effect in column experiments.

Calcium carbonate is not usually recognized as a mineral which complexes organic matter<sup>4</sup>. This conclusion is supported by the negative results of our co-precipitation and artificial sediment experiments. It is reasonable to assume, therefore, that any uptake capacity we have demonstrated in natural sediments is due to some other factor. This is probably a surface layer of some other class of organic compounds, perhaps lipid in nature.

It is well known that varying proportions of natural calcareous sediments are biological in origin. The proportion varies from almost entirely of biological origin as in some deep-sea sediments to a relatively small biological contribution in the Bahamas Bank and analogous areas<sup>5</sup>. Calcareous plants and animals are believed to be the major source of this calcium carbonate but the possible role of micro-organisms has recently been re-emphasized<sup>6,7</sup>. The presence of organic matter within the structure of these sediments is therefore not surprising. However, the observation that in a well-oxidized surface sediment so much organic matter is preserved appears to be novel. This organic matter constitutes a net loss from the environment, as it is well protected from the usual degradation processes by being bound within the sediment particles. The only likely means by which such organic matter would leave the sediments would be by the latter's physical alteration during geological time. The entrapped organic matter thus could constitute an additional reservoir of organic matter for various geochemical processes.

In conclusion, calcareous sediments have been shown to possess weak affinities for certain nitrogen compounds of low molecular weight. This weak ability to act as adsorbents, retardants, or concentrators of organic matter is probably due to other classes of organic compounds rather than the mineral itself. However, it has been demonstrated that these sediments do possess an appreciable amount of organic matter bound with their particle structure. It appears likely that this organic matter is the residue of the organic matrix used by the plant or animal to lay down the mineral. Thus if a calcareous sediment is found to have an appreciable amount of ninhydrin-

positive substances bound within its particles the sediment may be considered to be of possible biological origin.

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## CHEMISTRY

### Self-diffusion of Lead in Lead Oxide (PbO)

We have utilized lead-210 isotope and surface-counting technique to evaluate the self-diffusion coefficient of lead in lead oxide. Since lead-210 has a very weak  $\beta$ -emission (0.025 MeV) it is wellnigh impossible to estimate the activity with the conventional Geiger-Müller counters. Furthermore,  $\mu$ , the absorption coefficient, is so high that the radiations from within the bulk material do not penetrate the layers. However, we have successfully surmounted the difficulty by depositing lead-210 on one face of a pellet, allowing the daughter to build up, and following the movement of lead-210 by determining the amount of bismuth-210. We present here the relevant mathematical treatment and results.

There are three distinct possibilities when lead-210 is deposited on the pellet. They are: (1) lead-210 is in equilibrium with bismuth-210; (2) lead-210 is in excess of the equilibrium; (3) lead-210 is in deficit. In all these cases, after a length of time (30 days) the two isotopes will be in equilibrium. However, if a freshly purified lead-210 is used, only the second possibility exists.

If  $x$  be the equilibrium counting rate,  $y$  and  $z$  the amount of bismuth that is in excess or deficit at times  $t_1$  and  $t_2$ , then:

$$C_1 = x \pm y, C_2 = x \pm z$$

Where  $C_1, C_2$  are the actual counting rates at  $t_1$  and  $t_2$

Let us now review the three cases individually:

Case (i):

$$C_1 = C_2 = x = A_0$$

$$C'_1 = C'_2 = x' = A t$$

Case (ii). In this case if  $C_1 = x - y$  is the counting rate at  $t_1$  and  $C_2 = x - z$  the counting rate at time  $t_2$ , both before heating, then ( $x < y$ )  $z$  is given by  $z = y e^{-\lambda t}$ , where  $t = t_2 - t_1$  and  $\lambda = 0.693/t_{1/2}$ ,  $t_{1/2}$  being the half-life of bismuth-210:

$$C_1 = x - y \text{ (i) and } C_2 = x - y e^{-\lambda t} \text{ (ii)}$$

Multiplying equation (i) by  $e^{-\lambda t}$  and subtracting (i) from (ii) one gets:

$$C_2 - C_1 e^{-\lambda t} = x(1 - e^{-\lambda t}) \text{ (iii)}$$

Writing  $b$  for  $e^{-\lambda t}$ ,  $x$  can be expressed as:

$$\frac{(C_2 - C_1 b)}{1 - b} = x_1$$

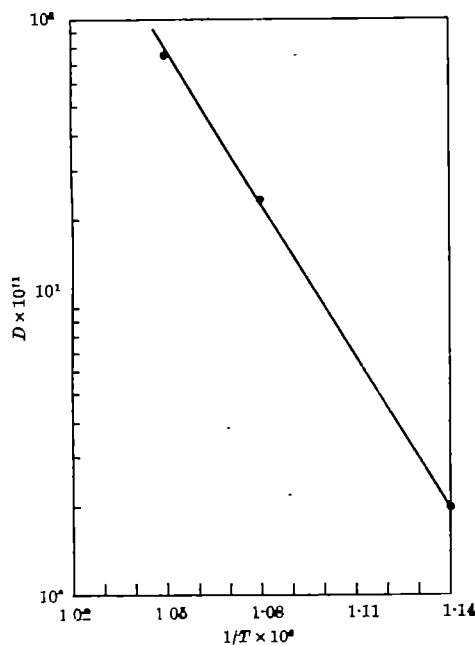


Fig. 1

Similarly, after heating for a time  $t$ ,  $x_2$  can be given by:

$$(C'_2 - C'_1b)(1-b) = x_2$$

Case (iii). Similarly for this case where  $C_2 < C_1$  the same relation holds.

If  $C_1$  and  $C_2$  are determined at  $t_1$  and  $t_2$  before heating and  $C'_1$  and  $C'_2$  determined at  $t'_1$  and  $t'_2$  after heating for a time  $t$  then  $x_1$  and  $x_2$  correspond to  $A_1$  and  $A_2$  of equation (1) (ref. 1).

The results thus obtained are given in Fig. 1 as  $\log D$  versus  $1/T$ . The plot yields a value of 64 kcal for the activation energy. This is in remarkable agreement with that obtained by Lindner<sup>2</sup>.

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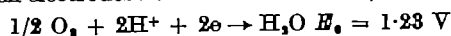
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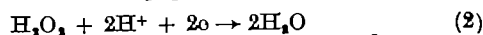
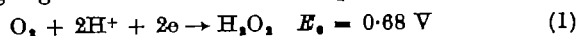
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### Reduction of Oxygen at the Platinum Electrode

THE formation of hydrogen peroxide is considered by some authors<sup>1-3</sup> as the possible source of the poor efficiency of oxygen electrodes in a fuel cell. Indeed, the reduction:



would proceed at a normal potential below 1.23 V if the foregoing reaction occurs in two steps:

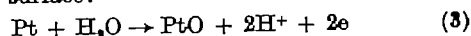


assuming that the second step is irreversible.

The following triangular (cyclic) polarograms prove that molecular hydrogen peroxide is not produced in detectable amounts at the surface of the electrode when oxygen is reduced in acid solution at a bright platinum wire. The experiments were performed according to standard voltammetric techniques. The working electrode

was a 3.6 cm<sup>2</sup> spectroscopically pure platinum wire and the comparison a 40 cm<sup>2</sup> Hg-Hg<sub>2</sub>SO<sub>4</sub> electrode. The two electrodes were separated by three sintered glass diaphragms. The volume of the solution in the working compartment was 10 ml. The cell filled with 4 M sulphuric acid electrolyte had a resistance of 10 ohms at 27°C (ref. 4).

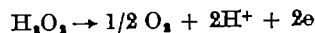
The significance of the shape of the triangular scanned polarogram obtained when an inert gas bubbles through the acid solution has been clearly established by Will and Knorr<sup>4</sup>. In this case, the oxidation current generated during the anodic sweep between 0.6 and 1.4 V versus the normal hydrogen electrode (NHE) is due exclusively to the building of a monolayer of platinum oxide on the bare platinum surface:



The reduction current during the following cathodic sweep is generated exclusively by the reduction of this oxide. The lack of symmetry of the polarogram as shown by the full curve of Fig. 1 is due to the irreversibility of reaction (3).

When oxygen instead of helium bubbles through the solution, the rate of reduction below 0.9 V rises above the value corresponding to the reduction of the platinum oxide layer. The increase in the reduction current, due to the reduction of oxygen, starts when approximately one-quarter of the platinum oxide layer has already been reduced to platinum. However, in the presence of oxygen the portion of the polarogram between 1.0 and 1.4 V remains identical to the shape of the polarogram obtained when helium is bubbled through the solution, as shown by Fig. 1.

On the other hand, the oxidation current between 1.0 and 1.4 V increases by approximately 6 per cent when a trace amount of hydrogen peroxide, as low as  $1 \times 10^{-4}$  mole/l., is added to the helium-bubbled solution. The same increase in current is observed when hydrogen peroxide is added to the oxygen-bubbled solution, as shown by Fig. 2. In both cases, the current is generated by the oxidation:



which proceeds together with reaction (3).

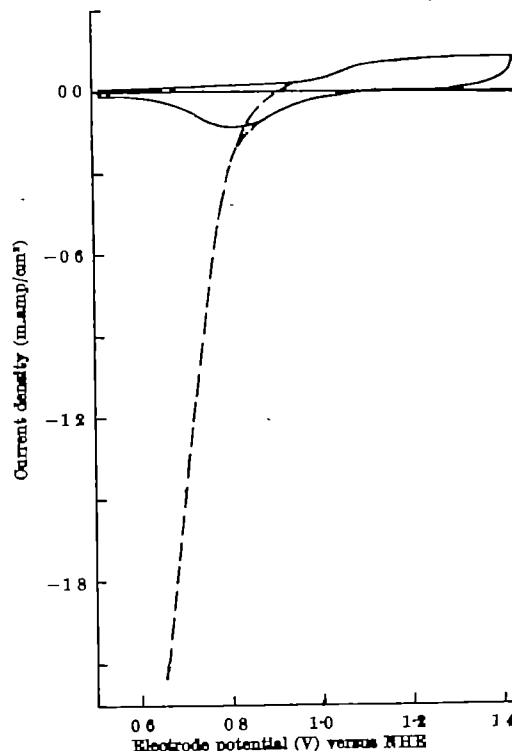


Fig. 1. Effect of the nature of bubbling gas. —, Helium; ---, oxygen; scanning rate, 5.4 V/min

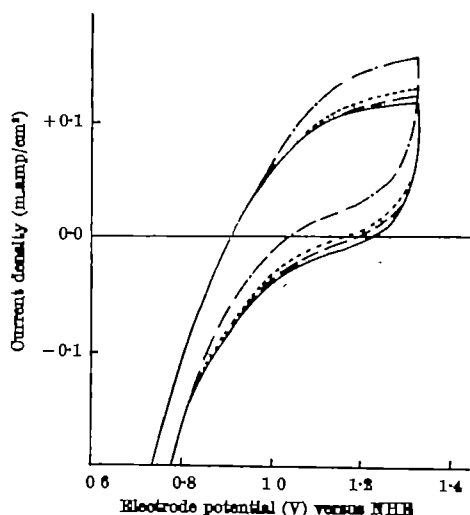


Fig. 2. Effect of hydrogen peroxide with oxygen-bubbled solution. —, No  $H_2O_2$ ; ---,  $1 \times 10^{-4}$  M; ····,  $5 \times 10^{-4}$ ; - · - ·,  $10 \times 10^{-4}$  mole  $H_2O_2$ /l.

Now, the number of moles of oxygen reduced during one cycle can be calculated from the integral of the appropriate portion of the current-voltage or current-time curve of Fig. 1 since the polarization varies linearly with time. This quantity is equivalent to 13 millicoulombs per  $cm^2$  or equal to  $2.3 \times 10^{-7}$  mole for the  $3.4 \text{ cm}^2$  electrode. Therefore, the current of oxidation above 1.0 V would increase in a measurable way if only 1 per cent of this amount of oxygen was converted in  $H_2O_2$ , provided that these  $H_2O_2$  molecules remain adsorbed on the electrode during the following anodic sweep. Even if the  $H_2O_2$  molecules were instantaneously released from the electrode, the coverage would never reach 50 per cent of the surface during the cathodic sweep. Otherwise the concentration of hydrogen peroxide liberated in the 10-ml. solution of the working compartment would reach  $1 \times 10^{-4}$  mole and could be detected by the increase in oxidation current above 1.0 V.

The described experiments suggest that hydrogen peroxide is not formed as an intermediate product of the reduction of oxygen in acid solution at the platinum electrode. Hydrogen peroxide observed in other experimental conditions appears most probably as a side-product of reduction of oxygen. Consequently, hydrogen peroxide formation cannot be held responsible for the overpotential of the oxygen electrode in an acidic fuel cell.

Furthermore, the foregoing experiments confirm the hypothesis of Breiter<sup>8</sup> that the reduction of oxygen proceeds only at the contact of bare platinum atoms since the reduction proceeds during the cathodic sweep only after approximately one-quarter of the monolayer of platinum oxide has already been removed from the surface. The importance of the surface which must be cleared from the monolayer of platinum oxide before the reduction of oxygen occurs suggests, furthermore, that this catalytic reaction proceeds only after a molecule of oxygen has been adsorbed by two adjacent straight platinum atoms.

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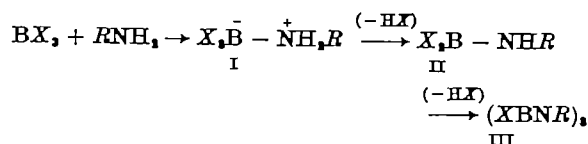
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## Reaction of Pentafluoroaniline with Boron Tribromide

THE reactions between boron halides and amines have been examined extensively<sup>1-3</sup>. In theory, it is possible to substitute successively the halogen atoms in a boron halide to form the derivative  $B(RNH)_3$  from a primary amine, and the derivative  $B(R_2N)_3$  from the secondary amine. Alternative reaction schemes are possible, and in practice, if the amine is not in large excess, the final product from a primary amine is usually a borazine.



Intermediates of type II are unstable though their existence has been inferred, but those of type I have been isolated. With the secondary amines, the reaction stops at the second stage, and compounds such as  $(CH_3)_2NBBR_2$  are well known both as simple monomers and as dimers with four-membered boron-nitrogen rings<sup>4</sup>.

We find that approximately equimolar amounts of pentafluoroaniline and boron tribromide react at 25° with the evolution of hydrogen bromide and the formation of a solid mixture. At 50° the mixture melts, more hydrogen bromide is evolved, and a colourless liquid remains. This is pentafluoroanilinoboron dibromide,  $C_6F_5NHBBr_2$  (found: B, 2.9; Br, 45.2;  $C_6F_5NHBBr_2$  requires B, 3.1; Br, 45.4 per cent). The compound melts at 23° and distills unchanged at 40°–50°. It is moisture sensitive and is decomposed by water to boric acid, hydrogen bromide, and pentafluoroaniline.

At 70° pentafluoroanilinoboron dibromide decomposes quickly, with the evolution of hydrogen bromide, to a white, crystalline solid which melts at 118°–120°, and has a composition close to  $C_6F_5NBBR$  (found: B, 3.2; Br, 26.3; calc. for  $C_6F_5NBBR$ : B, 3.9; Br, 29.4 per cent). The infra-red spectrum of this material shows a weak absorption at  $3,425 \text{ cm}^{-1}$  in the position of the strong N—H frequency of the dibromide, which suggests that this is the contaminant. There is also a strong absorption, nearly absent in the dibromide, at  $1,380 \text{ cm}^{-1}$ , which lies in the region ( $1,300$ – $1,490 \text{ cm}^{-1}$ ) expected for boron-nitrogen ring vibrations in borazines<sup>5</sup>. So far, it has not proved possible to remove the contaminant from the material, which is almost certainly *tris-N*-pentafluorophenyltribromoborazine.

In benzene, pentafluoroaniline reacts with boron tribromide at 10° to give a crystalline adduct which appears to be the addition complex  $Br_2B - \overset{+}{N}H_2(C_6F_5)$ .

Pentafluoroanilinoboron dibromide is the first example of a type II intermediate to be actually isolated from the reaction between a primary amine and a boron halide. Its reactivity and physical appearance suggest that it is monomeric, as the dimeric dihalides are air-stable solids. On standing, specimens appear to form the borazine slowly even at room temperature, so it is unlikely that the dimer will ever be isolated in a pure state.

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### Free Radical Formation in the Neutral Sulphitation of Wood at High Temperature

In a previous report<sup>1</sup>, it was shown that, when wood and cellulose, pretreated with sodium hydroxide and afterwards freeze-dried, were rapidly heated in the cavity of an electron spin resonance (ESR) spectrometer to a temperature between 180° and 190° C, rapid formation of free radicals (radical centres) was recorded, and it was found that within a relatively short time free-radical decay took place, following approximately an exponential function. After cooling, a limited number of free radicals remained in the specimens; these free radicals were quite stable over a considerable period of time and were considered to be trapped by a 'cage effect' of the polymers.

A question of interest was whether similar phenomena take place when wood, holocellulose and cellulose are treated in the same way but impregnated with water-free Na<sub>2</sub>SO<sub>3</sub> instead of NaOH. Such knowledge might contribute towards a better understanding of high-temperature neutral-sulphite pulping of wood.

The following materials were used: (a) black spruce sawdust (the fraction which passed the 40-mesh sieve but was retained by the 60-mesh sieve); (b) chlorite holocellulose prepared from the same black spruce sawdust by a series of mild chloriting stages similar to the procedure of Wise *et al.*<sup>2</sup>; (c) commercial, acetate grade, high alpha-cellulose (98 per cent) pulp.

These materials were subjected to 30 min impregnation at 83° C in a 10 per cent Na<sub>2</sub>SO<sub>3</sub> solution (pH about 8.6) in a ratio of 1 : 20. At the end of the impregnation period, the materials were separated from the liquor on a sintered glass crucible and then pressed to remove the remaining liquor excess. Later, they were freeze-dried to remove water; finally, they contained about 25 per cent Na<sub>2</sub>SO<sub>3</sub>. Weighed specimens were introduced into quartz capillaries; after evacuation and filling the capillaries with nitrogen at atmospheric pressure, the capillaries were sealed.

The filled capillaries were rapidly heated to 190° C in the cavity of a Varian Associates model 'V-4500' electron spin resonance spectrometer by a stream of hot nitrogen. Single line signals (first derivatives) were found and recorded (modulation 630, gain 630, range 16) with increasing time of heating. For comparison, the changes of the signal area (sq. cm) of the three specimens, calculated free of inorganics, and based on the same initial wood weight are presented in Fig. 1. The holocellulose yield

was about 71 per cent and that of the high-alpha-cellulose pulp about 40 per cent. Thus, the curves show how holocellulose and the high alpha-cellulose pulp obtained in these ratios from the same wood weight compare with the initial wood.

It can be seen that free radicals were formed with each material investigated, and that the concentration of the radicals trapped in the polymeric substances increased with increase of the heating time. However, after a definite reaction time (wood, about 25 min; holocellulose, about 20 min; high alpha-cellulose pulp, about 15 min) a levelling-off was observed. Probably, as polymer degradation progressed, the 'cage effect' of the degraded material approached a distinct limit value. In contrast to the alkaline pulping experiments<sup>1</sup>, no immediate formation of free radicals in very high concentrations at the very start of reaction, followed by free-radical decay according to an approximate first-order pattern, was observed. This seems to indicate that the mechanisms of alkaline delignification and high-temperature sulphitation of the wood are distinct phenomena.

As shown in Fig. 1, for equal reaction times, the ESR signal area as a measure of the free radical concentration decreased in the order: wood, holocellulose, high alpha-cellulose pulp. Remarkably, the holocellulose values were relatively high when compared with those of the wood; even if correction was made for the alpha-cellulose portions of the holocellulose, these values still accounted for about half those of the wood. This appears to constitute evidence that during reaction, besides thermal degradation and sulphonation of the lignin, considerable changes of the hemicellulose portions also took place, both resulting in free radical formation.

This work was carried out partly at the Pulp and Paper Research Institute of Canada, Montreal, and partly at the National Research Council, Ottawa, and I thank these organizations for their help, and Dr. J. R. Morton for his co-operation in recording the ESR spectra.

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### Synthesis of *Tris*-(hexafluoroacetylacetonato)-Chromium (III)

REDUCTION of Cr(VI) in the presence of chelating ligands offers a promising approach to synthesis of Cr(III) chelates in high yield. This principle was used by Aikens and Reilly<sup>1</sup> to devise a rapid chelometric method for determination of chromate. Reduction of chromate with bisulphite in a buffered solution of ethylenediaminetetraacetic acid (EDTA) gave quantitative formation of Cr(III)-EDTA. The quantitative yield of Cr(III) EDTA suggested that this might be the basis of a general method for synthesis of Cr(III) complexes. We have now applied this principle to synthesis of Cr(III) chelates of much lower stability as exemplified by *tris*-(hexafluoroacetylacetonato)-Cr(III).

Efforts to synthesize *tris*-(hexafluoroacetylacetonato)-Cr(III) in aqueous solution in a manner similar to the synthesis of Cr(III) EDTA were unsuccessful. Attempted synthesis in hydroxylic non-aqueous solvents, such as methanol with paraldehyde as the reductant, also failed. A successful synthesis was based on the heterogeneous reaction of hexafluoroacetylacetone with potassium dichromate. This reaction is interesting because evidently the ligand acts as the reductant.

0.5 g (0.0017 mole) of finely powdered potassium dichromate was placed in a 100-ml. round-bottom flask and covered with 4.16 g (0.020 mole) of the ligand. The flask was fitted with a water-cooled condenser and the

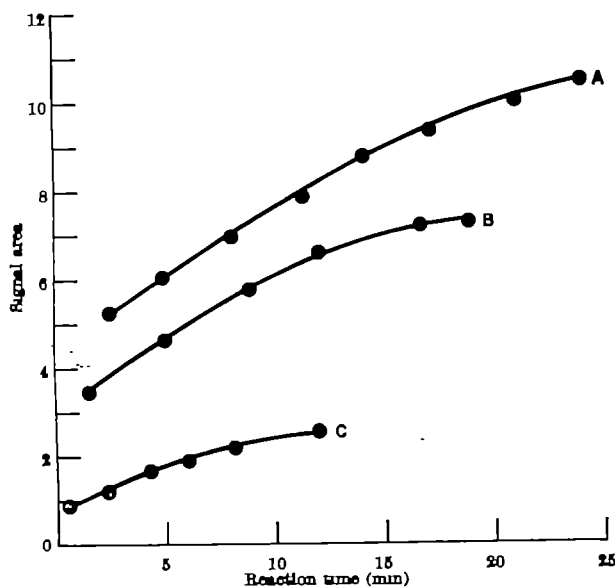


Fig. 1. Signal areas in relation to reaction time. A, Wood (0.015 g); B, holocellulose (0.0105 g); C, high-alpha-cellulose pulp (0.006 g).

solution was refluxed gently for 3 h, during which time the liquid changed from colourless to dark green. Fifty ml. carbon tetrachloride was added and refluxing continued for 30 min. The solution was filtered and the filtrate was evaporated to dryness by a stream of dry air. The yield of *tris*-(hexafluoroacetylacetonato) Cr(III) was 1.85 g (80 per cent based on potassium dichromate). Calculated for  $\text{Cr}(\text{C}_6\text{HF}_5\text{O}_4)_3$ : C, 26.76 per cent; H, 0.45 per cent; F, 50.80 per cent. Found: C, 27.04 per cent; H, 0.68 per cent; F, 51.30 per cent. The infra-red spectrum and melting point ( $84^\circ$ ) of the compound were determined and agree well with the data of Morris *et al.*<sup>2</sup>

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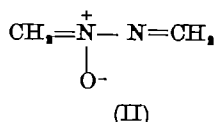
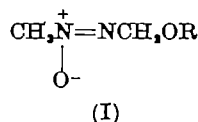
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## BIOCHEMISTRY

### Decomposition and Carcinogenic Activity of Azoxyglycosides

THE toxicity of leaves and seeds of cycads to animals and humans is well established (for review, see ref. 1). The toxic constituents are azoxyglycosides of which the simplest are cycasin (I;  $R = \beta$ -D-glucopyranosyl)<sup>2</sup> and macrozamin (I;  $R = \beta$ -primeverosyl)<sup>3,4</sup>. More recently, liver damage<sup>5</sup> and carcinogenesis<sup>6</sup> have been observed to follow ingestion of the seeds or extracts containing the unstable aglycone, methylazoxymethanol (I;  $R = \text{H}$ ) (ref. 5). The latter may theoretically decompose to the alkylating agent, diazomethane, a potent carcinogen<sup>7</sup>, and is indeed isomeric with hydroxydimethylnitrosamine through which dimethylnitrosamine may be transformed *in vivo* to the same active carcinogen<sup>8</sup>.

That cycasin and macrozamin may be effective methylating agents *in vitro* has now been demonstrated by the production of anisole (40–50 per cent yield, based on azoxyglycoside) when a solution of the glycoside in molten phenol is treated with a drop of concentrated sulphuric acid and warmed until evolution of gas ceases (2–3 min). No reaction occurs in the absence of strong acid, nor is any trace of anisole produced when azoxyglycoside is hydrolysed in hot dilute sulphuric acid containing a large excess of phenol. This behaviour is analogous to that of benzaldazine monoxide<sup>9</sup>, and formaldehyde monoxide (II) may be an intermediate, formed by loss of water from initially produced aglycone.



Azoxyglycosides are rapidly degraded by alkali to a variety of products including cyanide ion, ammonia, methylamine, nitrogen and formic acid; the sugar component is liberated even with cold dilute ammonia<sup>10</sup>. The first step is now formulated as (a) in the annexed scheme (Fig. 1). A little anisole (c. 2 per cent) is produced when cycasin is degraded by aqueous alkali buffered to pH 10 by an excess of phenol, so that the postulated intermediate, formaldehyde monoxide (II), may break down partly to formaldehyde and free diazomethane in alkali. Most of these products, however, are apparently diverted into

acetaldehyde, their product of (perhaps intramolecular) reaction (b), as shown by the appearance of iodoform (20 per cent yield) on treatment of cycasin or macrozamin with aqueous alkaline hypiodite. The presence of acetaldehyde has not been demonstrated directly but the same yield of iodoform was obtained from an equimolar mixture of *N*-nitrosomethylurea and formaldehyde on treatment with alkali followed by hypiodite, none being obtained by similar treatment of these compounds alone. The iodoform probably does not arise from direct iodination and cleavage of the 'active' methyl group (but see ref. 10) in the aglycone, for the electronically similar nitromethane yields no iodoform under the same conditions.

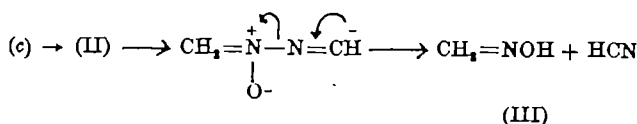
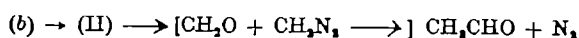
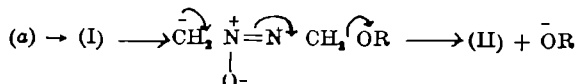


Fig. 1

The formation of cyanide ion in alkali is now postulated to follow an alternative mode (c) of decomposition of formaldehyde monoxide (II), which finds a close analogy in the base-induced cleavage of aldehyde quaternary hydrazones to nitriles and tertiary amines<sup>11</sup>. The other product expected in the present case, formaldoxime (III), has not been isolated but evidence for its presence is found in (i) the production of formic acid (45 per cent), the hydrolysis product of formaldoxime in dilute acid<sup>12</sup>, when the alkaline solution is acidified with dilute sulphuric acid and distilled<sup>3</sup>, and (ii) the slow deposition of formaldehyde-dimedone (26 per cent) when the alkaline solution is buffered to pH 4 with acetic acid and sodium acetate in the presence of dimedone. The small amounts of methylamine and ammonia produced from macrozamin in boiling alkali<sup>3</sup> may also arise from decomposition of formaldoxime.

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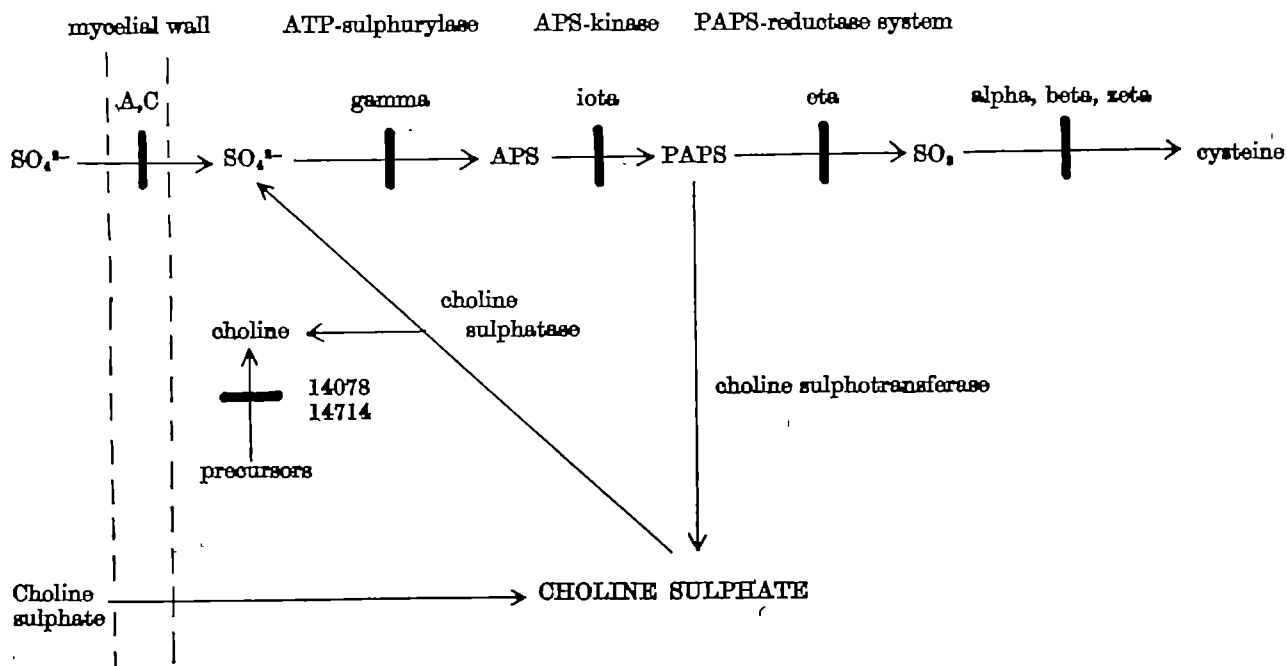
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### Mechanism of Choline Sulphate Utilization In Fungi

CHOLINE sulphate is synthesized by higher fungi<sup>1</sup>, algae<sup>2</sup> and plants<sup>3</sup>. In fungi it is found in high concentration in the mycelium<sup>4</sup> and in spores<sup>5</sup> and is thought to act as a store of sulphur<sup>6,7</sup> and possibly carbon and nitrogen. Synthesis of choline sulphate<sup>8,9</sup> involves activation of inorganic sulphate by the enzymes ATP-sulphate adenylyltransferase (*EC* 2.7.7.4), (ATP-sulphurylase) and ATP-adenylyl 3'-phosphotransferase (*EC* 2.7.1.25), (APS-kinase), to give adenosine 3'-phosphate 5'-sulphatophosphate (PAPS) and the subsequent transfer of the



sulphate group of PAPS to choline catalysed by 3'-phosphoadenylylsulphate: choline sulphotransferase (EC 2.8.2) (choline sulphotransferase)<sup>8</sup>. The mechanism of the utilization of the sulphur of choline sulphate for cysteine synthesis has been in some doubt. A reversal of the choline sulphotransferase reaction to give PAPS which could then be converted to  $\text{SO}_4^{2-}$  and then to cysteine has been ruled out<sup>9,10</sup>. Several workers have failed to detect a choline sulphatase in fungi<sup>11,12</sup>, and while this enzyme has been detected in *A. sydowii* and *P. chrysogenum*<sup>13</sup>, the activity of the enzyme was very low and its physiological significance uncertain. Evidence is now presented confirming the presence of choline sulphatase in fungi and establishing that this enzyme is obligatory for choline sulphate utilization.

A number of parathiotropic mutants<sup>14</sup> of *Aspergillus nidulans*, A.69, and mutants A and C of a biotin-less mutant of *Aspergillus nidulans* were examined. Growth requirements were ascertained on Czapek-Dox liquid medium in which sulphate-containing salts were replaced by the corresponding chlorides. Choline sulphate synthesis *in vivo* and *in vitro*, overall PAPS synthesis and choline sulphotransferase were determined by methods used previously<sup>8</sup>, and ATP-sulphurylase was determined by the molybdolysis technique<sup>15</sup>. APS-kinase was assayed by following the conversion of adenosine 5'-[<sup>32</sup>S]-sulphatophosphate (APS) to PAP<sup>32</sup>S and choline [<sup>32</sup>S]-sulphate in the presence of ATP, choline and choline sulphotransferase. Sulphate and choline sulphate transport through the mycelial wall was measured by suspending mycelia for 15 min at room temperature in media containing <sup>35</sup>SO<sub>4</sub><sup>2-</sup> or choline [<sup>32</sup>S]-sulphate, filtering and assaying the loss of radioactivity from the media. Choline sulphatase in mycelia was measured by a method similar to that used by Segal<sup>13</sup>.

Heterokaryosis tests confirmed Hockenbush's findings<sup>14</sup> that the mutants could be divided into four main groups:

I, gamma; II, iota; III, eta; IV, alpha, beta, zeta. A and C formed heterokaryons with all other mutants and thus constitute a fifth group. A scheme has been drawn up (above), based on the positions of the enzyme blocks as elucidated by *in vitro* assay (Table 1), which explains the growth requirements of the fungi and their ability to synthesize choline sulphate *in vivo*. The scheme envisages the obligatory hydrolysis of choline sulphate to inorganic sulphate and choline before its sulphur can be utilized for cysteine synthesis.

The two key mutants are iota and A. The former cannot grow on sulphate or choline sulphate, while A can grow on choline sulphate but not on sulphate. Mutant gamma lacks the enzyme ATP-sulphurylase and thus cannot utilize inorganic sulphate supplied as such or arising from the hydrolysis of exogenous choline sulphate although it will transport both sulphur sources into the mycelia. Mutant A does not grow on inorganic sulphate since it cannot transport sulphate into the mycelia. When grown on cysteine as a sulphur source it does not synthesize choline [<sup>32</sup>S]-sulphate from exogenous <sup>35</sup>SO<sub>4</sub><sup>2-</sup> but it does possess all the enzymes for choline sulphate synthesis. There is no block to the utilization of choline sulphate by this mutant since the enzymes are present for choline sulphate transport, hydrolysis and sulphate utilization.

The findings of others that choline sulphatase is absent<sup>11,12</sup> or present only in trace amounts<sup>13</sup> in mycelia are explained by the fact that the enzyme is repressed under conditions of sulphur sufficiency and it is only as all other sulphur reserves are being used up that enzyme is de-repressed and choline sulphate utilized. The *in vivo* operation of choline sulphatase and the absence of any alternative form of choline sulphate utilization were demonstrated in short-term experiments in which mycelia which have been starved of sulphur were incubated in media containing choline [<sup>32</sup>S]-sulphate. In A.69, <sup>35</sup>SO<sub>4</sub><sup>2-</sup>, [<sup>32</sup>S]-cysteine and other labelled sulphur compounds were

Table 1. GROWTH REQUIREMENTS AND ENZYME PATTERNS OF *A. nidulans* MUTANTS

Fungus	SO <sub>4</sub> <sup>2-</sup>	Growth on			Choline sulphate synthesis		ATP-sulphurylase	APS kinase	Choline sulphotransferase	Choline sulphatase	SO <sub>4</sub> <sup>2-</sup> transport	CS transport
		CS	SO <sub>4</sub> <sup>2-</sup>	Cysteine	<i>in vivo</i>	<i>in vitro</i>						
Wild-type A69	+	+	+	+	+	+	+	+	+	+	+	+
Gamma	+	+	+	+	+	+	+	+	+	+	+	+
Iota	-	-	+	+	-	-	+	+	+	+	+	+
Eta	-	-	+	+	+	+	+	+	+	+	+	+
Alpha, beta, zeta	-	-	+	+	+	+	+	+	+	+	+	+
A, C	-	+	+	+	-	+	+	+	+	+	-	+

produced in the mycelia, but in mutant gamma, which lacks ATP-sulphurylase, the only radioactive compound produced was  $^{35}\text{SO}_4^{2-}$ . A further point requiring clarification was whether the *in vivo* release of sulphate from choline sulphate was the result of the direct action of a sulphatase or only followed after the catabolism of the carbon skeleton. That a choline sulphatase was operative *in vivo* was implied by the demonstration that choline sulphate could act as a source of sulphur and of choline for two cholineless mutants (ATCO 14078 and 14714) of *Neurospora crassa*<sup>16,17</sup>.

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### Acid Nucleases of the Bovine Adrenal Medulla

THE chromaffin granules of the adrenal medulla are characteristic cell organelles which serve the function of amine storage. Earlier work has shown that these granules are distinct from mitochondria<sup>1</sup>.

A relationship between lysosomes and secretory granules has been discussed<sup>2</sup>, particularly where the secretion product of the granules is an enzyme. However, a relation between the chromaffin granules and lysosomes may also have to be considered<sup>3</sup>. It is known that the chromaffin granules, like the lysosomes, release their contents into hypotonic media.

A possible connexion between lysosomes and chromaffin granules is of particular interest in view of a recent report on the occurrence of a ribonuclease (RNase) in the chromaffin granule fraction<sup>4</sup>.

During a recent investigation of the soluble protein obtained from a lysate of chromaffin granules it was noted that the RNase was present in the soluble lysate and that the latter also contained a deoxyribonuclease (DNase), and we have examined some of the properties of these enzymes.

Yeast RNA and calf thymus DNA were used as substrates. After an incubation for 1 h at 37° C the RNA or DNA was precipitated and the acid-soluble nucleotides determined by measuring the absorption at 260 mμ; this was supplemented, for confirmation, by total phosphate analysis.

Purified fractions of chromaffin granules were lysed in *tris*-sodium succinate buffer of pH 5.9 and of low ionic strength ( $I \approx 0.015$ ), as recently described<sup>5</sup>. After lysis, more than 80 per cent of both RNase and DNase activities were recovered in the supernatant fluid on centrifugation. The soluble lysate was fractionated in the same buffer on a column of 'Sephader G-200'. The enzymes could be

separated from 50 per cent of the total soluble protein. The enzymic activities emerged in two overlapping peaks; the distribution coefficient,  $K_d$  (ref. 6), was 0.201 for RNase and 0.178 for DNase. The enzymatic activities for the peak fractions were determined and expressed in terms of μM of acid-soluble P/mg protein/h; these values were 2.74 for RNase and 2.72 for DNase. In the experiments to be described below, pooled fractions from each peak were used.

The RNase of the soluble lysate from bovine adrenal medulla differed from the RNase of bovine pancreas in that it acted on RNA 'core', and in its thermostability and in its pH optimum; the latter was 5.5 for the adrenal enzyme, as compared with 7.3 for the pancreas enzyme<sup>7</sup>. The pancreas enzyme is known to be very stable to heat. On the other hand, the rat liver lysosomal enzyme has a pH optimum similar to that of the adrenal enzyme and is thermostable<sup>8</sup>; it also acts on RNA 'core'<sup>9</sup>.

The optimal pH for the adrenal medullary DNase, in sodium acetate buffer of  $I = 0.136$ , was 4.6. This enzyme was not inhibited by  $\text{Cu}^{2+}$  concentrations up to  $2 \times 10^{-4}$  M, whereas the RNase of the adrenal lysate showed a 50 per cent inhibition with  $2.5 \times 10^{-4}$  M  $\text{Cu}^{2+}$ .

The observations show that the RNase described by Philippu and Schümann<sup>4</sup> is present in the soluble protein fraction and also that an acid DNase is present in the same lysate. The material used in this study was obtained from the bovine adrenal medullary 'large granule' fraction by ultracentrifugation over a sucrose density gradient. Although a possible contamination of the chromaffin granules by either mitochondria or microsomes can be excluded as a source of the enzymatic activities studied, the possibility remains that the two enzymes are lysosomal in origin. Two alternative interpretations offer themselves: either the presence of the two acid hydrolases is an expression of a close relationship between chromaffin granules and lysosomes, or the two enzymes are located not in the chromaffin granules but in lysosomes which might be present in the particulate fraction isolated from the chromaffin tissue.

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### Enzymatic Synthesis of the Sugar Esters of Hydroxy-aromatic Acids

GLUCOSE esters (1-O-acyl derivatives) of hydroxycinnamic acids have recently been shown to be widely distributed among higher plants<sup>1</sup>. They are also the most common derivatives produced when the free acids are fed to a wide variety of plants even in cases where the species examined contained either a different derivative of the acid in question or no trace of it at all<sup>2</sup>. In spite of this, however, comparatively little is known of the way in



which these glucose esters are synthesized. Although 1-*O*-anthraniloyl glucose has been shown to be formed from anthranilic acid and uridine diphosphoglucose (UDPG) in the presence of an enzyme preparation from *Lens esculentum*<sup>1</sup> it has been suggested that esters of phenolic acids are more probably synthesized from free glucose and the acyl coenzyme A derivative<sup>2</sup>. This hypothesis was presumably put forward because it has already been shown that many phenols form *O*-glucosides with UDPG in the presence of suitable enzyme systems<sup>3</sup>.

We report here that the glucose esters of the four commonly occurring hydroxycinnamic acids (*p*-coumaric, caffeic, ferulic and sinapic acids) are readily formed in good yield when the acids are incubated with a two-mole excess of UDPG and an acetone powder prepared from leaves of geranium (*Geranium zonale* cv. 'Paul Crampel') at pH 8.4 and 37°. Slightly smaller amounts were formed at pH 9.4 and 10.4, but no esters could be detected at pH 5.7 or 7.4. At pH 7.4, glucose esters were also formed from simple phenols (for example, *p*-cresol) incubated with UDPG in the presence of the same enzyme preparation from several plants<sup>4</sup>. No

higher breaking strength, whereas a compact matrix shows a lesser breaking strength. Up to now, attempts to correlate the varying degrees of egg-shell calcification with the mucopolysaccharide content have been unsuccessful. These attempts were carried out on egg-shells that had been decalcified with EDTA<sup>5,6</sup>.

We have now conducted experiments on non-decalcified egg-shells and investigated the correlation between the mucopolysaccharide content of the shells and their breaking strength. Fifteen hundred eggs were arranged in thirteen groups according to the breaking strengths and the mucopolysaccharide content of collective samples determined. Mucopolysaccharide was determined as uronic acid, and total organic material was determined as total nitrogen. The breaking strengths of the egg-shells was determined according to Rauch<sup>7</sup>, the uronic acid according to Dische<sup>8</sup> and nitrogen according to Kjeldahl.

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### Species Specificity in Reaction between Renin and Angiotensinogen

the most potent pro-

Table 1. *In vitro* FORMATION OF ANGIOTENSIN FROM HETEROGENEOUS MATERIALS AS COMPARED WITH HOMOLOGOUS MATERIALS

The amounts of product per 1 g of kidney are expressed in nanograms of synthetic isoleucine<sup>1</sup>-angiotensin-II precursor activity

Species of renin	Species of substrate	Amounts of formed angiotensin (ng)
Human	Human	53
Bovine	Human	8
"	Bovine	46
Hog	Human	12
"	Hog	20
Rat	Human	20
"	Rat	78

a common chemical feature, regardless of the species difference, in the binding site of the enzyme-substrate complex of the reaction.

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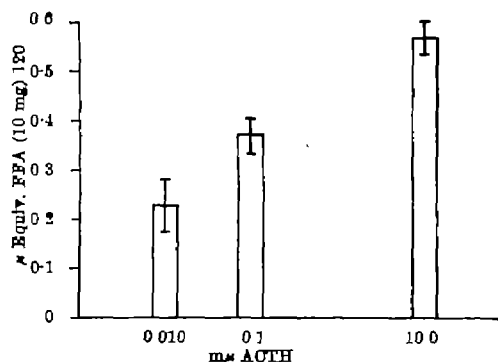


Fig. 1. The vertical bars indicate the mean ( $\pm$  S.E.) differences in lipase activity between untreated controls (27 samples) and homogenates treated with 0.01 (15), ( $P < 0.001$ ), 0.1 (15), ( $P < 0.001$ ) and 10.0 (16), ( $P < 0.001$ )  $\mu$ units of ACTH per flask. The ordinate is the increase in lipase activity attributable to ACTH. Mean lipase activity by the untreated controls (27) was  $1.46 \pm 0.05$   $\mu$ equiv. FFA (10 mg) 2 h.

in  $\mu$ -equiv. of free fatty acids released into the incubation medium during the time of incubation per 10 mg of fresh weight adrenal tissue.

As seen in Fig. 1, all doses of ACTH which were used in our experiments markedly activated the rate of adrenal lipase, and this stimulation was related to the doses of ACTH. These findings correlate well with many histological results which demonstrated the fall of adrenal lipids after administration of ACTH or stress<sup>4-6</sup> and confirmed the histochemical findings of activated adrenal lipase after administration of ACTH *in vivo*<sup>7</sup>.

It is not yet possible to explain the significance of these findings in relation to the mode of action of ACTH on adrenal steroidogenesis, but several possibilities may be suggested. The neutral fats are depleted by ACTH-activated adrenal lipase and the fatty acids are released. These fatty acids could: (a) be converted to acetoacetyl co-enzyme A and acyl co-enzyme A, which are considered as precursors of adrenal steroids; (b) be oxidized to CO<sub>2</sub> and NAD; NADP reduced during this oxidation could be used in hydroxylating reactions of adrenal steroidogenesis.

In conclusion, ACTH has a definite direct dose-dependent action on the activity of adrenal lipase in the rat adrenal; the relation of this action to adrenal function is not yet clear.

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### Arborization Patterns in Human Bile

FERN-LIKE or coralline arborization patterns have been observed in human bile allowed to desiccate and examined microscopically. Blumenkrantz and Kohan<sup>1</sup> call this phenomenon 'rhythmic crystallization', and suggest a relationship between paucity of arborization patterns and the presence of biliary lithiasis. Their work was carried out on bile obtained by duodenal intubation.

The occurrence of the arborization phenomenon in body fluids is thought to be due to an interaction of macro-

molecules of the mucin type together with electrolytes. The mechanism of formation of these patterns is postulated to be a retardation of the normal crystallization process of sodium chloride when macromolecules are present. In the absence of macromolecules, arborescent and dendritic phases of crystallization are transitory stages in the formation of typical salt crystals according to Blumenkrantz and Moreno<sup>2</sup>.

The arborization phenomenon is of clinical value in the study of mucus from the female genital tract. Arborization is quite marked during periods of oestrogenic activity, and has been shown to be the result of relatively high concentrations of NaCl in cervical mucus<sup>3</sup>.

In this study, human bile from individuals with and without gallstones was obtained by direct puncture of the gallbladder at surgery. Where possible, hepatic bile was obtained from the common duct simultaneously. Hepatic bile was also studied in post-operative patients with 'T' tubes. A drop of bile was placed on a glass slide, allowed to desiccate at 37° C and when, desiccation was complete, studied microscopically.

It was found that hepatic bile always produced the arborization patterns seen in Figs. 1a and 1b. The fern-like pattern tended to occur at the edges of the drop, while

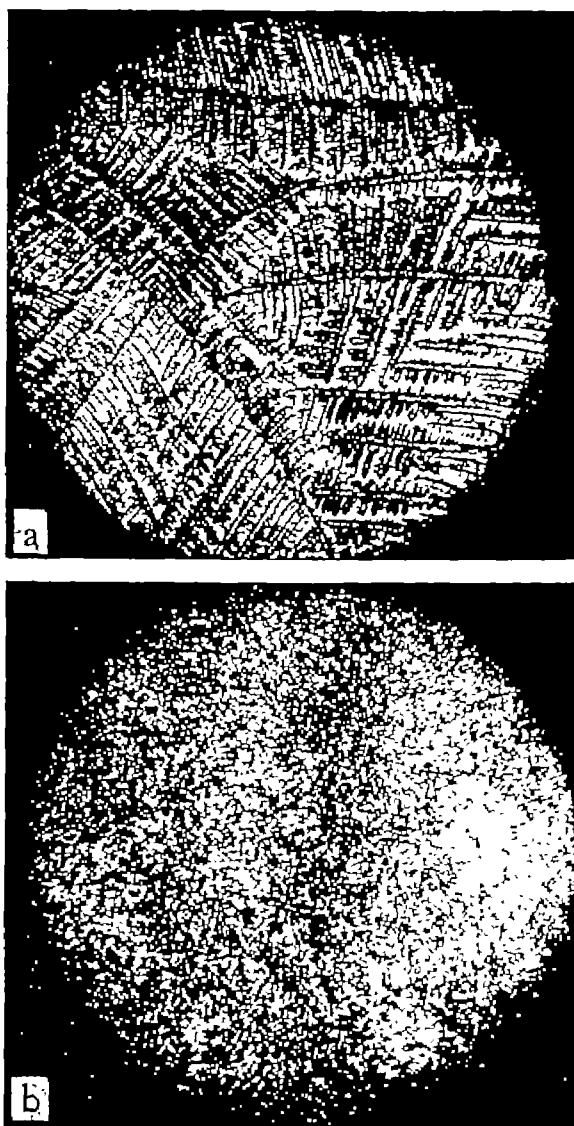


Fig. 1. (a) Fern-like pattern seen in desiccated hepatic bile ( $\times 48$ ); (b) coralline pattern seen in desiccated hepatic bile ( $\times 48$ ).

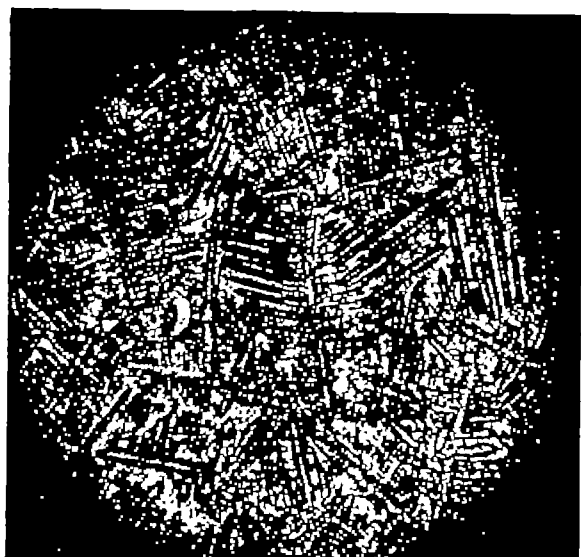


Fig. 2. Fern-like pattern produced in dialysed (electrolyte free) hepatic bile by the addition of NaCl ( $\times 48$ )

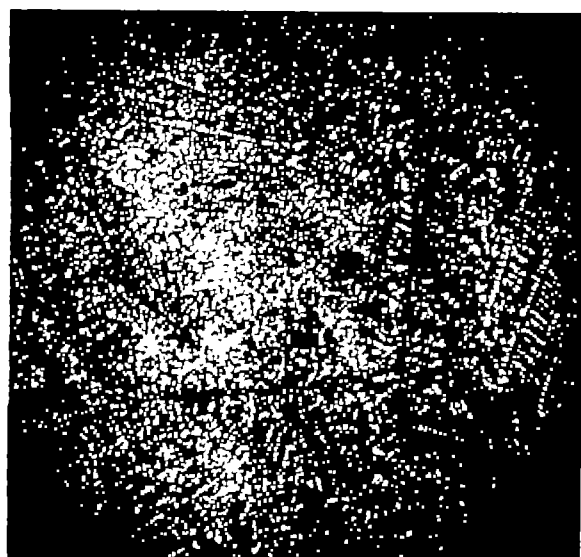


Fig. 3. Fern-like pattern reproduced in gallbladder bile by addition of excess NaCl ( $\times 48$ )

the coralline patterns occurred nearer the centre. On occasion the coralline patterns were absent, and fern patterns predominated. Dialysis of hepatic bile against distilled water caused the arborization patterns to disappear when the bile was desiccated. Addition of NaCl to dialysed hepatic bile caused the patterns to reappear, as seen in Fig. 2. Redialysis against isotonic saline gave the same result. The addition of KCl to dialysed hepatic bile caused a similar but not identical pattern to appear. The addition of  $\text{NaHCO}_3$ ,  $\text{MgCl}_2$  or  $\text{MgSO}_4$  to dialysed hepatic bile did not cause the appearance of arborization patterns. Since the gallbladder concentrates bile, hepatic bile was evaporated *in vacuo* so that it was concentrated up to ten times. The arborization patterns were unchanged.

Gallbladder bile gave little or no arborization pattern. A pattern similar to that in hepatic bile was seen in only one of 22 gallbladder bile specimens examined. This occurred in bile from a gallstone-containing gallbladder. Sparse arborization patterns were seen in two of nine normal gallbladder bile specimens, and in five of 13 gallbladders containing gallstones.

Gallbladder bile, in which the arborization pattern was absent, and hepatic bile, in which the pattern was quite striking, were obtained simultaneously from a patient undergoing operation for cholelithiasis. The addition of NaCl to the gallbladder bile produced the arborization pattern seen in Fig. 3. Dilution of the original gallbladder bile up to ten times with distilled water did not cause the arborization patterns to appear.

These specimens of bile were analysed for sodium, chlorine and potassium in an Autotechnicon 'Auto-Analyzer'. The results obtained agreed well with reported values except that chloride in gallbladder bile was somewhat higher than the values reported, though it was lower than in hepatic bile. The data are summarized in Table 1.

Table 1. ELECTROLYTE CONTENT OF GALLBLADDER AND HEPATIC BILE (Ranges from refs. 4, 5 in parentheses)

	Na m.equiv./l.	Cl m.equiv./l.	K m.equiv./l.
Gallbladder (arborization absent)	201.5 (122-275)	61.8 (15-30)	12.8 (4.7-12.5)
Hepatic (arborization marked)	145 (131-164)	105 (75-110)	6.3 (2.6-17.0)

These results demonstrate a physicochemical difference between gallbladder and hepatic bile. There is an inhibition of the arborization patterns on desiccation of gallbladder bile which is not related to simple passive concentration of hepatic bile. A possible explanation of this inhibition of arborization is that electrolytes may be bound in ionic aggregates or micelles during the process of concentration of bile in the gallbladder. We found no correlation between the arborization phenomenon in human bile and the presence or absence of biliary lithiasis when specimens were obtained directly from the gallbladder or common bile duct.

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### Changes of Histone Composition in the Developing Chick Embryo

THE possible role of histones as gene suppressors has been discussed by many authors<sup>1-3</sup>. If histones do have such a function, one might expect to find some qualitative or quantitative changes in the histone pattern during the course of development, reflecting steps in the processes of differentiation. However, up to now no conclusive evidence of this has been produced. Small changes have been demonstrated in the electrophoretic pattern of total nuclear histone from embryonic chick liver during development<sup>4</sup>.

The following results were obtained from chick embryos from an age of 2 days after incubation and onwards. A quantitative recovery and subsequent fractionation of the total amount of histone, nuclear as well as cytoplasmic, has been attempted.

Organs or whole embryos were homogenized in cold 0.14 M NaCl-0.01 M Na-citrate. The precipitated nucleoprotein was washed at least twice with the saline citrate and the histones were extracted with 0.25 N HCl. The yield was 93-95 per cent of the histone amount directly obtained from acid extraction of acetone-dried material. Acid contaminants, such as polysaccharides, had to be removed, to avoid interference with subsequent fraction-

ation on carboxymethyl cellulose (CMC) (ref. 7). These acid compounds were firmly attached on 'Dowex 1' during afterwashing with 0.1 N HCl, while the histones were quantitatively recovered. Hydrogen ions were removed by filtering on 'Sephadex G 25' with acetate buffer (pH 4, ionic strength 0.02). The recovery of histone after gel-filtration was more than 95 per cent and is higher than after dialysis. The fractionation of the histones was made on CMC (ref. 8). The total recovery was about 90 per cent. Only protein from the  $F_1$  complex soluble in 5 per cent TCA was regarded as highly lysine rich histone. To obtain a stable ratio  $F_1/F_2$ , the CMC-columns were always loaded with the same quantity of histone per weight of ion exchange mass. The three fractions were also characterized by the use of the Sakaguchi reaction. The arginine ratio was in conformity to that of the corresponding fractions from thymus histone. Histones from the early embryos (represented in Fig. 1) were run on micro-columns. All preparations except the fractionations were made at 0–3° C.

The ratio of histones to DNA (w/w) does not vary during embryogenesis. This ratio was  $1.24 \pm 0.019$  in whole embryos during the 4th–16th day of development. In another series of whole embryos, 2–5 days old, the ratio was  $1.10 \pm 0.050$  and without any trend. The somewhat lower value from these young embryos may depend upon the micro-method used for this material. In embryonic livers during the developmental period of 10–20 days, the histone DNA ratio was  $1.21 \pm 0.028$ , also without any tendency to change.

However, if the total histone is separated into the three fractions, lysine rich ( $F_1$ ), slightly lysine rich ( $F_2$ ) and arginine rich ( $F_3$ ), there can be seen a tendency for this histone pattern to change during the early embryonic period (2–5 days). There occurs a gradual transformation of an embryonic pattern in which the amounts of the three fractions are similar to an adult pattern where the amount of  $F_1$  is high and that of  $F_2$  is low. The same trend appears in both experimental series (Fig. 1). The cytoplasm of the early embryonic cells encloses yolk granules. The yolk contains basic protein with a relatively high amount of  $F_1$ . The ratio  $F_1 : F_2 : F_3$  is 47 : 34 : 19. However, we have found that this incorporated protein amounts to less than 10 per cent of the total histone measured in the cell, and therefore the observed change in pattern can only to a small extent be due to a gradual decomposition of cytoplasmic yolk granules.

Furthermore, the change from an embryonic to an adult histone pattern can also be observed as a trend in single organs, erythrocytes and liver, during a later embryonic period, 10–16 days (Fig. 2). In other organs (eyes, heart, brain) such a trend is absent or insignificant and

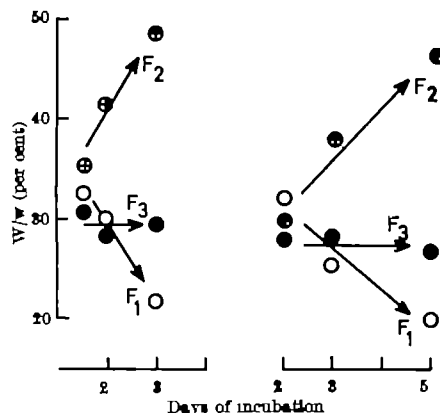


Fig. 1. The relative amount of the three histone fractions during the early development of the chick embryo. The change tendency for each fraction is marked by arrows. White circles,  $F_1$  (lysine-rich histone); cross circles,  $F_2$  (slightly lysine-rich histone); black circles,  $F_3$  (arginine-rich histone). Each point in the diagrams represents values from about 50 whole embryos

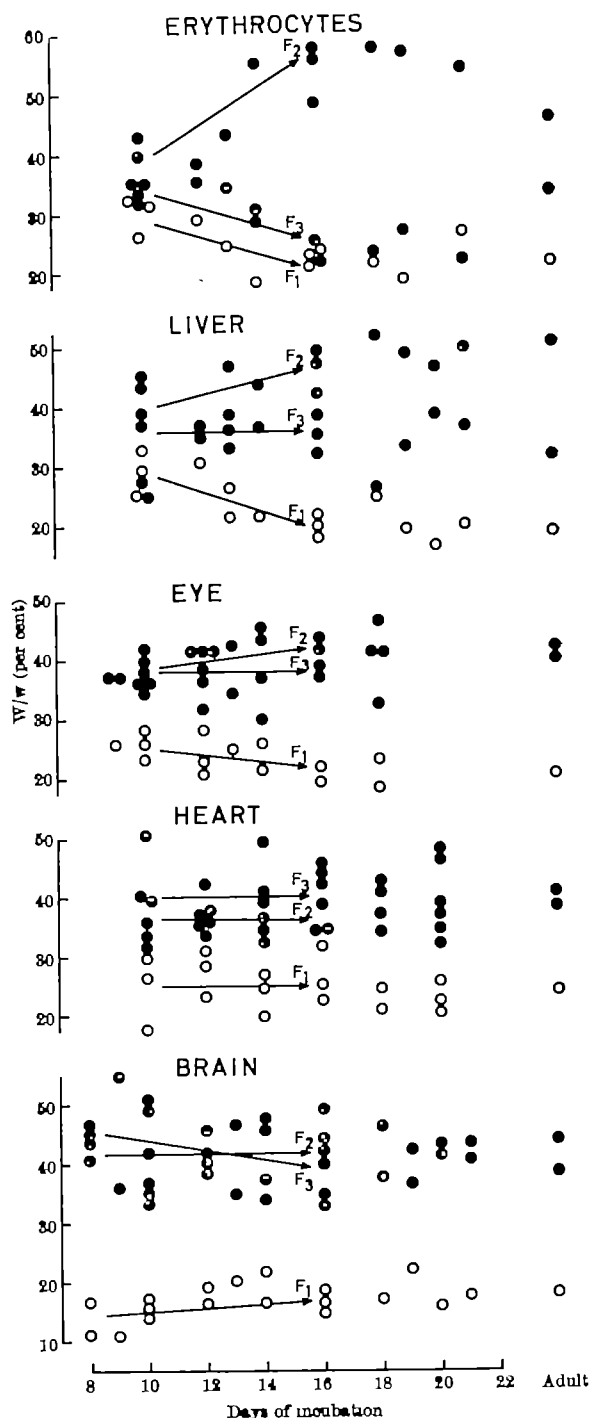


Fig. 2. The relative amount of the three histone fractions in different organs during the later development of the chick embryo. The change tendency for each fraction is marked by arrows. The designations are the same as in Fig. 1. Each point represents an estimation on organs from 20–100 embryos

the adult pattern is established before the 10th day. Whether this indicates that erythrocytes and liver continue to undergo differentiation after the other organs have ceased to do so is, of course, uncertain. We are well aware of the fact that the observed changes in the histone pattern may also reflect metabolic changes, particularly because the histones prepared for these estimations include cytoplasmic histones. However, the possibility that one of the ways in which histones influence differentiation is by masking RNA-templates should not be overlooked. Thus, RNA-associated basic protein in the cytoplasm of blastomeres from cleavage stages of sea-urchin

embryos disappears gradually with advancing development<sup>8</sup>, and it has been found in this laboratory that the non-incubated chick embryo has ribosome-associated basic proteins.

Finally, as can be seen in Fig. 2, the adult histone pattern is different in different organs. In erythrocytes and liver the relative amount of  $F_2$  is intermediate, while in the other organs  $F_2$  is very near in quantity to  $F_1$ . In electropherograms the histone pattern is different in erythrocytes, spleen and testes from chicken<sup>10</sup>.

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### Benzyl Thiocyanate Taint in the Milk of Dairy Cattle Ingesting *Coronopus didymus* Sm.

AN objectionable flavour is found in the milk of dairy cattle which graze on pastures infested with the cruciferous weed *Coronopus didymus* Sm. (bitter cress or land cress)<sup>1</sup>. This taint is of considerable concern in Queensland and New Zealand where the weed is widespread during winter; unlike many other fodder or weed taints in milk, it cannot be removed during the manufacture of cream or butter<sup>1,2</sup>. The taint in milk is characteristically a burning flavour with a sharp odour, while in butter it is a burnt flavour with a burning after-taste.

In earlier investigations<sup>3,4</sup> glucotropaeolin, a mustard oil glucoside<sup>5</sup>, was found to occur in this plant. Benzyl cyanide, benzyl isothiocyanate, dibenzyl disulphide and benzyl mercaptan have been isolated as products of the enzymatic or chemical breakdown of the glucoside. None of these compounds was found to give rise to the characteristic taint when fed to dairy cattle<sup>1,2</sup>, or when added to taint-free milk.

Recently, Virtanen *et al.*<sup>6</sup> found that on crushing the seeds of the cruciferous weed *Lepidium sativum* L. and incubating the crushed seeds in water, benzyl isothiocyanate is the initial breakdown product of the glucotropaeolin which is present in the plant. The isothiocyanate, however, was found to isomerize rapidly in the incubated extract, to give benzyl thiocyanate. This isomerization, observed also in a related *Lepidium* species, was apparently carried out by an enzyme present in the crushed seeds.

Similar experiments have now been carried out on seeds from *C. didymus*. Gas chromatograms and infra-red absorption spectra of extracts of the crushed seeds, after chromatography on alumina, have indicated that benzylthiocyanate ( $\nu$ -SCN 2158  $\text{cm}^{-1}$ ) (ref. 7) is produced in *C. didymus* by isomerization of initially produced benzyl isothiocyanate. The isomerization was apparently complete within 15 min of mixing the powdered seeds with water.

The characteristic off-flavour has now been reproduced in the milk of dairy cattle by either drenching a dairy cow with benzyl thiocyanate dissolved in peanut oil or by the addition of benzyl thiocyanate to taint-free milk. When a dairy cow was fed 3 g benzyl thiocyanate, a taint developed in her milk within 2 h, became very strong after 4–6 h and disappeared within 24 h. The characteristic taint carried through to the derived cream and butter. Addition of

0.16 p.p.m. of benzyl thiocyanate to taint-free milk produced a detectable taint, whereas 4 p.p.m. gave a very strong taint to the milk, and the characteristic burning flavour to the cream.

There is the possibility that benzyl mercaptan may contribute to the associated taint in butter, as has previously been suggested<sup>8</sup>, since benzyl thiocyanate could be reduced to the mercaptan by cysteine or similar reducing agent, during the pasteurization of cream before the manufacture of butter.

Evaluation of flavour of the milk and products in these experiments was carried out by experienced dairy produce graders from the relevant Queensland and Commonwealth Government Departments.

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### Mechanism of the Inhibitory Effect of Adenine Nucleotides on Porphyrin Synthesis by *Rhodospseudomonas spheroides*

EXCESS biosynthesis of porphyrins is strongly decreased by some adenine nucleosides and nucleotides (inosine, 5' AMP, 5' ADP, 5' ATP) *in vivo* as well as *in vitro*<sup>1–3</sup>. Experiments with *Rhodospseudomonas spheroides* have shown that ATP is the most active of these adenine derivatives, determining a more than 90 per cent inhibition<sup>4,5</sup> in a concentration of 3 m.moles/l. of medium. On the other hand, it was established that this effect is confined to the step of ALA formation<sup>3,6</sup>.

To explain this decrease of porphyrin synthesis we have first envisaged, on the basis of the tricarboxylic-glycine cycle of Shemin<sup>4</sup>, a negative feed-back reaction on ALA synthetase. Our recent experiments have shown that this hypothesis is not correct. In *Rhodospseudomonas spheroides* grown anaerobically for 24 h in the light in medium I of Lascelles<sup>7</sup>, ALA synthetase activity was even enhanced when ATP was added to the medium (Table 1).

Table 1. ALA SYNTHETASE ACTIVITY OF EXTRACTS IN MAXIMUM ALA FORMED PER MG PROTEIN OF EXTRACT\*

Exp.	Extracts of micro-organisms grown in presence of ATP (3 m.moles/l. of medium)	Extracts of micro-organisms grown without addition of ATP to the medium
1	66.6	18.3
2	45.0	11.4
3	23.0	4.3

\* 0.5 ml. extract was incubated for 30 min at 37° C and pH 7.4, with glycine, succinate, CoA, pyridoxal, Mg<sup>++</sup>, and ATP (following the technique of Grandik and Urate<sup>8</sup>).

We have therefore assumed that the adenylic derivatives enhance the formation of a physiological inhibitor of porphyrin synthesis, the existence of which in *Rhodospseudomonas spheroides* was reported by Kikuchi and colleagues<sup>7</sup>. This hypothesis seems to be confirmed by our experiments. *Rhodospseudomonas spheroides* suspended in Lascelles medium I to which ATP was added (3 m.moles/l. of medium) were incubated for 24 h semi-anaerobically, in the light. The micro-organisms were then gathered by centrifugation at 0° C and repeatedly washed with 0.9 per cent NaCl solution.

After freezing and thawing, or sonication, of the micro-organisms, extracts were prepared by diluted phosphate buffer (0.02 M, pH 6.8). These extracts added to a new suspension of *Rhodospseudomonas spheroides* significantly inhibited the porphyrin synthesis. Extracts prepared from *Rhodospseudomonas* incubated without ATP exerted no inhibitory effect (Table 2).

Table 2. PORPHYRINS SYNTHESIZED BY *R. spheroides* DURING 18 TO 24 H INCUBATION  
(In  $\mu\text{g}$  per ml. of medium)

Exp.	Without addition of extract to the medium	In presence of extract from <i>R. spheroides</i> incubated with ATP*	In presence of extract from <i>R. spheroides</i> incubated without ATP*
1	70.2	34.0	68.5
2	40.4	16.7	39.5
3	36.2	25.0	32.0

\* 1 ml. extract, containing 1 mg/ml. protein, was added to 10 ml. of Lasecelles medium I.

The experiments show clearly that *Rhodospseudomonas spheroides*, grown in a suitable medium containing 3 mmoles ATP, synthesizes to an increased degree an inhibitor of porphyrin which is extractable from the micro-organism by diluted phosphate buffer.

This inhibitor seems to be a protein and is presumably an enzyme. In fact, it is heat-labile and non-dialysable. Purification of this enzyme, which is in progress in our laboratory, will be necessary to establish its substrate. Our observations provide some evidence that the substrate may be succinic acid, one of the two simple precursors of porphyrins. Addition of succinate to *R. spheroides*, previously incubated in presence of ATP, re-establishes indeed to some extent the greatly decreased porphyrin synthesis. On the other hand glycine, the other precursor of porphyrins, is ineffective in this respect (Table 3).

Table 3. PORPHYRINS SYNTHESIZED BY *R. spheroides*  
(In  $\mu\text{g}$  per ml. of Lasecelles medium I)

Exp.	After 18 h incubation		After 24 h further incubation	
	Without ATP	With ATP*	With ATP* and succinate†	With ATP* and glycine‡
1	19.2	0.13	1.02	0.20
2	22.0	0.20	3.0	0.25
3	47.0	0.20	1.70	

\* 3 m moles/l. of medium.

† 0.001 moles/l. of medium.

‡ 0.005 moles/l. of medium.

A similar effect of succinate was observed when it was added to *R. spheroides* previously incubated with AMP. Our experiments seem to show that ATP and AMP added to *Rhodospseudomonas spheroides*, growing in Lasecelles medium I, anaerobically, in the light, enhance the formation of a presumably physiological inhibitor of porphyrin synthesis. This inhibitor seems to be an enzyme, the substrate of which remains to be established.

**Note added in proof.** In our latest experiments, using extracts obtained with a French press, which disrupts the cell membranes more completely than sonication or freezing and thawing, ALA-synthetase activity was not modified (or enhanced) after incubation of *Rhodospseudomonas spheroides* with ATP. These findings do not confirm the conclusions of our earlier observations.

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## PHYSIOLOGY

### An Inhibitory Component of the Response to Distension of Rat Ileum

THERE is good evidence that the longitudinal smooth muscle of small and large intestine of rat, mouse, guinea-pig and rabbit is supplied with inhibitory nerves arising from intrinsic neurones which are probably components of the myenteric plexus<sup>1,2</sup>. These inhibitory nerves can be excited by transmural stimulation of segments of intestine<sup>3</sup>, by 'field' stimulation of strips of intestinal muscle<sup>4</sup>, or by ganglion stimulants such as dimethyl-4-phenylpiperazinium iodide (DMPP)<sup>1,2</sup>. The relaxation of the longitudinal muscle mediated by these nerves can always be observed in the presence of atropine, 1  $\mu\text{g}/\text{ml}$ , which blocks any muscarinic excitatory effects due to stimulation of cholinergic nerve fibres within the wall of the intestine. Since the inhibitory response is unaffected by concentrations of guanethidine and bretylium which block the relaxation in response to paravascular nerve stimulation, it seems likely that the intrinsic inhibitory system is distinct from the sympathetic system. The conditions which cause reflex activation of the intrinsic inhibitory neurones, *in vivo*, remain to be clarified.

An example of the response of rat ileum, in the presence of atropine, 1  $\mu\text{g}/\text{ml}$ , to transmural stimulation with pulses of 200  $\mu\text{sec}$  duration at 5/sec is shown in Fig. 1a. In this preparation, as described previously<sup>5</sup>, the relaxation was not maintained throughout the period of stimulation. At this frequency and at even lower frequencies which would be unlikely to cause a diminished output of transmitter, rhythmic contractions began again after 5–10 sec. On the cessation of stimulation the tone and amplitude of the contractions were greatly increased. Fig. 1b shows that a very similar response occurs following a small dose of the ganglion stimulant DMPP. During the present experiments we have found that isolated segments of rat intestine give a similar relaxation in response to an increase in intraluminal pressure (Fig. 1c).

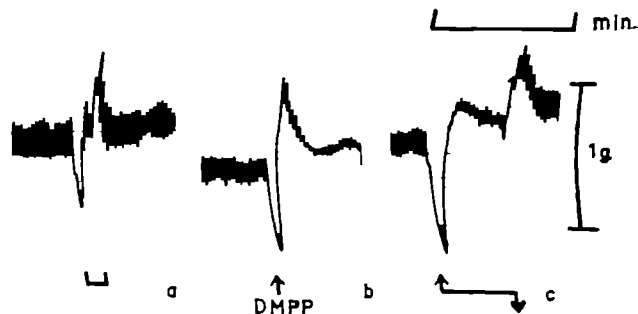


Fig. 1. Rat ileum preparation in atropine, 1  $\mu\text{g}/\text{ml}$ . Tension recorded isometrically from longitudinal muscle. a, Response to transmural stimulation with 200- $\mu\text{sec}$  pulses at 10/sec; b, responses to 1  $\mu\text{g}/\text{ml}$ . DMPP; c, response to increased intraluminal pressure by 2 cm water.

Segments of rat ileum, 2 and 3 cm long, were set up in a modified Krebs solution<sup>6</sup>, in a 70-ml. isolated organ bath at 35° C. One end of the preparation was occluded by a knot of silk thread which was attached to the recording device while the other was tied to a J tube of the type described by Trendelenburg<sup>7</sup>. A platinum wire was passed through the J tube into the lumen of the gut for transmural stimulation. Responses to distension were elicited by introducing Krebs solution into the J tube so that the intraluminal pressure was increased by 1.5–3 cm water. In most experiments the tension in the longitudinal muscle was measured with a force transducer. In some experiments the length of the preparation was recorded with an isotonic lever. In all cases, an increase in intraluminal pressure caused relaxation of the longitudinal muscle. Only a small fraction (less than 0.2 g) of



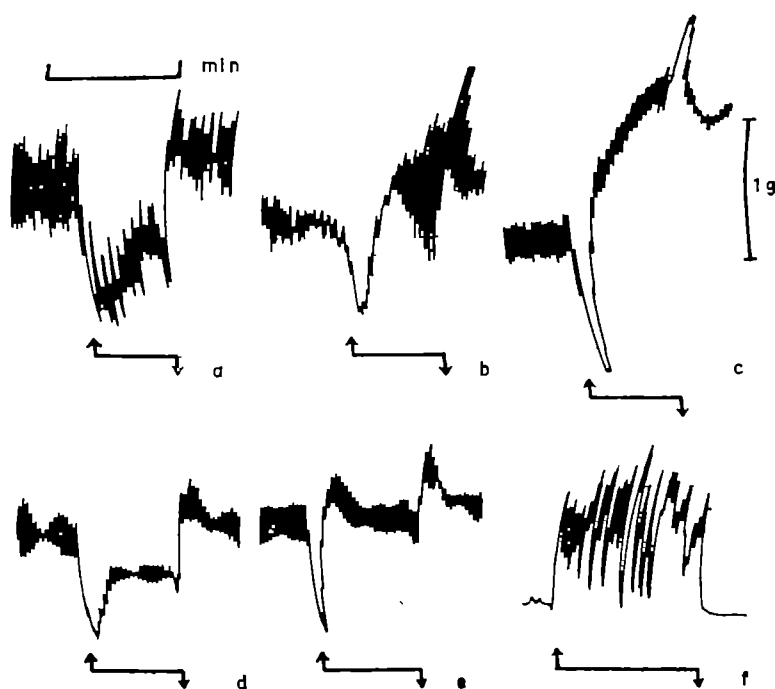


Fig. 2. *a-c*, Responses of five different rat ileum preparations to increased intraluminal pressures of 2.5 cm water. All in atropine 1 µg/ml. *d* and *e*, in hexamethonium, 100 µg/ml, and guanethidine, 50 µg/ml. *f*, response of guinea-pig ileum, in atropine 1 µg/ml, for comparison with rat ileum.

the decrease in tension recorded under isometric conditions was due to the increased intraluminal pressure itself, which constituted a force acting in the opposite direction from that generated by the longitudinal muscle. The absence of any other mechanical artefacts was confirmed by experiments in which procaine, 500 µg/ml., was used to block the reflex response.

The fall in tension elicited by distension was transient. If the increased pressure was maintained, some preparations regained their normal tone after 50–100 sec and some developed tensions which were greater than those recorded during the control period. The tone and beat of other preparations remained below normal throughout the period of distension but there was always some recovery. When the intraluminal pressure was returned to atmospheric pressure again, the longitudinal muscle developed increased tension which resembled the rebound contractions following transmural stimulation and DMPP. Some examples of the response of different preparations of rat ileum in the presence of atropine (1 µg/ml.) to an increase in intraluminal pressure of about 1 min duration are shown in Fig. 2.

These responses of rat ileum differ markedly from those of the guinea-pig ileum, an example of which is shown in Fig. 2*f*. It is well known that distension of the lumen of the guinea-pig ileum causes increased tension and large rhythmic contractions of the longitudinal muscle (the preparatory phase of the peristaltic reflex<sup>6</sup>). Although this response was blocked initially by atropine, 1 µg/ml., it returned during prolonged exposure to the drug, as reported previously<sup>4</sup>. We have occasionally seen a brief relaxation or a delay before the onset of contractions when recording from guinea-pig ileum under isometric conditions. However, it seems likely that the response of this preparation to distension is dominated by excitation.

The initial fall in tension, subsequent recovery and rebound contraction of the rat ileum in response to distension were unaffected by atropine (10 µg/ml.), guanethidine (100 µg/ml.) and hexamethonium (100 µg/ml.). Relaxation in response to transmural stimulation was also resistant to blockade by these drugs. Both responses were blocked by procaine, 500 µg/ml. The only way in

which the response to distension differed from the response to transmural stimulation was in its time course. Although both relaxations were transient, relaxation in response to distension was sometimes of longer duration than the relaxation in response to transmural stimulation.

In some preparations, especially those in which the relaxation was followed by a rapid redevelopment of tension (Fig. 2*b*, *c* and *e*), it was possible to 'block' the response to transmural stimulation by interacting this stimulus with an increase in intraluminal pressure. If the intramural inhibitory nerves were stimulated submaximally during the early part of the recovery phase of a maximal response to distension there was little or no subsequent fall in tension.

The similarity of the responses to distension, transmural stimulation and DMPP suggest that these three types of relaxation may be mediated by the same efferent pathway. We suggest that the afferent fibres which are sensitive to distension of the lumen of the intestine synapse with inhibitory intrinsic neurones which supply the longitudinal muscle of rat ileum.

The receptors of this synapse was unlikely to be of the nicotonic type, since the response to distension is not blocked by hexamethonium. On the other hand, these inhibitory neurones appear to be excitable by cholinomimetic ganglion stimulants. It seems possible that they may be supplied with cholinergic terminals from other intrinsic neurones or from autonomic fibres.

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### Characteristics of Excitatory and Inhibitory Synapses in the Central Nervous System of the Cat

ELECTRON microscopic investigations by Gray<sup>1,2</sup>, De Lorenzo<sup>3</sup> and Hamlyn<sup>4</sup> suggest that there are three types of patches of membrane density in the synaptic contacts of the central nervous system, of which only two are functional (Gray's type 1 and type 2). However, no criterion has yet been established to distinguish the excitatory synapses from the inhibitory ones. In the peripheral autonomic nervous system two types of synaptic vesicles (cholinergic and adrenergic) have been found, the former having a diameter of 500 Å with clear content, while the latter have a diameter of 1000 Å with granular cores<sup>5-7</sup>. So-called granulated vesicles have also been discovered by Shimizu<sup>8</sup> in the hypothalamus of mammals. Gray's classification hints that the type 1 synapse is excitatory, whereas the type 2 is inhibitory<sup>9</sup>.

In our experiments two different types of synaptic vesicle have been found in the somatic nervous system of the cat. The size and shape of each type of synaptic vesicles are quite different, making it easy to differentiate the excitatory synapses from the inhibitory ones. The thorax of a cat was opened under 'Nembutal' anaesthesia and a cannula was inserted into the ascending aorta



Fig. 1. Cross-section of dendrite in the molecular layer of the cat's cerebellum. The dendrite (D) is surrounded by several excitatory synapses (E), and only one inhibitory synapse (I). The bar indicates 0.1  $\mu$ .

through the left ventricle. The blood of the whole body was washed out through the cannula by Locke's solution. After confirming that the whole body had been perfused (no blood from the veins) the Locke's solution was replaced by formalin solution. The osmolarity and hydrogen ion concentration of the fixative were regulated carefully with sucrose and other chemicals. After the fixation of the whole body of the cat, the skull was opened. Thin sheets of the cerebellum were cut off. Small pieces of the out cerebellum were immersed in an ice-chilled osmium tetroxide solution for about 2 h. After routine dehydration and embedding thin sections were made by an LKB ultramicrotome. Electron microscopic investigations were carried out by an Akashi 80 electron microscope. The thick sections were used for the location of the molecular and Purkinje cell layers of the cerebellum using a phase-contrast microscope.

Two quite different types of synapses were easily identified. Fig. 1 shows a dendrite (D) in the molecular layer of the cerebellum of the cat. The dendrite is surrounded by several synapses, of which one type (E-type) contains spherical vesicles having a diameter of about 400 Å, while the other type (I-type) is filled with ellipsoidal vesicles, and the size of the latter vesicles is smaller than the former. The whole surface of the soma of Purkinje cells in the cerebellum is exclusively surrounded by

numerous synapses which have ellipsoidal vesicles in them. It has also been suggested electrophysiologically that the soma of Purkinje cells is densely innervated by inhibitory synapses. So it may be concluded that the synapses containing ellipsoidal vesicles are inhibitory in nature and correspond to Gray's type 2. The dendrites of the outermost part of the molecular layer of the cerebellum are innervated almost exclusively by E-type synapses which are equivalent to Gray's type 1. Electrophysiologically it is highly probable that the E-type synapses are excitatory<sup>3</sup>. Fig. 2, left, shows the schematic representation of a dendrite (D) and its synapses in the molecular layer of the cat's cerebellum. Two inhibitory synapses are indicated (I), while the others are all excitatory. Fig. 2, right, illustrates schematically two types of synapses, the one, the excitatory (E), the other, the inhibitory (I).

The method of synapse differentiation based on the shape and size of synaptic vesicles has enabled us to distinguish quite easily excitatory and inhibitory synapses on the surface of neurones in the central nervous system of mammals. The microtopography of different types of synapses of neurones in the central nervous

system, including the spinal cord, is being carried out. The identification and classification of various types of neurones in the central nervous system may be possible.

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### An Electronically Modified Wet Spirometer

In a recent investigation of respiratory muscles, recordings were made simultaneously from multiple indwelling electrodes, along with spirometric tracings, so that electrical activity could be examined in a series of conscious rabbits during quiet respiration. The electrical signals from the muscles and the spirometric tracing are shown on a film record (Fig. 1). Attention is directed especially to the spirometric tracing which appears as a horizontal band which widens with expiration and narrows with inspiration. A primary interest was to secure an accurate reflexion of the movement of air into and out of the lungs of the rabbit, a factor dependent on the accuracy of the spirometer. Although many spirometers are available, a simple, inexpensive electronic modification of a wet spirometer (400-c.c. Phipps and Bird spirometer, Richmond, Va.) was developed (Fig. 2). The electronic modification of the spirometer, its application, and a method devised to determine its latency from the subject of this communication.

The spirometer is designed so that its lower cylinder can be filled with water and its inverted floating cylinder filled with air. A metal tube within the lower cylinder is connected to an external primary T-tube and carries respiratory gases above the water line and into the

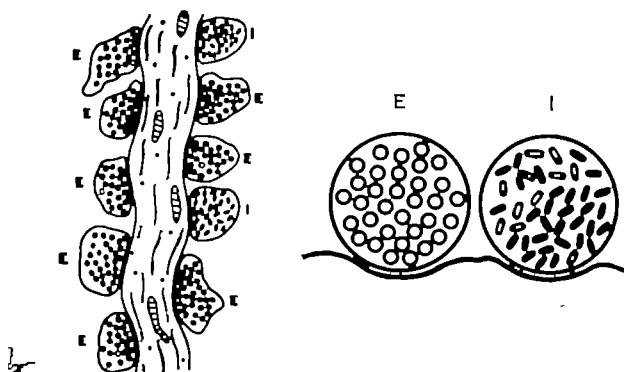


Fig. 2. Schematic representation of longitudinally sectioned dendrite (left) and two types of synapses, excitatory (E) and inhibitory (I).

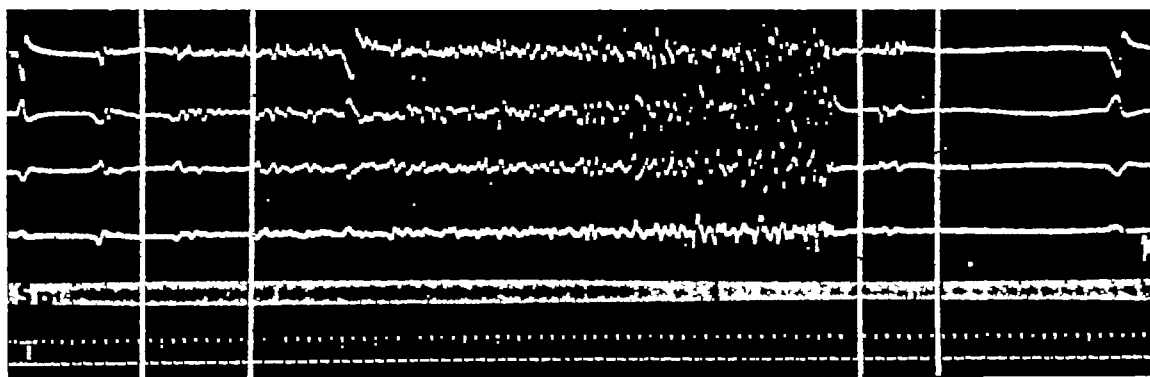


Fig. 1. An electromyogram from separate muscular slips of the diaphragm co-ordinated with spirometry (Sp) and 10-msec time marker (T). The spirogram appears as a horizontal band which widens with expiration and narrows with inspiration

floating cylinder. A secondary T-tube is inserted into another vessel containing soda-lime for the absorption of carbon dioxide. The two T-tubes are interconnected by a rubber tube 10 cm long and 5 mm inside diameter. The other end of the primary T is connected to an oxygen tank by means of a similar rubber tube. Another tube 35 mm long and 5 mm inside diameter leads from the remaining arm of the secondary T to the stem of a Y-tube the arms of which are equipped with a pair of rubber tubes 7.5 cm long. Flexible plastic foetal catheters 4 mm inside diameter and 5 cm long are inserted into the tips of the last-mentioned tubes. The catheters, which are passed into the rabbit's nares during recording sessions, are coated with a topical anaesthetic ointment ('Tromothane hydrochloride') which serves to desensitize the nasal passages as well as to prevent any possible leakage of air around the catheters.

The nasal catheters are well tolerated and the animal will rest in the sternal recumbent position for long periods of time. In an earlier experiment a dental plate with an embedded tube for the passage of air was moulded to fit into the mouth of the rabbit and was in turn connected to the spirometer. The respiratory excursion of the spirometer was nil and it was found that the rabbit breathed mainly through its nose. It was therefore imperative to employ the nasal route.

Under experimental conditions, the animal inspires 50–75 ml. of oxygen, moving the floating cylinder 1–1.5 cm during respiration. The frequency of the respiratory cycle varies from 40 to 50 per min. No evidence of resonance was detected in the system at this frequency and within the range of the volume of gas exchanged.

For the purpose of recording respiratory movements, a modification of the spirometer was devised in order to produce a fluctuating electrical signal capable of translating the excursions of the floating cylinder on a cathode ray oscilloscope. Two small inductance coils 1.5 cm in diameter and 1 cm wide were constructed. Each coil consisted of 150 turns of No. 28 enamel copper wire wound on a plastic spool. One of the coils was passed over a vertical rod which ascends from the floating cylinder and was securely fastened to it 1.5 cm above the top of the cylinder (Fig. 2). Preliminary tests showed that the weight of this coil does not affect the mechanical properties of the spirometer. The second coil was also passed over the vertical rod of the floating cylinder but was fixed in place, independent of that rod, by means of a plastic clip attached to the upright pulley support, 7.5 cm above the lower or movable coil. This arrangement was such as not to interfere in any way with the vertical movement of the rod and cylinder.

In preparing to use the apparatus for experimentation on a rabbit, sufficient oxygen is passed into the spirometer so that the movable coil approaches to within 3–4 cm of the fixed coil. An a.c. signal generator adjusted to

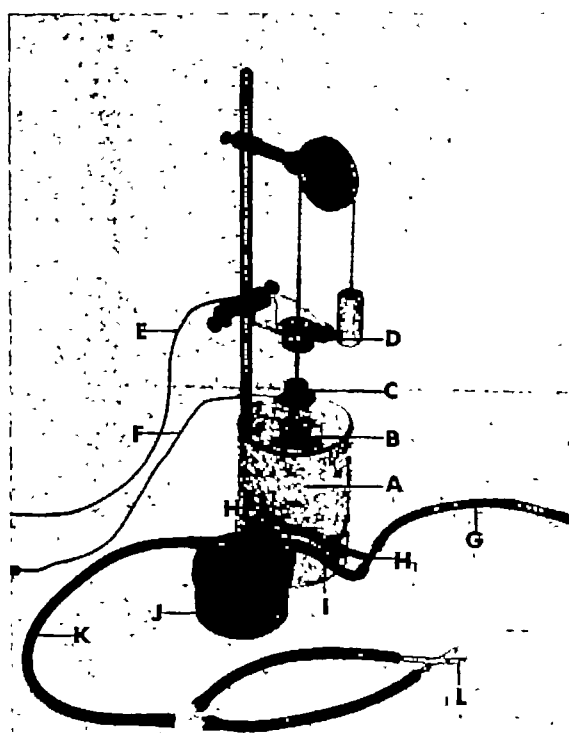


Fig. 2. An electronically modified wet spirometer. A, Lower cylinder; B, floating cylinder; C, movable coil; D, fixed coil; E, lead from fixed coil to one channel of a dual beam oscilloscope; F, lead from signal generator to movable coil; G, rubber tubing from oxygen tank to external primary T; H<sub>1</sub>, external primary T-tube; H<sub>2</sub>, secondary T-tube; I, rubber tubing interconnecting the primary T with the secondary T; J, vessel containing soda lime; K, rubber tubing connected to nasal catheters; L, nasal catheters

produce a 5,000-cycle sine wave was connected to the mobile coil. The fixed or pickup coil was connected to one channel of a dual beam oscilloscope. By variable inductive coupling the fixed coil picks up from the moving coil the 5,000-cycle signal applied to it. Since the output from the fixed coil is proportional to its distance from the moving coil, it follows that the electrical output is proportional to the volume of air fed into the spirometer during each respiratory cycle.

It was deemed important to determine the factor of latency in the spirometer. A method which was devised to determine any latency consisted of setting up the spirometer in the manner described here (Fig. 2) except that the nasal catheters were inserted into a rubber balloon instead of the nose of a rabbit (Fig. 3). The balloon was



NH band at  $3,300\text{ cm}^{-1}$ , a shifted, sharpened CO band at  $1,700\text{ cm}^{-1}$ , and a split  $\text{COO}^-$  band at  $1,550\text{--}1,650\text{ cm}^{-1}$ . The diffuseness of the monohydrate bands indicates a low degree of order, and this conclusion is supported by the crystal densities and solubilities. X-ray diffraction analyses (performed by Prof. T. Doynes of Villanova University) give densities of 1.372 and 1.312 and molecular weights of 348 and 370 (calculated: 349 and 367) for the anhydrate and monohydrate. The anhydrate has lower solubility in water, dimethylformamide, dimethylacetamide, dimethylsulphoxide and acetonitrile. It does not change into the monohydrate when exposed to an atmosphere of 100 per cent humidity, and undissolved crystals remain anhydrous after 24-h suspension in water.

A remarkable difference in  $\beta$ -lactam lability stems directly from the association of a molecule of hydrate water with the penicillin molecule in the solid crystal (Table 1). This difference, established by hydroxamate formation<sup>7</sup>, activity against *E. coli*, and infra-red analysis, is also demonstrable at room temperature and under various conditions of heat plus high humidity, such as autoclaving.

Table 1.  $\beta$ -LACTAM CLEAVAGE IN CRYSTALLINE AMPICILLIN

°C	H	Per cent hydrolysis	
		Anhydrate	Monohydrate
70	66	0	39
70	160	0	61
107	18	2	66
107	90	1	79
135	0-25	4	55

It is apparent from this study that, despite very firm binding within the hydrate crystal, the water molecules are sufficiently free to participate in a solid-state hydrolytic reaction. Whether the reaction is intramolecular or intermolecular, or whether this concept even has validity in such a case, will depend on more precise information on the site of water attachment and the structure of the crystal.

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### Effect of 3,4-Benzpyrene on the Formation and Hydrolysis of Conjugates

REPORTS in the literature postulating the presence of sex differences in conjugation, especially in glucuronide conjugation, led us to demonstrate this point on the Wistar strain of rats by raising the conjugation capacity with a carcinogen, 3,4-benzpyrene (Inscoc and Axelrod<sup>1</sup>). The purpose was to elicit the possible differences more clearly. In the same connexion, we studied both uridine diphosphate glucuronic acid (UDPGA) transferase and total conjugation, as we had found in earlier experiments that these processes do not necessarily have a parallel course. We also observed how, when a carcinogen of this kind shifts the conjugation activity to another level, certain hydrolytic enzymes associated with it, such as  $\beta$ -glucuronidase and arylsulphatase, also undergo a change. In addition, we used Sprague-Dawley rats from another laboratory besides our own Wistar strain.

The groups of Wistar rats from our own laboratory and Sprague-Dawley rats obtained from outside comprised 5-8 animals each.

UDPGA-transferase and *o*-aminophenol conjugation on the slice were analysed by the same methods as in earlier experiments<sup>2</sup>. Acetone powder was prepared from the tissues for the determination of hydrolytic enzymes. The powder was washed once with acetone and dried on phosphorus pentoxide in vacuum. The acetone-dried powder was then dissolved in water and the water-soluble and insoluble fractions were separated by centrifuging the suspension at  $18,000g$  for 30 min. Water-soluble sulphatases A and B, 4-nitrocatecholsulphate as substrate and  $\beta$ -glucuronidase were determined from the aqueous phase. *p*-Nitrophenylsulphate was used as the substrate of fraction C. The methods are modifications of those described in earlier studies<sup>3</sup>.  $\beta$ -Glucuronidase was determined according to Fishman, Springer and Brunetti<sup>4</sup>. The  $\beta$ -glucuronidase remaining in the fraction that was insoluble in water was determined in addition to sulphatase C.

The results are presented in Tables 1, 2 and 3. UDPGA-transferase activity rose distinctly under the influence of benzpyrene in both male and female rats. This applied to both our own Wistar strain and the Sprague-Dawley animals. No notable differences were observed between the activity of the different rat strains and no sex differences were seen in the elevated activities. The total conjugation of *o*-aminophenol in the liver was lower for both the male and female Sprague-Dawley rats than for the Wistar strain. In contrast, the activities in the duodenum did not differ. The situation in the kidneys appeared to be the same as in the liver. 3,4-benzpyrene did not produce a distinct increase in total conjugation in the Wistar strain, but the conjugation in the Sprague-Dawley rats rose to nearly the level of the Wistar animals. It had no major influence on hydrolytic enzymes. The sulphatase A + B fraction in the liver was roughly twice as great for the female as for the male rats. No definite sex differences in the strength of the hydrolysis of the conjugates were noted elsewhere. Most of the  $\beta$ -glucuronidase could be analysed from the water-soluble phase. This method also failed to demonstrate any difference between the animal strains used. On the other hand, it was possible to confirm

Table 1. HYDROLYSIS OF CONJUGATES

	Arylsulphatase type I		Arylsulphatase type II	
	Liver	Kidney	Liver	Kidney
Female controls	378 $\pm$ 64	180 $\pm$ 34	78 $\pm$ 23	127 $\pm$ 36
benzpyrene	333 $\pm$ 79	110 $\pm$ 38	99 $\pm$ 37	100 $\pm$ 17
Male controls	366 $\pm$ 156	183 $\pm$ 80	29 $\pm$ 11	113 $\pm$ 23
benzpyrene	508 $\pm$ 170	106 $\pm$ 43	35 $\pm$ 2	123 $\pm$ 21
	$\beta$ -glucuronidase, water-soluble		$\beta$ -glucuronidase, non-soluble	
	Liver	Kidney	Liver	Kidney
Female controls	186 $\pm$ 74	110 $\pm$ 24	71 $\pm$ 39	15 $\pm$ 5
benzpyrene	118 $\pm$ 13	103 $\pm$ 44	69 $\pm$ 30	16 $\pm$ 3
Male controls	144 $\pm$ 72	115 $\pm$ 6	53 $\pm$ 11	20 $\pm$ 11
benzpyrene	110 $\pm$ 33	83 $\pm$ 14	60 $\pm$ 34	21 $\pm$ 3

Arylsulphatases and  $\beta$ -glucuronidase in rat tissues. Sulphatase II as  $\mu\text{g}$  4-nitrocatechol/h/mg, sulphatase I as  $\mu\text{g}$  *p*-nitrophenol/h/100 mg and  $\beta$ -glucuronidase as  $\mu\text{g}$  phenolphthalein liberated/h/mg. 5 mg 3,4-benzpyrene given intraperitoneally in corn oil in one dose. Rats were killed 48 h later.

Table 2. GLUCURONIDE CONJUGATION WITH SLICES

	Wistar rats		Sprague-Dawley rats	
	male	female	male	female
Liver benzpyrene	316 $\pm$ 56	236 $\pm$ 37	257 $\pm$ 26	249 $\pm$ 25
controls	306 $\pm$ 63	210 $\pm$ 31	115 $\pm$ 25	90 $\pm$ 25
Kidney benzpyrene	190 $\pm$ 30	232 $\pm$ 28	145 $\pm$ 17	149 $\pm$ 9
controls	176 $\pm$ 23	234 $\pm$ 42	138 $\pm$ 11	145 $\pm$ 21
Duodenum benzpyrene	403 $\pm$ 94	488 $\pm$ 58	566 $\pm$ 61	488 $\pm$ 92
controls	427 $\pm$ 52	471 $\pm$ 42	527 $\pm$ 34	345 $\pm$ 53

Results as  $\mu\text{g}$  *o*-aminophenol glucuronide/100 mg dry weight rat tissue. Benzpyrene was given intraperitoneally in one 5-mg dose in corn oil.

Table 3. UDPGA TRANSFERASE ACTIVITY IN RAT LIVER

	Wistar rats		Sprague-Dawley rats	
	male	female	male	female
benzpyrene	228 $\pm$ 24	218 $\pm$ 15	207 $\pm$ 43	207 $\pm$ 50
controls	190 $\pm$ 23	147 $\pm$ 20	136 $\pm$ 29	150 $\pm$ 43

Results as  $\Delta\text{O.D.}$  of *p*-nitrophenol. Benzpyrene was given intraperitoneally in one 5-mg dose in corn oil.

the observation that benzo[a]pyrene has an effect on the transferase activity of UDPGA. An increase occurs also in the total conjugations.

The increase in conjugation capacity caused by this carcinogen is a theoretically interesting defensive reaction by the organism against a noxious agent. This manifest sex difference displayed by hydrolytic enzymes in fraction A+B is evidence of the role of regulation by hormonal factors in the conjugation processes. It may be said that the results obtained in the present study also show how different results can be obtained not only with different animal species but also with different strains of the same species.

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## HISTOCHEMISTRY

### An Evaluation of the Histochemical Demonstration of Certain Pyridine Nucleotide-linked Dehydrogenases

Farber, Sternberg and Dunlap<sup>1,2</sup> have questioned the validity of histochemical techniques for the demonstration of pyridine nucleotide-linked dehydrogenases. They have argued that since the actual reduction of the tetrazole results from its interaction not with a dehydrogenase but with a diaphorase (tetrazole reductase) specific for NADH or NADPH, strictly speaking the reactions are specific only for NADH- or NADPH-tetrazole reductase. Qualitative evidence was presented to show that of five pyridine nucleotide-linked dehydrogenases studied in rat kidney, only two staining patterns emerged, depending on whether the dehydrogenase required NAD<sup>+</sup> or NADP<sup>+</sup> in the reaction medium. While admitting that the tetrazole reductase was responsible for the actual reduction of tetrazole at the expense of reduced pyridine nucleotide, other workers<sup>3,4</sup> have concluded from similar experiments that each dehydrogenase has its own characteristic pattern of distribution. This point of view has been extensively accepted, but anomalies of formazan localization within the cell<sup>5,6</sup> and the observed escape of soluble dehydrogenases into the incubation medium<sup>7-12</sup> support the original conclusions of Farber and his colleagues.

The development of quantitative histochemical methods<sup>11,12-14</sup> for the estimation of dehydrogenase activity in which formazan production from neotetrazolium has been related to the total residual nitrogen of the stained, extracted tissue section has enabled glucose-6-phosphate (G6P) and 6-phosphogluconate (6PG) dehydro-

genases to be studied in frozen sections of rat liver. Although G6P dehydrogenase, or, more accurately, the G6P-neotetrazolium reductase enzyme complex, is fairly active in rat liver, preincubation of sections in 0.1 M glycylglycine buffer pH 7.6 for only a few minutes before incubating in the full reaction medium abolished the activity. It has been shown<sup>15</sup> that about 70 per cent of the total nitrogen of frozen sections of rat liver was lost during the first 5 min of incubation in an aqueous medium. In addition, direct measurements have established that G6P- and 6PG-dehydrogenases escaped into the incubation medium and reduced NADP<sup>+</sup> at the expense of substrate outside the section<sup>11,12</sup>, while in the preincubation experiments the enzymes were lost during the preincubation. These data suggest that cells of different types might be expected to show formazan deposition more in proportion to the relative activities of NADPH-tetrazole reductase at rate-limiting concentrations of NADPH than in proportion to their original dehydrogenase content.

This possibility has been tested in the following experiments. Rats were killed by asphyxiation with nitrogen, and pieces of liver and heart about 4 mm<sup>3</sup> were frozen in cold tubes<sup>17</sup> at -70° C and in hexane<sup>18</sup> at the same temperature respectively. Sections were cut in a cryostat at -25° C, the knife being chilled with solid carbon dioxide<sup>19</sup>, and mounted near the ends of microscope slides. For quantitative experiments the incubation media were as follows.

(a) *G6P-neotetrazolium reductase*: 0.1 M glycylglycine buffer, pH 7.6; NADP<sup>+</sup>, 2.0-2.5 mg/ml.; G6P, 4.5 mM.; neotetrazolium chloride, 1.5 mM.; cyanide, 10 mM.

(b) *6PG-neotetrazolium reductase*: the medium was identical, but 6PG was substituted for G6P at the same molarity.

(c) *NADPH-neotetrazolium reductase*: both substrate and NADP<sup>+</sup> were replaced by NADPH (2.0-2.5 mg/ml.). Sections were incubated singly at 37° C in microcells<sup>14</sup> containing 0.18 ml. incubation medium (internal diameter of cell, 8 mm). Sections of both tissues were mounted adjacently on the same slide and incubated together (double technique) in larger microcells containing 0.35 ml. incubation medium (internal diameter, 12.5 mm). Formazan was produced exclusively on the sections and not in the incubation medium. After washing and drying, the stained sections were quantitatively analysed<sup>13,14</sup>.

As shown in Table 1, the values found for the activities of G6P- and 6PG-neotetrazolium reductases were much higher in heart sections which were incubated with liver sections than when the heart tissue was incubated on its own. NADPH-neotetrazolium reductase was equally active in sections incubated either by the single or by the double technique. Control sections incubated in the absence of substrate or NADPH gave negligible reactions, and the activities were too low to measure.

Similar effects have been demonstrated qualitatively under slightly modified conditions. In recent histochemical studies nitro-blue tetrazolium<sup>20,21</sup> and tetranitro-blue tetrazolium (TNBT)<sup>22,23,24</sup> have been generally preferred to neotetrazolium on account of their greater ease of reduction and the fineness of the corresponding formazan

Table 1. ACTIVITIES OF NADPH-, GLUCOSE-6-PHOSPHATE- AND 6-PHOSPHOGLUCONATE-NEOTETRAZOLIUM REDUCTASES IN FROZEN SECTIONS OF HEART AND LIVER INCUBATED SINGLY AND TOGETHER. Numbers in parentheses indicate number of sections analysed per determination. Time of incubation, 2 h at 37°, except second group of NADPH-neotetrazolium reductase experiments (30 min at 37°)

Experiment	Enzyme complex studied	Enzymatic activities, µg formazan/µg residual nitrogen/h			
		Liver		Heart	
		Single technique	Double technique	Single technique	Double technique
I	NADPH-neotetrazolium reductase	4.1 ± 0.1 (2)	—	1.4 ± 0.02 (2)	—
	G6P-neotetrazolium reductase	2.9 (1)	1.0 ± 0.06 (4)	0.10 (1)	0.70 ± 0.05 (2)
II	NADPH-neotetrazolium reductase	6.8 ± 0.56 (3)	7.1 ± 0.27 (2)	1.1 ± 0.05 (3)	1.1 ± 0.04 (2)
	6PG-neotetrazolium reductase	0.90 ± 0.12 (3)	0.54 ± 0.10 (2)	0.025 (2)	0.26 ± 0.01 (2)

deposits, but the high degree of substantivity which is shown by the formazans makes these tetrazoles unsuitable for quantitative studies of the kind described. In view of the widespread use of these compounds, experiments have been carried out in which sections of rat liver have been either incubated alone or pretreated with 0.15 M saline for 5 min before being incubated singly or with untreated sections. The incubation media contained 0.5 mM TNBT, 0.1 M phosphate buffer pH 7.6, NAD<sup>+</sup> or NADP<sup>+</sup> (2.0–2.5 mg/ml.) and 50 mM substrate. Substrates tested with NAD<sup>+</sup> were DL-lactate, DL-β-hydroxybutyrate, L-glutamate, α-glycerophosphate and DL-malate; substrates tested with NADP<sup>+</sup> were DL-isocitrate, G6P and 6PG. NADH- and NADPH-TNBT reductases were also studied, the levels of reduced pyridine nucleotides being 2.0–2.5 mg/ml. For lactate, β-hydroxybutyrate and NADH-TNBT reductases the staining time was 15 min; for all other enzyme complexes the staining time was 60 min. The reactions were carried out at room temperature.

Preincubation in saline had no detectable effect on the activity of NADH-TNBT reductase; similar results were found with NADPH-TNBT reductase, but in some experiments very slight losses of enzymatic activity may have occurred. Without exception, all substrate-linked enzyme complexes examined showed extensive or complete loss of activity following incubation in saline. The degree of recovery of enzymatic activity by saline-pretreated sections incubated together with untreated sections in the same microcells depended on the particular enzyme studied. Of the NAD<sup>+</sup>-linked enzyme complexes, extensive recovery was obtained in the presence of glutamate, and some recovery was shown in the presence of lactate or α-glycerophosphate. Neither malate nor β-hydroxybutyrate caused any apparent recovery. In the case of the three NADP<sup>+</sup>-linked systems tested, significant deposition of formazan was found associated with all the saline-pretreated sections.

Formazan production by frozen sections of heart muscle was low in the presence of either G6P or 6PG as substrate, but was considerably increased by incubating heart tissue with an active section of frozen liver simultaneously in the same solution. Substrate-linked TNBT reduction was sharply decreased or even abolished by preincubation procedures, but enzymatic activity was partially restored in certain cases by incubating together with untreated sections in the same solution. Qualitative<sup>7,8</sup> and quantitative<sup>10,12</sup> investigations of lactate dehydrogenase have indicated that this enzyme is substantially lost into the incubation medium from fresh frozen sections; it has also been quantitatively shown that G6P dehydrogenase<sup>8,11,12</sup> and β-hydroxybutyrate dehydrogenase<sup>12</sup> are lost under similar conditions. The results recorded here strongly suggest that the dehydrogenases escaped rapidly from the sections into the incubation medium, where substrates were oxidized with the concomitant reduction of pyridine nucleotide. The reduced pyridine nucleotide diffused back to the sections, and was oxidized by the appropriate tetrazole reductase at the expense of a tetrazole. It is therefore inadvisable to compare pyridine nucleotide-linked dehydrogenase activities in cells on a section on the basis of the intensities of formazan deposition, since the amount of formazan produced is not necessarily related to the amount of dehydrogenase activity originally present in the cells. Farber<sup>7</sup> has pointed out the importance in histochemical investigations involving the use of tetrazoles of showing that the dehydrogenase and the reduced pyridine nucleotide coenzyme which it generates remain bound to the tissue during incubation. Since the retention of enzymes may depend to some extent on the method of tissue preparation and will certainly depend on the subsequent treatment, the advisability of testing dehydrogenase systems under consideration for loss of enzymatic activity either by the techniques described or directly or both cannot be overstressed.

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### Non-specific Alkaline Phosphatase Activity of Evaginating Epithelia

NON-SPECIFIC alkaline phosphatase activity has been demonstrated in the developing central nervous system, eye, lung and the allantois of the human embryo by Rossi *et al.*<sup>1</sup>, in the thyroid of the rat by McAlpine<sup>2</sup>, in the central nervous system, eye, ear and other sites in the chick embryo by Moog<sup>3</sup> and also in the pituitary and thyroid by Kaneko<sup>4</sup>. All the structures which show a high level of activity of this type develop by a process of evagination from an epithelium, and it has been suggested that alkaline phosphatase activity may be found in all epithelial evaginations during organogenesis<sup>5</sup>. No special examination of the association between phosphatase activity and evagination has been carried out, however. Forty-two chick embryos of known incubation time which formed a graded series from 30 h to 6 days were fixed in 70 per cent ethyl alcohol at 20° C. This method of fixation was found to be the most satisfactory and convenient. Whole embryos were embedded in paraffin wax and serial sections cut at 10μ. Alkaline phosphatase activity was demonstrated by means of an azo-dye method as described by Pearce<sup>6</sup>. The incubation period was 1 h.

Alkaline phosphatase activity was noted in the invaginating neural tube and its derivatives, the cerebral hemispheres, neurohypophysis and primary optic vesicle, and subsequently in the margins of the choroid fissure and the secondary optic vesicle. The renal tubules, dorsal and



ventral portions of the myotomes, biliary ducts, gall bladder, dorsal and ventral pancreatic anlagen likewise exhibit a high enzymatic activity. During the development of the ear, phosphatase activity was found in the otic vesicle, ductus endolymphaticus, lagema, semi-circular canal pouches, anterior recess of the utricle and ampullae. High levels were also found in Rathke's pouch, the anterior portion of the mesobranchial groove from which the thyroid is derived, the posterior laryngo-tracheal portion of the mesobranchial groove and afterwards in the trachea, mesobronchi, and entobronchi. Similar levels of phosphatase activity may be found in similar sites in the rat.

Further, when bronchial buds from a foetal rat were explanted *in vitro* and cultured for 4 days, new growth of irregular evaginating tissue also showed alkaline phosphatase activity. Under these conditions, the enzyme must have been formed by the evaginating tissue.

In all the evaginations examined, alkaline phosphatase activity was found during the active stage of the process, this activity being of a transient nature and disappearing as each evagination approached completion and was located on the concave aspect of each structure examined.

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## PATHOLOGY

### Behaviour of Intracerebral Autografts of Mouse Tail Skin pre-treated with a Single Application of 20-Methylcholanthrene

THURBORN grafts of tail skin from 30 mice of various strains and both sexes were implanted intracerebrally in an attempt to produce an expanding, space-occupying, intracranial lesion, and to investigate its effect on the surrounding brain, with particular reference to 'cerebral oedema'. The autografts, which were cut with a Castroviejo keratome set to a thickness of 0.2 mm, measured approximately 3 mm × 1 mm. Immediately after removal they were inserted into the right cerebral hemisphere with a blunt probe, through a small hole made over the lateral aspect of the skull with a dental burr. All operative procedures were performed under 'Nembutal' anaesthesia.

The grafts survived, and in most of the mice killed after 2-4 months had given rise to small intracerebral dermoid cysts containing non-laminated debris including keratin and hair shafts (Fig. 1). The implants were not associated with recognizable neurological signs during life. After 8-10 months most cysts showed atrophy of the epidermal lining and a varying degree of obliteration by a surrounding fibro-granulomatous and to a lesser extent gliotic reaction. It appeared that the cysts neither underwent continuous enlargement for more than 4 months nor had pronounced effects on the surrounding brain.

One of us<sup>1</sup> showed that the application of a single, minute dose of 0.1 per cent 20-methylcholanthrene (MCA) in acetone to a 10 mm × 3 mm strip of mouse tail



Fig. 1. Intracerebral dermoid cyst containing non-laminated debris (haematoxylin and eosin × 35)



Fig. 2. Intracerebral cyst containing compact laminated keratin (haematoxylin-Van Gieson × 35)

skin (equivalent to approximately 5 µg MCA per graft) accelerated the growth of the treated skin, but only when it was transplanted into another site (subcutaneous tissues of the flank); further, in the walls of some of the resultant cysts, keratinizing squamous carcinomas developed. If the treated tail skin was left *in situ*, repeated painting with similar or larger doses of MCA did not result in tumour formation.

We repeated these experiments on 24 mice, substituting intracerebral for subcutaneous transplantation, either 0.5 h or 48 h after painting with the same dose of MCA in acetone; in the 48 h group the treated site was covered with a glass tube. Control experiments on 12 mice using tail skin painted with acetone only gave results identical to the original experiments using untreated skin. In contrast, the growth of the MCA-treated grafts was accelerated and after 4 weeks prominent epithelial-lined cysts filled mainly with compact laminated keratin had developed (Fig. 2). Within 2 weeks there was a greatly increased number of mitoses in the basal layer of the cyst lining, as shown by the injection of colchicine (0.2 mg/100 g body-wt.) 6 h before death. Of the 9 mice killed between 4 and 12 months after transplantation, only 3 appeared normal before death, and these showed obliterated intracerebral cysts on histological examination. The remaining 6



Fig. 3. Keratinizing squamous carcinoma in the wall of a cyst (haematoxylin and eosin,  $\times 150$ )

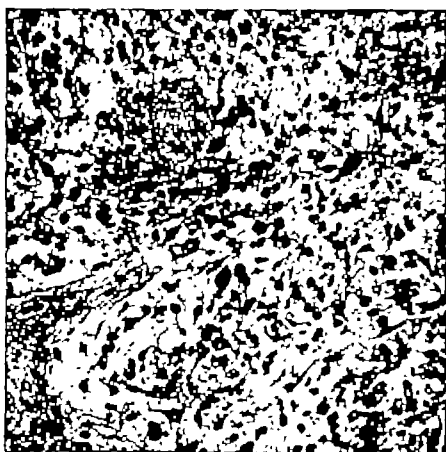


Fig. 4. Well-differentiated astrocytic glioma at site of implantation (haematoxylin and eosin,  $\times 150$ )

mice showed marked loss of weight and akinesia and in 3 there was swelling of the cranium; 3 animals showed histological squamous carcinoma in the walls of the cysts (Fig. 3); in 2 the grafts were replaced by an abnormal cellular proliferation which resembled astrocytic glioma (Fig. 4); and one showed a lipogranulomatous reaction which also included undifferentiated neoplasm. In all the 6 animals with tumours the surrounding brain tissue showed the histological features of well-marked oedema and consecutive gliosis; similar but less pronounced changes developed around the non-neoplastic cysts. There were no detectable differences between the grafts transplanted 0.5 h and 48 h after MCA treatment.

It is shown, therefore: (1) Pre-treatment of small Thiersch grafts of tail skin with a single minute dose of MCA before intracerebral transplantation frequently induces an expanding intracranial cystic lesion. (2) In some instances squamous carcinoma develops in the transplanted skin. (3) Reactive and sometimes neoplastic changes occur in the adjacent brain tissue.

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### Demonstration of 5-Hydroxytryptamine in Blood Platelets by Electron Microscope Autoradiography

MAMMALIAN blood platelets are known to contain 5-hydroxytryptamine (5-HT) and to concentrate 5-HT added to platelet-rich plasma *in vitro*<sup>1</sup>. Hughes and Brodie<sup>2</sup> suggested that 5-HT existed in an unbound form in the platelet. The maintenance of increased platelet 5-HT was believed to be best explained by the presence of an active transport system for 5-HT<sup>3,4</sup>. The finding that the amount of 5-HT in platelets is related to the content of adenosine triphosphate (ATP) has led to the suggestion that the amine is bound to ATP<sup>4</sup>, and investigations using density-gradient centrifugation of disintegrated platelets have demonstrated a fraction containing most of the 5-HT and ATP<sup>5</sup>. More recently, in experiments with platelets disrupted ultrasonically and subjected to differential centrifugation 80 per cent of the 5-HT was recovered in the sedimented fraction, suggesting that 5-HT was bound within granules<sup>6</sup>.

New methods for improving autoradiographic resolution and sensitivity<sup>7</sup> suggested that it should be possible to investigate the localization of platelet 5-HT at the ultra-structural level. To determine 5-HT loss from platelets during the fixation and dehydration used for electron microscopy 5-HT-3'-<sup>14</sup>C creatinine sulphate (specific activity 32 mc./mmole obtained from the Radiochemical Centre, Amersham) was dissolved in 0.15 M sodium chloride, and incubated with rabbit platelet rich plasma (PRP) according to the method of Hardisty and Stacey<sup>1</sup>. PRP was obtained by differential centrifugation after collection of carotid artery blood in 1/10 volume of citrate solution<sup>8</sup>. PRP specimens contained 150–250,000 platelets per cu.mm. The final concentration of 5-HT (expressed as free base) was 1.5  $\mu$ g/ml. PRP. After incubation for 90 min at 37° C the PRP was centrifuged, the supernatant plasma was decanted, and the platelet button was resuspended in 1 ml. cold 0.15 M sodium chloride. After further centrifugation the sodium chloride solution was decanted. 1.1 ml. of 0.02 N hydrochloric acid was added to the platelet button which was frozen and thawed twice. Aliquots of hydrochloric acid were plated on aluminium planchets and counted in an end-window Geiger-Müller counter, to determine the amount of 5-HT taken up by the platelets. To determine loss during fixation and dehydration the platelet button was fixed at 4° by the procedures indicated in Table 1, and afterwards dehydrated in graded alcohols, also at 4° C. Aliquots of fixative and alcohols were taken to determine 5-HT activity as a fraction of the total activity<sup>9</sup>. Representative 5-HT loss is shown in Table 1.

PRP for electron microscopy was incubated for 40 and 90 min with tritiated 5-HT creatinine sulphate (final concentration 1.5  $\mu$ g/ml.) and centrifuged at 4° C to obtain a platelet button (the tritiated 5-HT creatinine sulphate was supplied by Dr. E. A. Evans, Radiochemical Centre, Amersham, Bucks. Specific activity 7.7 c./mmole. Radiochemical purity 90 per cent. At least 80 per cent of the tritium was present in the indole ring). The platelets were resuspended in chilled 0.15 M sodium chloride and

Table 1. PERCENTAGE LOSS OF PLATELET 5-HT DURING FIXATION AND DEHYDRATION

Fixative	Loss in fixative	Loss in 0.1 M phosphate buffer wash Buffer wash unnecessary	Loss in alcohols	Total
1% Osmium tetroxide in Caulfield's buffer (30 min, 4° C)	13.0			20.3
3% Glutaraldehyde in 0.1 M phosphate buffer (40 min, 4° C)	2.5	1.5	19.0	23.0
3% Glutaraldehyde in 0.1 M phosphate buffer with 9% sucrose (40 min, 4° C)	2.2	3.3		
3% Glutaraldehyde (30 min) washed with 0.1 M phosphate buffer, followed by 1% osmium tetroxide (10 min)	10.0	1.8		

recentrifuged, and the sodium chloride was removed. Platelet buttons were then fixed as indicated in Table 1, with osmium tetroxide, glutaraldehyde or glutaraldehyde followed by osmium tetroxide. After dehydration in graded alcohols the tissue was embedded in 'Araldite'. Pale gold sections were cut on a Huxley (Cambridge) microtome, and processed for autoradiography by the method of Salpeter and Bachmann<sup>7</sup> using Kodak NTE emulsion. In certain cases sections were prepared according to the method of Koehler *et al.*<sup>10</sup> using standard glass slides to which NTE emulsion was applied with a pipette and then drained. The slides were dried in air, placed in boxes, and then exposed for 3-6 weeks in a light tight metal canister, containing silica gel, in an atmosphere of nitrogen. Slides were developed in Kodak 'Dektol' after gold intensification<sup>8</sup>. Sections were examined in a Philips '100B' electron microscope at 60 kV.

Background silver grains on control sections free of radioactivity were negligible in number. Silver grains were evident over platelets containing radioactivity (Fig. 1). The proportion of grains over platelets was compared to the area of the print occupied by platelets, as estimated by the method of Chalkley<sup>11</sup>. In three different fields the percentages of the total grains in the field which were over platelets were 91, 90 and 88 (mean number of grains per field: 225). These compare with percentage areas occupied by platelets of 58, 61 and 57 respectively. The difference ( $P < 0.01$ ) indicates a significant concentration of radioactivity within platelets. Developed silver grains were found in relation to dense granules, throughout the hyaloplasm, and at or adjacent to the limiting membrane (Fig. 2). The possibility that movement of 5-HT occurred during fixation and dehydration precludes any firm conclusion about platelet 5-HT localization, but experiments are in progress to eliminate or minimise such movement during processing.

Since aldehydes are known to form a tetrahydro-4-carboline derivative with 5-HT<sup>12</sup>, it is of interest that glutaraldehyde fixed platelets lost 5-HT mainly during alcohol dehydration, while osmium fixation resulted in a much larger loss during fixation. Although losses occurred at different stages the distribution of developed silver grains appeared to be similar in each case.

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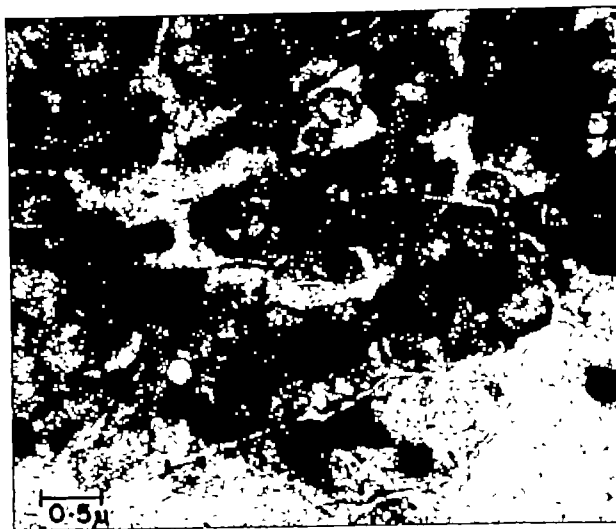


Fig. 2. Rabbit platelets. Osmium tetroxide fixation. Kodak 'Dektol' development. Arrows indicate developed silver grains within alpha granules. Lead citrate stain. ( $\times 18,000$ )

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### Beneficial Effect of Oxytetracycline in Cortisone-induced Wasting Disease

INJECTION of hydrocortisone into new-born mice results in the development of a wasting syndrome similar to that seen in mice after neonatal thymectomy<sup>1</sup>. McIntire *et al.*<sup>2</sup> suggested that the wasting disease seen in neonatally thymectomized mice is associated with an infectious process as the wasting syndrome does not appear in neonatally thymectomized germ-free mice. Administration of antibiotics to neonatally thymectomized rats has been shown to influence favourably the subsequent development of wasting disease in these animals<sup>3,4</sup>.

During experiments performed to investigate the effects on various immunologic functions of both neonatally cortisone-treated and neonatally thymectomized C3H and C57BL mice some animals from both groups received oxytetracycline in their drinking water. Groups of inbred C3H and C57BL mice of both sexes were given 0.1 mg/g doses of hydrocortisone acetate at 4 days of age. Littermates were thymectomized or sham-thymectomized at 1-2 days of age. Treated animals were caged with two groups of foster mothers, one of which had 5 mg per cent oxytetracycline added to the drinking water. The animals were weaned after 20-30 days.

All the animals receiving hydrocortisone initially showed retardation of development, evidenced by subnormal weight gain and poor growth of hair. However, the animals nursed by mothers receiving oxytetracycline in

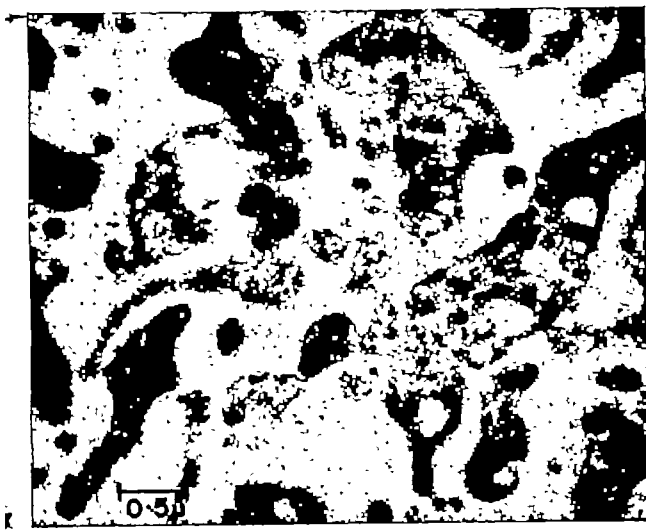


Fig. 1. Rabbit platelets. Glutaraldehyde fixation, followed by osmium tetroxide. Kodak 'Dektol' development, with gold intensification. Developed silver grains lie primarily over platelets. Unstained. ( $\times 18,000$ )

the drinking water soon began to show an accelerated rate of weight gain, better development of hair and heightened physical activity. The differences between some littermates were noticeable at ten days. Afterwards, the animals receiving terramycin developed relatively normally, while cortisone-treated animals not receiving terramycin developed severe wasting disease and began to die between 20 and 30 days. About this time the thymectomized animals also began to show signs of retarded development (Fig. 1).

Animals dying with cortisone-induced wasting disease showed lymphoid hypoplasia, poor development of sub-

cutaneous fat and connective tissue, and collections of polymorphonuclear leukocytes in the lungs and bronchi, wall of the small bowel (Fig. 2) and hepatic portal tracts. Pair-killed littermates having terramycin in their drinking water showed lymphoid hypoplasia but no prominent infectious disease histologically. Development of connective tissues in animals of this group older than about 30 days also did not seem impaired compared to sham-thymectomized animals of similar age.

Schlesinger and Mark<sup>1</sup> suggest that the cortisone-induced wasting syndrome may result from metabolic disorders induced by the loss of thymic factors normally antagonistic to steroids. Glucocorticoids are known to inhibit mitosis<sup>2</sup> and Lehtiharju *et al.*<sup>3</sup> have shown that steroids may differentially inhibit DNA synthesis in mouse tissues. It seems possible that inhibition of development of the lymphoid tissues of the mouse by steroids might allow infectious agents to invade mouse tissues during the first weeks of life. The beneficial effect of oxytetracycline shown here suggests that infectious processes could contribute to the poor development and early death of the mice initiated by the neonatally administered steroids.

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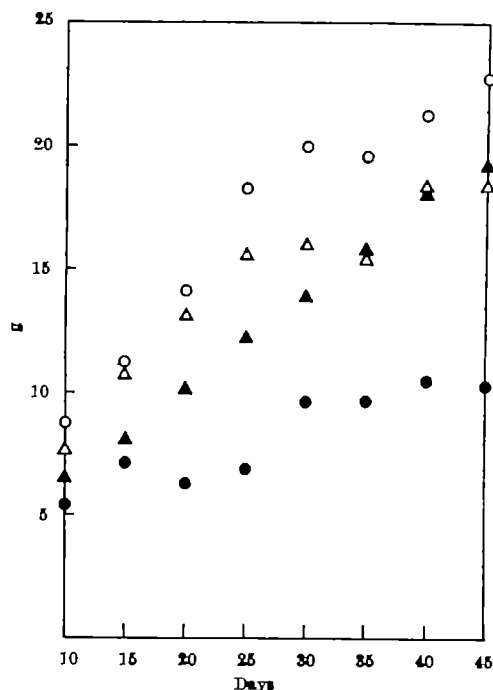


Fig. 1. Means of weights of C57BL male survivors at time-interval after birth. Sham-thymectomized, 1-2 days (○) (15 mice); thymectomized, 1-2 days (Δ) (11 mice); hydrocortisone (4 days) and terramycin (Δ) (12 mice); hydrocortisone, 4 days (●) (9 mice except mean of 3 at 30, 35 and 40 days, mean of 2 at 45 days). Similar results were seen for other sex and strains used.

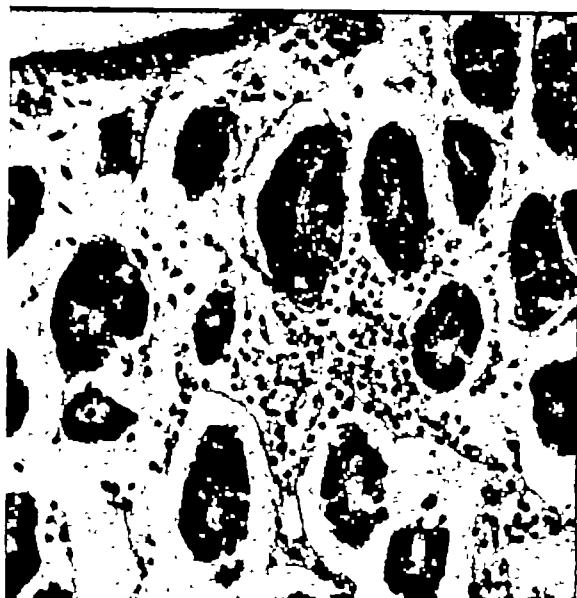


Fig. 2. Wall of small bowel C57BL mouse, 18 days of age, 0.1 mg/g hydrocortisone at 4 days. Note poor development of supporting connective tissue and muscularis. Collection of polymorphonuclear leukocytes and histiocytes in mucosa ( $\times 240$ ).

## Tissue Distribution of Zinc-65 in Tumour Tissue and Normal Tissue in Man

Zinc is a trace metal of physiological importance which is widely distributed in many enzyme systems. Changes in the activity of certain enzymes have been reported in normal and tumour tissues of tumour-bearing animals<sup>1</sup> and of man<sup>2</sup>, and in the serum of patients with neoplastic diseases<sup>3-7</sup>. Since the activity of some enzymes depends on the presence of specific trace metals, cellular derangement resulting in neoplasia may be linked with changes in trace metal concentration. Differences in zinc concentration in normal and tumour tissue in experimental animals and in man have been previously reported<sup>8-11</sup>.

In the work recorded here on man the uptake of zinc-65 in tumour tissue localized at different sites in the body was determined and was compared with that of the corresponding normal tissues. Samples of tumour tissue and of adjacent normal tissue were obtained at autopsy from nine patients with advanced neoplastic disease at time-intervals ranging from 1 to 71 days after the injection of a single intravenous dose of zinc-65 chloride of high specific activity. The age, the diagnoses of the patients and the type and localization of the different tumours in the body are listed in Table 1. The samples of tumour tissue obtained from patients 2, 3 and 6 were primary tumours, while the 11 samples of tumour tissue obtained from the remaining six patients represented metastatic tumour. The samples of normal tissue and of tumour tissue were wet weighed and 1-g aliquots were radioassayed in a well-type sodium iodide crystal  $\gamma$ -scintillation counter equipped with a single channel pulse height analyser at the zinc-65 peak<sup>12</sup>.

Tumour tissue localized in the liver concentrated considerably less zinc-65 than liver tissue uninvolved by tumour (Table 2), the ratio of zinc-65 concentration in

Table 1. LIST OF PATIENTS STUDIED: TYPE AND LOCALIZATION OF TUMOURS

Patient	Age and sex	Diagnosis	Days between <sup>65</sup> Zn injection* and death	Organ localization of tumour
1	61, M	Bronchogenic carcinoma	1	Liver, spleen
2	65, M	Bronchogenic carcinoma	6	Lung†
3	67, F	Hepatoma	11	Liver†
4	58, F	Carcinoma of breast	11	Liver, lung, ovary
5	90, M	Hodgkin's sarcoma	18	Muscle
6	56, M	Bronchogenic carcinoma	17	Lung†
7	33, F	Fibrosarcoma with bone involvement	20	Lymphnode
8	65, M	Carcinoma of colon	21	Liver, lung
9	70, M	Carcinoma of pancreas	71	Spleen, pancreas

\* A single tracer dose of 100  $\mu$ Ci <sup>65</sup>ZnCl<sub>2</sub> was given intravenously.

† Organ site of primary tumour; the remaining tumours were secondary tumours localized in the organs listed.

Table 2. UPTAKE OF <sup>65</sup>Zn IN NORMAL AND TUMOUR TISSUE IN MAN

Patient	Days*	Site of tumour	<sup>65</sup> Zn, % dose/g tissue (×10 <sup>3</sup> ) Normal tissue	<sup>65</sup> Zn, % dose/g tissue (×10 <sup>3</sup> ) Tumour tissue	Ratio Normal tissue/ Tumour tissue
1	1	Liver	58.0	14.0	4.0
2	11	Liver	6.0	2.0†	3.0
4	11	Liver	36.0	14.0	2.0
8	21	Liver	11.0	0.4	25.0
2	6	Lung	1.0	5.0†	0.2
4	11	Lung	2.0	2.0	1.0
6	17	Lung	3.0	3.0†	1.0
8	21	Lung	1.0	1.0	1.0
1	1	Spleen	14.0	11.0	1.4
9	71	Spleen	2.0	2.0	1.0
5	18	Psoas muscle	2.0	4.0	0.5
7	20	Lymphnode	2.0	2.0	1.0
4	11	Ovary	2.0	2.0	1.0
9	71	Pancreas	1.0	1.0	1.0

\* Time-interval between the intravenous injection of a single dose of <sup>65</sup>ZnCl<sub>2</sub> and tissue sampling at autopsy.

† Primary tumours; all other samples of tumour tissue were secondary tumours.

normal tissue as compared with that of tumour tissue ranging from 3:1 to 28:1. The zinc-65 uptake in both tumour tissue in liver and in liver tissue uninvolved by tumour was lower per gram in patient 3, who had a primary tumour of the liver, than in patient 4, who had metastases in the liver. The tissue samples of both these patients were obtained at comparable time intervals after the injection of zinc-65 (11 days). Despite these differences in zinc-65 concentration in normal liver tissue and in tumour tissue of these two patients, the uptake ratio of normal/tumour tissue was similar in patient 3, who had a primary tumour (hepatoma), and in patient 4, who had metastatic liver disease.

Tumour tissue located in organs other than the liver, for example, in the lung, spleen, lymphnodes, ovary and pancreas, had a similar or a somewhat greater uptake of zinc-65 than the corresponding normal tissues. Two of the samples of tumour tissue in the lung were primary tumours (patients 2 and 6, Table 2). The zinc-65 uptake in one of these tumours (patient 2) was 5 times higher than that of lung tissue uninvolved by tumour, while the zinc-65 uptake in the primary tumour of the lung in patient 6 was similar to the uptake in the metastatic lung tumours. The radioactivity in lymphomatous tissue localized in psoas muscle was twice as high as that of the muscle tissue not involved by tumour.

The high uptake of zinc-65 in normal liver tissue in man<sup>17</sup> may be due to a combination of factors, such as rich vascularization of this organ, the presence of zinc metallo-enzymes<sup>18</sup> and the binding of zinc to proteins<sup>19</sup>. The lower zinc-65 concentration in tumour tissue localized in the liver in man may be due to several factors such as deficiency of zinc containing enzymes in the neoplastic tissue, decreased vascularization and changes in protein content in tumour tissue. The low zinc-65 uptake in tumour tissue localized in the liver is in agreement with data on stable zinc concentration in man reported by

Olson<sup>11</sup>. Low levels of zinc have been reported in cirrhosis of the liver and in other hepatic dysfunctions<sup>20</sup>.

The concentration of zinc is high in prostatic tissue in man. However, the concentration of zinc in tumour tissue localized in the prostate gland is approximately one-third that of the normal prostate<sup>14</sup>. A report on the zinc content in three different types of tumour localized in the thyroid gland in man indicates that the zinc concentration may depend on the cytological type of the tumour<sup>13</sup>. In the latter investigations, the concentration of zinc was higher in a sample of reticulum cell sarcoma in the thyroid gland and in a sample of papillary carcinoma than in normal thyroid tissue while the zinc concentration was lower in recurrent thyroid carcinoma and in necrotic thyroid adenoma than in normal thyroid tissue. In some animal investigations, the zinc-65 uptake has been reported to be higher in tumour tissue as compared with non-malignant tissue in tumour-bearing mice<sup>8,10</sup>.

The results presented here and the reports in the literature indicate that tumour tissue which is located in organs that normally have a low concentration of zinc have a similar or a greater zinc-65 uptake than the tissue in which the tumour is located. On the other hand, tumour tissue located in organs which normally have a high zinc concentration, for example, the liver and the prostate gland, have a lower zinc-65 uptake than liver and prostatic tissue uninvolved by tumour. The zinc content of white blood cells has also been reported to be high<sup>13</sup>, while the zinc content in leukaemic leucocytes has been found to be decreased<sup>4</sup>. Recently, a decrease in the zinc content has been observed in leucocytes of patients with a variety of neoplastic diseases and this difference has been suggested as a test for the diagnosis of cancer<sup>21</sup>. In tissues in which zinc seems to have a special metabolic function, deficiency of this trace metal may lead to alterations in cellular function and structure and ultimately result in neoplasia.

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## RADIOBIOLOGY

## Migration of Spleen Cells into the Blood Stream following Antigen Stimulation of the Rat

A MARKED cellular hyperplasia occurs in the rat spleen in response to a single intravenous injection of a particulate antigen<sup>1-3</sup>. This hyperplasia is predominantly manifested in the red pulp, which exhibits a striking increase in the number of actively mitotic, large pyroninophilic cells within 2 days after antigen administration. The proliferation of these cells, which have been shown to be intimately associated with antibody formation in the rat<sup>4,5</sup> as well as in other species<sup>6</sup>, reaches a maximum on about the fourth day after antigen. Later, their number rapidly diminishes so that by the ninth day the spleen has largely regained its pre-stimulation pattern and few if any of these cells appear to differentiate locally into plasma cells.

The fate of these 'antibody-forming cells' has not been established with certainty, although there is morphological evidence suggesting direct migration into the blood stream<sup>7</sup>. At about the time of maximal hyperplasia, cells resembling small lymphocytes make a transient appearance in the red pulp. It has been suggested that the antibody-forming cells shed their cytoplasm and give rise to these small lymphocytes which then migrate from the spleen<sup>8,9</sup>. This interpretation was strengthened when lymphocytes exhibiting specific adherence of the bacteria used for immunization were demonstrated in the blood<sup>8</sup>. However, immune adherence gives only indirect evidence in support of the proposed cellular migration, particularly since the possibility of cellular contributions from extra-splenic sources was not excluded.

In the present experiment, cell migrations into the blood stream of the rat were investigated by using tritiated thymidine to label the proliferating pyroninophilic or 'antibody-forming' cells of the spleen. For selective labelling of the spleen cells, the spleens were first exteriorized with intact vascular pedicle and protected in a lead shield while the remainder of the body was subjected to X-irradiation, a technique described by others<sup>8</sup>. A dose of 900 r. was necessary to suppress adequately the incorporation of tritiated thymidine into the actively mitotic extrasplenic tissues such as gastro-intestinal tract, bone marrow and lymph nodes. Eighteen hours after X-irradiation, which is within the period of maximal lymphocytic suppressive effect of the irradiation, tritium thymidine (specific activity 0.36 c./mmole) was injected intraperitoneally at a dose of 25  $\mu$ c./100 g. Six hours after tritiated thymidine injection the animals were killed. Samples of spleen and blood buffy coat smears were fixed in Carnoy's fixative. The smears and spleen sections were covered with stripping film (Kodak 'AR 10') according to the method of Pelo<sup>10</sup>. Autoradiographs were stained with methyl green-pyronin after 25-30 days' exposure.

Twelve young male rats (Sprague-Dawley) were assigned to 4 experimental groups. The first group was injected intravenously with 1.0 ml. of a standard formalinized typhoid vaccine. Three days later the rats were irradiated with spleen-shielding. They were killed on the following day, 6 h after receiving tritiated thymidine. The second group was similarly treated except that physiological saline was substituted for typhoid vaccine. In the third group, which also received antigen, irradiation was performed without spleen-shielding. The fourth group, a control for the splenic histology, received antigen but not irradiation or tritiated thymidine.

The spleens of the antigenically stimulated rats which were irradiated with spleen-shielding exhibited a histological pattern qualitatively similar to that of the unirradiated controls. The only prominent difference was a decreased number of lymphocytes in the white pulp. The greatly hyperplastic red pulp contained many labelled cells in-

cluding most of the large pyroninophilic cells and the clusters of smaller cells resembling lymphocytes (Fig. 1). The large cells of the germinal centres also showed appreciable labelling, but the smaller lymphocytes of the white pulp were not labelled. The histological pattern in the saline-injected rats which were irradiated with spleen-shielding was essentially normal except for a decrease in the number of white pulp lymphocytes. The relatively small number of labelled cells included a few scattered large pyroninophilic cells in the red pulp and some of the large cells of the germinal centres. The spleens of the antigenically stimulated rats subjected to total-body irradiation without spleen-shielding were severely atrophic. Only rare cells, chiefly fibroblasts and histiocytes, were labelled.

In investigating the labelling of blood cells, lymphoid cells were classified so far as possible on the basis of morphological criteria used by others<sup>10,11</sup>. Cells were assigned to 3 categories: (1) small lymphocytes; (2) medium lymphocytes and large pyroninophilic cells; (3) unclassified cells including blast cells and 'basket cells'. Polymorphonuclear leukocytes, none of which was labelled, were excluded. In all, 2,000 lymphoid cells, 1,000 from each of two slides, were counted from each animal. Labelled cells from the blood of antigen-stimulated, spleen-shielded animals are shown in Fig. 2. A marked lymphocytopenia was observed in the rats subjected to total-body irradiation without spleen-shielding: labelled cells were not present in the blood of this group. The labelled cells in the blood of the two spleen-shielded irradiated groups are enumerated in Table 1. The two groups were compared with respect to the total number of labelled cells per 2,000 lymphoid cells using Student's *t*-test with four degrees of freedom. A significantly greater ( $P < 0.005$ ) percentage of labelled cells was present in the antigenically stimulated group.



Fig. 1. The labelling pattern of the splenic red pulp on the fourth day after antigen. Numerous large pyroninophilic cells clustered about an arteriole are heavily labelled, as are the small lymphocytic cells situated more peripherally. (Methyl green-pyronin,  $\times$  c. 670)





water. They are more acidic than the phenylcarbamate esters or the benzenesulphonamides, neutral, water-soluble, alkali metal salts being formed on treatment with the corresponding aqueous bicarbonate solution. The potassium salts have been extensively used in field trials. Estimation of residues in plant materials can be effected by the classical procedures in present use for estimation of sulphonamides, based on the presence of a diazotizable aromatic amine group. Both M and B 8882 and M and B 9057 are readily converted into sulphanilamide. Toxicity in experimental animals is low; acute oral  $LD_{50}$ 's in mice, rats, rabbits and hens range from 1,000–5,000 mg/kg.

M and B 8882, M and B 9057 and M and B 9555 kill some graminaceous species, including wild oats and some grass weeds, while M and B 8882 and M and B 9057 also kill dicotyledonous species. Levels of herbicidal activity, determined by pre- and post-emergence application using established methods<sup>6</sup> under greenhouse conditions, are set out in Tables 2 and 3. Tolerant crop species include linseed (flax), lucerne, potatoes, black currants, raspberries, sugar cane, oil poppy, and some crop grasses. The growth of cereals is affected at herbicidal rates.

The new herbicides are absorbed by the leaves and the roots of susceptible species at seedling and later stages of growth, unlike protham and chlortham, which are absorbed only by roots. The effects of foliar application develop slowly and are very characteristic. After two weeks, susceptible plants become chlorotic and lose vigour, then the growing points cease to grow, and after 5–6 weeks the plants die or are so seriously arrested that they offer little, if any, competition to tolerant plants. The chlorotic and inhibitory effects appear in apical and axial meristems remote from treated leaves, being especially well marked in the tillers of *Avena fatua*, the buds of rhizomes of *Agropyron repens*, and the apex of *Rumex* spp., following application of M and B 9057 or M and B 8882.

The following experimental observations suggest that the herbicides act by interfering with cell division and expansion; the inhibitory action on meristems (already described here); seeds soaked in lethal concentrations

produce swollen and stunted roots; young dicotyledonous plants sprayed with M and B 9057 develop a larger number of smaller leaves. The phenylcarbamates cause similar inhibitory<sup>7,8</sup> and morphological effects<sup>9</sup> when taken up by the roots of plants, and so does barban when applied to leaves of wheat and wild oats<sup>11</sup>. Although chlorosis is the most obvious symptom it is less intense than that caused by amitrole and therefore possibly of lesser importance in killing plants. Unlike the phenylcarbamates, none of these compounds inhibits the Hill reaction at concentrations of up to  $10^{-3}$  molar<sup>12</sup>.

Field trials in Great Britain, Canada<sup>13</sup> and the West Indies show that the three herbicides are of interest for controlling grass weeds, by both pre- and post-emergence application. Two of them, M and B 8882 and M and B 9057, are of interest also for killing composite, cruciferous and polygonaceous weeds. The situations in which they would be of practical value have not been fully explored, but the removal of grass weeds in certain grass crops appears to be possible. Practical rates of application have, in some instances, been reduced by the addition of a wetting agent to the spray solutions. An account of the chemical and biological properties of these sulphonylcarbamates will be published elsewhere.

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Table 2. HERBICIDAL ACTIVITY BY FOLIAR APPLICATION (LB/ACRE)

Plant species and growth stage (No. of leaves)	Dose of herbicide required for (a) > 90% redn. in fresh wt (b) 70–90% redn. in fresh wt. (c) 90% mortality				
	M and B 8882*	M and B 9057*	M and B 9555*	Chlor. protham	
<i>Avena fatua</i>	1–2	2	1.5	2	1.5
<i>Agrostis stolonifera</i>	1–2	1	—	2	1
<i>Agropyron repens</i>	10–18 in. high	16†	—	8†	—
<i>Alopecurus myosuroides</i>	1–2	4	3	8	2–4
<i>Deschampsia glomerata</i>	1–2	2	1	2	as 1
<i>Lolium perenne</i>	1–2	8	4–6	8	4
<i>Poa annua</i>	1–2	4	2	4	3
<i>Polygonum lapathifolium</i>	1–2	2†	—	1.5†	as 8†
<i>Stachys arvensis</i>	2–3	1†	—	1†	as 4†
<i>Tripleuroperum maritimum</i>	3–5	2†	—	1†	as 8†
<i>Trifolium spp. inodora</i>					No kill§
<i>Rumex crispus</i> †	6 in. diam. plant	2–4†	1–2†	—	—

\* Applied as potassium salt.

† Applied as free acid in aqueous acetone solution.

‡ Tested in the field.

§ At 8 lb./acre the highest tested.

Table 3. RHIZOMAL HERBICIDAL ACTIVITY IN SOIL (LB/ACRE)

Plant species	Dose required to kill 90% of germinating seeds			
	M and B 8882	M and B 9057	M and B 9555	Chlor. protham
<i>Avena fatua</i>	2	2	2	<1*
<i>Holcus lanatus</i>	1	1	1	—
<i>Poa annua</i>	1	1	1	<1*
<i>Chenopodium album</i>	2	1	1	1
<i>Polygonum lapathifolium</i>	1	1	2	2
<i>Stachys arvensis</i>	1	1	8	1
<i>Tripleuroperum maritimum</i>	<1*	1	8	4
<i>spp. inodora</i>				

\* Lowest rate tested.

## Non-essential Role of the Adrenal Medulla in the Hibernation of the 13-Lined Ground Squirrel

ALTHOUGH the adrenal cortical hormones are reported to be necessary for successful hibernation and arousal in European ground squirrels, *Citellus citellus*<sup>1</sup>, and in European hamsters, *Cricetus cricetus*<sup>2</sup>, the necessity and function of the adrenal medullary hormones are still in doubt. Most investigators agree, however, that these catecholamines, especially norepinephrine, are probably involved in chemical heat production by torpid and arousing hibernators<sup>3–5</sup>. Although totally adrenalectomized hibernating animals arouse in a nearly normal manner, a return to normal hibernating patterns is prevented unless adrenal cortical tissue is transplanted into the anterior chamber of the eye or unless cortisone or deoxycorticosterone acetate (DOA) is administered<sup>1,2</sup>. Presumably, then, the hormones of the adrenal medulla are not needed for at least short 'bout' of hibernation or for arousal, even though the importance of the sympathetic-adrenal system in spontaneous arousal has been repeatedly emphasized<sup>4,6–8</sup>.

The work recorded here was initiated to obtain more information on the influence of the adrenal medullary hormones in hibernation and arousal during an entire hibernating season.

Six male 13-lined ground squirrels (*O. tridecemlineatus*) were bilaterally adrenalectomized in July, but a remnant of adrenal cortical tissue was left *in situ*. Cortical regeneration occurred during the following seven weeks. Six control males were laparotomized, their adrenal glands being manipulated and slightly traumatized but not removed. On September 1 the animals were placed, one to a cage, in a cold room at  $6 \pm 0.5^\circ \text{C}$  with 2 h of light a day. The animals had food and water at their disposal at all times and were inspected each morning, five days per week, until the following May. Hibernation or arousal was recorded for each animal. While it is recognized that these animals may arouse from, and return to, a hibernating state within a 24-h period, nevertheless we believe that comparisons between the two groups are valid because any error would be common to all animals. The animals were autopsied at the termination of the investigation, the remnants of adrenal cortical tissue being examined histologically for any evidence of medullary tissue. None was found in any of the animals.

A total of more than 1,000 animal days (No. of animals  $\times$  No. of days of observation) was recorded for each group. For convenience and compilation purposes, the 9 months of observations were divided into 15-day periods, beginning September 1. The percentage of animal days spent in hibernation during each period was calculated and plotted (Fig. 1). Each 15-day period comprises approximately 55–72 animal days.

The medullectomized animals hibernated for 75.4 per cent of the entire period, while the controls hibernated for 86.3 per cent of the time. The difference between the two groups with respect to the proportion of time spent in hibernation was limited to the first six weeks. After this initial period, however, no significant differences could be seen between the groups in either percentage of time in hibernation or duration of bouts of hibernation. We conclude, therefore, that medullectomy disrupted the normal processes preparatory to hibernation and thereby delayed its onset. During January, sharp decreases in the amount of time spent in hibernation occurred in both groups and continued through the rest of the experimental period.

One medullectomized animal remained fully awake, and presumably homeothermic, after February 4. Another of these animals aroused permanently on March 18. Two of the remaining four were found dead on April 5; the other two died on May 7. In the control group, three animals were terminally aroused by April 12, one was fully aroused by May 22 and the other two hibernated sporadically through May and even into June.

The results of this investigation indicate that animals lacking the adrenal medullary catecholamines enter into,

and arouse from, hibernation in a normal manner, although the initial entry into hibernation is somewhat delayed in the medullectomized animals. Both the medullectomized and the control animals show the typical hibernation pattern, in which the duration of a 'bout' of hibernation and the percentage of time in hibernation are maximal in mid-winter<sup>10</sup>. We may assume, therefore, that the catecholamines of adrenal medullary origin are not required for successful hibernation or arousal. The concentration of catecholamines in adrenal medullary tissue is reportedly reduced or variable during hibernation<sup>11–13</sup>. Most of these investigators relate the changes found during hibernation to the frequency of arousal. In contradiction to the findings recorded by the authors just cited, Suomala and Uusipää<sup>14</sup> reported that the concentration of epinephrine within the adrenal medulla of the hedgehog increased during hibernation but decreased sharply immediately after arousal in the spring; Petrovic and Davidovic<sup>15</sup> noted a rise in the concentration of catecholamines within the adrenal medulla at the end of hibernation with a marked decrease in this concentration on terminal arousal. These and our results suggest that, at the time of spring arousal, the adrenal catecholamines may assume a special importance to the physiological well-being of hibernating animals. This may explain why four of six medullectomized animals died during the late stages of this experiment, while only one of the controls did so. Further experimentation on the importance of these compounds, particularly at the time of spring arousal, is needed.

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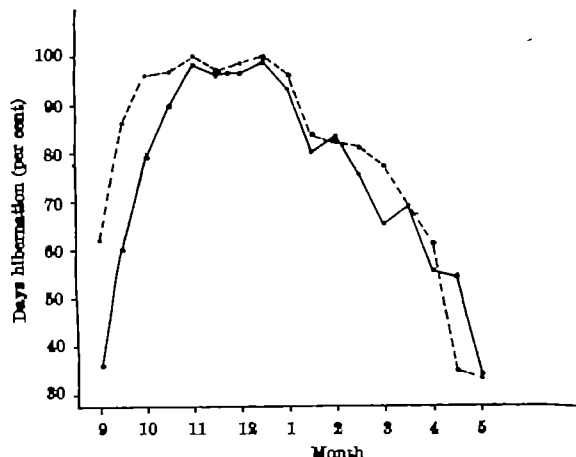


Fig. 1. Percentage of time spent in hibernation by medullectomized (—) or sham-operated control (---) 13-lined ground squirrels throughout a hibernating season, September–May.

## Secondary Walls in Phloem of *Pinus radiata* D. Don

Abbe and Crafts<sup>1</sup> showed that the development of secondary walls in the sieve cells of gymnosperms is apparently restricted to the Abietineae; there is no evidence of secondary wall in the sieve cells of Taxodineae, Araucarineae, Cupressineae and Taxineae. Moreover, the Abietineae develop no fibres, but the secondary walls of their sieve cells appear to be composed of relatively pure cellulose. Secondary walls occur in the sieve cells of white pine, *Pinus strobus* L. (Figs. 3 and 24 in ref. 1).

Barghoorn and Scott<sup>2</sup> examined the tracheids in thin sections of fossil material of *Pinus strobus* L. (Figs. 11 and 14) and *Sequoia* sp. (Fig. 16). They pointed out that the primary wall and the *S*<sub>1</sub> layer of the secondary wall are more resistant to degradative processes than the *S*<sub>2</sub> and *S*<sub>3</sub> layers of the secondary wall. In most of their pictures



Fig. 1. Transverse section of phloem photographed in ordinary light. Note the intact primary walls (b) for two contiguous cells. ( $\times 1,200$ )

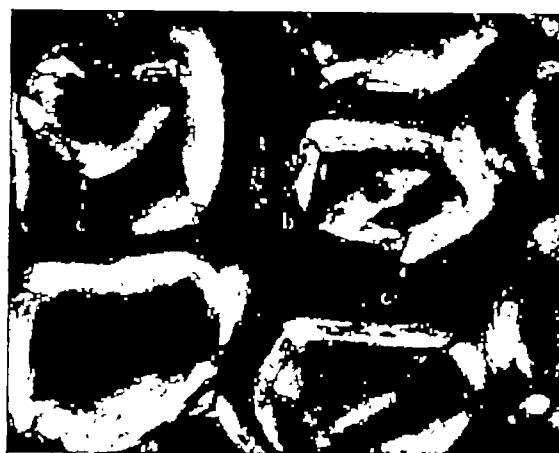


Fig. 2. Same as Fig. 1, seen between crossed nicols. The primary walls (b) are weakly birefringent; the collapsed material (c), because of the great birefringence, is interpreted as the secondary wall. ( $\times 1,200$ )

$S_2$  and  $S_3$  layers have collapsed away from  $S_1$  and the primary wall, leaving a gap.

In the present work cambial strips with a thick layer of phloem, preserved in formalin-acetic acid-water (10:5:85), were embedded in wax and sectioned at  $20\mu$ . The sections were placed in 25 per cent alcoholic hydrochloric acid for 2 h, washed with water and placed in 0.5 per cent ammonium oxalate for 15 min. Staining was done by Foster's ferric chloride-tannic acid method<sup>2</sup>.

Fig. 1 shows two adjacent sieve cells photographed in ordinary light. Collapsed material is present in each cell. Fig. 2 shows the same two cells between crossed nicols. The collapsed material (c) and the bounding walls (b) of each cell are both birefringent, suggesting that both are cellulose-containing wall layers. The weak birefringence of the bounding layer and the strong birefringence of the collapsed layer suggest that most or all of the secondary wall layers have collapsed away from the primary wall.

These results support Barghoorn and Scott's claim that secondary walls are less resistant to degradation than primary walls.

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### Influence of Compensatory Mechanisms and the Pineal Gland on Dark-Induced Gonadal Atrophy in Male Hamsters

It has been postulated that the gonads of male and female hamsters regress to a non-functional state as a normal seasonal response to short days and low temperatures in the autumn and winter<sup>1</sup>. Under laboratory conditions, the same results follow exposures to short photo-periods<sup>1-3</sup>. The functional change in the gonads is evidently controlled by the pineal gland, which itself responds to changing light periods, since pinealectomized animals show no regression of gonads when they are exposed to short photoperiods. A variable hypertrophic response of one gonad is a result of excision of the other. There are, therefore, probable hormonal processes which exert effects on the gonads opposite to those exerted by the pineal gland. In the course of our work, there arose a question as to whether the depressant action of the pineal gland could be counteracted by the compensatory processes which follows unilateral castration. The following experiments were designed to elicit an answer to this question.

Thirty-eight male hamsters were pinealectomized or subjected to a sham operation by techniques established in this laboratory<sup>4</sup>. In addition, all animals were unilaterally castrated by standard techniques and the excised testes were weighed to the nearest mg. Half of each group was exposed to daily light:dark (LD) cycles of 1:23 and the other half was maintained on LD cycles of 16:8. All animals received a standard rodent diet and water *ad libitum*. After four weeks the animals were killed and the weights of the remaining testes were obtained. The data were subjected to a two-factor analysis of variance and the means and standard errors of the means (S.E.M.) were calculated. The data were tested for significance ( $P < 0.05$ ) by use of Student's *t* test.

Previous experiments have demonstrated conclusively that four weeks of exposure to short daily periods of light are sufficient to produce atrophy of the male hamster gonad<sup>1,2</sup>. In the present experiment, however, it will be noted (Table 1) that the remaining testes of sham-operated animals in LD 1:23 regressed only slightly (not significant). The remaining gonads of the other 3 groups maintained or increased their weight as had been expected.

To ascertain whether dark-induced gonad atrophy could be reversed by pinealectomy or by compensatory mechanisms, animals which had been in LD 1:23 cycles for four weeks were unilaterally castrated and the weights of the excised testes were recorded. Half the animals were then pinealectomized and the other half were subjected to a sham operation and all animals were returned to LD cycles of 1:23. Four weeks later, these animals were killed and the remaining testes were weighed and compared with the weights of the previously removed testes.

Because the cranial surgery and unilateral castration were done at the same time, we could not divide the animals into pinealectomized and sham-pinealectomized groups having similar mean weights of the removed testes. Consequently, the initial mean weights of the testes in the two groups were significantly different (Table 2;  $P < 0.01$ ). This variable response at four weeks has been previously documented<sup>1</sup>. Because of this and because the response of the remaining testes was variable in the sham-pinealectomized group, the data for each individual are

Table 1. THE EFFECT OF PINEALECTOMY AND DIFFERENT LIGHT-DARK CYCLES ON THE SURVIVING TESTES OF HAMSTERS AFTER UNILATERAL CASTRATION

No. animals	Body wt. (g)		Testis wt. (mg/100 g)	
	Initial	Final	Initial	Final
Pinealectomized, LD 1:23				
8	106 $\pm$ 2.8	103 $\pm$ 3.3	1,250 $\pm$ 74	1,431 $\pm$ 130
Sham pinealectomized, LD 1:23				
8	107 $\pm$ 2.3	100 $\pm$ 3.0	1,253 $\pm$ 116	967 $\pm$ 200
Pinealectomized, LD 16:8				
11	110 $\pm$ 2.1	103 $\pm$ 1.6	937 $\pm$ 157	1,068 $\pm$ 162
Sham pinealectomized, LD 16:8				
11	104 $\pm$ 3.9	102 $\pm$ 2.1	1,168 $\pm$ 77	1,470 $\pm$ 63

Table 2. INFLUENCE OF THE PINEAL GLAND ON THE RESPONSE OF ATROPHIED TESTES OF UNILATERALLY CASTRATED MALE HAMSTERS EXPOSED TO SHORT PHOTO-PERIODS

(A) Pinealectomized		
Animal No.	Testes weight (mg/100 g)	
	Initial	Final
1	662	1,654
2	594	1,418
3	252	1,480
4	439	1,405
5	177	1,064
6	208	1,040
7	648	1,404
$\bar{x}$	448	1,352
S.D.	196	
P value	< 0.001	

(B) Sham pinealectomized		
1	829	441
2	732	1,684
3	439	750
4	904	968
5	1,235	1,226
6	1,295	108
7	750	286
$\bar{x}$	837	784
S.D.	395	
P value	No significant difference	

tabulated in Table 2 along with the means and standard deviations.

After pinealectomy (A, Table 2), the mean weight of the remaining testes increased significantly (three-fold) during the following four-week period, lending additional confirmation of the inhibitory role of the pineal gland on gonad functions. On the other hand, the mean weight of the remaining testes of the sham-operated animals was unchanged, or decreased slightly. While the remaining gonads of all pinealectomized animals increased markedly in weight, those of only three of the sham-operated animals increased while two showed significant decreases and the remaining two showed no change (B, Table 2).

The results recorded in Table 1 show that those processes which are set in motion to compensate for partial loss of testicular tissues can be superimposed on the actions of the pineal gland to prevent its primary inhibitory effects. In this case, the initially enlarged, functional testis did not regress in the sham-pinealectomized, unilaterally castrated animals even though the pineal gland is presumably functional (inhibitory) in short light cycles. However, testes already atrophied or affected by exposure to short photo-periods fail to respond or respond variably to compensating processes in the presence of a functional pineal gland (Table 2). In its absence, however, marked enlargement of the remaining testis can occur. We infer from these data that if normal functional testes are present, normal pituitary-gonad hormonal balance exists. Consequently, compensating mechanisms are called into play when one testis is removed. Such mechanisms appear to be adequate to prevent any gonad-inhibitory actions by the pineal gland. On the other hand, already atrophied or dark-affected gonads imply modified pituitary-gonad hormonal interrelationships. In this case, removal of one testis may or may not stimulate compensating mechanisms. Only when the pineal gland is removed can gonad recrudescence become consistently possible under these conditions.

Our results demonstrate that in reproductively functional male hamsters, compensatory mechanisms can offset the effects of an active pineal gland. Further, we have made observations (unpublished) which suggest that the inhibitory effects of short daily periods of light on male gonads can be considerably modified (reduced) when two or more animals are in the same cage or if a normal female is in the same cage with the males. Thus, social contact, olfactory stimuli, or hormonal compensating mechanisms all appear to potentially interfere with or modify the primary inhibitory effects of an active pineal gland in short photo-periods.

We may tentatively look on the pineal gland as an organ which is called into play when the environmental photo-periods dictate a reduction in reproductive func-

tions. However, other stimuli may apparently be superimposed on and reverse these actions under appropriate conditions so that survival and perpetuation of the species are ensured. The pineal gland appears to be only one of a spectrum of exteroceptive factors regulating reproductive cycles in a changing external environment.

This work was supported in part by the U.S. Army Edgewood Arsenal Chemical Research and Development Laboratories In-House Laboratory Independent Research Program. In conducting this research, we adhered to the "Principles of Laboratory Animal Care", as established by the National Society for Medical Research.

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### Incidence and Lobar Distribution of Bronchiectasis in a Colony of Wistar Albino Rats

In some recent dietary investigations using a closed colony of Wistar albino rats the most common pathological condition was an inflammatory and suppurative disease of the lungs. This disease, a form of bronchiectasis, occurs frequently as a natural disease in rats, both domesticated and wild<sup>1</sup>, and has previously been described<sup>2</sup>. In extent it varies from a small focus of inflammation in one lobe of the lung to the entire lung being shrunken and nodular, the tissue of each lobe being converted into large sacs consisting of distended bronchi filled with a compacted caseous mass. Nelson and Gowen<sup>1</sup> have reported the incidence of the disease in male and female rats but only from a total of 145 animals. Other reports give the percentage incidence for the entire colony regardless of age and sex<sup>3,4</sup>. In the work recorded here 1,238 rats were examined and as no dietary differences were found<sup>4</sup> the opportunity was taken to sum over diet to obtain a detailed picture of the incidence of this natural disease in the colony. Particulars of the maintenance of the colony are given by Hickman *et al.*<sup>5</sup>.

At post-mortem examination a note was made of the number of each sex exhibiting pulmonary disease and also the number and position of the lobes affected. The results were then divided into six six-monthly age groups (Fig. 1). Of the 621 males and 617 female rats examined, 418 males

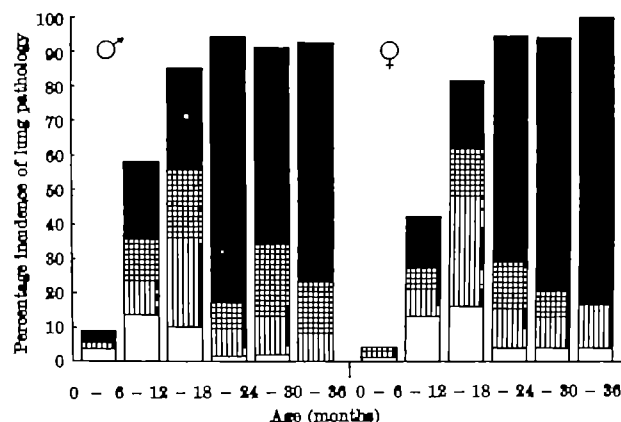


Fig. 1. Black, bronchiectasis in all 5 lobes of lungs; double hatched, 3-4 lobes affected; vertically hatched, 1-2 lobes affected; white, lungs inflated

and 406 females showed pathological changes in the lungs. Fig. 1 shows that only 4-9 per cent of the rats exhibited pathological changes in the first six months of life, and, of the 75 examined, only one animal under 2 months of age (a male of 50 days) showed any inflammation. By 2-4 months, however, the incidence rose to 10 per cent in the males and 3 per cent in the females. This difference in susceptibility between the sexes was maintained in the 6-12 months age group (males 58 per cent; females 42 per cent). Application of a  $\chi^2$  test to the summed results for the period 0-12 months (males: 60 diseased animals out of 221; females: 31 diseased animals out of 198) showed that the difference is statistically significant ( $P < 1$  per cent). Thus the male would appear to be more susceptible to pulmonary disease than the female during the first twelve months of life. By the time the animals were 12-18 months old there was very little difference in the incidence of the disease between the sexes (male: 85 per cent; female: 82 per cent), and at 18-24 months no difference at all (both 94 per cent). Viewed overall it is seen that animals are first contracting the disease up to an age of 18 months. After this period, when the incidence of lung pathology has risen to 91-94 per cent, the animals still unaffected (6-9 per cent) appear to remain immune (the column for females 30-36 months old provides the only exception, but since this is based on the data from only 24 animals, one of which provides 4 per cent of the total incidence, the value of the exception is doubtful). Although it appears that no more animals contract the disease after 18 months, the severity of the disease in each affected animal increases with time.

Bronchiectasis in rats has been attributed to a viral infection<sup>1,2</sup>. Cruickshank<sup>3</sup> believes that the disease is initiated by bronchial obstructions caused by the swelling of the mucosal lymphoid tissue. Mucus accumulating distally to the obstruction would then plug the bronchus and cause an inflammatory reaction, the combination of obstruction and sepsis eventually leading to bronchiectasis. He notes that the virus isolated by Nelson may well be the cause of the initial enlargement of the bronchial lymphoid tissue. In this connexion it is of interest to note that in the present experiment the occurrence of enlarged lymph nodes (0-18 months: males 3 per cent, females 1 per cent; 18-36 months: males 16 per cent, females 8 per cent) was significantly higher in males than in females ( $P < 1$  per cent in 18-36 months' age group); this clearly supports Cruickshank's theory. It is possible that the increased incidence of bronchiectasis in males, also noted by Nelson and Gowen<sup>1</sup> (although their results were insufficient for analysis), may be due, at least in part, to the usual methods of colony maintenance. In the present colony the animals were maintained at 3 to a cage except after mating when the females were placed in individual cages to produce their litters. The females were mated when 12-13 weeks old and on average spent five weeks in isolation, except for the presence of their litters, just at the age when animals were beginning to be found having the disease. If the initiation of bronchiectasis is due to an airborne virus the separation of the females into individual cages may reduce their chances of contracting the disease to an extent sufficient to cause a significant difference between the sexes in a time-incidence graph.

It is clear from an examination of the lobar distribution of the disease that the initial stages of bronchiectasis are not uniformly distributed throughout the lung (Table 1). The right superior lobe is scarcely ever affected by the initial stages of the disease whereas the median or post-caval lobe is affected more than three times as often. Examination of 25 healthy animals showed that the distribution of the weight between the lobes was: right superior 13 per cent, right middle 13 per cent, right inferior 19 per cent, right median or post-caval 11 per cent and left lung 35 per cent; there appeared to be no variation in percentage distribution with age. Thus the two lobes most frequently affected, the basal lobes (right inferior and median), are

Table 1. LOBAR DISTRIBUTION OF BRONCHIECTASIS IN THE LUNGS OF RATS WHEN ONE, TWO, OR UP TO FOUR LOBES ARE AFFECTED

No. of lobes affected	Location of infection					Total No. of lobes affected	No. of animals involved
	Right superior	Right middle	Right inferior	Median	Left		
<b>Males</b>							
1 lobe	1	7	9	9	11	37	37
2 lobes	4	15	19	25	17	80	40
Totals	5	22	28	34	28	117	77
<b>Females</b>							
1 lobe	2	13	8	13	17	53	53
2 lobes	11	17	19	19	8	74	37
Totals	13	30	27	31	25	126	89
<b>Combined sexes</b>							
1-2 lobes	18	53	55	55	53	243	166
1-4 lobes	80	149	152	156	133	670	295

both smaller than the less frequently affected left lung. Weight for weight the tissue of the median lobe is four times more susceptible to bronchiectasis than is that of the left lung. The variation in the incidence of disease between the lobes of the lung is not due to differences in lobe size and thus a higher probability of contraction of disease. It may possibly be attributed to the drainage of mucus into the basal lobes, which, if obstructed, would be the first to be occluded. The distribution of the disease from lobe to lobe may not be caused by the movement of infected material, but simply be due to the varying rates at which damage first becomes apparent in lobes initially affected to an almost equal extent.

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## ENTOMOLOGY

### DDT-metabolism in Resistant and Susceptible Stable-flies and in Bacteria

THE main cause of resistance to DDT in insects is an increased rate of detoxication, although other mechanisms (such as higher lipid content, or lower nerve sheath permeability) have been suggested<sup>1</sup>. Products of DDT-metabolism in insects are DDE, kalthan, and water soluble conjugates<sup>1</sup>, while yeast<sup>2</sup> and rat liver<sup>3</sup> metabolize DDT to TDE.

The following experiments were performed to investigate whether absorption, metabolism, or excretion of DDT may be a cause of resistance in the stable-fly (*Stomoxys calcitrans* (L.)). Strains of stable-flies developed resistance to DDT (30 times), methoxychlor (110 times) and TDE (extremely high level) when kept under selection pressure of DDT in the laboratory. The resistance to the three insecticides was mainly due to one gene allele<sup>4</sup>.

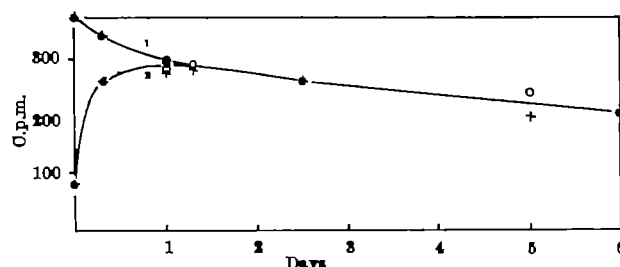


Fig. 1. Absorption and excretion of topically applied <sup>14</sup>C-DDT. The points represent the radioactivity per fly and are based on the average of 10 flies. The activity of the external tissues is added to the internal activity before plotting: 0, susceptible flies; +, resistant flies; 1, Internal + external activity; 2, Internal activity.

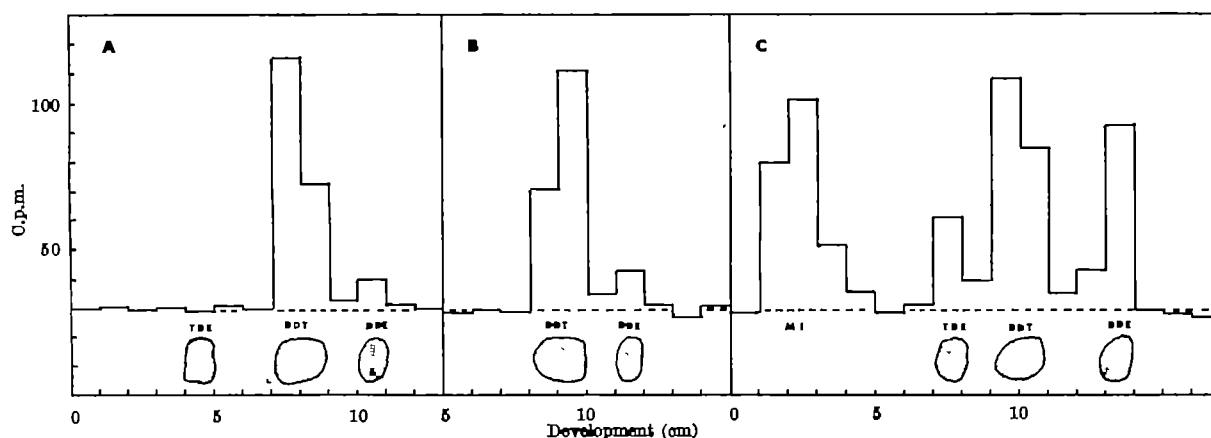


Fig. 2. The radioactivity on chromatograms developed in:  $\beta$ -methoxy propionitrile (stationary phase); isooctane (mobile phase). Carrier substances (10  $\mu$ g of TDE, DDT and DDE) were detected by the method of Mitchell<sup>7</sup>. The solvent front was invisible. The flies were kept 24 h after treatment with  $^{14}$ C-DDT before they, and the excrement, were extracted and chromatographed. A, resistant flies; B, susceptible flies; C, excrement of resistant flies

Resistant and susceptible flies were treated with 0.0164  $\mu$ g  $^{14}$ C-DDT in acetone by topical application and kept at 25° C in glass cages. Excrement was collected for the first 24 h after application and extracted with acetone. Fresh blood was supplied every day. At various intervals after the application, samples of 10 flies were removed, rinsed in petrol ether, ground in a mortar and extracted 3 times with acetone.

*Serratia marcescens*, *Alcaligenes faecalis*, and one unidentified bacterial strain were isolated from the excreta of flies kept in sterile cages. These isolated strains and laboratory strains of *Escherichia coli*, *Bacillus brevis* and *Aerobacter aerogenes* were grown in meat extract bouillon containing  $^{14}$ C-DDT under nitrogen atmosphere (anaerobically), in unshaken cultures (oxygen deficiency), and in fully aerated cultures at 25° C or 37° C. After 24 or 72 h sulphuric acid was added<sup>8</sup>. The radioactivity was completely extracted from the incubation mixtures with hexane. Radioactivity was determined in a 'Tracerlab' windowless flowcounter. Aliquots of external rinses and internal extracts were plated.

All extracts were purified by acetonitrile elution<sup>8</sup>, and subjected to paper chromatography on Whatman No. 1 paper. The solvent systems used were vaseline-ethanol-water-ammonia<sup>9</sup>, phenoxyethanol-isooctane<sup>9</sup>, soya bean oil-methanol-water<sup>1</sup>,  $\beta$ -methoxy propionitrile-isooctane<sup>9</sup>. Radioactivity in the chromatograms was located by cutting the paper into pieces 1  $\times$  2 cm and counting them individually.

Samples of resistant flies were also treated with 10  $\mu$ g TDE and killed after 10, 20 and 30 h. Similar extracts were subjected to paper chromatography in the same systems. The silver nitrate reagent<sup>7</sup> was used for the detection of spots.

There was no difference in the rate of absorption, detoxication or excretion of DDT by resistant and susceptible flies (Figs. 1, 2A and 2B). Both make only small amounts of DDE. TDE was the only substance detected on the chromatograms of extracts of TDE-treated flies.

In the excrement of  $^{14}$ C-DDT-treated flies, DDT, DDE, TDE and MI were found (Fig. 2C). MI was water soluble and could be hydrolysed by acid to give a substance less soluble in water.

When growing anaerobically or in oxygen deficiency, the facultative anaerobes *S. marcescens*, *E. coli*, and the unidentified strain, converted DDT almost completely to TDE (about 90 per cent) and DDE (5 per cent). In aerated cultures neither the facultative anaerobes nor the obligate aerobes converted any DDT to TDE or other products.

As there is no difference in the rate of absorption, metabolism and excretion by resistant and susceptible flies, the resistance mechanism must be of an unusual kind.

Bacteria from the intestines of the flies were able to convert DDT into two (DDE and TDE) of the three DDT-metabolites found in fly excrement. This further indicates that the cause of resistance to DDT, methoxychlor and TDE in stable-flies is not a detoxication mechanism, in spite of the high degree of resistance present.

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## Human Sweat Components Attractive to Mosquitoes

WHETHER human sweat is attractive to mosquitoes is a controversial problem. Howlett<sup>1</sup>, Rudolfs<sup>2</sup> and Rahm<sup>3</sup> concluded that human sweat was not attractive; however, Parker<sup>4</sup> and Brown<sup>5,6</sup> found the contrary. Recently, Brown<sup>7,8</sup> reported that lysine and alanine are attractive to *Aedes aegypti* (L.).

We have investigated the attractiveness of human sweat in a dual-port olfactometer modified from the design of Willis<sup>9</sup>. A charcoal-filtered, humidified air-stream is split into two equal portions and passed through glass coils submerged in a constant temperature water bath, which heats the air-streams to 27° C. Air flow rate measured by two glass-float flowmeters is kept at 2 l./min. Two glass tubes 150 mm  $\times$  30 mm with inlet and exit connections following the flowmeter hold the samples. The tubes are 3/4 filled with glass beads 4 mm in diameter. Five ml. of the test sample is placed in one tube and 5 ml. of distilled water in the control tube. The air-streams after passing through the sample tubes connect directly to two Buchner funnels 5 cm in diameter and placed side by side which exhaust into the test cage measuring 12 cm across, 10 cm deep, and 6 cm high. The two open ends of the test cage are covered with 40 mesh nylon net.

Attractiveness or repellency was determined by distribution of 20 mosquitoes in front of either port of the test cage. To determine the distribution, counts were made each min for a 10-min period. Cause for any

biased distribution of mosquitoes was checked by running water vapour in both air-streams prior to each test.

Female *Aedes aegypti* var. *quenslandensis* (Theo.) were used. The mosquitoes were fed a 5 per cent sucrose solution up until the time of testing and were deprived of a blood meal. Test mosquitoes were 8-10 days old and reared in the presence of males.

Our laboratory has demonstrated repeatedly with the olfactometer that air passed over human whole-body sweat is more attractive to female *Aedes aegypti* than a control stream of air (Table 1). In addition, after lyophilization the human sweat retains its attractiveness to female *Aedes aegypti* and the water removed by lyophilization is not attractive. When concentrations of lyophilized sweat in distilled water are high ( $\approx 1:1$ ), there is slight repellency. This agrees with Brown's statement<sup>6</sup> that sweat attracts at low vapour concentration and repels at a high concentration.

On dialysis of human sweat through 'Visking' cellulose for 10 days at 0°C, the attractive component(s) were separated into the dialysable fraction. In a typical dialysis using 100 ml. of sweat and  $2 \times 1,000$  ml. of distilled water, the weights of dialysable and non-dialysable fractions were 610 mg and 31 mg respectively. The non-dialysable fraction after lyophilization and redissolving in distilled water proved to be unattractive to female *Aedes aegypti* in the olfactometer.

Lyophilized human sweat was extracted with hexane, diethyl ether, acetone, isopropanol and ethanol; the extracts after removal of the solvent *in vacuo* were added to distilled water and evaluated in the olfactometer. The attractive component(s) were extracted only by diethyl ether and ethanol. The insolubility of amino-acids in diethyl ether would eliminate them as the attractive factor extracted. The residues left after exhaustive extraction at room temperature with diethyl ether followed by absolute ethanol were not attractive to female *Aedes aegypti*. Residues from the pure solvents used for extraction were found not to attract mosquitoes in the olfactometer.

Present efforts involve the use of thin-layer chromatography to separate and identify the attractive component(s) in human sweat. Table 2 shows the pattern obtained with chromatography of the ether and ethanol extracts of silica gel-G and kieselguhr thin-layer plates and gives some indication of the degree of complexity of the extracted materials.

It is somewhat surprising that the attractive component(s) present in human sweat are not volatile during lyophilization. However, in view of the low volatility of many of the sex attractants for insects we may assume that volatility in the usual chemical meaning is not of much importance in attracting insects.

Table 1. ATTRACTIVENESS OF HUMAN SWEAT FRACTIONS TO FEMALE *Aedes aegypti*

Sample	Identification	Concentration (wt./vol. water)	Olfactometer data.* No. of mosquitoes Control Sample port		
IV	Lyophilized whole-body sweat	1:100	5-4	14-6	46-0
IV	Lyophilized whole-body sweat	1:1,000	4-9	15-1	51-0
IV	Lyophilized whole-body sweat	1:10,000	4-4	15-6	56-0
III	Ether extract of lyophilized sweat	1:1,000	8-0	12-0	20-4
Ve	Ether extract of lyophilized sweat	1:1,000	7-6	12-4	24-0
Ve	Ethanol extract of lyophilized sweat	1:1,000	7-2	12-8	28-0
XIII	Sweat dialysate	1:1,000	6-1	12-9	30-0
VIII	Sweat dialysate	1:1,000	7-5	12-5	25-0
	Whole-body sweat	Undiluted	8-8	11-7	17-0

\* Each figure is the mean of 10 one-minute observations. The student *t* test was used for significance. All values reported are significant at the 1 per cent level.

† *IA* (Index of attraction) =

$$\frac{\text{No. of mosquitoes on sample side} - \text{No. of mosquitoes on control side}}{N \text{ (total No. of mosquitoes)}} \times 100$$

Table 2. THIN-LAYER CHROMATOGRAPHY OF ETHER AND ETHANOL EXTRACTS OF LYOPHILIZED HUMAN WHOLE-BODY SWEAT

Sample	Identification (extract)	Adsorbent	Developing solvent	R <sub>F</sub> values (I <sub>a</sub> detected)
XXXV	Ether	Silica gel-G	Benzene	Origin, 0-52, 0-67
LIX	Ether	Silica gel-G	Benzene	Origin, 0-19, 0-50, 0-62
III	Ether	Silica gel-G	Chloroform	Origin, 0-62, 0-68
LIX	Ether	Silica gel-G	Chloroform	Origin, 0-60, 0-67
III	Ether	Silica gel-G	Ethanol	0-62, 0-75
III	Ethanol	Silica gel-G	Chloroform	Origin, 0-09, 0-21, 0-52, 0-68
III	Ethanol	Silica gel-G	Ethanol	Origin, 0-07, 0-25, 0-66, 0-73, 0-76
LVI	Ethanol	Kieselguhr-G	Benzene/ethyl-formate/formic acid (5:4:1)	Origin, 0-15, 0-42, 0-64, 0-77, 0-91

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## MICROBIOLOGY

### Growth of Mammalian Cells in a Heat-stable Dialysable Medium

NEARLY all media routinely used for cell culture contain from 5 to 40 per cent serum of a particular species of animal. As one might expect, there has been much research into the nature of the fraction or fractions of serum which form the active part of this material<sup>1-4</sup>. Despite this the exact nature of the activity of serum or its fractions is unknown.

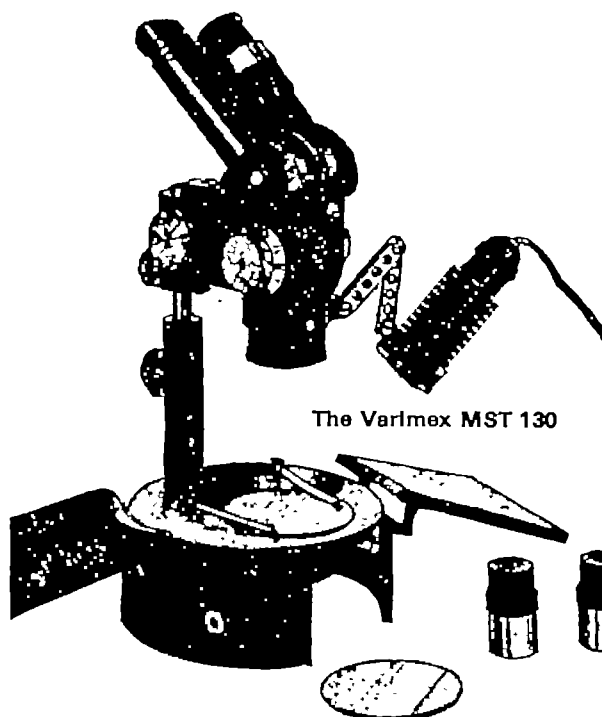
A wide variety of macromolecular substances have been used, either alone or in combination with smaller than normal amounts of serum, in addition to a chemically defined base medium, in attempts to simplify tissue culture media. These include various peptones, lactalbumin hydrolysate, yeast extract, insulin, tryptic hydrolysate of milk and inert substances such as methylcellulose or polyvinylpyrrolidone.

A very limited number of completely chemically defined media exist which support the growth of mammalian cells *in vitro*<sup>5-14</sup>. The most successful of these media are those developed by Evans *et al.*<sup>8-11</sup>. The drawback of media of this type lies in their complexity, some containing as many as 70 components, the extended period of cell adaptation needed, the very limited spectrum of cells which the medium will support, or the constant replenishment of the cell monolayer which is necessary. In addition, the growth rate seldom matches that of the serum-containing parent line.

The present report deals with a simple, heat stable, dialysable medium which promotes the rapid growth of a mammalian cell line. Growth in two basic media of varying complexity is reported for comparison.



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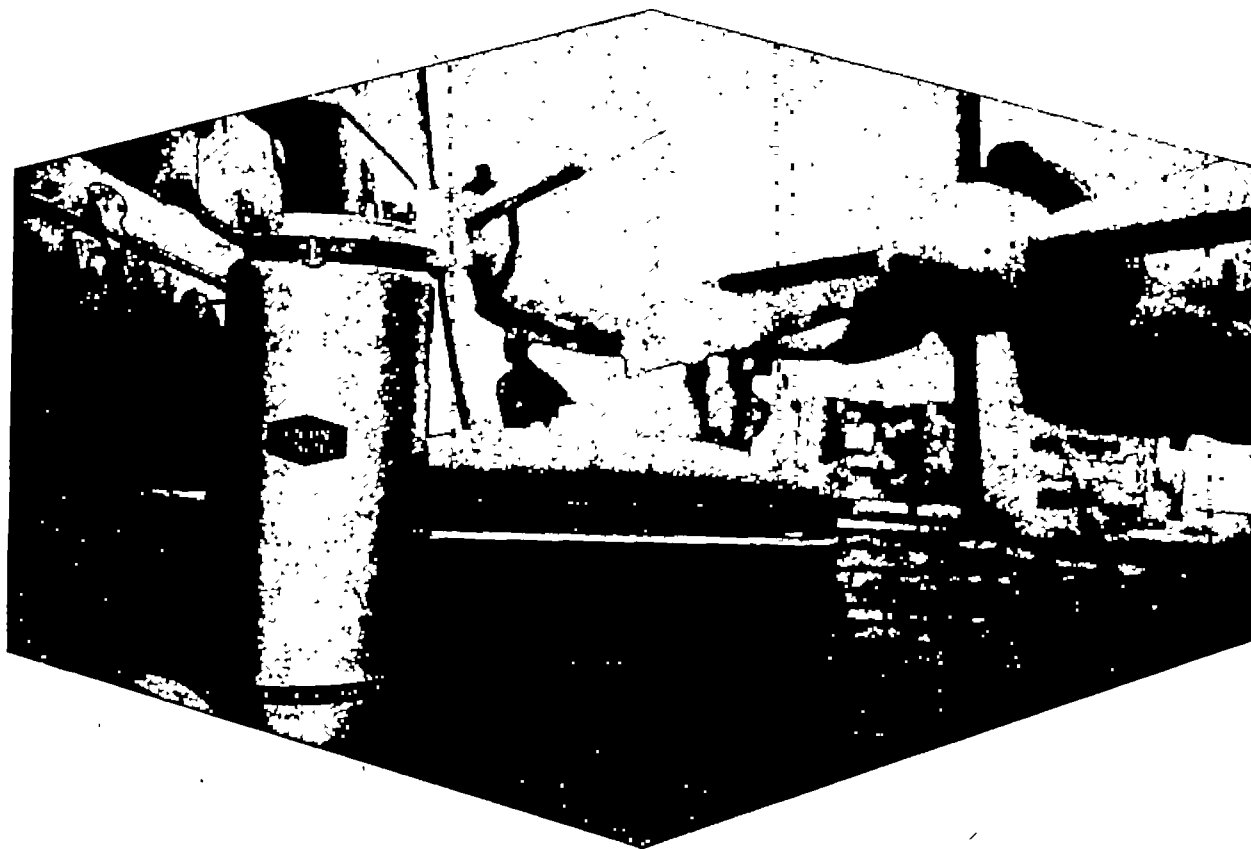
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The basic cell line was originally isolated from adult normal rabbit myocardium. The original medium consisted of 10 per cent calf serum, 90 per cent Medium 199 (ref. 15), 50 units of penicillin and 50 µg streptomycin per ml. These cells were subsequently adapted to growth on the same medium in which the serum was replaced by 0.5 per cent autoclaved whole Difco 'Bacto-peptone' plus 100 mg per cent of glucose. These cells have undergone more than 270 passages and are passed every 6-7 days.

While attempting to simplify the added peptone fraction it was found that a dialysate of this fraction substituted quite well for the crude peptone material. This dialysate was prepared by dissolving 30 g of dry Difco 'Bacto-peptone' in 270 ml. triple-distilled water, and autoclaving it. 150 ml. sterile triple-distilled water was placed in 50-ml. quantities in 3 sterile dialysing tubes and placed directly into the peptone. Dialysis was carried out in the cold for 24 h with agitation. At the end of this time the material inside the bag was collected, and routinely gave a yield of 6-8 ml. per tube. The dialysate was placed in screw-capped tubes and autoclaved before use. Portions of each lot were lyophilized in a Virtis centrifugal 'Bio-Dryer' until dry, removed, weighed and reconstituted with sterile water. The average dry weight yield was 53 mg per ml.

**Media.** The 6 media compared were: 1, Medium 199, 90 per cent; calf serum, 10 per cent. 2, Medium 199, 99.5 per cent; crude autoclaved peptone, 0.5 per cent. 3, Medium 199, 99.5 per cent; autoclaved peptone dialysate, 0.5 per cent. 4, Totally autoclaved No. 3 above. 5, Eagle's minimum essential medium (MEM) without glutamine, 99.5 per cent; autoclaved peptone dialysate, 0.5 per cent. 6, Totally autoclaved No. 5 above.

No adaptation period was necessary when crude peptone cells were transferred to the dialysate medium. The cells were simply transferred and passed on the selected medium.

Growth curves were measured by inoculating 10-150-ml. 'Pyrex' milk dilution bottles with a given number of cells in a total volume of 10 ml. of medium. At 0 h, and every 24 h for a total of 7 days, one bottle was collected by use of a 1 per cent trypsin solution, and haemocytometer counts were made. All lines had undergone at least 10 passages in the individual media before they were used in the growth experiments. Many of these experiments have been repeated 10-12 times with essentially the same result.

Typical growth responses may be seen in Table 1. It is quite apparent that there is little if any difference in the growth rates of the cells in any of these media.

It is also quite obvious that macromolecules are not necessary to attain rapid growth. With regard to heat stability of the base medium, it is most logical to consider the simplest one, in this case Eagle's MEM. Here, one would expect the least stable substances to be pyridoxal and folic acid. Whether or not these substances are needed by this cell line is not known. Since the cells will not survive more than one passage on any of the base media alone, it is quite certain that the dialysate is essential for growth. Preliminary studies on the dialysate have indicated the presence of the free amino-acids threonine, serine, aspartic acid, glutamic acid, proline, glycine, alanine, hydroxylysine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and

arginine, together with two unknown substances presumed to be peptides.

Preparation of media which contained added amounts of these free amino-acids in addition to either Medium 199 or Eagle's MEM medium did not result in cell growth. The addition of methylcellulose (0.12 per cent) to any of the base media used did not support cell growth.

The process of isolating, testing and identifying these 'peptides' is in progress. It is quite possible that obtaining these substances in a pure state, which will allow their incorporation in increased amounts without concomitantly increasing possible toxic substances, may allow for the production of a wide-spectrum, well-defined and stable medium for cell culture.

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## Fractions of Staphylococcal Enterotoxin B

In a recent paper<sup>1</sup> we showed that enterotoxin B isolated from *Staphylococcus aureus* strain S8 could be separated into two main protein fractions by starch-gel electrophoresis. After the completion of this work we received communications concerning this observation from Dr. M. S. Bergdoll (University of Chicago) and from Dr. E. J. Schantz (U.S. Army Laboratories, Fort Detrick). Dr. Bergdoll told us that he has demonstrated the presence of at least 3 toxic fractions of enterotoxin B which would react with the same antibody<sup>2</sup> and suggested that denaturation during purification of the enterotoxin may be responsible for these fractions. Dr. Schantz informed us that he has obtained the two main protein components by starch-gel electrophoresis of purified enterotoxin. However, he was unable to resolve these by ultracentrifugation or by free electrophoresis; amino-acid analysis results were also consistent with a single molecular species of one polypeptide chain<sup>3</sup>. Dr. Schantz suggests, therefore, that the starch-gel fractions are artefacts produced in the gel by the current and possibly by the reaction of impurities with functional (charged) groups on the protein. Accordingly we have tried to resolve our fractions by methods other than starch-gel electrophoresis and we have re-examined the purification procedure to find whether the fractions appear as artefacts as a result of any stage of purification.

In order to test the suggestion by Dr. Bergdoll that the fractions were produced by denaturation during purification we re-cycled the toxin through 'Amberlite IRC 50' and ethanol-precipitation<sup>1</sup> and then prepared starch gels of the re-cycled toxin. We compared the relative opacity of the resulting bands with a control of untreated toxin

Table 1. GROWTH OF RABBIT HEART CELLS IN VARIOUS MEDIA

Medium	0	1	2	3	4	5	6	7
10% Calf serum + 199	2.8	3.3	5.0	8.3	11.3	12.4	14.0	14.6
0.5% Peptone + 199	1.8	2.2	3.2	7.4	12.5	14.1	15.2	16.4
0.5% Dialysate + 199	2.0	2.3	3.1	6.4	11.2	12.8	13.9	14.8
0.5% Dialysate + autoclaved 199	1.9	2.3	3.0	7.1	—	11.0	—	14.5
0.5% Dialysate + Eagle's MEM	2.1	2.2	3.8	7.2	—	10.4	—	14.0
0.5% Dialysate + autoclaved Eagle's MEM	2.1	2.0	3.0	6.6	10.6	11.9	14.1	14.4

\* All dialysate autoclaved 10 lb. x 10 min.

using a 'Chromosean' apparatus (Joyce, Loebel and Co., Gateshead). Since there was no difference we concluded that the purification steps did not affect the toxin. If starch-gel electrophoresis causes denaturation of enterotoxin B we would expect to see, after developing the gels, not discrete protein bands but a continuous streak of protein, representing the denaturation of more and more of the original species during the run. However, we have prepared 17 batches of the toxin and have always obtained two discrete main protein bands. Also, when unstained portions of these bands were cut out and re-run on starch gels they ran as single bands in their original relative positions; thus there was no evidence of inter-molecular transformations.

Since it is apparent that neither the purification procedure nor the separating technique is responsible for the appearance of the two bands, it remains to be discovered what the molecular difference is between the two bands and how this has arisen. Resolution on starch gel is dependent on protein size and charge. The two fractions were not separated by chromatography on 'Sephadex' G75, G100 or G200, in *tris*-citrate buffer at pH 8.88. This indicates, therefore, that they are of close molecular weight, and that separation by starch gel is not due to size difference and must thus be mainly dependent on charge. The two proteins, when extracted from starch gels using Gordon's cell<sup>4</sup>, were found to be serologically identical. Both proteins were precipitated at the same titre by an antiserum to one of them; serological identity was confirmed using Ouchterlony plates<sup>5</sup>.

The relative toxicity of the two fractions has been compared in pigs and young cats. We showed previously that starch-gel band 2 did not cause vomiting in pigs<sup>1</sup>. However, we find that both fractions cause vomiting when administered intraperitoneally to young cats, which are more sensitive to enterotoxin than pigs. However, the minimum vomiting dose of protein corresponding to band 2 was at least 3 times greater than that of band 1.

In conclusion, the two protein fractions are extremely similar and the only differences appear to be of charge and toxicity. We suggest, therefore, that the fractions that we have resolved on starch gels differ in secondary or tertiary protein configuration and that they do not appear to be artefacts of the purification procedure.

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## VIROLOGY

### A Common Mode of Antiviral Action for Ammonium Ions and Various Amines

AMMONIUM ions and various aliphatic amines have been reported<sup>1-4</sup> to inhibit the growth of influenza virus in tissue culture. Recently, 1-adamantanamine was reported<sup>5</sup> to inhibit the multiplication of certain strains of influenza virus by slowing or blocking the penetration of virus into the host cells. In this communication we present results showing that ammonium ions and various aliphatic amines inhibit influenza virus growth by the same mechanism as 1-adamantanamine.

To demonstrate the nature of the action of the compounds, we have used the procedure of Davies *et al.*<sup>6</sup>. In this procedure, a virus inoculum in tissue culture systems is exposed to a compound for various periods of time. To some of the cultures, specific antiserum is added 15 min

before the end of each exposure period. The tissue sheets are then washed to remove the compound and the antiserum; fresh medium is added and the cultures are re-incubated. The amount of viral growth in the cultures treated with the compound indicates whether or not the compound has inactivated the virus or interfered with its adsorption to the host cells. Antiserum neutralizes extracellular virus, and the amount of viral growth in the cultures treated with the compound and antiserum indicates whether or not the compound has prevented penetration of the cells by the virus.

To produce optimal antiviral effect, ammonium chloride, the aliphatic amines and 1-adamantanamine must be added at the time of viral inoculation and must remain present during the entire incubation period. If the compounds are removed early in the incubation period, subsequent viral growth is not inhibited<sup>4-6</sup>. As shown in Fig. 1 (curve B), exposure of the viral inoculum to ammonium chloride for periods as long as 4 h did not result in inhibition of viral growth. This result indicates that ammonium chloride did not inactivate the virus on contact, and did not interfere with its adsorption to the host cells. Addition of antiserum to control cultures at the time of virus inoculation, or 15 min thereafter, greatly decreased subsequent viral growth. However, when the antiserum was added at 45 min or later, it had no effect on the viral growth (curve C). In contrast, addition of antiserum to tissue cultures containing ammonium chloride substantially reduced the viral growth, even when it was added as late as 3.75 h after virus inoculation (curve D). These results indicate that in the absence of ammonium chloride the virus was no longer susceptible to neutralization by antiserum 45 min after the virus was added to the tissue culture. In the presence of ammonium

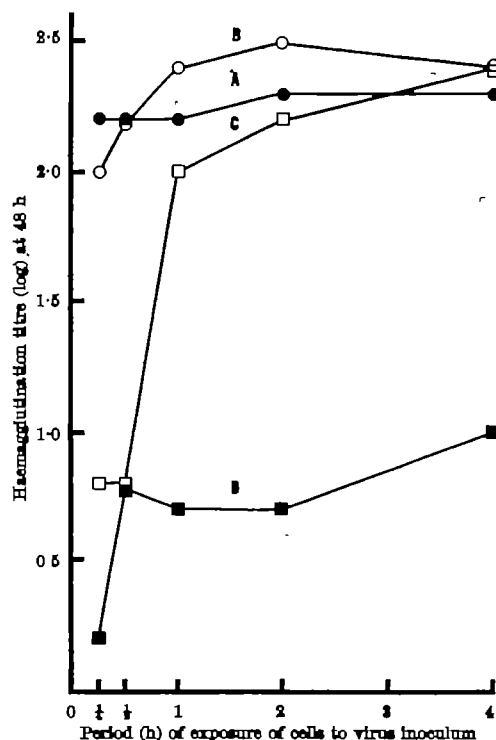


Fig. 1. Effect of  $\text{NH}_4\text{Cl}$  and antiserum on an influenza A PB-8 virus inoculum as measured by its subsequent growth in monkey kidney tissue cultures. The cultures were inoculated with 0.1 ml. of a  $10^{-5}$  dilution of stock PB-8 virus,  $\text{TCID}_{50} = 10^{-4.5}$  ml.  $\text{NH}_4\text{Cl}$  (final conc. 250  $\mu\text{g}/\text{ml}$ ) was added 15 min before the virus (curves B and D). Antiserum (0.1 ml. of 1/5 dilution of serum) was added 15 min before the end of the indicated exposure period (curves C and D). The final vol. of the cultures was 2 ml., pH 7.2. At the end of each exposure period the tissue sheets were washed twice, fresh medium was added and incubation continued. The cultures were incubated at  $37^\circ\text{C}$  for 48 h, at which time haemagglutination titres were determined by Balk's procedure (ref. 6) adapted to use the microtitre technique (Cooke Engineering, Alexandria, Va.). Curve A, control; curve B,  $\text{NH}_4\text{Cl}$ ; curve C, antiserum; curve D,  $\text{NH}_4\text{Cl}$  + antiserum.

chloride, however, the virus remained susceptible to neutralization by the antiserum for as long as 3.75 h. Since ammonium chloride did not inactivate the virus, nor interfere with its adsorption to the cells, it must be concluded that the compound slowed or blocked the penetration of the cells by the virus. This effect on penetration is not complete. The small amount of viral growth in the tissue cultures treated with ammonium chloride and antiserum indicates that a small amount of virus had penetrated the cells in the presence of the compound.

Several amines, including propylamine, diethylamine, triethylamine, 1,3-diaminopropane HCl, 1,4-diaminobutane 2HCl and 1,5-diaminopentane 2HCl, were tested. At concentrations of 250 µg/ml. these compounds produced effects similar to those shown for ammonium chloride.

Ammonium chloride and the various aliphatic amines had no inhibitory effect against the Lee strain of influenza type B. In this respect, also, they were similar to 1-adamantanamine, which is inactive against strains of influenza B (ref. 5).

Since influenza virus neuraminidase may be involved in the infection process<sup>1</sup>, the effect of the various compounds on this enzyme was examined. PR-8 virus concentrated by high-speed centrifugation served as source of enzyme and malolactose (General Biochem. Inc., Chagrin Falls, Ohio) as substrate. Sialic acid released by enzyme action was determined by the Aminoff<sup>2</sup> procedure. Neither ammonium chloride, nor any of the other amines, tested at 1,000 µg/ml., affected neuraminidase activity.

Electron microscope studies suggest that intact influenza particles are taken into the cells by phagocytosis<sup>3</sup>. Our results show that ammonium chloride and various amines prevent this engulfment process. That this effect can be nullified by simply washing the cells suggests that the compounds may interfere with the penetration process by causing ionic changes at the cell surface. The failure of the agents to block a virus of influenza type B indicates that viruses of type A and B may penetrate the cells at different sites or differ in their tolerance of changes in ionic conditions. Of the various amines, only 1-adamantanamine shows antiviral activity in animals. This activity may be related to the fact that the compound reaches the site of infection in the body without undergoing changes.

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### Growth of Foot and Mouth Disease Virus in Organ Cultures of Mouse Pancreas

THE use of organ culture techniques in the investigation of animal virus diseases allows the examination of the histo-pathological processes of infection to be made under conditions which resemble more nearly the *in vivo* state than the monolayer type of cell culture, but which permit a considerable degree of control of the organ or tissue environment. As yet, this kind of investigation has

not been widely made though observations have been reported on herpes simplex virus in skin<sup>1</sup> and also with polyoma and vesicular stomatitis viruses in mouse metanephric rudiments<sup>2</sup>. Preliminary work with foot-and-mouth disease virus (FMDV) has shown that in organ cultures of guinea-pig metatarsal pad<sup>3</sup>, virus multiplication will take place for up to four days, but only to a level at least 1,000-fold lower than in the metatarsal pads of the intact animal.

In adult *P* strain mice infected with unadapted FMDV the pancreas is the principal site of virus multiplication and a pancreatitis occurs<sup>4</sup>. In suckling mice, however, virus multiplication occurs mainly in the skeletal muscles and pancreatitis is absent<sup>5</sup>. The experiments reported here were undertaken to obtain more information about the susceptibility of the pancreas of the suckling mouse to this virus.

Pancreases to be cultured were removed aseptically from freshly killed 6-7-day-old *P* strain mice and placed in Eagle's basal medium containing 10 per cent calf serum. To initiate infection a known number of pancreases were incubated at 37° C for 1 h in 1 ml. of medium containing 10<sup>5.5</sup> ID<sub>50</sub> of FMDV (type O; strain VI). A sample of medium containing virus but no tissue was similarly incubated. Titrations of residual virus after incubation with and without pancreases using 5-7-day-old *P* strain mice gave end points not significantly different from each other (calculated by the method of Reed and Muench<sup>6</sup>). If allowances are made for the error of such titrations, the maximum amount of virus which could have been absorbed by the batch of tissues would not be greater than 10<sup>3.7</sup> ID<sub>50</sub>, and this would be considerably reduced by thermal inactivation during incubation. Uninfected control pancreases were similarly incubated in virus-free medium. The infected pancreases were washed twice with phosphate buffered saline (pH 7.2) at 37° C for 2 min, in groups of 3 on rayon strips mounted on stainless-steel gauze. Each group of three pancreases was placed in a separate wide-mouthed transfusion bottle containing 30 ml. of medium which was then gassed for 2-3 min with 5 per cent carbon dioxide in oxygen and incubated at 37° C. The medium consisted of 80 per cent Eagle's basal medium<sup>7</sup>, 10 per cent calf serum and 10 per cent 14-day chick embryo extract and was changed on the third day.

The virus content of each group of three infected pancreases was determined at intervals for up to five days, the pancreases being washed in phosphate-buffered saline (pH 7.2) and stored at -20° C pending titration. Parallel cultures for histological studies were fixed in 10 per cent neutral buffered formalin, dehydrated and embedded in paraffin wax. Sections 7µ thick were cut at intervals of 20-30µ through the pancreas and stained with haematoxylin and eosin. In all 45 infected and 16 uninfected pancreases were cultured.

Virus was found to be present up to the third day *in vitro*, but was recovered only once after this time. Table 1 summarizes the results of two experiments. It is evident that the pancreatic explants were able to support virus multiplication and the total amount of virus rose to a

Table 1. QUANTITY OF FOOT AND MOUTH VIRUS RECOVERED FROM 6-7-DAY-OLD MOUSE PANCREASES CULTURED *in vitro*

Exp. No.	Hours after infection	Virus content of pancreatic tissue (3 pancreases = 60 mg approx)*	Log ID <sub>50</sub> of culture medium (80 ml.)	Total virus (tissue and medium)	Ratio of virus in medium to virus in tissue
1	18	2.3	Not determined	—	—
	20	3.3	Not determined	—	—
	48	3.2	3.1	3.5	2.3
	50	4.3	Not determined	—	—
	74	0	0	0	—
	120	0	Not determined	—	—
2	19	2.8	3.5	3.6	5.1
	20	2.4	Not determined	—	—
	48	3.0	3.5	3.6	3.1
	50	4.2	4.5	4.7	3.2
	67	2.4	Not determined	—	—
	72	0	0	0	—

\*Mean weight of 6 pancreases (6-7 day old mice) was found to be 20 mg

peak at about 50 h, much of the virus being released into the medium. Histologically, lesions attributable to FMDV could not be detected. Apart from occasional areas of necrosis in older cultures, both infected and control pancreases appeared fully viable, but with continued culture the acinar lumen increased in size and the cells became flattened and atrophic, forming duct-like structures (Figs. 1 and 2). Zymogen granules decreased in number during the period of culture. This is in contrast to the active synthesis of zymogen granules observed in cultured foetal pancreases<sup>6,7</sup>.

It can be concluded that the 6-7-day-old mouse pancreas cultured *in vitro* produces moderate yields of FMDV but without specific cytopathic changes. A similar growth of FMDV without the development of cytopathic effects has been observed in polygonal cell monolayers derived from bovine tongue epithelium<sup>10</sup>.

The transfer of results obtained in tissue culture to the whole animal can only be made with caution; but it

is possible that some multiplication of FMDV may occur in the pancreas of the suckling mouse *in vivo* in spite of the absence of demonstrable pathological lesions there.

I thank Dr. J. B. Brooksby and Dr. H. Platt for their advice and Mrs. D. Read for the histological preparations.

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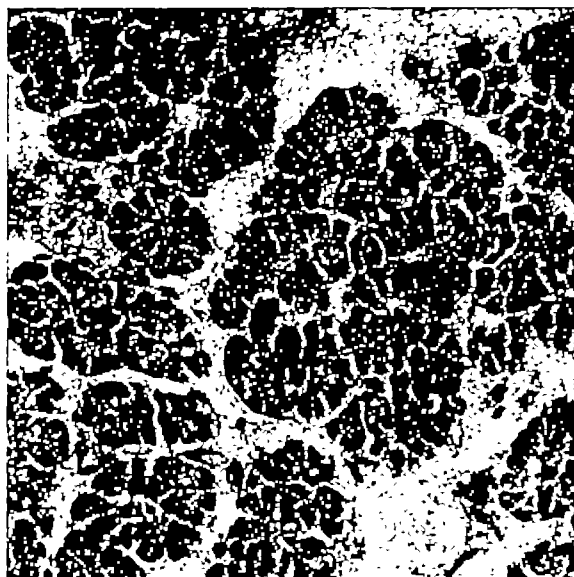


Fig. 1. Longitudinal section of pancreas of 6-7-day-old mouse. Control material not cultured. Haematoxylin and eosin. Acinar cells filled with zymogen granules. ( $\times 110$ )

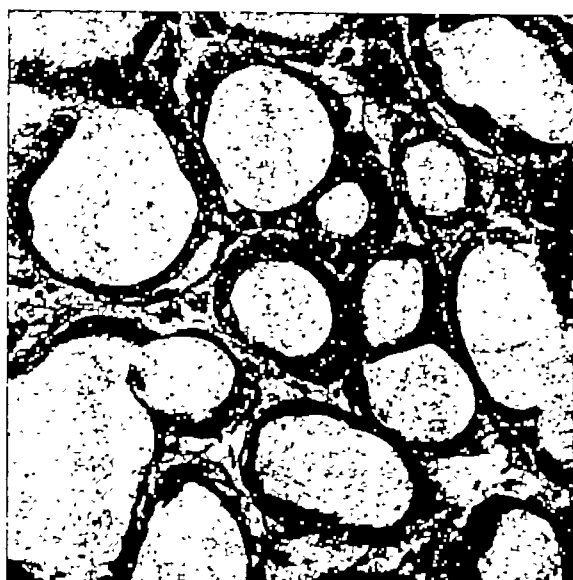


Fig. 2. Longitudinal section of pancreas of 6-7-day-old mouse. Cultured for six days. Haematoxylin and eosin. Note greatly enlarged lumen of acini with flattened acinar cells. ( $\times 110$ )

## VETERINARY SCIENCE

### Diurnal Variation in the Diet selected by Free-grazing Sheep

THE use of oesophageal fistulae has facilitated work on the diet selected by grazing ruminants. Three experiments at this laboratory with free-grazing sheep have shown marked diurnal changes in the nitrogen content of the diet selected by Merino wethers.

In experiment A, ten two-year-old Merino wethers fitted with oesophageal fistulae continuously grazed a *Phalaris tuberosa*/white clover (*Trifolium repens*) pasture for two months during summer. During the last five days, samples of approximately 20 g of dry matter were collected through the fistula for two 20-min periods starting at 0830 h and 1500 h. The sheep were not deliberately fasted before the samples were collected. The samples were dried at 70°C in a forced draught oven and analysed for nitrogen and organic matter. The mean nitrogen contents expressed as a percentage of the organic matter for the morning and afternoon samples were 3.38 and 3.99 per cent respectively, the difference being significant ( $P < 0.05$ ). There were also significant differences between sheep ( $P < 0.001$ ) and a significant day  $\times$  time of day interaction ( $P < 0.001$ ). The difference between sheep has been observed on many occasions, even when, as in these trials, the sheep were of similar sex, breed and age, and had been originally drawn at random from a single flock.

In experiment B, five Merino wethers similar to those used in exp. A were run on a ryegrass (*Lolium perenne*)/white clover pasture for several months during winter. Samples were taken on two separate days, starting at 0830, 0910, 1140, 1430 and 1710 h. The nitrogen content of samples collected at different times throughout the day differed significantly ( $P < 0.01$ ), rising rapidly during the morning and levelling out during the afternoon. It was possible to fit a significant quadratic regression equation ( $P < 0.01$ ) to this trend:

$$y = 3.01 + 0.1187x - 0.00783x^2 \quad (1)$$

where  $y$  is the mean nitrogen content of the sample expressed as a percentage of the organic matter, and  $x$  is the time elapsed in hours from dawn (0830 h). The values are shown in Fig. 1.

Although there were significant differences between sheep ( $P < 0.01$ ) and between days ( $P < 0.05$ ), and a significant interaction between days and time of day ( $P < 0.05$ ), the slope of the relationship between dietary nitrogen concentration and time of day could be combined between sheep and between days.

In experiment C, a *Phalaris tuberosa*/cocksfoot (*Dactylis glomerata*)/white clover sward was continuously grazed

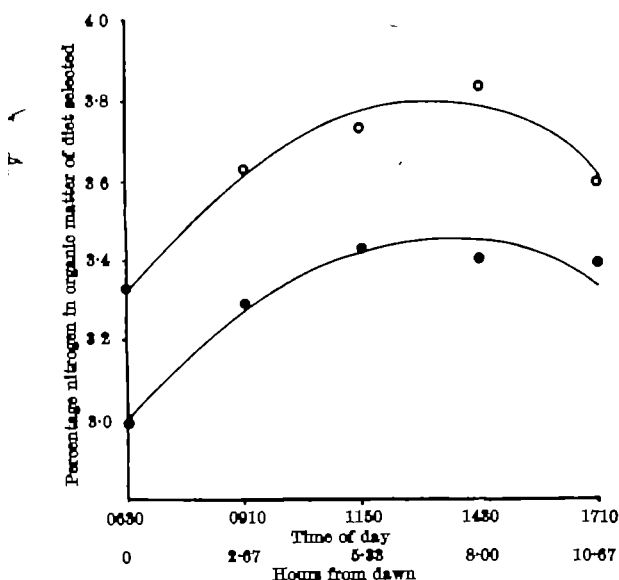


Fig. 1. Relationship between nitrogen content of diet selected by grazing sheep and time of day. ●, Exp. B; ○, exp. C.

at four stocking intensities ranging from 1 to 13 sheep per acre for eighteen months, resulting in a wide range in herbage availability. On each pasture a fistulated Merino wether similar to those used in the previous experiments had been grazed with the other sheep. A second fistulated sheep was then introduced, which was identical with the first except that it had previously grazed a ryegrass/lover pasture. Ten days later, samples were taken from all fistulated sheep on two separate days at similar times to those used in exp. B.

As observed in exps. A and B, there were significant differences ( $P < 0.05$ ) between samples collected at different times of day, and again it was possible to fit a significant ( $P < 0.05$ ) quadratic equation to this trend which is shown in Fig. 1:

$$y = 3.32 + 0.143x - 0.0108x^2 \quad (2)$$

There were also significant differences in the nitrogen content of the diet between the different pastures ( $P < 0.05$ ) and between the sheep which had been grazed on the experimental pastures for many months and those which had not ( $P < 0.01$ ); the mean value for the former was 3.72 per cent and for the latter 3.52 per cent nitrogen. This phenomenon cannot at present be explained, but similar differences have been observed on other occasions.

The regression coefficients given in equation (2) did not differ significantly between paddocks or between the two groups of sheep. Furthermore, those derived in exp. B could be combined with those in exp. C, the pooled coefficients being  $+0.131$  for  $x$ , and  $-0.00939$  for  $x^2$ .

Information on diurnal trends in selection by grazing ruminants is limited. Taylor and Deriaz<sup>1</sup> report investigations in which samples of herbage were collected from one steer fitted with a rumen fistula by manually collecting the feed boluses as they entered the reticulo-rumen. They found no consistent trend throughout the day under continuous grazing. Since in the three experiments reported here such trends were statistically significant and of similar magnitude, it would be of value to define those conditions in which they do occur. Until these conditions are clearly established, it would seem prudent to consider the possibility of diurnal trends when designing experiments to examine selection by grazing sheep. This may also be necessary when a representative sample of the daily intake is required, but it may be difficult to establish an optimum sampling time for this purpose because sheep tend to graze at specific times of day<sup>2</sup> and possibly at different rates at these times. Further work will be necessary to ascertain whether the changes observed reflect

diurnal changes in the composition of the herbage organic matter, or in the parts of the plant or species of plant selected.

J. P. LANGLANDS

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<sup>1</sup> Taylor, J. C., and Deriaz, R. H., *J. Brit. Grassl. Soc.*, 18, 29 (1963).

<sup>2</sup> Arnold, G. W., *J. Brit. Grassl. Soc.*, 17, 41 (1962).

## SOIL SCIENCE

### Contribution of Organic Matter to the Cation Exchange Capacity of Soils

THE cation exchange capacity of soils is due to inorganic constituents such as clay minerals, hydrous oxides, primary and secondary minerals and to organic matter. Estimates of the contribution of organic matter are usually made by determining the cation exchange capacity of soils before and after destruction of organic matter. Using this approach, Gorbunov<sup>1</sup> estimated that organic matter accounted for between 30 and 80 per cent of the cation exchange capacity of plough-depth layers of a number of Russian podzol soils.

I have recently re-examined this problem in a more direct fashion, that is, by determining exchangeable hydrogen (which in this case is equivalent to cation exchange capacity) in extracted and purified organic matter and found that the cation exchange capacity due to organic matter was considerably higher than that indicated by the 'difference' method.

For this purpose, soil samples taken by  $B_1$  horizons of imperfectly drained podzols, such as Armadale, are especially suited because: (a) practically 100 per cent of the organic matter can be extracted; (b) the extracted organic matter can be purified with relative ease; (c) we have assembled a considerable amount of information on the distribution of functional groups in and chemical properties of this type of organic matter<sup>2</sup>.

The soil sample contained 10.0 per cent organic matter, 10.1 per cent  $Al_2O_3$ , 2.9 per cent  $Fe_2O_3$ , 76.5 per cent  $SiO_2$ , 1.07 per cent 'free'  $Al_2O_3$ , and 0.91 per cent 'free'  $Fe_2O_3$ . It contained 10 per cent clay, which was predominantly illite. The cation exchange capacity was measured with N ammonium acetate solution adjusted to pH 7 (ref. 3), before and after destruction of the organic matter with increments of hydrogen peroxide. Methods of extraction and purification of organic matter were the same as those described previously<sup>2</sup>. Exchangeable hydrogen in the extracted and purified organic matter was determined by shaking with an excess N ammonium acetate solution at pH 7 and measuring the acetic acid liberated.

As shown in Table 1, the method based on difference measures only one-fifth of the cation exchange capacity that might be due to organic matter. Since for each milliequivalent of exchangeable hydrogen in the organic matter 3.6 millimoles of Fe+Al were available in this soil, it is very likely that the remaining exchange sites were blocked by ions or hydrous oxides of these constituents. In addition, reactions of organic exchange sites with silicon and/or compounds of silicon are also within the realm of possibilities. Conversely, we have been able to eliminate practically all the exchangeable hydrogen in the extracted and purified organic matter by interaction with sufficient ions or hydrous oxides of iron and aluminium. We have obtained results similar to those shown in Table 1 with soil samples taken from other podzol  $B_1$  horizons.

From the information presented here the following is suggested: (1) Reliable estimates of the contribution of organic matter to the total cation exchange capacity



Table 1. CATION EXCHANGE CAPACITY VALUES FOR ARMADALE B<sub>2</sub> SOIL AND ORGANIC MATTER

Material	Mequiv./ 100 g soil	% Cation ex- change capacity
'Difference' method		
Unirradiated soil	18.7	100.0
Soil after destruction of organic matter (= inorganic portion)	5.7	30.5
Due to organic matter	13.0	69.5
Organic matter extracted and purified	5.7	8.1
Inorganic portion		
Extracted and purified organic matter (humic + fulvic acids)	65.0	91.9
	70.7	100.0

cannot be made by the 'difference' method. Because in most predominantly inorganic soils only 20–30 per cent of the organic matter can be extracted, meaningful estimates of the organic exchange capacity can be made only in those soils in which either the bulk of the organic matter can be extracted, such as podzol B<sub>2</sub> horizons, or in organic soils where most of the inorganic constituents can be extracted from the organic matter. (2) The results in Table 1 suggest that in the case of soils containing appreciable amounts of organic matter two types of cation exchange capacities should be considered: (a) 'measured' cation exchange capacity as determined by exchange with NH<sub>4</sub><sup>+</sup> or any other ion one might choose to use; (b) 'potential' cation exchange capacity, that is, 'measured' cation exchange capacity plus cation exchange capacity due to blocked organic exchange sites which are inaccessible to or do not react with exchange ions. These blocked sites can be unlocked and measured by methods such as have been used in this investigation. Thus, the 'potential' cation exchange capacity of soils might be several times greater than the 'measured' one. From the foregoing a practical application is suggested: if organic exchange sites presently blocked by inert iron and aluminium compounds could be filled with ions more beneficial to plant nutrition, soil productivity might be increased.

M. SCHNITZER

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Canada Department of Agriculture,  
Ottawa, Ontario, Canada.

<sup>1</sup> Gorbunov, N. I., referred to by Flaig, W., Sochting, H., and Bouteleupacher, H., *Landbauforschung Volkswirtschaft*, 18, 18 (1963).

<sup>2</sup> Schnitzer, M., and Desjardins, J. G., *Soil Sci. Soc. Amer. Proc.*, 25, 362 (1962).

<sup>3</sup> Pech, M., Alexander, L. T., Dean, L. A., and Reed, J. F., *U.S. Dep. Agr. Circ.*, 757 (1947).

## MISCELLANY

### Probability of Life

PROF. H. F. BLUM has given reasons for believing that the probability of the development of life up to the present human cultural level on our planet is unlikely to exceed  $10^{-18}$  (ref. 1). His arguments may well be valid if they are supposed to apply to our specific civilization with its Ford cars and hybrid maize and antifluoridation societies. There is, however, a simple fallacy involved when Prof. Blum suggests that this minute figure should "give our imagination pause in peopling the universe with living things, particularly with 'intelligent' life approaching closely the characteristics of men".

The probability of any set of bridge hands being dealt twice running to the same four people from well-shuffled packs is in the region of  $10^{-31}$ . There is no comparable improbability that the second deal will yield something usable for the purpose of playing bridge.

Since the beginning of life on this planet, Prof. Blum suggests that not less than  $10^9$  successful mutations have gone to the making of the present million or so living species.

Hence he deduces that the probability of biological evolution having reached its present state is of the order

of  $10^{-9}$ . Even if we grant this numerical deduction for the present particular set of species—though for this  $10^{-9}$  seems incredibly large—it neglects the fact that if any one of the successful mutations had not occurred, some other mutation would have been successful. Without going into any numerical detail at all, it is surely clear that, once a mutable life has started, the probability that some assortment of living species should exist at any future time is not  $10^{-9}$  but unity (assuming that no catastrophe great enough to sterilize the entire planet occurs). Following Prof. Blum in treating man as a special case, there seems no reason for thinking that the rest of the present living species are unexpectedly complex in number or variety; that they are more than a very ordinary bridge hand.

The development of a human level of intelligence might be considered seriously as a highly improbable event, but such palaeontological evidence as we have does not support such a consideration. Creatures in widely separated groups, and even in different phyla, have shown a continuous increase in brain size with time, so that though specific mutations may have been improbable, the kind of mutation leading to this was not. Even at the immediately pre-human level there seem to have been at least three evolutionary lines which learnt the use of tools.

Finally, Prof. Blum multiplies his improbability by a further factor of  $10^{-4}$  to represent the improbability of the 'cultural mutations' leading to our present state. Again, if he is considering the probability that the world should have the particular pattern of nations and cultures that it has, his factor of  $10^{-4}$  is probably far too large, but surely some kind of advanced culture had a probability approaching unity once the use of tools by creatures of human intelligence was established some tens of thousands of years ago.

A different path for some of the most vital 'cultural mutations' might have changed the rate of development—in either direction—as well as the final form, in quite major ways, but the ultimate development of a highly complex civilization was surely inevitable.

We do not yet know enough to be at all confident of the probability that life should arise in the first place in suitable conditions—Prof. Blum sets this as  $10^{-8}$ —but once this is done, though there may well be  $10^{18}$  or more major routes by which it can develop to organized intelligence, the chance of one of these routes being eventually found is surely very large indeed.

J. H. FREMLIN

Department of Physics,  
University of Birmingham.

<sup>1</sup> *Nature*, 206, 131 (1965).

DR. FREMLIN's analogy to probabilities of bridge hands seems far from applicable to evolutionary processes of the kind considered in my article. Before dealing a bridge hand the pack is thoroughly shuffled to avoid retention of patterns of arrangement of the cards. In evolution each step is predicated on an existing pattern which has been formulated in the course of previous evolutionary steps, the pattern being copied (by one means or another) and so retained between steps. Thus, the probability of a given step is to be reckoned in terms of existing pattern, not in terms of a shuffled arrangement as for a bridge hand. The analogy to computer operation used in my article seems more suitable and leads to a very different point of view.

HAROLD F. BLUM\*

National Cancer Institute,  
U.S. Department of Health, Education  
and Welfare.

\* Present address: Princeton University, P.O. Box 704, Princeton, New Jersey.

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

**RESEARCH ASSISTANT (honours graduate) IN THE DEPARTMENT OF APPLIED MATHEMATICS**—The Registrar, University College of Wales, Aberystwyth (August 10).

**LECTURER IN COMPUTER SCIENCE IN THE DEPARTMENT OF MATHEMATICS**—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (August 13).

**LECTURER (preferably with interests in fluid mechanics or heat transfer) IN MECHANICAL ENGINEERING**—The Secretary, The University, Edinburgh (August 14).

**UNIVERSITY DEMONSTRATOR (with an appropriate honours degree and post-graduate teaching and research experience) IN ANIMAL PHYSIOLOGY AND GENETICS**—The Secretary, School of Agriculture, University of Cambridge, Downing Street, Cambridge (August 18).

**KEEPER (with an appropriate university degree and/or the Museums Association Diploma, and preferably museums experience) OF ARCHAEOLOGY IN THE MUSEUM AND ART GALLERY DEPARTMENT**—The Town Clerk, Town Hall, Bolton (August 20).

**LECTURER IN EPIDEMIOLOGY AND PREVENTIVE MEDICINE**—The Secretary of the University Court, The University, Glasgow (August 20).

**LECTURERS OR ASSISTANT LECTURERS (3) (with interests in any of the branches of electrical engineering, but one of the posts may be particularly concerned with physical electronics, in connection with the recently inaugurated honours course in physics and electronic engineering) IN MICROELECTRONICS IN THE FACULTY OF SCIENCE**—The Registrar, The University, Manchester, 13, quoting Ref. 153/65 (August 20).

**RESEARCH ASSISTANT IN THE DEPARTMENT OF CIVIL ENGINEERING, to work on problems of creep in concrete**—The Registrar, King's College (University of London), Strand, London, W.6.2 (August 23).

**RESEARCH ASSISTANT (with relevant research experience of some aspects of turbulent flow) IN THE DEPARTMENT OF AERONAUTICAL ENGINEERING to take a leading part on a research project supported by the Science Research Council on the turbulent mixing of cold and hot jets with an external stream**—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (August 23).

**ASSISTANT DIRECTOR OF RESEARCH (with a degree in agriculture or agricultural science, or suitable postgraduate qualifications in these subjects, and substantial experience of the agriculture of developing countries) IN COMPARATIVE AGRICULTURE**—The Secretary, School of Agriculture, The University, Downing Street, Cambridge (August 25).

**RESEARCH MICROSCOPIST (with an honours or ordinary degree or an equivalent qualification, and experience in the use of the electron microscope in biological research) IN THE DEPARTMENTS OF BIOLOGICAL CHEMISTRY AND PHYSIOLOGY, to take charge of the electron microscope serving these two departments, and, for the present, certain others**—The Secretary, The University, Aberdeen (August 27).

**LECTURER OR SENIOR LECTURER IN GEOGRAPHY AT THE UNIVERSITY OF TASMANIA, AUSTRALIA**—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 27).

**TECHNICAL STAFF EDUCATION OFFICER (with a university degree in one of the physical or biological sciences, broad experience of science teaching, and good experimental technique) IN THE FACULTY OF SCIENCE**—The Registrar, The University, Hull (August 27).

**COMPUTER MANAGER (graduate with experience of computer operation and administration) IN THE COMPUTING CENTRE**—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (August 28).

**ASSISTANT LECTURER (with qualifications in probability theory and statistics) IN THE DEPARTMENT OF MATHEMATICS**—The Secretary, University of Lancaster, Bailrigg House, Lancaster (August 31).

**EXPERIMENTAL OFFICER (with a degree or equivalent qualification or considerable experience in the field of single crystal and powder phosphor preparation) for the supervision of a crystal growing laboratory in the Physics Department**—The Registrar, The University, Hull (August 31).

**LECTURER/ASSISTANT LECTURER (preferably with experience in the field of magnetic resonance, gas discharges or studies on the mechanical properties of materials) IN THE DEPARTMENT OF PHYSICS**—The Registrar, The University, Keele, Staffs (August 31).

**SENIOR LECTURER OR LECTURER IN PHILOSOPHY AT VICTORIA UNIVERSITY OF WELLINGTON, NEW ZEALAND**—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 31).

**SENIOR RESEARCH ASSOCIATE (graduate in agriculture, veterinary science or zoology, with some postgraduate experience in helminthology) IN PARASITOLOGY, to work on the biology of parasites of the respiratory system of sheep**—The Registrar, University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne, 2 (August 31).

**TUTOR IN THE DEPARTMENT OF PURE MATHEMATICS**—The Registrar, University College of Wales, Aberystwyth (August 31).

**ASSISTANT LECTURER OR LECTURER IN MATHEMATICAL STATISTICS IN THE DEPARTMENT OF APPLIED MATHEMATICS**—The Registrar, The University, Liverpool, quoting Ref. CV/196 (September 1).

**LECTURER (science graduate) IN BACTERIOLOGY AT QUEEN'S COLLEGE**—The Secretary, University of St. Andrews, c/o Queen's College, Dundee (September 1).

**LECTURER (with experience in soil mechanics and concrete technology and allied experience in related fields) IN CIVIL ENGINEERING MATERIALS, to take charge of the Materials and Building Science Section of the Department of Civil Engineering**—The Registrar, The University, Newcastle upon Tyne 2 (September 1).

**RESEARCH ASSISTANT (scientific or medical graduate) IN HUMAN ALLERGY IN THE DEPARTMENT OF PHARMACOLOGY for work involving research into pharmacological and biochemical aspects of human allergy**—The Professor of Pharmacology, University College, Gower Street, London, W.C.1 (September 1).

**LECTURERS OR SENIOR LECTURERS IN THE SCHOOL OF BOTANY, UNIVERSITY OF MELBOURNE, AUSTRALIA**—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 4).

**DATA PROCESSING OFFICER to assist in the mechanization of financial and other administrative processes**—The Deputy Secretary, The University, Southampton (September 11).

**OFFICIAL FELLOW AND TUTOR IN PURE MATHEMATICS**—The Master, St Catherine's College, Oxford (September 11).

**CLINICAL LECTURER IN THE DEPARTMENT OF PSYCHIATRY**—The Assistant Registrar, The Medical School, University of Birmingham, Edgbaston, Birmingham, 15 (September 14).

**CHAIRS (2) OF PATHOLOGY AT THE UNIVERSITY OF MELBOURNE, AUSTRALIA**—The Association of Commonwealth Universities (Branch Office), Marl-

borough House, Pall Mall, London, S.W.1 (Australia and London, September 15).

**SENIOR LECTURER/LECTURER IN THE SCHOOL OF ANATOMY, UNIVERSITY OF NEW SOUTH WALES**—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 17).

**LECTURER (with a first degree in mathematics, physics or engineering and appropriate postgraduate experience) IN CYBERNETICS IN THE DEPARTMENT OF APPLIED PHYSICAL SCIENCES**—The Registrar (Room 23, O.R.B.), The University, Reading, quoting Ref. AP8.13 (September 18).

**LECTURER/SENIOR LECTURER IN METALLURGY AT THE UNIVERSITY OF NEWCASTLE, NEW SOUTH WALES, AUSTRALIA**—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (September 20).

**LECTURER (suitably qualified person with special interests within any of the main branches of chemistry) IN CHEMISTRY AT THE UNIVERSITY OF QUEENSBURY, CHRISTCHURCH, NEW ZEALAND**—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 20).

**LITERARIAN**—The Secretary, The University, Aberdeen (September 20).

**MYCOLOGIST (with a good honours degree) to work on soil-borne diseases of cereals**—The Secretary, Rothamsted Experimental Station, Harpenden, Herts, quoting Ref. 1051/61 (September 20).

**POST-DOCTORAL FELLOWS (2) (organic chemists with a Ph.D. or equivalent degree in chemistry) IN ORGANIC CHEMISTRY AT THE UNIVERSITY OF QUEENSBURY, CHRISTCHURCH, NEW ZEALAND, to carry out synthetic and kinetic research on derivatives of ascorbione**—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 20).

**PROFESSOR OF STATISTICS IN THE SCHOOL OF MATHEMATICS, UNIVERSITY OF MELBOURNE, AUSTRALIA**—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 20).

**CHAIR OF EDUCATION AT THE UNIVERSITY OF MELBOURNE, AUSTRALIA**—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 1).

**SENIOR LECTURER (with special interests in physical oceanography, including dynamic oceanography and/or marine geology) IN OCEANOGRAPHY, and a LECTURER (preferably with a special interest in enzymology) IN BIOCHEMISTRY**—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (October 1).

**CHAIR OF PHILOSOPHY AT MONASH UNIVERSITY, MELBOURNE, AUSTRALIA**—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 5).

**SENIOR LECTURER IN MEDICINE (Neurology)**—The Secretary, The University, Edinburgh (October 13).

**CHAIR OF GENETICS**—The Registrar, University College of Swansea, Singleton Park, Swansea (October 20).

**AGRICULTURAL CHEMIST (national of the United Kingdom or the Republic of Ireland, with an honours degree in chemistry or agricultural chemistry) IN ZAMBIA, to supervise routine chemical analysis for agricultural purposes and to undertake development or modification of analytical methods, with special reference to foods and feeding stuffs**—The Appointments Officer, Ministry of Overseas Development, Room 301, Hans House, Stag Place, London, S.W.1, quoting R.O. 214/122/010.

**ASSISTANT EXPERIMENTAL OFFICER OR EXPERIMENTAL OFFICER (graduate with a pass degree in agriculture or agricultural science) to assist in investigating the role of conservation in intensive livestock feeding**—The Secretary, The Grassland Research Institute, Hurley, Maidenhead, Berkshire.

**ASSISTANT PHYSICIAN, RADIO GRADE (previous hospital experience is not essential) to work in the Radiotherapy Department and the Clinical Isotope Laboratory**—The Group Secretary, Saint Mary's Hospital, Milton Road, Portsmouth.

**ASSOCIATE PROFESSORS (2) (with an honours degree and experience in the planning and direction of groups engaged in research) IN CHEMICAL TECHNOLOGY AT THE UNIVERSITY OF BUENOS AIRES, ARGENTINA (one from February 1966, and the other from February 1967)**—The Recruitment Division, The British Council, 65 Davies Street, London, W.1, quoting Ref. C/65/UNI/60-61.

**CHAIR IN COMMUNICATION THEORY; and CHAIR IN ACOUSTICS**—Dr G. L. d'Ombra, Chairman, Electrical Engineering Department, McGill University, Montreal, Canada.

**DEMONSTRATOR (with an honours degree in mechanical engineering or physics, or ordinary degree, H.N.D. or H.N.C. and some experience in a relevant subject) IN THE THERMODYNAMICS AND FLUID MECHANICS DIVISION OF THE MECHANICAL ENGINEERING DEPARTMENT, to carry out research into separated supersonic flows and assist in the fluid mechanics laboratory teaching in the department**—The Registrar, College of Science and Technology (University of Manchester), Backville Street, Manchester, 1.

**RESEARCH SPECIALIST (with experience of transistor techniques) to take charge of the electronic workshop in the Department of Veterinary Physiology**—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh.

**FELLOWS AND RESEARCH ASSISTANTS IN THE ASTBURY DEPARTMENT OF BIOPHYSICS. Fields of study include the biochemistry of proteins and protein polysaccharide systems; the electron microscopy of tissues and macromolecular systems**—The Registrar, The University, Leeds, 2.

**GRADUATE PHYSICAL CHEMIST to carry out research into the control of polymer properties, through graft copolymerization**—The Director, Arthur D Little Research Institute, Inveresk, Musselburgh, Midlothian.

**LECTURER OR ASSISTANT LECTURER (preferably with research interests in forensic chemistry, toxicology or enzymology) IN CHEMISTRY IN THE DEPARTMENT OF PHYSIOLOGY**—The Secretary, The Royal Veterinary College (University of London), Royal College Street, London, N.W.1.

**MASTER (preferably with a knowledge of or interest in S.M.P.) to teach Mathematics to all levels**—The Director of Studies, Nautical College, Pangbourne, Berkshire.

**POST-DOCTORAL FELLOW IN ORGANIC CHEMISTRY**—The Registrar, The University, Manchester, 13.

**POST-DOCTORAL FELLOW IN SOLID STATE PHYSICS for work on the transport and optical properties of molecular crystals**—The Registrar, University of Leicester, Leicester.

**RESEARCH ASSISTANTS (2) IN THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, Southampton College of Technology, for work on separatory and kinetic problems associated with nitrogen and sulphur compounds in petroleum**—The Chief Education Officer (Ref. 56/BA), Civic Centre, Southampton.

**RESEARCH FELLOW (graduate in engineering, science or mathematics) IN THE DEPARTMENT OF AIRCRAFT DESIGN, to carry out theoretical and experimental work in the field of optimum structures**—The Registrar, The College of Aeronautics, Cranfield, Bedford.

SENIOR PHARMACIST (full or part-time)—The Physician Superintendent, Oakwood Hospital, Maidstone, Kent.

SENIOR RESEARCH FELLOW or RESEARCH FELLOW (suitably qualified graduate interested in theoretical work in support of concurrent experimental programmes) IN THE DEPARTMENT OF BIOMEDICAL AND CONTROL ENGINEERING, to be concerned with Government-sponsored research into techniques of device analysis and synthesis using analogue networks in conjunction with analogue/digital computer methods—The Registrar, The College of Aeronautics, Cranfield, Bedford.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

- Proceedings of the Royal Irish Academy. Vol. 64, Section A, No. 4: The 1-Method for Acoustic Rectangular and Skew Plates. By Patrick M. Quinn. Pp. 49-84. 10s. Vol. 64, Section A, No. 5: Correlation of Spectroscopic and Electrical Properties of Glow Discharges Through Iodine. By B. S. Stewart, G. A. Woolsey, J. M. Brown, J. B. M. Coulter and K. G. Hume. Pp. 85-91. 1s. 6d. Vol. 64, Section B, No. 4: The Upper Carboniferous (Wamurian) Stratigraphy North-East of Castle Island, Co. Kerry, Ireland. By Timothy P. Brennan. Pp. 41-64 + plates 5 and 6. 6s. 6d. Vol. 64, Section B, No. 5: The Lower Carboniferous Stratigraphy of the Buttevant Area, Co. Cork. By R. G. S. Hudson and M. R. Philcox. Pp. 65-79 + plates 7, 4s. Vol. 64, Section B, No. 6: The Relation Between the Extracellular Concentration of Glucose and the Combination of Glucose in Normal and Diabetic Tissue. By J. M. O'Connor. Pp. 81-88. 1s. 6d. Vol. 64, Section B, No. 7: Notes on the Hemiptera, Coleoptera, Diptera, and other Invertebrates of the Burren, Co. Clare, and Inishmore, Aran Islands. By I. Lanebury. Pp. 89-115. 5s. (Dublin: Royal Irish Academy, 1965.) 46
- Admiralty Tidal Handbook, No. 3: Harmonic Analysis for Short Period Observations. Pp. 24. (H.D. 529.) (London: Hydrographer of the Navy, 1964.) 21s. net 46
- HMI Electronics, Ltd. Cathode Ray Tubes. Pp. 12. (Hayes, Middx.: HMI Electronics, Ltd., 1965.) 46
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## SCIENTIFIC AND INDUSTRIAL RESEARCH

THE final report of the Council for Scientific and Industrial Research, covering the year 1964\*, is marked by the same calm dignity which characterized its previous annual report amid the near hysteria with which the Trend Report had been received in some quarters. The report is brief, about a third of its pages being devoted to a retrospect of the Department's work, and this makes it the easier to pay fitting tribute to the achievements of the Council and of its predecessor, the Advisory Council for Scientific and Industrial Research, appointed in July 1916. The work of the Department has contributed greatly to the place which science and technology now hold in the Nation's affairs, and while the establishment of the Council was due largely to the initiative of Lord Balfour, it was fitting that on July 14, when the House of Commons considered in Committee the Civil Estimates for the Ministry of Technology, Sir Henry Legge-Bourke, at least, should pay tribute to those who have worked over the years in the Department and achieved these results.

As the Research Council itself observes, most of the broad lines of its present activities were foreshadowed forty years ago by the Advisory Council for Scientific and Industrial Research, and it is in the tactics used to pursue this programme and to maintain flexibility to meet changing circumstances that marked developments have occurred. Support for training graduates in the methods of research, and support for research projects in universities and colleges, have gone hand in hand since 1916, although the scale of this support did not increase between the two World Wars, only 119 training awards being current in 1939-40, compared with 132 in 1920. After 1945 development was much more rapid, and in 1959-60 525 research grants totalling £916,000, and 2,464 training awards, were current—an almost twenty-fold increase since 1939-40. On November 1, 1964, there were almost 1,200 current grants, totalling more than £13 million, and more than 5,000 training awards.

The National Physical Laboratory has always been the largest of the Department's establishments, although some of the Laboratory's research divisions have since formed, for example, the nucleus of the National Engineering Laboratory and the Radio Research Station. The report does not refer to the National Chemical Laboratory, which in future is to become part of the National Physical Laboratory, and, while there is a reference to the Building Research Station as one of the Department's oldest and largest establishments, no reference is made to the problem of its future location, on which a final decision has yet to be taken. The Council does, however, comment that during the Department's existence the general policy of the Government has been to place civil research outside the administrative responsibility of policy-making departments. The one notable exception—aviation research—has not been a momentous success. Expenditure on the research stations increased from £346,000 on six stations in 1925 to almost £10 million on fifteen stations in 1964.

Concerning the growth of the co-operative industrial research associations, which now number more than fifty

compared with eighteen at the end of July 1920, the Council comments on the various arrangements for grants. However, the report does not discuss the functions or work of the associations generally. Nevertheless, the Council does comment on the ways of devising new forms of Government support and the use of multi-disciplinary teams to tackle many-sided problems and offset specialization in the basic training of research workers. The techniques of operational research have evolved during the Department's lifetime and in recent years have influenced the organization and methods of many applied research establishments and the ways in which they have sought to promote innovation in industry. In the past decade the Department's own Industrial Operations Unit has made a valuable contribution to higher productivity by demonstrating to a wide range of industry how operations can be simplified. Special reference is made to the studies of its Economics Committee in such industries as machine tools and shipbuilding and to the useful action they have promoted. This section of the report is strictly factual, and is supplemented by a tabulated list of developments over the years, which completes the account of the Department given by Sir Harry Melville in the monograph in the new Whitehall Series.

Dealing with activities during the year, the report points out that applications for research grants increased almost eight-fold between 1956-57 and 1963-64, and expenditure increased about ten-fold. It is estimated that these research grants at present represent some 10 per cent of recurrent expenditure by universities, from public funds, on scientific research. The Council suggests that this is a reasonable fraction, but it is growing. Accordingly, it is becoming more and more necessary continuously to examine and to discuss the division of responsibility between the universities and the research councils. Moreover, the proportion is high enough to justify the close concern manifest over the effect of any change in the organization of civil science. The Council also emphasizes the importance of directing attention to the problems of securing the best balance between different scientific disciplines in the work supported at universities now that science has become such an important instrument of national policy. The Council suggests that it is inevitable that the Government, through the University Grants Committee and the Research Councils, should offer (and the universities be prepared to accept) more guidance on how, by choice of subject and allocation of effort, universities can make their most effective contribution in scientific research. This is particularly necessary when the cost of research in one field is becoming very large and absorbing a significant part of the available resources, for example, in nuclear physics.

To encourage this development, the Council notes that its Research Grants Committee appointed four new sub-committees for technology to consider applications for grants, to select promising areas for investigation and to keep in touch with developments in Government laboratories and industry. During the academic year 1963-64 the Research Grants Committee considered 980 new applications for grants, totalling £10.5 million (excluding major capital grants), compared with 639 applications, totalling £6.3 million, in the previous year. Supplementary

\* Department of Scientific and Industrial Research. Report of the Research Council for the year 1964. Pp iv+70. (Cmd. 2706.) (London: H.M.S.O., 1965.) 5s. 6d. net. See also page 668 of this issue of *Nature*.

applications relating to existing major capital grants amounted to £821,000. The value of grants awarded has increased, but the gap between the number of good applications and the number of awards was slowly increasing. The 789 grants made totalled £5.4 million, and included 205 grants in applied science and technology, which were valued at £1.8 million: this compared with 517 grants amounting to £3.7 million in the previous year. The new sub-committee appointed the previous year in the field of computing science has already begun to influence this field of research, and the largest grant so far made is £110,000 to the University of Cambridge to establish a research group in computing science. This centre is only the first of a number that the Committee hopes to see established and working in this field. The Human Sciences Committee considered 61 new applications for grants, totalling £480,000, and made 42 awards, amounting to £221,000. This included, as an innovation, a grant for a regional study of the interaction of industrial, economic, social and demographic changes, which is to be carried out by four departments of the University of Liverpool.

To encourage further graduate training of people with industrial experience, the Council instituted during the year a series of new awards known as the Department of Scientific and Industrial Research Industrial Fellowships. These are intended mainly for postgraduate training in technological subjects and are designed for older men, normally of 26-30, who have at least three years' postgraduate industrial experience in British industry and who intend to return to work in industry after completing their training. The Fellowships carry a substantially higher rate of reward than the normal studentship, and are comparable with the salary that the Fellow could normally expect to command in industry or in a university, but within a maximum of £2,000; the Council hopes that the scheme will commend itself to industry as a co-operative enterprise. The Council also notes a further increase in the demand for studentships and fellowships, including a further increase in the number of new ones held abroad.

As regards the research stations, the Council comments on the report of the Review Committee which was appointed in 1963 to consider the functions of the Water Pollution Research Laboratory. While the Committee was satisfied with the work of the Laboratory, it saw a need for additional basic research, particularly in microbiology and in physics and physical chemistry, for in the Committee's view advances in basic knowledge in these areas were most likely to lead to radical improvements in the processes for treating sewage and industrial effluents. Arrangements are being made to implement the recommendations of the Review Committee, and during the year the Laboratory's study of the conditions of the polluted Thames atmosphere, which had occupied fifteen years, was completed. The closing stages of the work were greatly accelerated by the use of the *Deuce* computer at the National Physical Laboratory, where the effect of changes in the polluting load during recent years are still being followed and compared with those predicted from knowledge of such loads and of other factors which influence the condition of the water. The methods of computation developed at the Laboratory should be of value to the Port of London Authority in controlling discharge of polluting matter to the estuary and should make possible, for the first time in Britain, control of pollution by quantitative prediction of the effects of individual discharges.

The report notes, but does not comment on, the report of the Brundrett Committee on Technical Assistance for Overseas Geology and Mining. However, it does outline recommendations of the sub-committee appointed by the Steering Committee of the National Engineering Laboratory to examine and report on the needs of industry for improvements in design procedure, particularly in computer-aided design and in programming for numerically controlled machine tools. The sub-committee recommended that the National Engineering Laboratory should be the focal point for this work, and that steps should be taken immediately to make available a nationally recommended programme for continuous control of machine tools in two axes simultaneously, with step-by-step movement in a third orthogonal axis interposed. Moreover, the sub-committee believed that such an arrangement would stimulate further discussion of industry's needs and help to formulate similar proposals for a comprehensive programme. It also recommended full evaluation of the American Automatically Programmed Tools Programme from the technical and economic point of view, and the development of a general generator programme for post-processors. These recommendations have been endorsed by the Steering Committee. The report also notes the large increase in the computing capacity of the National Physical Laboratory during the year.

Considerable thought has been given by the Council to grant policy towards research associations. However, beyond asking the question whether the concept of an indefinite financial partnership between Government and industry is justified in all circumstances, the general or inherent effectiveness of the research associations is not considered, although the Council does suggest that the concept of Government partnership with vigorous and useful research associations should remain an important feature of policy. It believes also that the main function of a research association is as an essentially industrial research laboratory, to develop all the skills that it can in the interests of its industry and so secure continuing and expanding industrial financial support within the industry for co-operative research. Nine contracts have now been placed under the Advanced Computer Techniques Project, and the field of techniques originally envisaged is largely covered. Considerable importance is attached by the Council to the success of this project in establishing direct co-operation in this field between Government and industry.

During the year, the Council developed a campaign to implement the recommendations of the Feilden Committee on Engineering Design. Although the Committee on Engineering Design, when reviewing the position, thought that progress in implementing its recommendations in the education field had been disappointingly slow during the year, the Council suggests that this may be partly due to changes in organization which are taking place. The Committee itself was formally dissolved in October 1964. In this, its last report, the Council refers rather more fully to information activities and to the report of the working party which the Department was asked to set up by the Advisory Council on Scientific Policy regarding a national organization for scientific and technical information. The functions recommended for such a body by the working party are essentially those to be exercised by the Office for Scientific and Technical Information which the Government has since set up within the Department of Education and Science. While the Research Council comments that arrangements had been agreed to ensure the closest liaison between the

proposed new organization and the National Reference Library for Science and Invention, which is the responsibility of the British Museum, there is no reference to the continued delays in the establishment of this reference library. Neither here nor elsewhere is there any sign in official circles of the real urgency for either the new reference library or a national library policy.

Generally speaking, then, the final report of the Council for Scientific and Industrial Research is purely factual, with few exceptions such as have been noted. It makes little reference to future policy, nor does it comment on a number of the steps which the Government has taken with regard to the organization of civil science, on which some uneasiness, to say the least, is felt in scientific circles. That uneasiness could easily grow if there is undue delay in dealing with many of the tasks which the Council for Scientific and Industrial Research left uncompleted. What remains to be said is, however, a word of congratulation to the Council and its predecessor, and to those who have worked during the years to contribute to the growing understanding of the important place which science holds in our national life, not only in purely industrial but also in many social and economic matters. The extent to which science has now become a matter for national policy is in great part due to the success of the organization initiated forty years ago, largely as a result of Lord Balfour's foresight and understanding.

## EINSTEIN SPACES

### Einstein-Räume

(Mathematische Lehrbücher und Monographien. Herausgegeben von der Deutschen Akademie der Wissenschaften zu Berlin Institut für Mathematik. 11, Abteilung Mathematische Monographien, Band XVI.) Von A. S. Petrow. Pp. x+394. (Berlin: Akademie-Verlag, 1964.) 58 D.M.

THIS is a translation into German by Prof. H.-J. Treder of the Russian original, published in 1961. Prof. Petrow has himself revised and enlarged the original for this translation. The translator has increased the bibliography so that it now contains more than 500 references, and has supplied an appendix on the newest developments in the invariant representation of free gravitational fields and the propagation of gravitational waves.

The first chapter deals with tensor analysis in a Riemannian  $n$ -space; this material is conventional, except for a very detailed discussion of the principal axes of a tensor and an account of Lie groups. In Chapter 2 Einstein's field equations are set down after some historical preliminaries, and an Einstein space defined as one in which the usual vacuum field equations are satisfied. The chapter ends with a useful account of known exact solutions of the vacuum field equations, with or without the cosmological term: here we find the fields of Schwarzschild, Kottler, Weyl, Levi-Civita, Bach, Brinkmann, Kasner, Delsarte, Chou, Narlikar and Karmakar, Einstein and Rosen, Buchdahl, Verma and Roy, and some others.

Chapter 3 contains the matter for which Prof. Petrow is justly famous: Einstein spaces are classified. But here arises a source of petty confusion. It is really the 4-index Riemann tensor that is being classified, but in general that tensor has 20 independent components and defies classification. The vacuum field equations reduce that number from 20 to 10, and the classification proceeds. But then the author shows that if we take another related 4-index tensor (actually the Weyl tensor), it has only 10 independent components anyway (irrespective of

the field equations), and can be classified in just the same way. In fact, the title of the book is a bit of a misnomer, so far as the essentials of the classification-process are concerned: that process is more powerful than the title seems to imply.

Chapters 4 and 5 are devoted to groups of motions, and the known solutions mentioned above are reviewed in this light. Chapter 6 deals with conformal transformations of Riemannian spaces, and Chapter 7 with the Cauchy (or initial-value problem) *in vacuo*, for a mass-stream (or dust-cloud) and for a perfect fluid. In Chapter 8 special types of gravitational fields are discussed. These include separable fields ( $ds^2$  splits into a sum of quadratic forms with no co-ordinate occurring in more than one), fields which are separable as the result of conformal transformation, fields with central symmetry and axial symmetry, harmonic fields, cylindrical gravitational fields, and gravitational fields with boundary conditions. Many exercises are distributed throughout the book.

In the days when physicists made crucial experiments with the aid of sealing-wax and string, their physical concepts were merely refined analogues of things in the world about them. Those happy days are gone. Unless one is a mathematician, enjoying mathematics for its own sake, the choice is between a guilty conscience and a headache when it comes to the concepts on which gravitational theory is based. Most people prefer to batten down their consciences and use Newtonian concepts—absolute space and time, action at a distance with the inverse square law, the laws of motion—all indefensible. The conscientious mind must grapple with a mathematical theory which is impeccable in concept, but so complicated that what little comes out of it may seem scarcely worth the trouble. Some people compromise: working with Einstein's concepts, they try to fit Newtonian language to the results—for example, they see a 'force' in the quadratic term in the equations of a geodesic.

Prof. Petrow does not go in for compromise of that sort. With a clear conscience and no headache, he weaves the mathematical structure pertaining to Einstein's theory, without much apparent concern about physics. Nevertheless, I think he has hit the nail on the head for physicists by insisting that the 4-index Riemann tensor lies at the heart of the matter. This tensor is the gravitational field, just as Maxwell's vectors are an electromagnetic field. For a long time physicists did not like Maxwell's theory, but they have learned to think as he did. Any physicist who wants to think seriously about gravitation must think in terms of the Riemann tensor. Indeed, there is here a good analogy. A Maxwellian field *in vacuo* may be classified in terms of its two Lorentz-invariants ( $E \cdot H$  and  $E^2 - H^2$ )—when these both vanish we have the familiar radiation field. Petrow's classification of the Riemann tensor *in vacuo* (or of the Weyl tensor in general) is, algebraically, a much more erudite affair, but in principle it is similar—it is a question of seeking invariants.

This book is an obvious must for serious students of general relativity. It should be translated into English.

J. L. SYNGE

## PALAEARCTIC GRASSHOPPERS

### Locusts and Grasshoppers of the U.S.S.R. and Adjacent Countries

By G. Ya. Bei-Bienko and L. L. Mishchenko. Part 1: Pp. 400+xxi. Part 2: Pp. 291+xxi. Translated from the Russian. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1963.) 160s. the two volumes.

SINCE its appearance in 1951 the original version of this publication has been the standard work on the Acridoidea of the U.S.S.R. The language in which it was



published has, however, severely limited its usefulness to Western European entomologists, and this translation will be widely welcomed.

The two volumes deal with the three families Tetrigidae, Eumastacidae and Acrididae, and the areas treated includes Scandinavia, Germany, Austria, northern Yugoslavia, the satellite countries of Eastern Europe, Turkey, northern Persia, north Afghanistan, Mongolia, Palearctic China, Korea and northern Japan, as well as the whole of the U.S.S.R. As a result of this very broad scope a total of 833 species are covered by the work and over 40 per cent of them are known only from outside the U.S.S.R.

Vol. 1 begins with a useful introduction of some 70 pages, dealing with such topics as the morphology, biology, ecology and economic importance of grasshoppers and locusts, and this is followed by a bibliography with over 200 entries. The remainder of this volume is devoted to a systematic treatment of the Tetrigidae, Eumastacidae and all but two subfamilies of the Acrididae; these two subfamilies, Acridinae and Oedipodinae, form the subject of the second volume.

By far the greatest value of this work lies in the fully illustrated identification keys, which are provided for every level in the classification from family to subspecies, and are the result of years of painstaking study. As many characters as possible have been incorporated into these keys, and for categories below the level of genus no further descriptions are given.

Bibliographical references are given to all original descriptions and to other works of taxonomic importance. The account of each species includes a few measurements, a brief indication of its distribution, and references to any work on its biology. Drawings of whole insects are given for many species, representing a fair proportion of the genera; these drawings were excellent in the Russian edition (though not always well printed), but in the Israeli edition they are reduced in size and badly reproduced. Almost all the shaded illustrations have suffered in this way and represent the worst feature of the translated edition; they are, however, irrelevant to its main purpose.

Unlike their original counterparts, the two volumes are printed on good quality paper and are adequately bound. The Canadian orthopterist Dr. R. L. Randell has acted as technical consultant to the translators, and mistakes in translation resulting from the technicalities of entomology are few. The Russian abbreviations used for labelling the anatomical drawings in the introduction have been replaced by numbers; this has been carried out successfully in most cases, though there are a few minor errors.

In so competently bringing this valuable work, which comes near to covering all the Palearctic Acridoidea, to the English-speaking world, the Israel Program for Scientific Translations is to be warmly congratulated.

DAVID R. RAGGE

## SOCIAL HABITS OF WATER MOLECULES

### Water and Solute-Water Interactions

By Prof. J. Lee Kavanau. Pp. 101. (San Francisco and London: Holden-Day, Inc., 1964.) 5.50 dollars.

ASK the reflective person what water consists of, and the reply will probably be on the lines of two parts hydrogen to one part oxygen. Address the same question to one of to-day's science graduates and the answer will of course be more sophisticated (being very likely embellished with murmurings about dipole moment and ionization), but little enlightenment in terms of any structural arrangement among the myriads of jostling molecules can be expected. It is not that our teachers or students have been

remiss, but that no one knows for certain what a snapshot of water on a molecular scale would look like. It is not for lack of speculation either that our knowledge is so doubtful, for there has been great theoretical activity in the field of ice and water structure in the past twenty years, and to say the present outlook for solving the principal problems is promising is to do the minimum justice to the situation.

No devotee believes that water in bulk and in any state of aggregation consists of molecules so estranged as to form no mutual attachments, however temporary. After all, ice is crystalline, cold water is denser than ice, and steam is not a perfect gas. Modern approaches to the structure of water do not view the liquid as if it were a highly concentrated steam (although Callendar suggested water dissolved steam), but they favour instead a retention of ice-crystallinity in the liquid. One idea is that the act of melting makes some lattice-bond rotations so irregular that the continuity of the lattice cannot persist for more than a few molecular diameters. Increase of temperature then twists the lattice apart still more. On the other hand, it may be the intermolecular vibrations that overcome the unity of the lattice, so that individual molecules break free in increasing numbers as the temperature is raised and take up intermediate positions in the array that remains. Another view allows the water molecules a much greater degree of promiscuity, while satisfying a continual compulsion to assemble in very short-lived clusters of about 50 molecules each. Taking this concept to an extreme produces a molecular orderliness where there are only neat clusters, and water can justifiably be described as its own hydrate.

Prof. Kavanau had all these considerations in mind when he recently completed a monograph on the structure and functions of biological membranes, for he included a substantial discussion of water structure and of molecular and ionic segregation in aqueous solution. His discussion has now been published separately and on the whole has withstood extraction from its original context very well. A certain amount of biological connotation survives, but this is neither intrusive nor irrelevant, and the text can be read with profit by chemists and physicists. Prof. Kavanau's treatment is largely descriptive and avoids mathematics, but sets out to comment in detail on the successes and shortcomings of present-day theories. The text has been carefully produced and is well supported by up-to-date references and indexes. The reader should not be dismayed by some apparent confusion between atoms and protons in the opening pages, and need not be dispirited by Prof. Kavanau's concluding passages, where there is a glimpse ahead to formidable problems concerning the role of water in biochemical processes.

E. C. POTTER

## GLASS-CERAMICS IN U.S.S.R.

### Catalyzed Crystallization of Glass

Editor-in-Chief: E. A. Porai-Koshits. (The Structure of Glass, Vol. 3.) Authorized translation from the Russian by E. B. Uvarov. Pp. 208. (New York: Consultants Bureau, Inc., 1964.) 20 dollars.

CATALYZED Crystallization of Glass differs from Volumes 1 and 2 in this series which were reports of meetings where very varied topics were brought together under the heading of the "Structure of Glass". The concentration on the one topic has resulted in a volume which leaves a better overall impression than the earlier volumes. In all 44 papers are divided into four sections: (1) general aspects of the crystallization of glass (7 papers); (2) two-component systems (5 papers); (3) the  $\text{Li}_2\text{O}$   $\text{Al}_2\text{O}_3$   $\text{SiO}_2$  system (18 papers); (4) other three- and four-component systems (14). The book may therefore be taken as a



progress report on the development of glass-ceramics in the U.S.S.R. Glass-ceramics were introduced by Corning Glass Works in the late 1950's as 'Pyroceram'.

Glass ceramics are poly-crystalline materials in which the crystals are extremely small and in which very little glassy phase remains. The material is prepared as a glass and the article fabricated by glass-forming techniques; by subsequent heat treatment it is converted into a crystalline ceramic body. The art in formulating the glass composition is to arrange that a multitude of nuclei can be produced by heating at one temperature; on these nuclei crystals are caused to grow by subsequent heating at a higher temperature. At the present time, nucleation arising from the formation of minute drops of one liquid in another appears to find most support. This immiscibility often occurs as a metastable condition in a glass below the liquidus temperature of the normal phase-equilibrium diagram.

The review of V. N. Filipovich entitled the 'Initial Stages in the Crystallisation of Glasses and Formation of Glass Ceramics' covers the statistical mechanics of immiscibility in a binary mixture, and the importance of the region of composition where the second differential of the free-energy with respect to the concentration is negative (the so-called 'spinodal region') is stressed. Porai-Koshits considers that in order to obtain the uniform fine crystallization desired for glass ceramics crystallization must be preceded by the formation of regions with the stoichiometric composition of the future crystals, although sub-microscopic immiscibility does not always cause the glass to crystallize. This must be so because the opalescence which is caused by the liquid-liquid separation can be made to disappear by heating at temperatures well below the liquidus.

Porai-Koshits and his colleagues describe work on the detection of small-scale heterogeneities in glasses by low-angle X-ray scattering.

The low-angle scattering results reported indicate that very useful information can be obtained. For example, the scattering intensity at an angle of 10 min from specimens of a sodium borosilicate glass are given as 300, 65 and 20 pulses/min for heat treatment of 8 h at 600° C, 5 h at 530° C and 5 min at 750° C. The glass heated at 750° C was transparent and it was calculated from the X-ray scattering to have heterogeneities of 50 Å; this is said to be confirmed by the fact that pores of 50 Å radius are formed after leaching. Another interesting observation is that in glasses in the Na<sub>2</sub>O, SiO<sub>2</sub> system maximum heterogeneity is obtained at 11.5 per cent Na<sub>2</sub>O.

The light-scattering work reported is interesting; generally it is in agreement with the findings from low-angle X-ray scattering. Useful results are reported for Li<sub>2</sub>O—SiO<sub>2</sub> and Na<sub>2</sub>O—SiO<sub>2</sub> glass systems.

The rest of the volume is marred by lack of details of the compositions of the glasses studied. A standard Li<sub>2</sub>O—Al<sub>2</sub>O<sub>3</sub>—SiO<sub>2</sub> glass is referred to without further details; additions of TiO<sub>2</sub> are made between 2.0–11 per cent and these are described as though they were minor additions and TiO<sub>2</sub> is referred to as a catalyst. Podushko and Kozlova assert, without any proof, that the effect of TiO<sub>2</sub> is to cause the temperature regions for nucleation and crystallization to overlap.

Some glass-ceramics in the Li<sub>2</sub>O—Al<sub>2</sub>O<sub>3</sub>—SiO<sub>2</sub>+TiO<sub>2</sub> system can be produced which are transparent although nearly completely crystalline. Florinskaya and her colleagues suggest that the crystals in the transparent material are different from those in the opaque material although both have been prepared from the same glass.

Various papers describe the study of nucleation and crystal growth by the use of infra-red and Raman spectroscopy, differential thermal analysis, changes in physical properties with heat treatment, and measurement of dielectric losses. Altogether it is an interesting volume which shows that a very considerable effort is allocated

in the U.S.S.R. to the development of this new technology. Generally the translation and printing are good; six or so minor misprints were noted, but these did not appear to be misleading.

R. W. DOUGLAS

## MATHEMATICS OF DECISIONS

### The Theory of Decision-Making

An Introduction to Operations Research. By W. Sadowaki. Translated from the Polish by Eugene Lepa. Translation edited by H. Infeld and P. Knightfield. Pp. viii + 292. (London and New York: Pergamon Press; Warszawa: PWE—Polish Economic Publishers, 1965.) 60s. net.

THE science of operational research (operations research in America) is one which is assuming increasing importance, as decisions made by management have to be based on an ever more complex array of facts. Over the past quarter of a century mathematical methods, designed to assist in the efficient utilization of available resources, have been evolved, and there is now a well-developed mathematical theory covering the best defined problems of operational research.

Although some early studies in linear programming were carried out in the U.S.S.R., the major developments in the science have taken place in Western countries, and it is interesting to find a book on this subject originating in a socialist society. However, the contents of this book are as relevant in the West as in Poland.

The author declares that the work is intended chiefly for economists, and the use of mathematics is therefore limited, although a slight knowledge of differential calculus is assumed. A mathematician will not be satisfied, as many proofs are omitted; this in itself is not of great importance although one would have liked to have seen proofs included in an appendix, but it is unfortunate that the mathematical theory which is used is at times suspect, and, while the results obtained are correct, it would be a pity if economists were led to believe that methods presented here were mathematically sound. In particular, the description of the simplex method suffers because one set of variables is used alternately with two different meanings; thus on one page there appear apparently conflicting equations, and while the author does not confuse them, the inexperienced reader may well have difficulty. On another occasion, after stating his intention to prove a formula, the author, when faced with a formula similar to the one he wishes to obtain, but including an extra term, states that this term causes some difficulty, and thereupon makes the assumption it is zero: such a proof would be better omitted.

The presentation of the material is well thought out, and, were it not for defects in the English edition, would make for easy reading. The larger part of the book is devoted to linear programming, which is treated in an elementary manner, with an abundance of examples. The development is good, but unfortunately in his attempt to avoid complicated mathematics the author has not presented the simplex algorithm in its usual form, in which it is more suitable for computational purposes. The principle of duality and the significance of the dual variables are well explained, although the translation is bad and obscures the meaning in places. The method quoted for the solution of the transportation problem is rather tedious. Although the benefits of computers are mentioned in the introduction there is little reference to their use in the body of the work; indeed the methods presented throughout the linear programming section are more suitable for calculation with pencil and paper than with computers.

Shorter chapters follow, on the theory of queues, theory of games, statistical methods, and dynamic programming, the first three of which are well explained, usually by

means of examples. Dynamic programming is a subject too often obscured by difficult mathematics, and it is to be regretted that the example given here has been needlessly complicated for non-mathematicians by the use of a cost function itself involving an integral. Each chapter is concluded by a good review of the relevant literature, as it existed in 1959. The final chapter contains a brief survey of applications of operational research in Poland, where, it would appear, little work had been carried out by the date of the Polish edition.

Although this book may well have fulfilled a need when it was originally published in Polish, it does not contribute to the literature in English. Its merits have been lost by poor translation and frequent misprints, both factors which needlessly confuse the reader, and could have been rectified by an editor with a knowledge of the subject.

ANTHONY WRAN

## PROGRESS IN GERONTOLOGY

Advances In Gerontological Research

Edited by B. L. Strehler. Vol. 1. Pp. xi+410. (New York and London: Academic Press, 1964.) 96s. 6d.

THIS first volume of *Advances in Gerontological Research* contains a mixed collection of ten papers, mainly by American authors, which vary in length and the amount of detail thought to be appropriate. The chosen topics are wide-ranging, but concentrate more on the general biological than on the purely medical aspects of ageing. The psychological and sociological sides of the subject are omitted altogether, but can be expected to appear in subsequent volumes.

The opening paper by Bondareff is primarily concerned with the microscopic pathology and cytology of neurones of different ages. Only a short paragraph is devoted to the neurophysiological aspects of ageing processes in the central nervous system, and indicates how much less is known about functional changes in the nervous system with age than about morphological changes. Generally, Bondareff finds little undisputed evidence that important and causal changes with age can be identified in the central nervous system.

The two contributions from Europe come from Rumania (Oeriu) and the U.S.S.R. (Medvedev) and deal with the roles of proteins and nucleic acids in ageing processes. Both take a very broad view of the scope of ageing studies and extend their surveys to the early stages of embryonic development. Medvedev, in particular, shows how much we now depend on advances in molecular biology to provide hypotheses and ways of looking at the ageing process, either in terms of the accumulation of transcription errors or of the 'noise' engendered by the unmasking of genes at predetermined and later ages.

Oeriu ranges even more widely in a catalogue-type of review providing information about changes with age in the tissue contents of protein, enzymes and vitamin co-enzymes and in amino-acid patterns. He believes that the accumulation of disulphide bonds inhibiting enzyme activity is important and illustrates in detail his experimental findings that cysteamine and folic acid can restore the biochemical balance towards the situation characteristic of younger ages.

The three other general reviews are by Casarett, Clark and Blumenthal and Berns. Casarett critically discusses the extent to which exposure to irradiation can mimic or accelerate natural ageing changes and, while finding differences between the two processes, believes that the similarities are sufficient for the irradiated animal to be a useful model system. He, too, puts forward a theory of ageing in which he sees both irradiation and natural ageing having their effects as a result of increasing connective tissue barriers between the circulation and the parenchymal cells.

Clark's contribution on genetics and ageing is, perhaps, the most readable of all. He emphasizes the importance of genetic factors, the opportunities for research which genetically controlled material provides and discusses the relevance of somatic mutation to ageing studies.

Blumenthal and Berns's topic is auto-immunity and they too are concerned with the importance of somatic mutations. Their discussion centres mainly around possible ways in which modifications in immunological reactivity may lead to the diseases of old age and attempts to bridge the gap between the concepts of natural and pathological old age.

The remaining articles are by Andrews on changes in nuclear morphology, a short informative note by Sinex on cross-linkages especially in collagen and elastin, and two by Björkerud and the editor, Strehler, on the isolation of lipofuscin granules from heart muscle and the chemical and enzymatic properties of the particles, and on the histochemistry and ultrastructure of age pigments. There is considerable new and detailed information here about techniques, biochemical findings and histochemical appearances together with speculations on the origins of the granules. One wonders, however, whether the likelihood that these granules are more than an epiphenomenon of ageing is sufficiently great to justify the allotment of about a fifth of the book's pages to this topic.

Most of the present-day views on the causes of ageing are aired somewhere in these reviews. The authors vary considerably in their approach to the problem of how to prepare an article for this type of book and some succeed better than others. Some degree of overlapping and a few trivial proof-reading failures occur. Not everyone will want to read straight through the volume, but anyone who delves into its pages is bound to come up with some fresh ideas and some new pieces of information.

In the past, reviewers of books on ageing have often begun by emphasizing the unpopularity and lack of scientific respectability of ageing studies. To be included in the *Advances* series can be taken as good evidence that the subject has at last arrived. But in addition to marking this event, an important thing about the book is that it makes one realize how amorphous and pervasive a subject ageing is and how dependent workers are on advances in other fields. More depressingly, it serves to show one how little real progress has been made in coming to grips with the main problems.

P. L. KROHN

## BIOLOGY OF RESPIRATION

The Biology of Respiration

By Sir Victor Negus. Pp. xi+228. (Edinburgh and London: E. and S. Livingstone, Ltd., 1965.) 60s. net.

THE *Biology of Respiration* by Sir Victor Negus is a disappointing book on a number of counts. In the first place, the title promises considerably more than the contents offer. I anticipated that this would be August Krogh brought up to date, incorporating the mass of work on the comparative physiology of respiration carried out since the publication of his classic in 1941. In fact, the contents are very much restricted in scope. About three-quarters of the text is concerned with the external apparatus of respiration and is clearly centred around the unique contributions of this distinguished oto-rhinolaryngologist. When he is on home ground, Sir Victor is superb without qualification, and this remarkable account of fastidious dissection of the upper airway systems of innumerable species, admirably illustrated, carefully, thoughtfully and crisply presented would, by itself, justify the purchase of the book. This part of the book is marred only by compression; for example, it might be thought that the complex air sac system of birds deserves more than half a page of text and the mechanics of pulmonary ventilation in mammals more than a brief mention.

However, there is more to respiration than the passage of air in and out of the upper respiratory tract, or of water over the gills of fish; the most serious criticism of the book is that there is little attempt to correlate anatomical adaptations with physiological function. Thus, for example, there is nothing on foetal respiration; in discussing diving mammals and birds, the discussion barely goes beyond the use of stopwatches, and the names Scholander and Irving are never mentioned. There is no mention of adaptations to altitude nor any discussion of the relation between respiratory gas exchange and the size and metabolism of mammals.

Instead of this, the author has inserted two highly compressed chapters on respiratory gas exchange and the neural control of respiration which apply only to man or common laboratory mammals. The latter chapter, in particular, provides a synopsis that is potentially misleading for the novice and irritatingly dogmatic for the professional.

Where space is at a premium, a statement such as "The effects of changes in arterial pH upon pulmonary ventilation do not, however, depend on the alteration of pH *per se*, but are determined by the extent to which the pH change is caused by or gives rise to coincident alterations in arterial PCO<sub>2</sub>," is not only ambiguous but positively misleading since no mention has been made of the medullary pH-sensitive chemoreceptors or of the role of the pH of the cerebro-spinal fluid.

The bibliography contains 98 references of which only about a half are primary sources.

There is a very real need for a comprehensive book with this title, a book which would gather together the work done so far and emphasize where the tracts of ignorance lie; Schmidt-Neilsen's recent *Physiology of the Desert Animal* is an admirable model. It might require a reincarnated John Hunter, to whom Sir Victor is clearly much indebted, to do justice to such a labour. In his absence, Sir Victor and Messrs. E. and S. Livingstone, Ltd., might consider expanding this book with Sir Victor's unique contribution as the nucleus. Zoologists and respiratory physiologists would then be much in their debt.

M. J. PURVES

## THE PROTURA

### The Protura

A Revision of the Species of the World with Keys for Determination. By Prof. S. L. Tuxen. (*Actualités Scientifiques et Industrielles*, No. 1311.) Pp. 360. (Paris: Hermann, 1964.) 54 francs.

THE Protura, a group of small Arthropods usually placed for convenience with the Insecta, were not recognized at all until 1907 when Silvestri described the order and the first species; even now there are fewer than 150 known species. The original descriptions, with a few exceptions, are inadequate, resulting in names being applied to different species by different workers and in the formation of many synonyms. The only remedy for this is to interpret conclusively all the specific names by examining the type material—designating lectotypes where necessary and erecting neotypes where the types are lost. This, together with a re-assessment of taxonomic characters, has been done by Prof. Tuxen in *The Protura: a Revision of the Species of the World with Keys for Determination*.

In this, 48 pages are devoted to morphology, partly based on original work by Prof. Tuxen, with emphasis on the characters used in the classification. Post-embryonal development is dealt with in three pages, while techniques of collecting, examination and preservation are outlined in one. A list is provided of references to ecology and distribution (arranged geographically) together with a systematic list of all the known names, ten pages of

references to the literature, and indexes to names and subjects. The rest and greater part of the book contains the systematic discussion, keys, figures and descriptions of the species, including discussion of the intra-specific variation of some of the characters; all are clearly and conveniently presented.

Thus, for the first time a critical re-assessment of all the morphological and systematic information about the Protura is contained in one volume and it should now be possible to determine Protura material correctly. However, in using the keys it becomes apparent that for identification the specimens must be in good condition and some familiarity with the order is necessary, the arrangements of the sensillae and setae of the foretarsus being especially difficult to resolve; in this respect it is unfortunate that some of the figures of the foretarsus, as mentioned in the preface, have been so much reduced. One other criticism that might be made is the number of genera which have been used for the relatively small number of species, but in Prof. Tuxen's opinion this is a necessary, perhaps only temporary, step to survey a group in which relationships and evolutionary lines are difficult to assess.

It is hoped that this work with its high standards of taxonomy will induce similar standards in the work of subsequent taxonomists of the group and encourage the collection of information on the biology and ecology of the Protura. Further, their distribution may prove to be of considerable zoogeographical interest.

THELMA CLAY

## MORE ABOUT COCONUTS

### Coconuts

By Dr. Reginald Child. (*Tropical Agriculture Series*.) Pp. vii+216+25 plates. (London: Longmans, Green and Co., Ltd., 1964.) 42s. 6d. net.

IT is often surprising to observe, in quite different parts of the world, that coconuts are so badly grown, or so indifferently maintained. Of course, it can be appreciated that, with rising costs of labour, the crop is probably one on which only marginal sums can be spent. That, at least, is the general assumption. However, in these days of scientific agriculture, from the application of which other major tropical crops have certainly profited, it might reasonably be expected that more could be done to improve this important and valuable crop. The potentialities are there: very considerable areas could be utilized, in simple ways, for the cultivation of the coconut, if only . . . ! In fact, there are regions where scientific agronomy is being practised to advantage.

Accordingly, the handy volume prepared by Dr. R. Child, formerly director of the Coconut Research Institute of Ceylon, and produced as one of the *Tropical Agricultural Series*, under the editorship of Mr. D. Rhind, is to be welcomed. (The last considerable monograph on this crop, *The Coconut Palm*, was prepared for the Indian Central Coconut Committee by Dr. K. P. V. Menon and Dr. K. M. Pandalai several years ago.) A further circumstance which adds to the value of the new volume is that the relevant research and technical literature, which is often not too readily accessible to the grower who does not happen to be closely associated with a scientific department or institution, is presented in condensed form. Indeed, in a special chapter, the author has been at pains to inform his readers on this point, for, as with other pan-tropical crops, the literature tends to be somewhat scanty and widely scattered. In fact, various journals and reports are now being produced regularly in Ceylon, the Philippines, India, Malaya, Fiji, etc., and in Paris for regions within the French economy. There has also been recognition of the need for co-operative scientific work in tackling the problems of cultivation and protection—and

they are considerable—of this valuable and potentially extensible crop.

Dr. Child has dealt, in short, informative and readable chapters, with the history and botany of the crop, its cultural requirements, the importance of selection and breeding in the establishment of plantations, and with the many other problems of care, maintenance, fertilizing, processing and products. Pests, of quite diverse kinds, from insects to crabs, and diseases, which are often difficult to diagnose, which afflict the coconut, are described and illustrated. Indeed, a very considerable amount of information has been condensed into some two hundred odd pages. The book is helpfully illustrated by line drawings in the text and by photographic plates of good quality.

Especially in these days of expensive technical books, this volume impresses me as being really good value, both for the practical grower and the research reader. Nor is the coconut without its mythology and mystery: let the curious reader find out for himself about 'coconut pearls' to which a short appendix is devoted.

C. W. WARDLAW

## MECHANISMS OF HORMONE ACTION

Actions of Hormones on Molecular Processes

Edited by Gerald Litwack and David Kritchevsky. Pp. xi+588. (New York and London: John Wiley and Sons, Inc., 1964.) 128s.

THE editors are to be congratulated on their idea of collecting nineteen articles relating to the mechanism of hormone action at the molecular level, for interest in this subject has grown rapidly in recent years. The coverage of subjects is, naturally, not comprehensive, but their selection is a good one. The hormonal influence on transport across cell membranes is discussed by Riggs, and there then follow four articles on various aspects of thyroid hormone action. Gonadotrophins and the sex hormones are discussed in four chapters, while corticosteroids, insulin and the catecholamines merit three articles each. There is also a discussion of gastric and the duodenal hormones. It is of interest that in many of the articles the emphasis is on hormonal action mediated via messenger RNA synthesis.

The articles are excellently written accounts, serving as good reviews of the literature, and many of the authors have taken advantage of the opportunity offered by the editors to speculate. Such personal views are often useful if only in promoting thought and experiment to counter an unpopular opinion. The book is clearly printed, the figures are admirably clear, the type is pleasing to the eye, the index is useful and appears to be both comprehensively and sensibly compiled.

But, and this is an important qualification, there was obviously a delay of about two years between the time when most of the articles were written and that when the book was published. Such a delay may be of limited consequence in some subjects but in rapidly developing fields—and this book was clearly envisaged as being in one—a delay of this length can be disastrous. One of the contributors has added an addendum to his thoughtful, elegant and comprehensive chapter (which clearly took a great deal of work and thought to write), in which he says that during the delay between writing the chapter and reading the proofs (18 months) it had become a period piece and that to attempt to bring it up to date would entail expensive revision. He is perhaps a little too hard on himself, but it is difficult not to sympathize. When asked to contribute a review article, many scientists feel an obligation to do so in order to tie together the diffuse and voluminous literature on their subject or to put their own view. No doubt, too, we respond to the implied flattery of the invitation. A review article,

especially one which is a thoughtful selection and discussion of relevant papers, requires a great deal of hard work to write, and it is a great pity that the hard work should be, to an extent, wasted by delays in publication.

A. KORMER

## MICHAEL FARADAY

Michael Faraday

By L. Pearce Williams. Pp. xvi+531. (London: Chapman and Hall, Ltd., 1965.) 70s.

IN his warm and human biography of Michael Faraday, Prof. Williams has traced Faraday's development as a man and a scientist. After describing Faraday's early days, he has chapters on his education as a chemist and philosopher, and on the "Fallow Years" between 1820 and 1830 when he gave up his researches in order to devote himself to the interests of the Royal Institution, raising it in popular esteem and strengthening its financial position. Then came the great years of the discovery of electromagnetic induction, and the author describes the development and clarification of Faraday's conceptions in three chapters on "The Nature of Electricity".

The chapter on "Faraday in the World" is of particular interest because it contains an account of Faraday's study of the art of lecturing. He had as a first model Humphry Davy, a master of that art. "Faraday had been preparing himself to replace Davy for a decade and it was his ability to reach a lay audience which was, over the course of some thirty-seven years, to give the Royal Institution that popularity and financial security it had so long needed." Faraday's advice to a lecturer about the importance of speaking his discourse, not reading it, reveals his combination of courtesy and firmness in a delightful way, "as we are sure that you would do it better the less you read, so I venture to express a hope that you will not read more than you may find necessary for your own convenience".

Another fascinating chapter deals with the origin of the field theory. Prof. Williams enables us to follow Faraday's lines of thought by the frequent well-chosen extracts from his papers and diary. There is, however, a blemish in this chapter which ought to be corrected in a further edition. A series of figures on p. 439 *et seq.*, which purport to illustrate Faraday's ideas on the distortion of a magnetic field by paramagnetic and diamagnetic bodies, are false in that they represent lines of force with physically impossible contours. Faraday's original rough sketches (Experiment 10,832, April 1850, in his *Diary*) and the finished drawings (2,807, October 1850, in "Experimental Researches in Electricity") are beautifully executed even though Faraday had to extrapolate in exaggerating the effect of a diamagnetic. His intuition led him to their right form, although he had no mathematics. The pictures in the book, on the other hand, show the lines of force which disobey the laws Faraday had so brilliantly established. Then again, on p. 393, in referring to "the setting of his glass across the magnetic lines of force", the author appears to suggest that a diamagnetic rod sets itself at right angles to the force in a uniform magnetic field, whereas, of course, it sets itself in a parallel direction like a paramagnetic, a phenomenon so elegantly explained by Faraday's conception of a field as opposed to that of induced poles. The familiar experiment in which a bismuth rod sets itself across the magnetic gap is explained by the ends of the rod being drawn to places where the field is weaker.

A number of books have been written about Faraday, but in the present case the author must have consulted more of the original sources than any previous biographer, and the result is a very complete and scholarly record, which is a notable addition to our knowledge of nineteenth-century science.

W. L. BRAGG

## HEADS OR TAILS

By DR. R. T. GREEN

Department of Psychology, University College, London

IN the social sciences, recourse is often made to non-parametric or distribution-free tests because the data are not amenable to the more traditional statistical procedures. Of these, the binomial or sign test is one of the earliest, simplest and most flexible. It can be applied to any binary series of the heads and tails variety to test the null hypothesis that the coin is true; that is, that the two outcomes have an equal probability of occurrence. Although the mathematics is straightforward, there has been a measure of disagreement over the epistemology involved. Since the test can be applied only to two-category outcomes, what is to be done when the coin lands on its edge (an indeterminate outcome) or is knocked off the table before we note its face (an unobserved outcome)? With a coin this type of outcome is unusual and the consequences trivial. In the social sciences the neutral category is apt to occur more often and the problem cannot be ignored.

For example, let us take a simple experiment in perception. We ask our subject to inspect a curved line presented in a manner to exclude all other structured stimuli. After five minutes we remove the curved line, replace it with a straight one, and ask the subject if the new stimulus is straight or curved and, if curved, in which direction. Thus, the subject is offered a three-category response. On the null hypothesis that the inspection of a curved line for five minutes will not systematically bias the perception of a straight line viewed immediately afterwards we would predict that he will not favour either direction of curvature and is just as likely to report the one as the other, regardless of how likely he is to choose the middle category of response. That is to say, if we run 100 subjects and 90 of them report the second line as straight we would expect the other 10 to split equally between the remaining two categories. Suppose, however, all 10 respond in the same direction. Are we then entitled to reject the null hypothesis at the 0.002 level of confidence on the grounds that this is the likelihood of getting 10 heads or 10 tails in a row? There are some statisticians who would challenge such a procedure and a second example is perhaps necessary to sharpen the issue still further.

Let us suppose we are engaged in an investigation of political imagery and that we are comparing the attitudes of working-class Conservatives with those of working-class Labour supporters. We have set up a hypothesis that the Conservatives will choose as an ideal Prime Minister someone who makes a good father figure, while the Labour supporters will be less inclined to make such a choice. This time we are careful to design the experiment so that each subject can be unequivocally designated as a positive or negative instance. The null hypothesis, of course, is that the choices of the two groups will not differ significantly in this respect. Our definition of working-class is a standard one based on occupational level, but not being altogether naïve in such matters, we take the trouble—after obtaining the imagery scores—to discover whether or not the respondent regards himself as working-class. To our chagrin, a number of subjects who sweep the roads, deliver milk, and mix cement, yield evidence that they see themselves as members of the middle class. We discard them on the grounds that we have no predictions to make concerning the psychodynamics of such difficult people. Are we justified in

treating the remainder of the sample as representative of the universe we are interested in and conducting a  $2 \times 2$  contingency analysis of the positive and negative cases for the two political affiliations? Note, we could in principle have saved ourselves the trouble of measuring the attitudes of these odd people we eventually discard simply by discovering their class attitudes at the start. It just so happens that we prefer not to follow this procedure in the interests of good experimental design.

Returning to the coin analogy, what we wish to do is test the null hypothesis that two coins are equally biased without wishing to assume that both are true. Again, in order to carry out the desired statistical test it is necessary to discard the unobserved and indeterminate outcomes. Since this is just a more complicated version of the first problem of testing the trueness of a single coin, we shall consider the arguments relating to the simpler case first.

The simplest and the most attractive solution is to discard the neutral cases no matter how frequently they occur, as had been suggested by Dixon and Massey<sup>1</sup> and Siegel<sup>2</sup> in a manner which is casual enough to imply that no difficulties are involved. Objections have been raised against this procedure, however, and if we are to use it we must demonstrate that we do not thereby invalidate the basic requirements for applying this test. Putter<sup>3</sup> has shown how the way we choose to deal with the neutral category affects the exact power and asymptotic efficiency of the test. What does not appear to have been done is to consider the epistemology lying behind the choice of one procedure or another when applying the sign test in particular instances. For once, the most attractive procedure turns out to be the most logical, as the following three arguments demonstrate.

(1) If we were kept in ignorance of the outcome of, say, half the total number of throws we would be obliged to carry out our analysis on the remaining throws of known outcome. Any sample of throws we take is of necessity, and by definition, only a small part of an infinite universe of possible throws. The unobserved throws not included in the sample may all be indeterminate and land on their edge for all we know, but this does not and cannot affect the treatment of the sample we actually have. This is the essence of the binomial test. We define our sample, and the test says whether or not the procedure producing that sample is biased. If we define our sample as that which specifically excludes unobserved or indeterminate outcomes, then the test cannot refer to any events outside our sample as defined. The distinction between unobserved and indeterminate outcomes is of no logical significance in this context.

(2) If it is argued that to be conservative we should allocate all indeterminate outcomes to the direction favouring the null hypothesis, this can be shown to lead to an absurdity. It sounds reasonable only if we confine this procedure to samples in which the coin lands on its edge relatively infrequently. A thick coin makes nonsense of the argument, as it could reverse a significant bias in one direction and make it appear as an even more significant bias in the other. For example, if on eight consecutive tosses having an observed determinate outcome the coin comes up heads every time (it is a double-headed coin if one wishes) we would reject the null hypothesis at the 1 per cent level of confidence, ignoring the indetermin-

inate throws when it lands on its edge. If it is a very thick coin and there have in fact been, say, one hundred indeterminate throws, and we allocate these against the emerging bias, we are obliged to reject the null hypothesis at a far higher level of confidence, and conclude that the coin is biased in the opposite direction from that in which it really is biased. To split the indeterminate outcomes in the proportion of the determinate outcomes, as is sometimes recommended, only helps to undermine the null hypothesis. The only procedure that is genuinely conservative is to split the indeterminate outcomes 50:50, with an odd member going to the smaller total of determinate outcomes.

(3) The fallacy of including the indeterminate outcomes in the analysis at all is best seen by considering the properties of thick biased coins. Let us take a coin which is tapered across its edge as if it were a truncated cone. Such a coin is patently prone to bias, although the degree and direction of bias will be a complex function of the angle and thickness of the edge and the sizes of its faces, apart from its elastic properties and the nature of the surface on which it lands. Without getting involved in the dynamics and geometry of the process, it would obviously be quite straightforward in principle to manufacture such a coin with any degree of bias we chose.

Let us suppose that we produce a coin with a 60:40 bias, in the sense that out of one hundred observed and determinate throws it will on average reflect this bias. Now the number of times we actually have to toss this coin in order to arrive at one hundred observed determinate outcomes will be a function of the way the coin is made. It could be made to produce one hundred determinate outcomes in 102 throws or in 200, but the question as to the direction and degree of bias with respect to the two faces can be settled by reference only to the observed determinate outcomes.

In effect we are dealing with a three-faced die and confining our interest to the relative probabilities of occurrence of only two of its faces. The third outcome is of no relevance to the null hypothesis that the two faces in which we are interested have an equal probability of appearing. The only relevance that the third outcome has is with regard to the amount of work we have to do in order to confirm or refute this null hypothesis. A thick coin has to be tossed a greater number of times in order to obtain a sufficient number of determinate outcomes with which to test the null hypothesis that is of interest to us, whereas a thin coin involves us in far less wasted effort obtaining outcomes not falling into the sample as defined.

These arguments may be summarized by considering a true roulette wheel of 100 pockets, any of which may be red, black or white. If we confine ourselves to asking whether there is an equal number of red and black pockets, then any trials in which the ball comes to rest in a white pocket must be ignored. According to the definition of the sample universe in which we are interested these trials are not trials at all. So far as the mathematics is concerned, it makes no difference whether there are 49 red, 49 black and 2 white pockets, or one red, one black and 98 white pockets. The null hypothesis with regard to red and black can be tested only by reference to those trials on which the ball comes to rest in a black or red pocket. The fact that in order to get a sample of 100 trials we have to spin the wheel a little more than a hundred times in the first case, and something like 5,000 in the second, is entirely irrelevant to the legitimacy or otherwise of the mathematics. If there are in fact twice as many red as black pockets we shall detect this degree of bias in just as many trials, but for a very different number of spins, whether there are two red and one black pocket, or 60 red and 30 black pockets.

The same arguments hold if someone upsets the wheel, or switches out the light and removes the ball, in a

random sequence during the course of our experiment. Our sample size is thereby reduced, and we have to make that many more spins to restore it. But, provided that the process is random, then the mathematics of the binomial theorem still apply to those events that we define as belonging to the sample universe. That is, they are determinate and observed. The indeterminate outcomes we have elected not to take an interest in, and the unobservable and unobserved we may not include in our analysis. If there is some systematic bias in the way that the light is switched out, or the wheel is upset, then this process must be investigated in some other way. The null hypothesis we choose to test makes the implicit assumption that the indeterminate and unobserved outcomes do not in fact suffer from such a systematic bias. The possibility that such a bias does exist is always with us, but it holds just as much for the tosses or spins that are unobserved because we have not made them as for those that are indeterminate because the coin lands on its edge, or unobserved because someone switches out the light.

It is not difficult to see that these same arguments hold if we are interested in testing the null hypothesis that two roulette wheels carry red and white pockets in the same proportions, regardless of how many pockets either has *in toto*, or what proportion of the pockets is white. The interesting question is why there would appear to be anything to argue about over this matter of discarding the neutral cases. Possibly the answer is to be found in one of the chestnuts that statisticians are fond of using to show how easy it is for common sense to lead our reasoning astray. The conundrum runs like this: "A woman has two children. One of them is a boy. What are the odds that the other is also a boy?" (It is assumed that there are an equal number of boys and girls in the universe under consideration, and there is no Markov chaining.) The correct, and slightly surprising, answer is 2:1. If we are surprised this is because we have been addressing ourselves to the wrong universe. The common error is to assume that we are dealing with the entire universe of women with two children, in which case the answer would obviously be 'evens'. In fact part of this universe, the quarter containing women with two daughters, has specifically been excluded, and we are left to deal with a universe in which 2/3 fall into a boy-and-girl category and 1/3 in a two-boy category. Had we been told: "A woman has two children. The first born is a boy. What are the odds that the other is also a boy?", then the answer would indeed be 'evens', because another part of the women-with-two-children universe has been excluded, and the remaining universe under consideration contains boy-then-girl and boy-then-boy in equal proportions.

The social scientist, in setting up his null hypothesis, is entitled to address himself to whatever universe he elects to investigate. If he wishes to exclude from his analysis subjects who report straight lines as straight, or working-class respondents who see themselves as middle class, then he is perfectly entitled to do so. His conclusions will, of course, be limited in that they will refer only to the universe to which he addresses himself, and he will not be able to make statistical statements about respondents lying outside his chosen universe, but there is nothing wrong in this. Provided he recognizes the limitations he has imposed on himself then he cannot be taken to task for using the sign test or 2x2 contingency analysis on his remaining data.

Reverting to the first example, if he wishes to confine his interests to those subjects who report a straight line as other than straight, and if 10 such subjects all report the straight line to be curved in the opposite direction to the one they inspected for five minutes, then he is entitled to reject that particular null hypothesis at the 0.002 level of confidence. The sort of statement he would end up making after running his 100 subjects would be



that: "Most people do not seem to be affected by the prior inspection of a curved line, but if they fall into the minority that is affected then it is highly probable that they will be affected in the particular direction revealed by the experiment".

With the second example the same arguments hold. Provided the experimenter confines his conclusions to working-class respondents who see themselves as working-class, he is quite within his rights to confine his analysis to these respondents. Nor does it matter whether he discards the 'neutral' cases before or after making his imagery assessments. This is just a question of administrative convenience, and is not on a par with deciding what to do with the results after having had a look at them and discovering that the 'neutral' cases are swamping the data.

The procedure is suspect only if it is *post hoc*. Statisticians have good cause to be wary of the experimenter who decides what tests he will make after having collected and inspected his data. If there are a hundred possible comparisons that could be made, then, on a purely chance basis, five of these comparisons will appear to be 'significant' at the 5 per cent level of confidence, and it would be picayune, to say the least, to seize on these as satisfactory evidence for rejecting some handy *ad hoc* hypothesis. See Cochran and Cox<sup>3</sup>, Snedecor<sup>4</sup> and Tippett<sup>5</sup>. (This is not to say that all statistics are necessarily invalid when used *post hoc*.)

Similar considerations apply to the experimenter who neglects to specify the universe in which he is interested before collecting his data. If he fails to foresee the possible consequences of including the neutral category and decides to exclude subjects who 'land on their edge', so to speak, only after inspecting his data, then he lays himself open to the same sort of suspicion as the experimenter who neglects to specify which groups he intends to compare. By tampering *post hoc* with his universe in various ingenious ways he could 'prove' that blue-eyed men are taller than brown-eyed men, or vice versa, simply

by adjusting his universe to exclude the awkward instances.

Perhaps it would not be too misleading to assert that the issues at stake in the real world, when we come to assess the value of an experimenter's work and the legitimacy of the statistics he uses, are not so much mathematical or even epistemological, but fundamentally psychological. A man who is known to have a theoretical system which he defends against all attacks at any cost must expect to have his procedures, experimental and statistical, examined with more care, simply because of his known propensity to shape the facts to fit into his paranoid delusional system. On the other hand, the competent experimenter with no theoretical axe to grind, who has shown himself content to shape his theories to the facts, or even abandon his theories if the facts demand it, earns our trust and respect. Because he is not irrevocably committed to an entrenched theoretical position, but is committed to 'discovering and facing up to the facts, we are more inclined to trust both his data and his treatment of them. Where the experimenter places his emotional investment, in a theory or the facts, is a highly relevant piece of information. Every scientific worker acquires a reputation somewhere along this dimension, and other workers habitually use this knowledge to weigh the value of his contribution. This may not sound much like scientific method as it is described in the text-books but, as has been observed before, whoever uses scientific method it is certainly not the scientist.

I thank Dr. A. R. Jonckheere for his advice.

<sup>1</sup> Dixon, W. J., and Massey, F. J., *An Introduction to Statistical Analysis*, 248 (McGraw-Hill, London, New York, 1961).

<sup>2</sup> Siegel, S., *Nonparametric Statistics*, 71 (McGraw-Hill, New York, Toronto, London, 1956).

<sup>3</sup> Putter, J., *Ann. Math. Statist.*, 30, 368 (1955).

<sup>4</sup> Cochran, W. G., and Cox, G. M., *Experimental Designs*, 75 (Wiley, New York, 1950).

<sup>5</sup> Snedecor, G. W., *Statistical Methods*, 251 (Iowa State College, Iowa, 1956).

<sup>6</sup> Tippett, L. H. C., *The Methods of Statistics*, third ed., 78 (Williams and Norgate, London, 1941).

## CORAL REEFS, ATOLLS AND GUYOTS

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THE problem of coral reefs and atolls to-day is not so much a lack of information but perhaps a new approach in the interpretation of existing observations. Recent deep borings into barriers and reefs have increased knowledge greatly, but the new data do not appear to be consistent with existing hypotheses on reef origin. A new explanation is presented here which emphasizes the role of the predominant swell and the consequences to be expected when a massive structure, sitting on a saturated sedimentary foundation, suffers continual vibration. This hypothesis appears to cover adequately the existing knowledge of coral formations and points out the likely discoveries when further holes are drilled across an atoll complex. The major differences between guyots and atolls are accounted for in terms of subsidence resulting from wave action. The explanations presented do not invoke sea-level changes. The implications of this are noted.

Coral reefs in general and atolls in particular have intrigued geologists and lay persons alike, either through personal observation or through the literature published about them. They have been the subject of some lengthy treatises, of which those by Davis<sup>1</sup> and Wiens<sup>2</sup> are the most comprehensive. In spite of this concentrated attention since the middle of the nineteenth century, the

mode of origin of atolls in particular remains somewhat of a mystery. Gullcher<sup>3</sup> has been led to remark: "Coral reefs form one of the most complex questions in geomorphology".

Coral reefs can be established and maintained under a wide variety of conditions, due to the large number of species available; but optimal growth of the most prolific types requires that the temperature, depth, turbidity and salinity of the water be within certain limits. Temperature controls the geographic distribution of coral and depth determines the specific sites for reefs, mainly through its control of light intensity. Salinity requirements are readily met in the oceans and it is only by the occurrence of fresh water, as from river mouths, that coral growth could be impeded.

Turbidity can be a controlling influence by its reduction of sunlight in general and by the settlement of suspended particles on the reef structure, or its allied plant life, with the consequent reduction in photosynthesis. This factor could assume importance during the early stages of reef development, when the majority of the reef is within the zone of particle suspension. It is apparent, therefore, that the greater the wave agitation, or the finer the bed material, the deeper the platform required for coral development. In fact, conditions could be envisaged



where the combination of wave action and silty soil might require such great depths before turbidity were reduced that the light intensity would be insufficient for the establishment of a colony. This indicates that mud could serve as a foundation only in zones of little or no wave action.

Rock, on the other hand, when also exposed to persistent wave action, could also prove an unlikely site for coral development. This is because the waves, reflected from the relatively steep slopes, develop into standing oscillations or clapoti, the high water velocities of which would tend to wash young colonies away. But even where a reef survives this ordeal, the wave forces imposed on the mature structure would most likely be too great for the relatively weak contact it has with the rock face. This attachment consists mainly of a cementing action of calcium carbonate which would not extend laterally in proportion to the horizontal development of the reef at the higher levels. Sooner or later the whole structure would be torn from the face and be pounded to pieces by the waves.

Thus, for areas where wave incidence is high, the most likely foundation material will be sand or some coarser sediment. This can be accreted or eroded in quite shallow depths with minimal turbidity, so that platforms within the range for coral colonization (namely, 35–40 m) can readily be provided in open ocean conditions. The fragmented material of volcanic mounds in a marine situation would appear to satisfy the requirements for suitable coral reef support.

#### Theories of Origin

Hoffmeister and Ladd<sup>4</sup> have pointed out that theories concerning barrier reefs and atolls may be divided into two main classifications, namely: (a) those invoking a change in sea-level relative to the foundations, and (b) those based on a static situation.

**Changing levels.** Darwin<sup>5</sup> proposed that fringing reefs were established around the edge of volcanoes which had emerged from the sea. When these mounds afterwards subsided the coral continued to grow until a stage was reached where the foundation was some hundreds or thousands of feet below sea-level. Dana<sup>6</sup> and Davis<sup>7</sup> supported this thesis strongly, contending that the valleys and embayments, formed on the existing volcanic islands, proved the effectiveness of sub-aerial erosion. Semper<sup>8</sup> submitted that ocean currents channelled the rising mass of a volcanic island. This had also been considered and rejected by Darwin.

Daly published many articles; but elucidated his thesis fully in 1915 (ref. 8), which proposed glacial control of sea-level. Accepting prior reef formation, he contended that the lowering of sea-level exposed many of the reefs, which afterwards died. Wave action disposed of the coral and then eroded the volcanoes to the water line, after which the sea rose 35–40 fathoms to give the present uniform depth in the atoll lagoons. This theory has received much criticism by Davis<sup>7</sup>, Kuenen<sup>9</sup> and Shepard<sup>10</sup>. A similar hypothesis was published by Vaughan<sup>11</sup>, except that initial colonization of platforms was envisaged during a low stand of sea-level, thus obviating Daly's required destruction of the atolls.

Kuenen<sup>9,12,13</sup> has attempted to combine the merits of the subsidence and glacial theories. Although he is strongly influenced by the former, he introduces eustatism as a necessity from other fields of evidence. He is not supported strongly in this respect; for example, Shepard<sup>10</sup> has been led to state: "It is difficult to assess accurately the importance of glacial control of sea-levels in the development of the atolls".

Stearns<sup>14</sup> proposed that anticlines, either rising or stationary, could be the major repositories for coral reefs. In other areas isostatic lowering could account for the great depth of atoll foundations. In more recent times, he contended, eustatism has assumed greater importance.

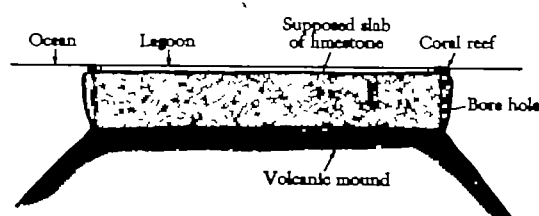


Fig. 1. Section of atoll as proposed by Menard and Ladd<sup>14</sup>

**Static levels.** Tyerman and Bennett<sup>15</sup> considered that volcanoes had been bevelled by tides until the reefs had risen to protect them. In many cases the islands had disappeared before this protection was afforded them. Rein<sup>16</sup> thought that organic debris could have accumulated on the submarine volcanoes which had not reached depths for coral colonization. Murray<sup>17</sup> extended Rein's hypothesis to include dissolving of the interior as the reef extended laterally, ostensibly to account for the lagoon. Murray also proposed that platforms could be produced by wave abrasion.

Wharton<sup>18</sup>, who had surveyed many atolls, was impressed with the limited depth of the lagoons in spite of the steep exterior slopes of the foundations. For him this did not comply with a concept of subsidence so he submitted an explanation<sup>19</sup> of wave abrasion, suggesting 30–40 fathoms as the general limit of wave power for fairly friable material. Davis<sup>7</sup> was very critical of these views, but an analysis of his arguments illustrates strong prejudices.

Agassiz<sup>20,21</sup> suggested that the platforms beneath barrier reefs were produced by wave action. He believed that lagoons could still be excavated after the reef had partially formed due to the topping of the waves. Hoffmeister and Ladd<sup>4</sup> have submitted an antecedent platform theory, having earlier attacked both the subsidence theory<sup>22</sup> and the glacial theory of Daly<sup>23</sup>. These platforms were purported to have accrued from erosion, deposition, volcanic eruption or earth movements. Once they existed at suitable depth and in an appropriate biological environment, these shoals could foster coral growth, so that rise in sea-level "is not essential for the formation of a flourishing barrier or atoll". Ladd<sup>4</sup> has recently been associated with a hypothesis that shallow water limestones have accumulated on ancient volcanic caps which have necessarily subsided under the load. A diagram is included in this reference showing a limestone cap a mile in thickness on which the reef is located (Fig. 1). Whence this vast volume of limestone was derived is not stated, but it should be realized that in the case of Eniwetok Atoll, which is some 20 miles in diameter, the volume of this disk would be in the order of 1,200 cubic miles.

Hass<sup>24</sup> has submitted that a reef subsides like a piece of cake after its central zone has become extinct through lack of nutrients and sediment asphyxiation. This view is based on the apparently false premise that "the depth of the lagoons seemed to bear a direct relationship to the size of the atoll".

From all these divergent opinions the writers of textbooks and monographs have been unable to perceive a unifying theme. Guilhaud<sup>25</sup> noted the lack of attention to wave forces: "The exact effects of swell and currents remain to be determined. These factors are undoubtedly important, but how do they fit in with eustatic oscillations and subsidence?" Wiens<sup>26</sup> concludes: "From the preceding discussion it can be seen that answers to the origin and evolution of coral reefs and atolls still are inconclusive in many respects".

#### New Theory of the Origin of Atolls

Consider a volcano which has just emerged from the sea and has become dormant or extinct. It is likely to be

circular in plan with a crater depression at its centre. The following analysis, however, does not depend on this latter assumption. As the lava was discharged at or near the surface of the sea it would have been cindered, and that ejected through the air likewise would have been fragmented, forming tephra. The speed and duration of the volcanism would determine the volume and distribution of debris and also the volume of the hard core, which had cooled more slowly.

Ocean swell would immediately attack this friable surface, aided and abetted by any local wave system. This erosion has been shown, from recent cases of volcanic action<sup>24</sup>, to be extremely rapid, especially where swell is involved, rather than local storm sequences which are relatively infrequent. Once a platform of reasonable size had formed, both through erosion and deposition, the waves would break further from the beach so formed and normal shoreline processes would ensue<sup>25</sup>.

The width and type of apron would vary around the periphery of the volcanic island, the orientation being determined by the swell waves, which arrive persistently from one direction<sup>26</sup>. As seen in Fig. 2a, the bench on the swellward boundary would be erosional, tapering at the sides and turning to deposition on the leeward margin. A sloping beach profile would soon be established on the zone receiving the brunt of the swell waves. It is on the seaward edge of this ledge that the optimum depth for coral colonization will be reached first. As noted previously, this implies reduction of turbidity as much as appropriate light and temperature conditions. Thus, even when similar depths are reached at the sides and to leeward the continued transmission of debris in these regions would preclude reef establishment.

**Initial reef.** On the swellward profile it is only the seaward edge that can provide a permanent footing, because inshore the steepening of the waves is still creating too much disturbance. A crescent-shaped reef will thus emerge as in Fig. 2b. In its infancy it will not influence the incoming swell, which will continue to erode and distribute the volcanic debris. When the reef reaches part way to the surface, it will induce wave-breaking, so that material could still be denuded between the reef and the island. The waves will diffract at the extremities and so enter this partially formed shadow zone; this will also occur when the reef is fully developed. As seen in Fig. 2c the opposing wave trains could create standing waves or clapotis, which produce excessive bed movement. These will, in association with waves breaking over the structure, continue the bevelling of the shoreward zones. It can be appreciated that any coral which had found a footing inside the marginal reef would have difficulty

in surviving the prolonged agitation of volcanic ash and tephra in this area.

The waves, diffracted by the reef and refracted by the shoal conditions (Fig. 2c), are still able to attack the island structure, the leading edge of which is thus forced back from the initial reef. No protective coral colonies can be established at the sides or leeward until all the erosion has occurred to a stage where turbidity is reduced to survival level. In the event of coarser than normal basaltic material becoming exposed to the waves, reefs could be established and so afford some protection. This would result in an almost-atoll, or even a volcanic island surrounded by a barrier reef.

There could well be several false starts to the initial reef since, at these early stages, large land-slides could ensue with concomitant turbidity of the surrounding sea. These could reduce and kill the existing colonies. The persistent swell, however, will cleanse the same region before all others, so that it again becomes the location for atoll genesis. At the sides and on the leeward face of the island the eroded material is deposited with very little compaction. Intermittent submarine slides could therefore be expected, which would have an adverse effect on any corals that may have survived these turbid conditions. In time, however, sediment movement would decline and the next site for coral would be the side margins (Fig. 2d). An important control is effected by the diffracting waves in the leeward region, which prevent reef formation until nearly all the volcanic mound has been reduced below sea-level, at least to the depth where sediment suspension is negligible.

As soon as the initial reef assumes a reasonable height above the bevelled mound, waves will be reflected with varying degrees of efficiency. Partial or complete clapotis will thus be established on the swellward side of the reef. Large oscillations of the water particles near the bed will result and mound material will be worked downhill or towards the side extremities of the reef. While the platform is deepened the sediment transported to the ends of the curved reef could form spits on which the colony could expand. It is at these points, where the reef is thickest, that the reef changes direction. Wiens<sup>4</sup> has accounted for this accretion by oceanic and lagoonal currents, but it is more likely to be from wave action.

**Reef extensions.** By the time the reef has extended itself to the sides of the volcanic island, the debris from wave abrasion of the initial reef will have started to arrive from the seaward face and be swept by the swell into the shadow zone of the leeward extremities. This promotes inward curvature of the reef as illustrated in Fig. 2e. Storm sequences may redistribute some of this spit material into the normally sheltered zone and so produce isolated mounds on which the reef could form. This results in a number of gaps or passes between coral patches in this zone. As these gaps decrease in size and number the flow of water through them, due to tidal changes or plain outflow of wave swash into the lagoon, increases to a point where colonization is precluded because of bed movement.

Through the processes described it is apparent that the atoll should assume an oval or U-shaped outline, with numerous passages in the zone sheltered from the persistent swell (Fig. 2f). This is confirmed by the published reports on the many hundreds of atolls existing in the Pacific Ocean and recorded on a statistical basis by Wiens<sup>4</sup>.

Should a crater have existed in the original island structure a stage will be reached where the wave erosion would cause a break-through of the sea into the depression. By this stage the reef development may be well advanced, but its survival as an atoll will hinge on the intensity of the disturbance during this crater inundation. If the volcano had a smouldering centre the contact of the sea-water would produce masses of steam and ash, which would be distributed over hundreds of square miles of

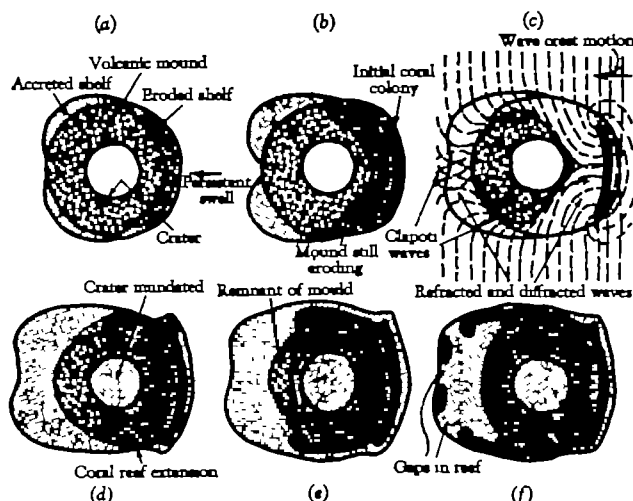


Fig. 2. Progressive development of an atoll from a volcanic mound

ocean. Even a cold crater would contain much fine ash which would be disturbed once waves entered the area. It is possible, therefore, that coral colonies of nearby and even distant atolls could be asphyxiated or retarded in growth.

The break-through of the crater edge is likely to take place on the swellward side, after which the crescent-shaped mound will continue to be denuded by subsequent swell (Fig. 2d). The crater will become the repository of much of the rim material. A significant change in the atoll development may occur at this stage due to a fresh pattern of accretion. The new zones of shallow water will influence the refraction pattern of the waves and so cause sediment transport which could distort the oval- or U-shape trend.

Should the reef be completely destroyed, the remaining part of the volcanic island will again be exposed to the full force of the oceanic swell. Its degradation may then be so swift that it might be bevelled below the survival limit for the coral, turning the mound into a flat-topped sea-mount or guyot. Such a result could also be produced without a crater catastrophe if the mound material had been exceptionally fine or friable throughout. It would then have been more easily eroded, in the style of a mud, and have been reduced to a depth greater than normal before turbidity was at a level suitable for coral subsistence. In the case of a reef having been established under these adverse circumstances, it could suffer more hazards from sub-aerial erosion and subsidence than when the volcanic debris was coarse. Any remnants of coral reef found on guyots should generally be found on the swellward end, since this is the zone where reef development would have commenced.

**Reef stabilization.** Returning now to the case of a thriving crescent-shaped coral colony on top of a partly bevelled volcanic cone, wave reflexion will increase as the reef builds up from the terrace bed. The higher the reef the more perfect the clapoti. As already mentioned, this standing-wave action agitates the bed more vigorously, with virtually infinite depth penetration, so that the seaward margin of the basaltic base is steepened and deepened. If this excavation were carried out too quickly the reef could well be undermined, causing it to topple towards the approaching swell. However, this is less likely if the reef has developed into a crescentic shape (Fig. 2b) and acts as a structural unit, for in this case the centre of gravity is well back from the reef edge.

But another, and more significant, effect of these clapoti is the continual pressure reversals applied to the seaward face of what is virtually a vertical wall. These produce vibrations which are transmitted to the foundation and assist in the settlement of the structure, the vertical load of which is constantly increasing from the addition of new reef. Since the coral on the seaward face is more prolific than that on the leeward side the extra weight provided is an eccentric load. From considerations of differential soil pressures it is apparent that the reef will tend to subside towards the oncoming swell.

The initial reef colony may not be very wide, but will slowly broaden out, mainly towards the open sea. The continual sinking, combined with its growing process, will ultimately form a wedge-shaped body, probably of a form as illustrated in Fig. 3a. The speed of settlement into the volcanic mound will vary greatly from barrier reef to atoll and between examples of either. It is dependent on the weight and height of the reef, the strength of the persistent swell and the nature of the foundation material. It will reduce with time since the soil resistance will increase while the wave forces remain ostensibly the same as those when the reef first reached the sea surface. There is an element of self-adjustment in this penetration process because the exposure to wave action is reduced as the reef disappears into the foundations, so moderating the vibrations which aid in this subsidence.

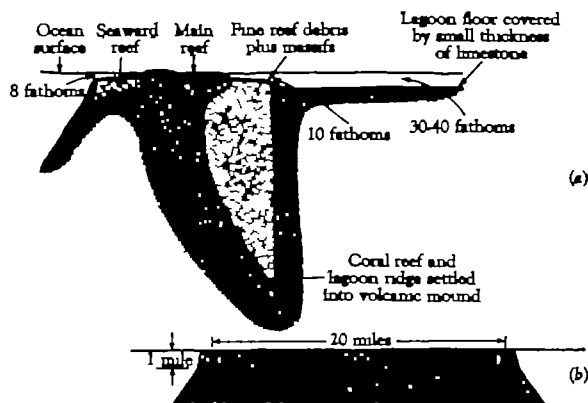


Fig. 3. a, Suggested penetration of coral wedge into a wave-bevelled mound; b, approximate proportions of Eniwetok atoll

In order to force its way into the footing the wedge-shaped reef (or any shaped reef for that matter) must displace some part of the foundation. Although part of this displacement will be in the form of soil compaction, the bulk will be provided by shear strain in the mound material. In this respect, the location of the reef near the edge of this conical foundation provides ideal penetration conditions. The outer slope is a zone of weak shear strength, and the inequality between it and the resisting forces available on the inner side causes the structure to deflect seawards as it is vibrated into the mound. This augments the turning tendency induced by the eccentric loading previously mentioned.

The saturated conditions under which this penetration is effected make comparisons with normal soil foundation problems rather tortuous. It appears reasonable to suggest, however, that the submerged condition of the sediment lowers its shear strength, due to the buoyant force of the water reducing its effective density from 2.65 to 1.45, for example. The pulsations, extending down the face of the wedge, produce pressure fluctuations in the pores of the soil which cause a reduction in its shear strength.

In time the reef should reach a stable depth at which the resistance balances the vertical load and the vibrational forces. It should be possible to determine by model experiments, or perhaps theoretical analysis, the optimum depth to which a reef of given dimensions would need to sink before it reached such stability or pseudo-stability. The rate of subsidence over time would be interesting to compute, if only qualitatively. Should it be uniform, and the age of atolls were considered, values in the order of one or two centimetres per annum would probably be sufficient to account for the thousand or so metres of penetration observed in some of the larger atolls.

The stabilizing effect of the crescentic outline has already been mentioned, but when the reef has more or less completed its encirclement of the disappearing or submarine volcanic mound, it is likely to act as a structural unit during settlement. The zone facing the predominant swell will be heavier and will be subjected to greater wave forces than the leeward section; it will, therefore, settle sooner and deeper. Such tilting of coral atolls might not be readily visible because slow subsidence is made good by further reef growth. A sense of proportion is provided by Fig. 3b, which gives approximate proportions for Eniwetok Atoll. It may be possible for the central axis of an atoll to act as a pivot so that the leeward region of the reef is lifted above its normal level. This would not be the case for isolated sections of reef on the sheltered periphery of the atoll, because they are not physically attached to the main body.

The root-type development described is necessary for the continual stability of the reef. If it did not exist the atolls could well be vibrated from the top of the volcanic

summit. Munk and Sargent<sup>11</sup> have stated: "Against the windward side of Bikini Atoll alone it is estimated that waves dissipate 500,000 horsepower, one-fourth the power generated at Hoover (Boulder) Dam". If the reef were just to sit on a flat slab, which in turn rested on the basaltic base, it would be only the frictional resistance between these three elements that could oppose the tremendous horizontal forces imposed in this pulsational manner.

Barrier reefs around islands or along continental margins will vary structurally from atolls only when the wave or sedimentary conditions are significantly different. The greater exposure of atolls to oceanic swell provides for deeper penetration than for barrier reefs. In the latter case, also, the more uniform development of the reef reduces the possibility of eccentric loading and the accompanying tilting of the structure. Even though it is not of the same order, the settlement of these reefs into the sedimentary shoal is an essential aspect of their stability.

This new thesis of atoll origin differs from those previously presented, both in the nature of the reef support and in the mechanism by which subsidence can be effected to great depths. The significance of the foundation conditions has been alluded to by other workers. For example, Ladd and Tracey<sup>12</sup> concluded that: "The origin of coral reefs will probably never be settled to the satisfaction of many investigators until a great deal more is learned about the foundations of existing reefs". Guilloher<sup>13</sup> also, in listing difficulties associated with atoll origin, has commented: "There are also the problems of the relative importance of corrosion and mechanical abrasion in the lowering of old reefs, and of the exact importance of this lowering".

#### Explanation of Specific Features

The theory of origin presented here should account for all features of coral atolls, the major ones being the lagoon floor and its boundary, lagoonal islets, the spur and groove system of the main reef, the seaward terrace and the steep submarine slopes of the volcanic mounds. Data on many of these features have been expanded in recent years by boreholes drilled into the margins of some atolls. Seismic surveys have also been undertaken to ascertain the foundation conditions for these coral structures.

**Lagoons.** The lagoons enclosed by reef, either partially or fully, differ as between the continental margin and the atoll situation. The former vary in depth from shore to reef since they are normal beach profiles partially encircled by a sand spit, on which the reef has established itself. Atolls, on the other hand, have lagoons which are more uniform in depth, ranging from 30 to 50 fathoms, with no correlation between this dimension and the width.

Around the edge of any atoll lagoon is a terrace of calcareous debris at about 10 fathoms depth. Much of this is fine sediment which is transported around the periphery by waves generated inside the lagoon by local winds. The floor of the lagoon is covered by calcareous mud and skeletal material containing calcite and aragonite. Hundreds of coral knolls rise from it to the surface, varying from pinnacle formations to massive patches of reef, which may cover many acres.

As seen in Fig. 3a, the continual outward tilting provides space on the lagoon side of the reef for debris to be deposited. This calcareous material becomes physically locked and cemented to the growing coral. Consequently it acts integrally with it and hence settles with it into the foundation. The boreholes so far drilled through the atolls have probably penetrated extensive depths of this detrital material, after passing through some growing reef and before reaching extinct original reef at a lower level.

As the coral progressively settles into the volcanic mound the supporting forces increase and the subsidence tends to zero. Horizontal growth becomes the main

feature of development, kept in check only by abrasion on the seaward face and deposition on the lagoonal face. Throughout this process the lagoon floor remains ostensibly the same. It may suffer slight lowering as material is worked into the void left by the outward tilting of the reef, but this is more than compensated for by the detrital products of carbonate precipitation, debris thrown over the main reef, and the coral knolls growing in the lagoon.

**Islets.** Islets are concentrated in certain sectors of the atoll periphery. They consist mainly of debris, as already mentioned above. Wiens<sup>14</sup> has graphed the incidence of these in 125 Pacific atolls and concluded that they were concentrated on the windward zone, although this was not clear from the diagrams. In this respect 'windward' must be differentiated from 'swellward', because the former controls the movement within the lagoon by the waves generated within it, while the latter determines the supply point of the bulk of the material through swell action. The various possible combinations of lagoon waves and persistent ocean swell are shown in Fig. 4, where it is seen that islets can vary widely in length and breadth. The occurrence of coral knolls on the lagoon may drastically affect the wave pattern within a lagoon and could therefore act as offshore breakwaters<sup>11,15</sup> to concentrate accretion behind them.

When an islet consists mainly of the residue of a volcanic island it is likely to be located at the leeward end of the atoll, away from the persistent swell. This would have been the last section to disappear, or to remain, when the final enclosure was being effected by the coral. Such islands would tend to be higher and more substantial than the normal islets which are constructed only to the 'reach' of the waves. It might be stressed that grouping many atolls together in a statistical averaging process, in order to assess the importance of certain factors in islet formation or location, serves little purpose unless the swell and local wind data are known for each. Even the general direction of oceanic swell may not suffice if an atoll is situated behind others so that only diffracted waves can reach it, necessarily from a changed direction.

**Spur and groove system.** This feature is most pronounced on the wave-exposed sections of atolls. The grooves run more or less radially across the reef from the seaward face to the lagoon. They extend down to the lagoon floor-level and vary in width from 2 to 12 ft. with a spacing of 10-50 ft. Between the grooves on the outer face the coral grows strongly, giving the impression of spurs protruding from a wall.

The reef structure as depicted in the present hypothesis is oval or U-shaped in plan and of a wedge section. It has penetrated a volcanic mound which has been bevelled flat to a depth of about 40 fathoms by the ocean swell. This penetration has extended hundreds or even thousands of feet with an inclination which has decreased with time.

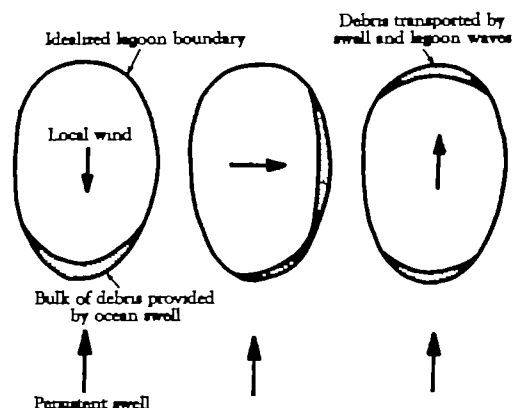


Fig. 4. Effect of oceanic swell and local winds on islet distribution in atoll lagoons

giving a curved cross-section (Fig. 3a). However, this progressive enlargement of the periphery during subsidence would have caused cracking in the reef, similar to that occurring in a cup to which an outward force is applied. These cracks would be small initially, but once the zones of weakness were established they would be maintained. As each subsidence, either spasmodic or continual, occurs, the cracks widen. Even so, their contained angles are extremely small, for example, a 12-ft. wide groove in a 4,000-ft.-deep reef includes an angle of only  $0.25^\circ$ . These grooves are concentrated on the swellward region of the reef where subsidence and tilting are more pronounced. They do not appear to extend below the lagoon floor-level because debris is deposited in them from the lagoon and the seaward face of the reef.

**Seaward terrace.** On the seaward side of atoll reefs are near-horizontal terraces, which vary in width around the periphery and differ greatly from one atoll to the next. They can extend from a few yards to hundreds of yards in width at a depth of about 8 fathoms, which is slightly shallower than the lagoon itself. The terraces are more pronounced on the swellward margin and may, in fact, not exist in the leeward zone.

As mentioned previously, the outside of the volcanic mound is exposed to standing waves, resulting from wave reflexion on the reef, and so is steepened and deepened. But coral fragments of large dimensions accumulate on this sloping face and soon build up a calcareous apron to this basaltic base. This deposit will grow until a depth is reached at which the standing waves will not permit settlement and any new material is swept clear. Thus a terrace of cemented coral could extend from about 40 fathoms up to 10 fathoms approximately, which is the general level of this feature. The protection against wave erosion afforded by this calcareous apron is not available on the leeward sections of the atoll and hence continued agitation of the basaltic base results in very steep slopes to the mound in this area.

**Submarine slopes.** The slopes of the submarine mounds vary around the atoll, being some  $37^\circ$  on the swellward side and an average  $6^\circ$  steeper on the leeward faces. At some reef projections the steepest slopes are encountered, sometimes approaching  $68^\circ$ . These slopes are greater than those occurring around volcanic islands, and it is this observation that provides the clue regarding the importance of standing waves or clapoti, produced by reflexion of the swell from the reef face. These waves agitate the mound material to much greater depths than do the progressive waves approaching the beach profile of normal volcanic islands. This disturbance of the particles causes a downhill movement which results in a steeper grade beyond the limit of the seaward terrace. In the leeward zone of the reef clapoti are generated by the swell waves propagating around either side of the atoll (Fig. 2c). In this region, however, the terrace is slight or non-existent so that the reef itself acts as the fixed point from which the bed profile begins. The sea at these seaward faces of the reef is therefore extremely deep.

**Borehole data.** A number of holes have now been drilled into barrier reefs and atolls. The information has been summarized by Wiens<sup>1</sup> and Shepard<sup>10</sup>. The Great Barrier Reef of Australia exhibited a sandy base at about 400 ft. depth and a reef in Bermuda extended down 250-odd ft. Earlier borings on the atolls did not reach the basaltic base, but in 1952 two holes in opposite margins of Eniwetok atoll fortunately displayed it<sup>23,24</sup>.

One of the surprising aspects of the material extracted from the bores, either from the Funafuti<sup>25</sup>, Bikini<sup>26</sup> or the Eniwetok Atoll, was that shallow water Foraminifera was found in all of it, even from depths of about a thousand metres. This, however, confirms the contention that the reef itself and debris from it becomes buried at the depth of the lagoon floor and hence carries water organisms with it that inhabit this depth. In the bores drilled so far these

marine indicators have a distinctive lagoonal origin. The holes, it should be stated, have tended to be on the inside edge of the reef, where detrital matter has accumulated under conditions of sea exclusion.

Other characteristics of the structures penetrated have been the high proportion of unconsolidated material, the presence of weathered layers of reef and the inclusion of skeletal material of terrigenous sources. The accretion of coralline debris in the lagoon occurs with very little compaction by waves so that the resultant rock contains many cavities. In fact, beneath large massifs of coral thrown over the reef by the swell and deposited on the lagoon margin, there are caverns due to the inability of sediment falling vertically to fill areas underneath them. These large sections of reef which have been transported across the reef may have been exposed to the atmosphere long enough for weathered qualities to be incorporated in them before burial. This has been assumed to indicate emergence of the reef above sea-level<sup>27</sup>.

The nutrient-rich volcanic soils soon have flora and fauna established on them even though they are subject to excessive erosion. When land-slides occur through denudation, vegetation and animals alike would be thrown into the sea. A small proportion would become lodged in the reef, especially in the sheltered lagoon zone, and so be buried with it in the volcanic mound. Only those elements which have a skeletal constitution that can withstand the decaying process would be available for historical demarcation. Ladd and Tracey<sup>28</sup>, for example, have located a snail shell deep in a reef structure. Such terrigenous material could also float long distances across the ocean to be deposited in the detrital margin of a distant lagoon.

The hole drilled on the swellward margin in the Eniwetok atoll struck the basaltic base 450 ft. below that on the leeward section. This can be explained by the longer time of penetration of this portion of reef and the greater weight and vibration present there. Even if the atoll were to act as an integral structure the tilting of the 'ring' is only of the order of 1 in 240, a very modest grade in differential settlement (Fig. 3b).

Shepard<sup>10</sup>, in summarizing the findings of the two Eniwetok drill holes, records: "The sections were very similar down to 1,400 ft. but strikingly different below. The deeper hole encountered Globigerina-rich limestone, apparently deposited in somewhat deeper waters than those in which the lagoonal sediments of the shoaler hole were laid down". As noted earlier, the deeper hole was on the swellward side of the atoll, which had grown first. Thus, when it was undergoing tilting, prior to the lagoon being enclosed, deep-water oozes could have penetrated the void left on the inside of the reef. These would have become deposited in the limestone, which, nevertheless, was of shallow water origin. The leeward reef would not have suffered such wave action and hence would not have tilted and provided entrance for deep-water organisms. As the reef completed its encirclement, any outward deflexion of the swellward reef would not have been accompanied by the incursion of deep water. Thus the top 1,400 ft. of both bores were similar, indicating that this depth of subsidence had occurred after the lagoon had been more or less completely surrounded.

The sinking of the wedge-shaped reef into the basaltic debris must have some effect on the zone in close proximity to it. Only one of the holes in Eniwetok Atoll penetrated the basement. It was reported<sup>29</sup> that a flow structure was exhibited in the olivine basalt. The alteration of the basalt indicated that it was carried out below the ocean-bottom rather than in hydrothermal conditions. This is satisfied by the subsidence theory submitted since the point of the wedge is considered to be well removed from the surface of the mound, as illustrated in Fig. 5. Compare the limestone slab concept of Fig. 1.

There is an urgent need to drill through a lagoon floor to establish the thickness of the calcareous debris, the

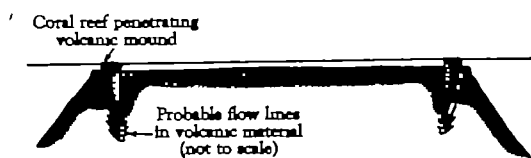


Fig. 5. Suggested section of atoll showing probable strain lines in the foundation material

volcanic debris and the depth to the core of the volcano. Apart from any large massifs of reef which may have been carried into the crater during its inundation, the calcareous matter in any such bore should be insignificant compared with the basaltic content, which should begin just below the marine limestone of the lagoon floor. A hole drilled into the seaward terrace should exhibit reef debris down to about 40 fathoms after which basalt should appear for extended depths. The location of the bore on the periphery will dictate the nature of the basalt, either as *in situ* or accreted material. In any future bores the compaction and flow of the foundation material should be examined thoroughly.

**Seismic information.** Seismic surveys of atolls have been conducted with a view to determining the characteristics of the foundations. These have not been extremely successful because the readings have been interpreted in terms of a thick slab of limestone resting on top of a volcanic mound, above which the reef is supposed to sit. Irregularities in refraction arrival times have indicated differing degrees of consolidation for material beneath the lagoon. This could occur even when fragmented basalt existed throughout the whole mound due to the differing conditions under which it might have been placed. For example, it could be present through volcanic action alone, be deposited by wave action, or be dumped during the crater inundation. The core rock beneath could exhibit yet another velocity. The thicknesses and slopes of the various strata could vary significantly from one volcanic mound to the next, depending on the height and nature of the eruption and wave forces exerted on it. The fragmentary nature of much of this material could well be interpreted from seismic tests as calcareous sediment of similar compaction.

The existence of the atoll as a cylinder or of a conical shell, extending down some hundreds of feet into the volcanic mound, could result in spurious reflexions in any seismic survey. The near horizontal shootings would be influenced more than those of steeper angle. But even results obtained from borsholes in the reef could produce anomalous results, if thought to emerge from an extensive slab of limestone.

### Guyots

Guyots are closely related to atolls and therefore any explanations submitted for the origin of one should readily account for existence of the other. Hess<sup>22</sup> and Hamilton<sup>23</sup> have provided extensive descriptions of guyots. In general they can be considered dome-topped mounds at about 800 fathoms with sides that concave upwards. Corals as well as basalt have been dredged from their tops and across their breaks in slope. It is thought that guyots were originally a group of basaltic islands which became eroded by waves and had coral colonies established on them. Having not developed fully they were purported to have been submerged below the level of subsistence, after which the guyots were carried down to their present level by isostatic adjustment or other sub-crustal forces. Emery, Tracey and Ladd<sup>24</sup> have outlined the differences between atolls and guyots and the following observations are taken from their paper and explained.

Volcanic rocks occur at the rims of guyots but only low on the flanks of atolls. Because of the variable fineness and erodability of volcanic mounds some may never provide stable foundations for coral colonization, especially

in an adverse wave climate. Thus, even before or just after reef formation, the mound may be eroded below subsistence-level. Because of the lack of a protective apron, generally provided by reef debris, the basalt becomes eroded to wave base which, over geological time, could be considered to be about 65 fathoms. Further spreading of this friable material would be accomplished by standing waves produced in the area by reflexion from adjacent atolls. Tidal oscillations in mid-ocean should not be underrated as a means of moving this fine sediment.

Side slopes on the upper reaches of atolls are much steeper than equivalent zones of guyots. As explained previously, the terrace protects the top of the atoll mound but the clapotis, set up around most of the reef periphery, help remove material from the seaward edge. The incidence of standing waves near guyots is not so great and hence the material rests more or less at the angle of repose.

**Profiles.** The guyot has a rounded profile compared with the sharp reef edge of atolls. It is likely that any reef on a guyot is only partly formed, so that it does not project far from the surface of the mound. During the ensuing subsidence it may sink into this foundation. The final distributing agent at the greater depths will therefore smooth the surface from a dome at the centre to a smooth curve at the edge. Atolls, on the other hand, have a non-erodable edge in the form of reef or seaward terrace.

The tops of guyots are smoother than the knoll-filled lagoons of atolls. From previous discussion, it will be recalled that coral growth does not commence until sediment movement is negligible. Hence, until the boundary reef has encircled the lagoon, conditions could not exist for knoll formation. Since a guyot is not likely to have reached this stage of maturity the final touches to the coral complex could not be expected.

The highest point of a guyot is at its centre, whereas that of an atoll is at its edge. The coral reef, which survives on an eroding mound to form an atoll, provides a fixed framework about which denuding or aggrading processes proceed. It also provides debris which is deposited at the boundary of the lagoon. Coral growth and carbonate precipitation add material for the lagoon floor. The resulting accretionary pattern is therefore a saucer-shaped basin.

Considering the possible reasons for the present depths of guyots, the foregoing authors<sup>22</sup> found little satisfaction in eustatism, sinking of the entire sea floor or isostatic adjustment under individual mounds. Age cannot provide the answer either, because similar Foraminifera are found on both atolls and guyots. However, wave-induced slumping appears to account for most of their features. The rapid erosion effected by surface waves on recently erupted volcanic material<sup>25</sup> serves to indicate the consequences that could be expected over thousands of years on basaltic debris when it is not protected by a calcareous sheath.

### Eustatic Implications

The theory of atoll origin outlined here does not invoke change in sea-level at any stage. This complements the approach of many other research workers, as noted in the historical background outlined. However, I may be a little less concerned with this situation than most writers on the subject, since I have yet to be convinced of eustatism by the varied but often anomalous data submitted in its defence. It is hoped to discuss this issue in detail in the near future.

For the present, the explanations submitted for coral development should be judged on their merits, particularly for their rationality and harmony. Observations already recorded should be checked against them in order to prove their validity or otherwise. Perhaps proposed programmes of research could include activities specifically



chosen to test some of the predictions made, as, for example, those in respect of data from future boreholes.

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## NEWS and VIEWS

### The Council for Scientific and Industrial Research

THE final report of the Council for Scientific and Industrial Research, for the year 1964, records gross expenditure during the year ended March 31, 1964, of £23,178,017, compared with £19,479,540 for the year ended December 31, 1963 (Pp. iv+70. (Cmd. 2705.) London: H.M.S.O., 1965. 6s. 6d. net. See also p. 671 of this issue of *Nature*). This is reduced to £20,759,733 by various receipts from industry and other sources for services rendered, of which £1,159,141 was from other Government departments and £914,414 from industry, £213,275 being received by the National Physical Laboratory. The net increase on 1963 was £3,528,186, while staff rose from 6,732 to 6,992 during the calendar year. Grants for special researches rose to £3,877,313, compared with £3,297,186 in 1963, contributions to the European Organization for Nuclear Research from £1,700,000 to £1,980,000, and there is a new item of £585,236 for the European Organization for Space Research and Preparatory Commission.

Postgraduate training awards totalled £2,522,920 (£1,735,067 in 1963) and the 1,741 new research studentships brought the total current at the end of the year to 4,142; there were also 735 advanced course studentships and 180 research fellowships, of which 709 and 111, respectively, were new. Of the 4,142 current research studentships, 547 were in biology and biochemistry, 291 in chemical engineering and metallurgy, 1,181 in chemistry, 202 in electrical and 357 in other fields of engineering, 271 in geology, 113 in human sciences, 353 in mathematics, and 827 in physics. The advanced course studentships were distributed mainly in engineering, other than electrical (149), mathematics (184), and physics (107), with 75 in the human sciences, 53 in chemistry, 44 in chemical engineering and metallurgy, 42 in geology, 41 in electrical engineering and 40 in biology and biochemistry. Of the research fellowships, 66 were in chemistry, 42 in physics and 34 in biology and biochemistry.

Grants for special researches totalled 1,335, 617 being new, and of the 1,335, totalling £15.9 million, 302, valued at £2.15 million, were in chemistry, 223 (£2.08 million) in technology, 204 (£1.27 million) in biology, 161 (£1.13 million) in physics, 109 (£1.34 million) in metallurgy and

materials, 107 (£935,844) in geology, and 49 (£3.14 million) in nuclear physics. An earmarked grant of £34,325 over three years was made to the Water Research Association for an investigation of the feasibility of desalting sea and brackish waters for water supply in Britain, and one of £39,685 to three research associations for a joint three-year investigation into industrial dust hazards.

There were increases in the net expenditure on all branches of the Department's work during the year, the major increases being for the National Physical Laboratory (£2,486,304 gross; £2,164,502 in 1963); the Geological Survey and Museum (£754,785; £649,807); radio research (£479,157; £379,041); road research (£1,204,067; £1,086,674); and building research (£948,366; £858,271). For fire research the corresponding figures are £179,539 and £170,605; for forest products research, £228,756 and £216,682; for hydraulics research, £313,125 and £266,868; Laboratory of the Government Chemist, £541,669 and £524,375; National Chemical Laboratory, £451,724 and £431,416; National Engineering Laboratory, £1,086,286 and £1,006,609; Torry Research Station, £279,218 and £252,709; Tropical Products Institute, £234,921 and £230,437, receipts making the former figure a credit of £88,088; Warren Spring Laboratory, £557,172 and £538,554; and Water Pollution Research, £218,768 and £199,932. Expenditure on the National Lending Library for Science and Technology increased from £335,827 to £380,803; on headquarters administration from £678,775 to £750,099; and annual grants to research associations from £2,073,565 to £2,384,446. Special assistance to industry decreased from £81,209 to £63,049.

### Chemical Physics in the University of Manchester :

Prof. G. Allen

DR. G. ALLEN, at present lecturer in chemistry and reader-designate in the University of Manchester, has been appointed to a newly established chair of chemical physics at that university. Dr. Allen was educated at Tupton Hall Grammar School, Claycross, Derbyshire, and the University of Leeds, where he obtained a B.Sc. degree in 1949 with first-class honours in chemistry. In 1952 he was awarded a Ph.D. degree by the University



of Leeds for his work in physical chemistry and in the same year was appointed post-doctoral Fellow at the National Research Laboratories, Ottawa, where he remained until 1954, when he was appointed assistant lecturer in chemistry at the University of Manchester. He was promoted to lecturer in 1956 and reader-designate earlier this year. His research work has been mainly in the field of spectroscopy and polymer science and he has published numerous papers on these and other subjects in learned journals.

#### Psychology In the University of Reading:

Prof. Magdalen D. Vernon

PROF. MAGDALEN D. VERNON, who has been professor of psychology in the University of Reading since 1956 (*Nature*, 178, 777; 1956), having been appointed lecturer in 1946 and later senior lecturer and reader, retires from her chair in 1967.

Dr. M. Treisman

DR. M. TREISMAN has been appointed to succeed Prof. Vernon. He studied medicine in the University of the Witwatersrand during 1947-52. He then practised in South Africa until 1954, when he entered the University of Oxford to read psychology and physiology, holding the Theodore Williams scholarship in physiology. He obtained first-class honours in these subjects in 1956. During 1956-59 he carried out research, and was a Senior Hulme Scholar at Brasenose College, and during 1958-60 he was a Wellcome Associate of the Royal Society of Medicine. He obtained his D.Phil. in 1962. Dr. Treisman was appointed junior lecturer in experimental psychology in the University of Oxford in 1959, and four years later was promoted to his present position of lecturer. Dr. Treisman's research has been mainly in the fields of sensation and perception. He has been interested in the central factors affecting sensory discrimination, and especially in applying statistical decision theory to elucidate how information given in one modality can affect discrimination in another. This has led to work on auditory masking, discrimination during sleep, and other problems. He has also worked on the scaling of subjective magnitudes, the re-interpretation of certain traditional sensory 'laws', and on temporal discrimination.

#### Geography In the University of Cambridge:

Prof. H. C. Darby

PROF. H. C. DARBY, who has been appointed to the chair of geography in the University of Cambridge, has held the chair of geography at University College, London, since 1949. He was previously John Rankin professor of geography at the University of Liverpool (1945 until 1949). Prof. Darby was appointed to a lectureship at Cambridge in 1931 and elected Ehrman Fellow at King's College the following year. During the Second World War, following a short period in the Army, he was attached to the Intelligence Department of the Admiralty. While at Liverpool, he also held a Leverhulme Research Fellowship. Since his appointment to London, he has been a visiting professor to the universities of Harvard, Chicago and Washington State. Prof. Darby has made the field of historical geography very much his own and has greatly stimulated interest in the subject on both sides of the Atlantic. His earlier work was on the medieval Fenland and the draining of the Fens. As editor and contributor, he is in the process of publishing a reconstruction of the Domesday geography of England. Four volumes of this study have already appeared. His contributions to geography were recognized in 1963 by the simultaneous awards of the Victoria Medal by the Royal Geographical Society and the Daly Medal by the American Geographical Society. Prof. Darby has been president of both the Institute of British Geographers and Section E of the British Association for the Advancement of Science.

He has demonstrated a practical as well as an academic interest in the landscape and resources of Britain, serving as a member of the Royal Commission on Historical Monuments and of the National Parks Commission. He is also a member of the recently formed Water Resources Board.

#### Medical Research Council:

Second Secretary

IN view of the rapid expansion of its work and its increasing involvement in public affairs, the Medical Research Council has, with the agreement of the Secretary of State for Education and Science, decided that the Secretary of the Council shall in future be reinforced by a Second Secretary who shall act as his deputy and alternate in all respects. Because of the Council's increasing concern with the development of biology, it is believed that the Second Secretary to the Council should be recruited from the field of men who have had experience of research, and have distinguished themselves, in the biological sciences. Accordingly, Prof. J. A. B. Gray, professor of physiology in University College, London, has been appointed to this post. Sir Charles Harington has agreed to occupy the post of Second Secretary in an acting capacity until Prof. Gray takes up duty some time in 1966.

Prof. J. A. B. Gray

PROF. GRAY was educated at Clare College, Cambridge, and University College Hospital. He qualified M.B., B.Chir. in 1942 and, after service in the Royal Navy, worked in the Department of Physiology at the National Institute for Medical Research. Thereafter he went with Sir Lindor Brown to the Department of Physiology in University College, London. He was awarded an Sc.D. (Camb.) in 1962 for his work on the mechanisms of sensory receptors, and the title of professor in physiology was conferred on him by the University of London in 1959. From 1957 until 1963 he was secretary of the Physiological Society, and since 1960 he has been dean of the Faculty of Science in the University of London.

#### Research Units

THE Medical Research Council has set up a Demyelinating Diseases Research Unit in the University of Newcastle upon Tyne. The Unit will continue and expand investigations of the mechanisms of demyelination with special reference to the possible virological as well as allergic aspects of multiple sclerosis which were previously undertaken by the Research Group with the same title. The Unit remains under the honorary direction of Dr. E. J. Field, senior lecturer in neuropathology in the University of Newcastle upon Tyne, and Prof. H. G. Miller will continue as the Unit's honorary clinical adviser.

Dr. Wallace Fox has been appointed director of the Council's Tuberculosis and Chest Diseases Research Unit, following the retirement of Dr. P. D'Arcy Hart. The title of the Unit has been expanded to take account of its increasing work in the general field of chest diseases, including carcinoma of the bronchus.

Mr. John Bleby has been appointed director of the Council's Laboratory Animals Centre at the Medical Research Council Laboratories, Carshalton, following the resignation of Dr. W. Lane-Petter.

#### Research Groups

UNDER its scheme of Research Groups, the Research Council is able to provide additional support for the development of research programmes in university departments. The financial provision made available in this way enables the university to expand its staff and other resources devoted to the research in question, on the understanding that, after a stated period, it will assume responsibility for the further support of the work. The Council has agreed to provide the following university institutions with support for the establishment of research

groups: *University of Bristol*: Research Group in Neurosecretion in the Department of Pharmacology (Prof. H. Heller) for research on the ultrastructure and function of the hypothalamo-hypophyseal system, and on the distribution and identification of neurosecretory products and their actions and metabolism throughout the vertebrate phylum. *University of Edinburgh*: Research Group on Bacterial Enzyme Variation, in the Department of Molecular Biology (Prof. M. R. Pollock), for research on the enzymatic basis of variation and adaptation in micro-organisms. *St. Mary's Hospital Medical School, London*: Research Group on the Structure and Biological Activities of Antibodies and Protein Antigens in the Department of Immunology (Prof. R. R. Porter), for research on the structure of antibodies and the means by which their structure enables them to combine specifically with antigens.

#### Academician Nikolai Vavilov Prize

THE Soviet Academy of Sciences has instituted an Academician Nikolai Vavilov Prize, to be awarded for outstanding achievements in genetics, selection and plant breeding every three years, on November 26—Vavilov's birthday. Academician Vavilov was responsible for many major discoveries in biology, and for laying the foundation for many new lines of research in botany, plant breeding, genetics and selection. A large plant collection of more than 300,000 varieties was amassed under his direction, representing the flora of the world; this has formed an experimental base for developing new varieties of farm crops in the U.S.S.R. and abroad. Nikolai Vavilov was the first president of the Soviet Agricultural Academy and headed the plant breeding institute. It is good to record that Vavilov has now, posthumously, been restored, by the Russians, to the honourable place which he rightfully deserves in Soviet science—a place which he has always held in the eyes of Western scientists.

#### The University Grants Committee

To fill vacancies on the University Grants Committee, Mr. Anthony Croeland, the Secretary of State for Education and Science, has announced four new appointments. The new members are: J. W. Atwell, managing director of G. and J. Weir, Ltd., Glasgow; Prof. C. E. H. Bawn, Grant Brunner professor of inorganic and physical chemistry, University of Liverpool; Prof. R. C. Cross, Regius professor of logic, University of Aberdeen; Prof. J. Diamond, Beyer professor of mechanical engineering, University of Manchester. Three members of the Committee, whose terms of office had expired, have been re-appointed. They are: Sir Eric Ashby, Master of Clare College, University of Cambridge; Prof. F. W. Rogers Brambell, Lloyd Roberts professor of zoology, University College of North Wales, Bangor; Prof. C. H. Philips, professor of oriental history and director of the School of Oriental and African Studies, University of London.

#### National College of Rubber Technology

At a meeting held at the Department of Education and Science between representatives of the Department and the University Grants Committee, the Inner London Education Authority, Loughborough University-designate, the Northern Polytechnic, the National College of Rubber Technology and the industry, it was agreed that a postgraduate centre of polymer technology, developing the work at present done at the National College of Rubber Technology, should be established in the new University at Loughborough. The University Grants Committee has invited the University-designate at Loughborough to submit detailed plans as soon as possible for the necessary buildings and equipment for 100 students in the first instance. This is on the understanding that the industry will meet the cost of residential accommodation. It is proposed that the National College shall come

to an end when the new centre has been established, but the Department of Education and Science and the Inner London Education Authority will make arrangements for work, up to and including degree level, to continue and to be developed as necessary in London in order to match the needs of the industry.

#### New Steering Committee for Hydraulics Research

THE Minister of Technology has appointed a Steering Committee to be responsible for the general supervision of the research programme and work of the Ministry's Hydraulics Research Station at Wallingford, Berkshire. This Committee has now established three specialist committees on coastal engineering and harbours, on rivers and estuaries, and on hydraulic structures and hydro-electrics, and an Inter-Departmental Liaison Committee will be appointed to maintain and strengthen links with other Government Departments. These changes came about owing to the fact that the previous arrangements, whereby the Council for Scientific and Industrial Research was advised by the Hydraulics Research Board on the work of the Station, automatically came to an end when the Council and the Department of Scientific and Industrial Research were dissolved on March 31, 1965.

The members of the Steering Committee for Hydraulics Research are: Dr. B. K. Blount (chairman); Prof. J. Allen; Dr. E. Lee; T. A. L. Paton; R. C. H. Russell (director of hydraulics research); E. B. Wright (secretary). The Committee's terms of reference are: "To be responsible to the Minister of Technology for the general supervision of the work of the Hydraulics Research Station and for the allocation of effort thereon within the financial resources assigned to the Station and such other limits as the Minister may impose".

The three specialist committees are to advise the Steering Committee on the programme of work of the Station in the fields for which they are responsible. The chairman of each of the committees is: R. C. H. Russell (coastal engineering); Prof. J. Allen (rivers and estuaries); T. A. L. Paton (hydraulic structures and hydro-electrics). The other members of the committees have yet to be nominated.

#### Voluntary Services Overseas

IN a written answer in the House of Commons on July 9, Mr. A. E. Oram, the Parliamentary Secretary to the Ministry of Overseas Development, stated that the number of graduate volunteers serving overseas under the Voluntary Service Overseas Scheme had risen from 36 in 1962-63 to 255 in 1963-64, to 507 in 1964-65, and was expected to reach 900-950 in 1965-66 and 1,300 in 1966-67. On July 13, also in a written answer, Mr. Oram stated that of 113 short-term appointments, mainly for agricultural graduates, which the Ministry of Overseas Development or the Department of Technical Co-operation had been asked to fill in Africa, during mid-1962 to mid-1965, 48 were to research posts and 65 to advisory or executive posts. Of these 113 officers, 36 had had formal training in tropical agricultural subjects as postgraduate students, and most of the others would have acquired specialized knowledge of tropical agriculture before selection. He estimated that the Department would probably make about 60 appointments in the next 12 months, of which about one-third would be for research.

#### University Foundation of Brussels

THE forty-fourth annual report of the University Foundation of Brussels covering the year ended September 30, 1964, in which grants totalling 6.3 million francs were made, includes a tribute to its honorary President, Baron Holvoet, on his ninetieth birthday (Pp. 190. Bruxelles: Fondation Universitaire, 1965). Study grants totalling 561,000 francs were made to 19 students at the universities of Brussels, Louvain, Liège and Ghent, and five subse-

diaries of 60,000 francs for studies abroad in the universities of Paris, London and Cambridge and at the College of France. Subsidaries to scientific works amounted to 743,000 francs, and to scientific periodicals 2.4 million francs, while scientific associations received grants amounting to 903,000 francs and another 30,000 francs went to university associations. Progress is reported with the *Index of Scientific Publications*, of which two volumes are to appear in 1965, and the report includes a first list of periodicals which it is proposed to microfilm, as well as notes on relations with other institutions. Under the heading "Belgian American Educational Foundation" is given a list of bursars nominated in 1964-65.

#### Euratom Information

*Euratom Information*, issued by the Centre for Information and Documentation, Euratom, Brussels, is printed in German, French, Italian, Dutch and English. No. 3, Volume 3, March 1965, lists scientific and technical publications issued in connexion with the Euratom/United States Joint Research and Development Programme, or the Joint Nuclear Research Centre, including reports of the European Atomic Energy Community. Lists of patents and of research contracts are provided, and each section has indexes of authors, inventors, firms and organizations.

#### Scientific Research in British Universities and Colleges

THE latest edition of *Scientific Research in British Universities and Colleges* for the year 1964-65 has been published by H.M.S.O. on behalf of the Department of Education and Science and the British Council (Vol. 1: *Physical Sciences*. Pp. xix+368. 37s. 6d. net. Vol. 2: *Life Sciences*. Pp. xviii+404. 40s. net. London: H.M.S.O., 1965). This invaluable publication outlines scientific research in progress in British universities, colleges of advanced technology, national colleges, regional technical colleges in England and Wales, and equivalent colleges in Scotland and Northern Ireland. So far as possible, all research undertaken within faculties of science, medicine, engineering, technology and agriculture is included, as well as work of a scientific nature occurring within other faculties. This year's publication differs somewhat from previous years' editions. Because of an increasing amount of material, the publication has been arranged in two volumes and the arrangement is now according to subject. Volume 1 contains the physical sciences, both pure and applied, and Volume 2 the life sciences, including medicine and agriculture. Certain inter-disciplinary subjects (biochemistry, etc.) are included in both volumes. Each volume is divided into broad subject fields and within each field the material is arranged by university/college departments. As in previous editions, each entry includes the head of department and the names of permanent staff actually engaged in supervising research. The material for the universities was collected by the British Council and for the colleges by the Department of Education and Science. While the new style of the publication offers many advantages, it would have been a considerable help if the page numbers had been dropped to the base of the page and if the page headings had been brought to the outer margins; this arrangement would have made reference from the indexes considerably easier.

#### Sir William Jackson Hooker

AMONG botanists throughout the world, the name of Sir William Jackson Hooker is remembered for his inspired directorship of the Royal Botanical Gardens at Kew, and for passing on the good work to an equally eminent son when he died on August 12, 1865. Hooker senior was educated at Norwich School, and being of independent means was able to devote a lifetime to science. He travelled extensively in Europe in his

research work, and later travel brought him perpetuation in Mount Hooker in the Rocky Mountains as well as in the order of mooses, Hookeriales. On returning from an expedition to Iceland the ship in which Hooker was travelling caught fire and much of his collected material was lost. However, his memory was so keen that he was still able to publish a review of the work which he had carried out. He set up at Halesworth, Suffolk, a herbarium which rapidly gained international renown. Later he gave the herbarium, together with his fine library, to Kew. Although it was his son, Sir Joseph Dalton Hooker, who graduated M.D. at Glasgow, Hooker senior became linked with that University through his election to the chair of botany in 1820 at the age of thirty-five. In 1841 came his appointment as director of Kew, and it was there that Hooker's genius showed itself to the full. Through him the great collections of algae, lichens, ferns and other plants were greatly enriched. Apart from published works such as his two-volume *Musci Exotici* dealing with foreign mooses, and his editing of the *Flora Londinensis* of William Curtis—works greatly enhanced by Hooker's abilities as artist and illustrator—he found time to edit many journals, including the *Botanical Magazine* and the *London Journal of Botany*. His son kept up the good traditions of the Hooker family both through his expeditions and published work, and is remembered, among other things, for his encouragement of a raw youth named Charles Darwin.

#### Perfumes against Pests

PESTS of the same family as carrot fly feed on the blossom of Umbelliferae and may be controlled by spraying the headlands. This observation suggested the possibility of using scents to attract and trap insects as an alternative to pesticides, and the Henry Doubleday Research Association undertook a pioneer experiment to test the idea (*Perfumes Against Pests*. Pp. 51. Bocking, Braintree, Essex: Henry Doubleday Research Association, 20 Convent Lane, 1965. 3s. 6d.). A range of twelve of the cheaper oils, seven from the order Umbelliferae and the remainder from plants used extensively for flavouring, were diluted with isopropyl alcohol and added to 10 per cent solutions of brown sugar. The solutions were placed in jars covered with a 3/16-in. square hole mesh, to exclude bees and wasps, and the traps were usually sited at a sheltered position a short distance from the crop so as to attract the adult pests before egg laying. For fruit pests, the traps were hung in trees or set between bushes. The catches were analysed and tabulated and some preference trends indicated that coriander oil was best for cabbage root fly, onion fly and celery fly and had a broader spectrum than sweet fennel oil. Promising results were also obtained with parsley seed, dill, cumin seed and caraway oils, and from an apple blossom synthetic scent, and there were suggestions that both Barbados and Demerara sugars were attractants. The Association is prepared to supply outfits for further study.

#### Rutherford Memorial Lecture and Scholarships

UNDER the terms of the scheme to commemorate Lord Rutherford of Nelson, the Council of the Royal Society has made the following appointments. *Rutherford Memorial Lecturer*, Prof. P. I. Dee, professor of natural philosophy in the University of Glasgow, to be Rutherford Memorial Lecturer for 1965 in Canada, and to deliver the Lecture in Saskatoon on September 29, 1965. *Rutherford Scholarships*, Mr. S. D. Bradshaw, of the University of Western Australia, Perth, to a Rutherford Scholarship for three years, to work for the first year at the University of Sheffield (with arrangements for the subsequent years to be made later) on comparative studies on the factors controlling the secretion of adrenal hormones in mammals and reptiles with particular reference to the

role of the pituitary gland and the kidney; Mr. B. J. Frost, of the University of Canterbury, New Zealand, to a Rutherford Scholarship for three years to continue working in the Department of Psychology, Dalhousie University, Halifax, Canada, on an investigation of the interaction of excitatory and inhibitory processes in colour vision.

#### The Paul Instrument Fund Awards

THE Paul Instrument Fund Committee has made grants as follows: £1,000, as a supplementary grant, to Prof. W. R. S. Garton, professor of physics, and Dr. R. C. M. Learner, research associate at the Imperial College of Science and Technology, for completion of the construction of a high-resolution, broad-range spectrograph; £3,200 to R. L. Gregory, university lecturer in experimental psychology and Fellow of Corpus Christi College, Cambridge, for the development and building of an instrument designed to minimize atmospheric and other disturbances in astronomical telescopes; £2,500 to Dr. W. J. Jones, of the University Chemical Laboratory and Fellow of Trinity College, Cambridge, for the construction of laser apparatus for the excitation of Raman spectra; £1,850 as a supplementary grant, to Sir John McMichael, professor of medicine in the University of London and director of the Department of Medicine, Postgraduate Medical School of London, for the continuation of the development of a miniature electromagnetic flowmeter.

The Paul Instrument Fund Committee, composed of representatives of the Royal Society, the Institute of Physics and the Physical Society and the Institution of Electrical Engineers, was set up in 1945 "to receive applications from British subjects who are research workers in Great Britain for grants for the design, construction and maintenance of novel, unusual or much improved types of physical instruments and apparatus for investigations in pure or applied physical science".

#### University News:

##### Keele

THE following appointments have been made: R. T. Beattie (lecturer in the Department of Psychology); Dr. D. B. Cook (post-doctoral Fellow in the Department of Theoretical Physics and Theoretical Chemistry); T. F. Webber (Research Fellow in the Department of Biology).

##### London

DR. A. N. DAVISON has been appointed to the chair of biochemistry tenable at Charing Cross Hospital Medical School. The following readers have also been appointed: Dr. A. P. Mathias (biochemistry, tenable at University College); Dr. J. L. Knill (engineering geology, tenable at the Imperial College of Science and Technology). The following titles have been conferred: *Professor*, Dr. M. Freedman (anthropology, in respect of his post at the London School of Economics and Political Science); Dr. A. L. Greenbaum (biochemistry, in respect of his post at University College). *Reader*, Dr. J. D. Billimoria (chemical pathology, in respect of his post at Westminster Medical School); Dr. A. J. Smith (geology, in respect of his post at University College); Dr. W. M. Watkins (biochemistry, in respect of her post at the Lister Institute of Preventive Medicine).

##### Manchester

THE following lecturers have been appointed: B. Cox and Dr. T. R. Wilson (pharmacology).

##### Newcastle upon Tyne

THE following lecturers have been appointed: Mr. D. Brook (statistics in the Department of Mathematics); Dr. R. H. Paim and Mr. R. Virden (biochemistry); Mr. B. J. Mifflin (plant science).

##### Sheffield

THE following lecturers have been appointed: Dr. G. Coleman (biochemistry); Dr. T. F. Mortimer (pathology); Dr. W. T. Raynes (chemistry). The title of reader in botany has been conferred on Dr. J. Webster.

##### Southampton

THE following appointments have been made: *Reader-ships*, Dr. A. W. Bright (electrical engineering); Dr. L. G. E. Bell (zoology); *Senior Lectureships*, W. A. Matthews (psychology); *Lectureships*, K. J. Binns (electrical engineering); Dr. J. A. Betts and D. R. Wilkins (electronics); Dr. F. A. Bostock, Dr. M. S. P. Eastham, I. J. Ketley, L. G. Proll, Dr. B. S. Westcott, N. L. Biggs, G. M. Phillips and P. J. Taylor (mathematics); Dr. R. D. Wills (physics); Dr. W. T. Drabble, Dr. B. J. Parsons and Dr. G. N. Woodruff (physiology and biochemistry); Dr. F. S. Billett (zoology).

#### Announcements

THE Alvarenga Prize for 1965 has been awarded by the College of Physicians of Philadelphia to Dr. Harry F. Harlow, director of the Primate Research Center, University of Wisconsin, Madison, for his work on social deprivation in monkeys.

At the annual general meeting of the Royal Statistical Society on June 16, Mr. L. H. C. Tippett was elected president for the year 1965-66. Mr. B. P. Emmett, Miss S. V. Cunliffe and Dr. J. A. Heady were re-elected honorary secretaries and Mr. W. Rudoe was elected honorary treasurer. Prof. C. R. Rao was awarded the Guy Medal in Silver of the Society.

A REGIONAL meeting of the Association of Applied Biologists will be held in Newcastle during September 14-17. Further information can be obtained from F. Blackburn, Department of Plant Science, the University, Newcastle upon Tyne 1.

THE 452nd meeting of the Biochemical Society will be held jointly with the Italian Biochemical Society in Santa Margherita during September 7-8. Further information can be obtained from the Biochemical Society, 20 Park Crescent, London, W.1.

AN international colloquium on "The Optical Properties and Electronic Structure of Metals and Alloys" will be held in Paris during September 13-16. Further information can be obtained from Prof. F. Abelès, Institut d'Optique, 3 Boulevard Pasteur, Paris 15.

A GENERAL discussion of the Faraday Society on "Intermolecular Forces" will be held at the University of Bristol during September 14-16. Further information can be obtained from the Assistant Secretary, the Faraday Society, 6 Gray's Inn Square, London, W.C.1.

A MEETING of the British Radio Spectroscopy Group on "Magnetic Resonance in Metals" will be held in the University of Leeds during September 13-14. Further information can be obtained from Dr. E. F. W. Seymour, Department of Physics, the University, Leeds 2.

AN international conference on "The Microwave Behaviour of Ferrimagnetics and Plasmas" will be held at the Institution of Electrical Engineers during September 13-17. Further information can be obtained from the Secretary, Institution of Electrical Engineers, Savoy Place, London, W.C.2.

A CONFERENCE on "Machines for Environmental and Materials Testing", organized by the Institution of Mechanical Engineers and the Society of Environmental Engineers, will be held at the Manchester College of Science and Technology during September 8-10. Further information can be obtained from the Conference Section, Institution of Mechanical Engineers, 1 Birdcage Walk, London, S.W.1.

## RECENT DEVELOPMENTS IN THE OIL INDUSTRY OF RUMANIA

IT is rare indeed these days that an opportunity occurs to obtain first-hand information of contemporary developments, both technical and economic, of a major industry in an 'Iron Curtain' country, more especially when this is presented in English and, perhaps what is even more surprising, by means of an elaborate series of photographs surveying indoor and outdoor activities within that industry. That this has been most successfully achieved in the case of the oil industry in Rumania is attested by a recent publication entitled *Oil—Natural Gas—Petrochemistry in Rumania*\*. The purpose of this publication is adequately summed up in the introduction: "The present album includes a brief survey of the development of the Rumanian oil industry, as well as facts and figures related to its present-day evolution; the photos—representing the images of the main sectors of the oil and petrochemical industry and socio-cultural achievements in this field—illustrate the upsurge taken in recent years by the oil industry as well as its future prospects". The photographs, in black-and-white and in colour, are excellent; they account for considerably more space than the descriptive text, the latter being essentially brief summaries of progress over the years, but with obvious emphasis on what has happened since the advent of the Rumanian People's Republic.

"A Brief History of the Rumanian Oil Industry" is the first heading; this traces events back to 1857 when production of kerosene was in fact the industry as then established, the first kerosene refinery plant being built near Ploiești, while Bucharest, the capital, was the first town in the world to be illuminated with kerosene. The gasoline stage dates from 1898 with production from oil-extraction pits about 250 m deep. With introduction of mechanical methods of drilling, oil production steadily rose, to be seriously interrupted by the First and later the Second World Wars. Nationalization of the industry began in 1948 and this is proclaimed "... the starting point for the impetuous development of the oil industry in Rumania". The second heading, "Well Drilling and Oil and Gas Production", records the fact that, compared with the crude oil production of Rumania for 92 years before 1948, "... in 15 years alone (1949–1963) more than 145 million tons were produced, i.e., some 90 per cent of the overall production before nationalization". Modern geological and geophysical methods greatly contributed to the discovery and exploitation of new and rich oil and natural gas pools in Oltenia, Western Wallachia and Moldova. Apart from oil, methane has proved one of the richest natural resources of the country; its occurrence is widespread and at 99.4 per cent pure methane it is claimed that Rumanian natural gas is unique in this respect throughout the world; output at 10,387 million m<sup>3</sup> of methane in 1963 was at that time a record for the country; since then, to meet industrial and household demands for the gas, the methane transportation pipeline network is being expanded to 4,000 km.

The section on "The Oil-Processing Industry and Petrochemistry" notes that, in recent years, important units for processing crude oil have been commissioned, among which are specifically mentioned the refineries at Dâmbovită and Onești in Moldova, the oil plant at the Teleajen refinery, and the catalytic reforming complex at the Brazi refinery. In the petrochemical field, the oil industry now produces (from methane, etc.) ammonia, high-concentration nitric acid, ammonium nitrate, methanol, formaldehyde, urea and acetylene; from the urea, aminoplasts and adhesives for the wood-processing industry are obtained. The range of methane carbon blacks

has been increased and a polymethacrylic resin 'Stiplax' is now in production. Further petrochemical plants are being built, to include new nitrogen fertilizer plants, plastics production, for example, polyvinyl chloride, polyethylene and polypropylene, polystyrene, phenol-formaldehyde resins, polyurethanes, etc.; manufacture of synthetic yarns and fibres is being developed, as also is Rumania's synthetic rubber output based on oil gas; and the 'Danubiana' factory in Bucharest is geared to a production of 1.1 million car and tractor tyres in 1965.

Under the heading "Research Work and Designing in the Oil Industry" the 'indoor' activities of more than 3,000 research workers and designers are briefly discussed; the relevant centres include: the Research Institute for Oil Drilling and Extraction at Cimpina; a Designing Institute for Oil-wells at Ploiești; a Designing Institute for the Oil-Processing Industry, again at Ploiești; here also is the Petrochemical Research Institute; and there is a Research Institute for the Chemical Utilization of Methane at Medias. Photographs of some of the laboratories concerned, equipment, apparatus, pilot plants, etc., leave little room for doubt as to the modern set-up and efficiency of this more academic aspect of the industry.

"Rumania—an Exporter of Oilfield Equipment and Oil Products" is the title of a section of this album devoted to commercial interests of the industry in its trade with other countries. We learn that Rumania now has commercial relations with more than 40 countries as regards its oil products and that new export markets are being opened up in Europe, Asia, Africa and Latin America. Rumania has built a 750,000-ton oil/year refinery at Gauhati, Assam State, India; it claims that "Rumania is now able to design and deliver the necessary equipment and plants for modern refineries of any capacity, working on any raw material and turning out any required product"; her co-operation with the oil industry in Afghanistan, Bulgaria, Burma, Ghana, Indonesia and the United Arab Republic is also noted.

No communication from a Communist country would be complete without some reference to cultural facilities and this publication ends with a section, fully illustrated, dealing with "Living Conditions of Oilworkers". The information given here is impressive, particularly as regards housing accommodation and conditions, social amenities, education, medical care, and recreation. In these respects the industry is true to the modern conception of care of all people concerned in its development and progress, as is such a traditional feature now of the major oil companies operating all over the world. It would indeed be interesting, if not instructive, if only half the information contained in this book were available in the same form from other oil-producing countries behind the 'Iron Curtain'; the aid that such reading (and in this case seeing as well) imparts to better understanding of these countries is both ethically and technically invaluable.

Not content, however, with this challenging essay into world publicity, 1965 sees the first issue of a new official bulletin entitled *Chemistry, Oil and Gas in Rumania*†. Here again the purpose is abundantly clear: to keep the Rumanian oil industry in the forefront of technical and commercial developments and to spread its potentialities world wide. This bulletin is also printed in English; whether in other languages is not disclosed. A synopsis of the contents of Vol. 1, No. 1, is indicative of its scope, which does in fact spread the net wider than just the oil industry. "... the interested circles will find surveys

\* *Oil—Natural Gas—Petrochemistry in Rumania*. Edited by A. S. Bandu, Ana Georgescu, M. Portarescu and S. Sigartan. Pp. 146. (Bucharest: Meridiane Publishing House, 1965.)

† *Chemistry, Oil and Gas in Rumania: News and Commentaries*, No. 1, 1965. Pp. 48. (Bucharest: Centrul de Documentare al Industriei Petrolului si Chimiei, 1965.)

of the articles published in the speciality journals, news related to the research and designing activity, brief plant information, trade notes and statements of visitors of our oil and chemical sector as well as other materials illustrating the steady progress witnessed by the Rumanian oil and chemical industries'. It is significantly added that this Centre will supply, on demand, "articles, summaries and other additional information translated into one of

the international languages, accompanied by photos". This bulletin is in mimeograph type with no illustrations save coloured advertisements on the inside covers; but the contents—research and design, short news, commercial information, patents—books and reviews, etc., if this pattern is followed in future issues are bound to attract attention and to command a keenly interested following of international oil technologists. H. B. MILLNER

## ATOMIC ENERGY IN AUSTRALIA

THE twelfth annual report of the Australian Atomic Energy Commission\*, for the year ending June 30, 1964, deals with the production of uranium in Australia, research activities at the Lucas Heights Research Establishment and in Australian universities, and generally with international relationships and nuclear power developments in various countries.

The financial statement records that capital expenditure was reduced by some £A175,000, but that research expenditure increased by about £A270,000 to nearly £A3 million, largely because of the introduction of improved salary scales for the staff and the increased cost of recruitment of staff. The net operating and capital expenditure for the year was £A3,473,862, an increase of £A287,000 over the previous year. The advance to the Rum Jungle Project was reduced, however, from £A1.58 million to £A720,000.

Uranium continued to be in over-supply throughout the world, but the major cut-backs in production required to stabilize the industry in the Western World are now completed. It is expected that since output will continue to fall as individual mines close down, no additional drastic changes will be necessary, and that in the early 1970's world requirements for nuclear fuel will increase again quite rapidly. During 1963 about 1,200 short tons of uranium oxide were produced and sales during this period were valued at approximately £A5.5 million. Only one sales contract remained in operation in June 1964, and although the United Uranium N.L. expected to complete production during 1964, the final deliveries under the £A5 million contract with the United Kingdom Atomic Energy Authority will extend into 1965. Treatment of stockpiled uranium ore at Rum Jungle is expected to continue at the present level for several years.

The Commission's main research programme is devoted to the investigation of the technical and economic feasibility of a high-temperature, carbon dioxide-cooled, beryllium oxide-moderated, power reactor of the pebble-bed type. The fuel elements consist of a dispersion of thorium oxide and commercial plutonium oxide in beryllia, and the core of the reactor comprises a large number of spheres or pebbles, about 1 in. in diameter, of the fuel material. Four different engineering designs of the reactor system are being studied and these are shown diagrammatically in the annual report. Although considerable effort has been devoted to the various engineering and theoretical physics problems involved, including the development of computer codes for core nuclear studies, the Commission specifically states in the report that there is no commitment to construct a power reactor in Australia.

At the Lucas Heights Research Establishment the high neutron flux materials testing reactor, *Hifor*, continued operation at a power output of 11-MW (thermal) on a 28-day cycle for the whole year, except for a two-month major overhaul period. The small low-power reactor, *Moata*, was used mainly for experiments associated with the performance of systems moderated with beryllium oxide. Alterations were made in the control rod read-out

system, and to the internal graphite reflector. New facilities completed or installed at the Establishment included a large single cell capable of remote-handling of up to 600,000 c. of cobalt-60, and a 3-MeV positive ion Van de Graaff accelerator for fast neutron physics experiments.

The Establishment maintained a good safety record during the year. No serious accident, either radiological or industrial, occurred. A wide range of radioisotopes was produced for use in medicine, research, agriculture and industry, and in addition to supplying Australian requirements, radioisotopes were exported to Japan, India, New Zealand, South Africa and the Philippines. The development of sources of gamma-excited monoenergetic X-rays and their application in industry were given particular attention. Special projects included the production of sources of caesium-134 and thulium-170 for radiographic purposes, and the investigation of the effects of neutron- and gamma-radiation on non-metallic bonding materials such as epoxy resin. Large sources of high specific activity of cobalt-60 can be produced in *Hifor*, and during the year the Commission supplied the Royal Adelaide Hospital with a 1,300-c. source, the Royal North Shore Hospital in Sydney with a 2,160-c. source, and under the Colombo Plan, the Cancer Institute of Madras, India, with a 3,025-c. source. The only significant change during the year in the demand for specific radioisotopes was an increased request for radiography sources in smaller sizes and cylindrical sources of iridium-192 of 1-mm length and 1-mm diameter with an activity of up to 8 c., and similar sources of cobalt-60 with an activity of 1.5 c. were produced to meet this requirement.

The first international conference on "Beryllium-oxide", attended by scientists from six countries, was held in Newport, New South Wales, during October 21–25, 1963. The conference was sponsored and organized by the Commission, and 56 papers were presented for discussion, including 27 from the Australian Atomic Energy Commission Research Establishment. The topics discussed covered aspects of fabrication, structure, physical and mechanical properties, radiation damage and gas-solid interactions. The last day of the conference was devoted to a symposium on the utilization of beryllium oxide in reactors. The proceedings of the conference have been published in a special issue of the *Journal of Nuclear Materials* (1964). In addition to a wide range of booklets, leaflets and other publications on atomic energy, the Commission also published a quarterly journal, *Atomic Energy in Australia*, a quarterly *Radioisotope Newsletter*, and a special 55-page booklet entitled *An Introduction to Nuclear Science* for use in schools. The Commission's film "Atoms for Everyday" was shown on the national television network and a major exhibition, "Australia and the Atom", featuring many aspects of atomic research, production and mining of uranium, nuclear power and the use of radioisotopes, was presented in Brisbane and Newcastle. The Research Establishment held open days during September 26–28, 1963, and some 6,000 persons visited and inspected it.

Research contracts totalling more than £A51,000 were placed with Australian universities. Details of these are

\* Australia. Twelfth Annual Report of the Australian Atomic Energy Commission for the year ended 30th June, 1964. Pp. 80. (Geogee, N.S.W.: Australian Atomic Energy Commission, 1964.)



listed in an appendix to the report. The Commission, together with the ten Australian universities, constitutes the corporate membership of the Australian Institute of Nuclear Science and Engineering, which is governed by the Institute Council consisting of one representative from each of the universities and four from the Commission. All member universities are engaged in training nuclear scientists and engineers and in related research. 112 projects were supported by research and training grants of the Institute, and the expenditure amounted to about £A100,000. Plasma physics, in particular, received special support. An informal conference on plasma physics was held during November 1963, and the research effort at three universities was assisted by providing on loan a special high-speed camera capable of photographing the formation and decay of plasmas. Two other informal

specialists conferences were held at Lucas Heights under the auspices of the Institute—a "Heat Transfer and Fluid Flow" conference during August 1963, and a "Radiation Chemistry" conference during October 1963.

In conjunction with the University of New South Wales, the Commission is to establish an Australian School of Nuclear Technology with headquarters at the Lucas Heights Research Establishment. The facilities of both the Research Establishment and the University will be available to the School. The aim is to promote formal training and education in nuclear technology; and full-time courses of varying length in the science and technology of nuclear reactors, the production and application of radioisotopes, radiological safety and health physics, etc., will be open to students from Australia and overseas.

S. WAINTROUB

## BRITISH CHEMICAL REFERENCE SUBSTANCES

IN late 1963, the General Medical Council and the Pharmaceutical Society of Great Britain agreed to set up a joint authority to prepare and distribute chemical reference substances needed to carry out certain tests and assays described in the *British Pharmacopoeia*, the *British Pharmaceutical Codex* and the *British Veterinary Codex*. The Joint Committee held its first meeting in March 1964, and established a number of panels. Each panel was charged with the task of establishing one or more of these reference substances, and, since these substances might be of value for other purposes, the panels were asked, should it prove impracticable to obtain absolute purity, to ascertain so far as possible the amounts of all impurities.

The first two "British Chemical Reference Substances" have now been made available: digoxin and 2-*t*-butyl-4-methoxyphenol (the most active isomer present in butylated hydroxyanisole); these are required for the *Addendum* 1964 to the *British Pharmacopoeia* 1963, which became official as from June 1, 1965. The reports to the Joint Committee of the panel responsible on the preparation and purity of these substances have now been published.

The digoxin reference substance is required in the assay of digoxin and of its two preparations, the injection and the tablets, and for purposes of comparison (in place of the previously used authentic specimen) in the infra-red identification test. From its examination of the reference substance, the panel concluded that the substance is at least 99.7 per cent pure, containing not more than a total of 0.3 per cent of gitoxin, acetyldigoxin, digoxigenin and digitoxin.

The 2-*t*-butyl-4-methoxyphenol reference substance is required for the assay of butylated hydroxyanisole and in the infra-red identification test. In this case, a purity

of at least 99.85 per cent has been achieved, the balance being made up of very small amounts of 3,3'-di-(*t*-butyl)-2,2'-dihydroxy-5,5'-dimethoxybiphenyl (*bis*-BHA), 2,6-di-(*t*-butyl)-4-methoxyphenol, 4-methoxyphenol and a trace of an unidentified impurity. As well as its pharmaceutical uses, butylated hydroxyanisole has extensive usage in the food industry as an anti-oxidant in oils and fats, and the establishment of this reference substance will be welcomed in fields outside of pharmacy.

Samples of these reference substances are distributed by the Pharmaceutical Society and are available on application to the Assistant Director, Department of Pharmaceutical Sciences, 17 Bloomsbury Square, London, W.C.1, at a cost of £4 10s. for 0.3 g of the digoxin, and £3 for 0.2 g of the 2-*t*-butyl-4-methoxyphenol. Copies of the panel's reports can be obtained from either of the joint secretaries, Mr. T. O. Denston, 44 Hallam Street, London, W.1, and Dr. K. R. Capper, 17 Bloomsbury Square, London, W.C.1.

Because there may be some confusion between "British Chemical Reference Substances" and "Authentic Specimens", both of which are distributed by the Pharmaceutical Society, it is appropriate to explain that the reference substances are samples which have been purified so far as is practically and economically feasible, any remaining impurities having been identified and a limit set on their presence. They are used in assay procedures and to serve as the reference material in tests used to limit the presence of certain impurities in pharmacopoeial and *Codex* substances. Authentic specimens, on the other hand, are only tested samples of good quality commercial material which are needed for certain tests, notably infra-red identification tests, for which a comparison material having the purity of a reference substance is not essential.

## PESTICIDES IN THE ENVIRONMENT

AN advanced study institute on "Pesticides in the Environment and their Effects on Wildlife", sponsored by the North Atlantic Treaty Organization, was held at Monks Wood Experimental Station during July 1-14.

The main purpose of the Institute was to enable those working on the effects of pesticides on wildlife to exchange ideas and to discuss future research. Thirty-four papers were read, and dealt with the background of the wildlife problem and with field and laboratory studies of the effects of pesticides in terrestrial, freshwater and marine environments. Seventy-one scientists from Government

and university laboratories attended the meeting, including chemists, toxicologists and zoologists of eleven nationalities.

The papers and discussions showed that pesticide residues have been detected in a wide spectrum of physical and biological samples from diverse environments, indicating that contamination is widespread. In some instances, harmful effects on wildlife populations were clearly demonstrated, but more frequently the effects of residues are unknown.

It was concluded that in order to understand the effects of pesticides more fully, there was need for more work,



including: routine collection of data; experimental research; use of present knowledge; and dissemination of information. Details are as follows:

**Routine collection of data.** Carefully designed surveys are needed to obtain valid estimates of animal populations to assess effects of changing environmental conditions. Furthermore, it is necessary to measure the accumulation of chemical residues in many physical and biological components of the environment to assess the present and future levels of contamination. Existing studies of residues in Belgium, Canada, Eire, France, the Netherlands, Sweden, the United Kingdom and the United States provide the basis for further research of this kind. Special emphasis should be placed on persistent compounds, and on aquatic species which seem to be especially valuable as indicator organisms. Multiple-detection methods with associated techniques for confirmation of identity should be used wherever possible. Detailed information concerning the total quantity of pesticide used in different countries must be obtained to understand residue data properly, and to predict future trends.

**Experimental research.** There is a need for developing and standardizing analytical procedures, censusing methods and other techniques. Investigators should pay full attention to the use of critical and confirmatory techniques which have already been worked out, and to the statistical treatment of accumulated data. More

work is required on pesticides other than organochlorine compounds. In particular, improved methods of chemical analysis for organophosphorus pesticides and their metabolites should be investigated. The dynamics of pesticides in different environments need to be investigated more thoroughly, including decomposition, physical and biological dispersal and accumulation. Additional investigations are required to resolve the difficulties in using results obtained from laboratory studies to assess possible effects on populations in the field. Long-term field studies are particularly necessary for assessing the effects of persistent chemical compounds and their metabolites.

**Use of present knowledge.** It is necessary to ensure that the great body of existing data be applied, starting at the stage of introduction of a new pesticide, and in considering the introduction of extensive control programmes. Special consideration should also be given to rare species and those of restricted range and special vulnerability.

**Dissemination of information.** To implement the development of research outlined above, it was hoped that members of the Conference would continue to exchange information, and that international meetings of a similar kind to this Advanced Study Institute would be held at regular intervals. An annual list of current research projects would be particularly valuable.

## NOCTILUCENT CLOUDS OVER NORTH AMERICA

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IN 1962, when the work reported here began, only one recorded sighting of noctilucent clouds from North America was known. This was the observation reported by Vestine in 1934 (ref. 1). The scarcity of sightings from North America led to the belief that noctilucent clouds did not occur here as frequently as over Europe and the U.S.S.R., where hundreds of such observations have been made during the past eighty years.

A careful literature search made in 1962 uncovered eleven additional records of North American sightings of noctilucent clouds, and an observation programme conducted at College, Alaska, during the summer of 1962 resulted in five more sightings. On the basis of these findings it was concluded<sup>2</sup> that noctilucent clouds occurred over North America more frequently than previously supposed, and that the scarcity of recorded observations was probably due to the lack of informed and interested observers.

To investigate the apparent frequency of occurrence, spatial extent and lifetime of noctilucent clouds over North America, an extensive network of noctilucent cloud observing stations was established in 1963 over Alaska and Canada which in 1964 was extended to Greenland and Iceland. Co-operation in the set-up of this network of stations was given by the Canadian Department of Transport, the Icelandic Meteorological Service, the U.S. Weather Bureau, the U.S. Federal Aviation Agency and the U.S. Air Force Weather Squadrons. Fig. 1 shows the locations of the stations participating in this programme. A *Noctilucent Cloud Observation Manual*<sup>3</sup> was prepared and issued to all participating stations to acquaint the observers with noctilucent clouds. During the summer of 1963, only 33 Alaskan stations and 15 stations in western Canada took part in the programme. This past summer (1964) all the stations shown in Fig. 1 took part, as well as the crews of the Pan American World Airways and Alaska Airlines aircraft flying the Fairbanks-Seattle route.

During 1963, with 48 stations participating, 25 displays of noctilucent clouds were sighted over North America.

During 1964, with 88 stations participating, 57 displays were sighted. These numbers represent reliable sightings. A number of additional reports had to be discarded because of insufficient evidence of a definite display. A complete listing of the nights of the 99 displays of noctilucent clouds reported over North America to date is given in Table 1. It also gives the number and latitude and longitude range of the stations which reported noctilucent clouds on these nights. The low-latitude, artificially produced noctilucent clouds reported by Meinel<sup>4</sup> are not included in Table 1. The date of the display, given by a single number, refers to the night beginning on that date and ending on the following one. The observations listed in Table 1 are presented in Fig. 2 in the form of a histogram and a smoothed curve. The

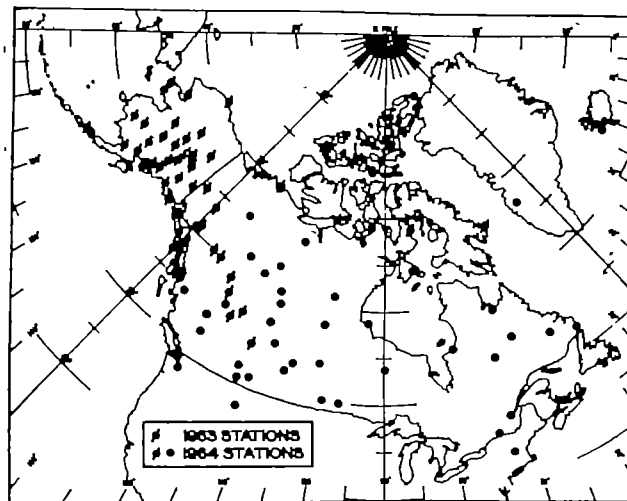


Fig. 1. Network of noctilucent cloud-observing stations over North America, Greenland and Iceland

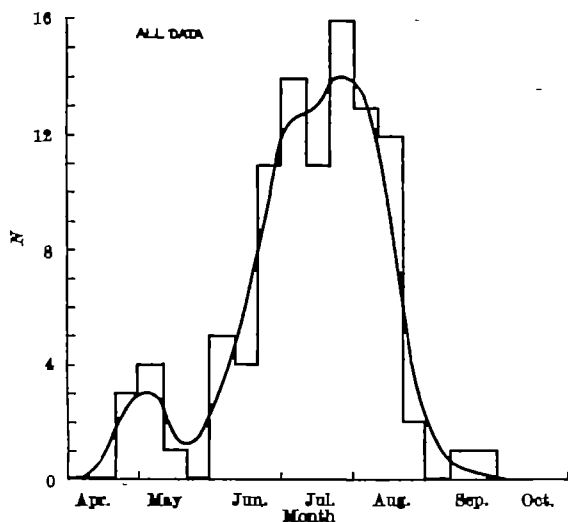


Fig. 2. Apparent frequency of occurrence of noctilucent clouds over North America based on all the 99 sightings made so far. The histogram represents the number of noctilucent cloud sightings per 10-day interval. The smoothed curve is based on the relation  $N = (a + 2b + c)/4$ , where  $b$  is the frequency in the interval in question and  $a$  and  $c$  are for the adjacent intervals.

histogram represents the number of sightings per ten-day interval. The smoothed curve was derived from the histogram using the smoothing relation  $N = (a + 2b + c)/4$ , where  $b$  is the frequency for the period in question and  $a$  and  $c$  are the frequencies for adjoining periods. This smoothing relation serves to correct at least partially for cloudy nights and other factors, and should represent the actual relative frequencies with greater accuracy than the histogram. The observations given in Table 1 are also

divided according to the number of sightings for different latitude ranges in intervals of 5 degrees. These frequencies are given in Fig. 3 in the form of a histogram and a smoothed curve.

The salient features of the North American observational data of noctilucent clouds are: (1) noctilucent clouds have been observed from late April through late September; (2) the peak of activity is around the end of July; (3) noctilucent clouds occur far more often after the summer solstice than before (27 before and 72 after); (4) the lowest and highest latitude sightings are 45.5 and 76.3 degrees, respectively; (5) the optimum latitude range for observing noctilucent clouds is 50–65 degrees; (6) noctilucent clouds occur as frequently over North America as over Europe and Russia.

Since the observation of noctilucent clouds is restricted to twilight periods when the Sun is between 6° and 16° below the observer's horizon, it is not possible at present to determine the true lifetime of these clouds. Sometimes extensive displays of noctilucent clouds recur for two or more successive nights, but whether they are parts of a distribution that is continuous throughout the intervening day-times is uncertain. Lifetimes of 5–6 h have been measured for two widespread displays—August 11 and August 12, 1963—observed over Alaska and Canada. Particular parts of noctilucent clouds may last for only a few tens of minutes. For example, one well-defined portion of the August 11, 1963, display formed and decayed in a clear portion of the sky in a period of about 30 min.

Sometimes the whole boundary of a noctilucent cloud display can be seen and its area estimated; this may be only a few tens of thousands of km<sup>2</sup>. The extent of sky at the level of 82 km visible from one station is a circular area about 1,000 km in radius; but only a part of this

Table 1. DATES OF NOCTILUCENT CLOUD DISPLAYS OBSERVED OVER NORTH AMERICA

Date	Station(s) observing display			Date	Station(s) observing display		
	No.	Lat. (N)	Long. (W)		No.	Lat. (N)	Long. (W)
20 July, 1963	1	54-6	113-3	5 June, 1964	1	56-2	68-3
26 June, 1966	1	53-6	113-5	9 June, 1964	2	51-0-58-1	101-6-114-1
27 June, 1966	1	53-6	113-5	10 June, 1964	1	53-4	60-5
28 June, 1966	1	53-6	113-5	11 June, 1964	2	57-1-58-4	134-6-135-3
27 July, 1967	2	61-1-61-2	145-5-150	12 June, 1964	1	58-2	68-3
7 Aug., 1967	1	61-2	150	15 June, 1964	1	53-8	90-1
1 June, 1968	1	51	97	20 June, 1964	1	54-8	66-8
13 Aug., 1968	1	62-5	114-6	21 June, 1964	2	52-2-53-5	106-6-106-6
8 July, 1969	1	50	63	23 June, 1964	1	60-2	120-2
8 Aug., 1969	1	61-2	150	24 June, 1964	2	53-9-60-2	123-5-123-2
23 July, 1961	1	52-2	106-6	28 June, 1964	8	53-5-60-1	90-1-117-3
13 Sept., 1961	1	76-8	170-6	29 June, 1964	10	49-7-53-8	90-1-123-7
6 May, 1963	1	61-2	150	30 June, 1964	11	48-4-60-6	55-9-151-5
11 Aug., 1963	1	61-2	150	1 July, 1964	5	53-6-58-8	113-5-123-7
13 Aug., 1963	1	64-9	148-0	4 July, 1964	3	52-2-58-2	66-8-106-6
14 Aug., 1963	1	64-9	148-0	5 July, 1964	10	53-2-60-6	60-5-135-3
16 Aug., 1963	1	64-9	148-0	6 July, 1964	4	53-4-59-5	60-5-139-7
1 May, 1963	1	56-4	124-6	7 July, 1964	1	54-8	66-8
26 June, 1963	1	52-2	106-6	8 July, 1964	8	53-5-60-1	66-8-124-6
23 June, 1963	1	61-2	150	10 July, 1964	1	56-6	123-7
1 July, 1963	1	54-8	66-8	11 July, 1964	11	51-0-60-6	66-8-125-3
3 July, 1963	1	58-7	94-1	12 July, 1964	8	51-0-60-2	106-6-120-2
4 July, 1963	1	58-7	94-1	13 July, 1964	8	53-6-60-8	111-2-120-2
5 July, 1963	1	58-7	94-1	15 July, 1964	3	55-4-59-6	94-1-131-5
8 July, 1963	2	59-4-59-5	124-6-130-7	16 July, 1964	15	52-2-64	106-6-156-6
9 July, 1963	1	61-5	144-5	17 July, 1964	1	61-1	135-6
11 July, 1963	1	61-1	150-6	20 July, 1964	4	59-5-64-5	139-7-150-1
13 July, 1963	3	61-2-61-6	150-159-6	21 July, 1964	7	47-6-64-9	66-8-123-0
13 July, 1963	1	61-2	150	22 July, 1964	8	47-6-64-9	66-8-145
23 July, 1963	3	57-58-4	124-6-135-7	23 July, 1964	8	53-2-62-5	68-3-129-2
25 July, 1963	1	63	143	24 July, 1964	11	53-2-64-9	60-5-148
27 July, 1963	1	64-9	148	25 July, 1964	1	60-1	113-2
31 July, 1963	7	53-6-60-2	113-5-120-2	26 July, 1964	6	53-2-64-9	70-8-148
1 Aug., 1963	1	60-2	120-2	27 July, 1964	4	54-6-61-1	123-7-156-6
5 Aug., 1963	4	58-8-64-9	123-7-148	28 July, 1964	8	58-2-63	68-8-151-5
6 Aug., 1963	5	60-6-66-2	125-8-152-1	29 July, 1964	3	58-6-62-1	111-2-145-4
8 Aug., 1963	1	63	142	30 July, 1964	4	58-1-61	94-1-113-2
11 Aug., 1963	17	56-4-65-2	122-7-153-9	1 Aug., 1964	2	58-2-63-8	68-3-153-0
12 Aug., 1963	23	58-4-66-2	125-8-161-7	2 Aug., 1964	13	54-64-9	68-3-145
16 Aug., 1963	1	63-66	10-60	3 Aug., 1964	8	57-3-66-9	94-1-133-6
18 Aug., 1963	2	64-5-64-9	148-149-1	6 Aug., 1964	4	63-6-64-9	21-1-148
23 Sept., 1963	1	64-9	148	7 Aug., 1964	5	61-1-64	22-6-156-6
23 April, 1964	3	45-5	73-7	8 Aug., 1964	8	61-68-5	22-6-162-6
23 April, 1964	3	53-4-64-9	60-5-148	9 Aug., 1964	5	58-7-64-9	94-1-159-6
30 April, 1964	2	53-4-53-5	60-5-105-6	11 Aug., 1964	4	53-6-66-9	113-5-168-0
3 May, 1964	3	58-8	111-2	13 Aug., 1964	10	58-8-71-3	132-7-165-4
9 May, 1964	2	53-4-58-2	60-5-68-3	20 Aug., 1964	1	58-7	94-1
11 May, 1964	1	59-7	154-9	29 Aug., 1964	2	53-8-64-1	90-1-139
17 May, 1964	1	58-2	68-3	30 Aug., 1964	1	53-8	90-1
2 June, 1964	1	58-7	94-1				

can be illuminated by sunlight against a dark sky. Twenty-six of the displays observed over Alaska and Canada were too extensive to be all visible from any one station. Combination of the data from many observers indicate that the areas covered by eight of these displays (August 11, 1963; August 12, 1963; June 29, 1964; July 5, 1964; July 11, 1964; July 16, 1964; July 24, 1964; and August 2, 1964) were more than 2 million km<sup>2</sup>. It is possible that noctilucent clouds might at times cover all longitudes around the pole and have an area of order 10<sup>8</sup> km<sup>2</sup>; but this, if true, would be difficult to establish from observations of the kind presently available because they are restricted to twilight, and only stations in a sector of about 90° in longitude can see them at the same time.

A prominent feature of many noctilucent cloud displays is the presence of well-defined parallel bands. These have been identified with systems of waves of various wave-lengths. Only a few wave-lengths have been measured in the past; they range between 7 and 100 km (refs. 5, 6). We have recently developed a simple method for measuring the wave-lengths from individual photographs of noctilucent clouds. The method is based on the constancy of the height of noctilucent clouds at 82 km, and requires good photographs which contain several points the elevation and azimuth angles of which are known. From the photographs, the elevation angle to the pair of bands in question is measured as well as the incremental angle between them. A knowledge of these two quantities is sufficient for determining the wave length. Photographs of three displays of noctilucent clouds observed over North America have been analysed by this method so far. A total of 248 wave-lengths were determined from these photographs and the frequency distribution obtained is shown in Fig. 4. As seen from this curve, the wave-lengths range from about 5 to 100 km, the most frequent value being about 10 km.

Two mechanisms have been suggested to account for the wave structure in noctilucent clouds: internal gravity waves<sup>7</sup> and interface waves<sup>8</sup>. A comprehensive discussion of these mechanisms by Haurwitz<sup>9</sup> indicates it is likely that the short wave-lengths of about 10 km are due to interface waves, and that the longer wave-lengths are due to internal gravity waves. More data on the temperature distribution, wind shear at the mesopause, as well as simultaneous velocity and wave-length measurements are needed to determine with more certainty the cause of the waves.

An examination by Vestine<sup>1</sup> of the noctilucent cloud data for the years 1885–1933 showed that there was no

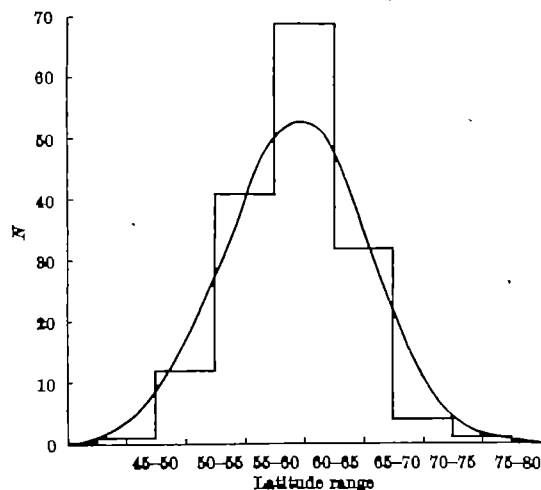


Fig. 3. Apparent frequency of occurrence of noctilucent clouds over North America as a function of latitude range. The histogram represents the number of noctilucent cloud sightings per 10-day interval. The smoothed curve is based on the relation  $N = (a + 2b + c)/4$ , where  $b$  is the frequency in the interval in question and  $a$  and  $c$  are for the adjacent intervals.

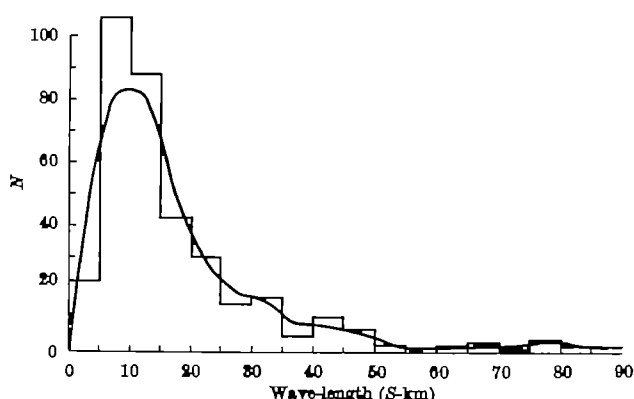


Fig. 4. Frequency distribution of wave-lengths in three noctilucent cloud displays (248 measurements).

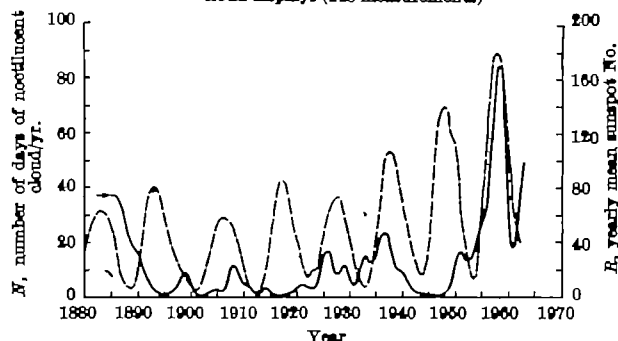


Fig. 5. Fluctuation of the apparent noctilucent cloud activity compared to the sunspot cycle for the period 1885–1964. —, Noctilucent clouds/yr.; ---, sunspot No./yr.

apparent correlation of noctilucent cloud activity with either volcanic activity or meteor showers, but there was some evidence of a correlation with years of sunspot minima. Vestine pointed out that this apparent correlation might be fortuitous since the number of noctilucent cloud observers and interest in these clouds have varied widely over the years. Quiroz<sup>10</sup> re-examined the possible correlation of noctilucent cloud activity with sunspot cycle using a partially complete list of 711 sightings for the period 1885–1963 and found no correlation. We have recently compiled a complete list of the 1,034 noctilucent cloud sightings reported during the period 1885–1964 and compared the frequency of occurrence of noctilucent clouds with the sunspot cycle data for the same period. These results, given in Fig. 5, show that there is no correlation of noctilucent cloud activity with sunspot cycle. A physical relationship between the two, however, is not improbable, since solar-induced variations in the density and temperature of the atmosphere near 80 km could well have an important effect on incoming dust particles and their subsequent redistribution.

This work was supported by U.S. National Science Foundation grant GP-1759. The participation of the personnel of the agencies named here in the observational work is gratefully acknowledged. I thank Mrs. Carol Echols for her assistance in the data analysis and the computational work. I also thank Mr. Yngvar Goteas, Dr. Sydney Chapman and Dr. Bernhard Haurwitz for their advice.

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<sup>9</sup> Haurwitz, B., *Geophysical Institute Rep. UAG-R160* (Univ. of Alaska, December 1964).

<sup>10</sup> Quiroz, B. S., *Tech. Rep. 181, Air Weather Service, U.S. Air Force* (August 1964).

# PHASE MODULATION OF GEOMAGNETIC MICROPULSATIONS

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THE Geomagnetism Group of Lamont Geological Observatory has recorded variations in the total intensity of the magnetic field with a rubidium vapour magnetometer. High-resolution power spectral analyses of large amplitude, narrow-band *Pc2* micropulsations have been performed by digital computer. A relationship was found in the frequency domain between the *Pc2* activity and longer period *Pc5* micropulsations which indicates that the shorter period signals were phase modulated by the longer period variations.

the natural spectrum, Fig. 2 presents the pre-whitened spectrum with the six dB slope removed. Power density is in units of gammas<sup>2</sup>/c/s. In Fig. 2 there is a series of peaks, somewhat equally spaced in frequency, between 0.015 and 0.04 c/s, which are incorrectly spaced to be due to side lobes of the hamming spectral window.

The high-amplitude, narrow-band *Pc2* micropulsations began on this day at 1,000 h local time and continued until 1,400 h. Fig. 3 shows 1-h spectra of the first and last hours of the 4-h period. Frequency-time analysis of the

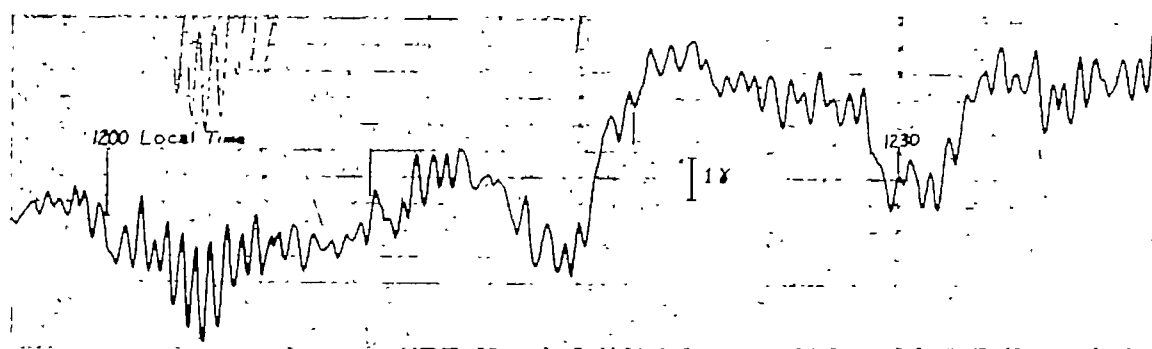


Fig. 1. Rubidium magnetometer recording of *Pc2* micropulsations superimposed on long period *Pc5* variations. Lebanon State Forest, New Jersey, 1156–1240 local time, Oct. 25, 1963

The magnetic variations were recorded at a station in the Lebanon State Forest on the New Jersey coastal plain. Part of the data were recorded in digital form on punched paper tape at a sampling rate of once per second. In other cases, analogue records were taken to the laboratory and digitized on an electromechanical instrument. In addition, part of the data were also recorded simultaneously at stations separated several hundred kilometres from Lebanon.

Power spectral analyses were performed on sections of records showing continuous, high-amplitude micropulsations. The typical geomagnetic power spectrum has an approximately six dB per octave rise in power towards long periods<sup>1</sup>. In this investigation, the data were pre-whitened before the spectral analysis was performed by use of a filter which attenuated at six dB per octave towards the long periods and also cut sharply the periods less than the folding period, thus reducing aliasing errors. Smoothing of the raw spectrum was done with a hamming window.

Fig. 1 is a photograph of the rubidium magnetometer record during part of the interval the spectrum of which is shown in Fig. 2. The photograph shows 30 sec period micropulsations superimposed on longer period variations. Fig. 2 shows the spectrum of a 2-h and 13-min section of record, during 11.37–13.50 local time, during an interval when the *Kp* index was at the relatively low values of 1+ and 2+. The spectrum was computed using 3,504 data points at 2.28-sec spacing. Three hundred and fifty lags were used. The frequency resolution was 0.00063 c/s. Since some of the spectral peaks are very marginal and were obscured by the steeply rising curve of

entire interval confirmed what is suggested by Fig. 3, namely, that the average period of the signals drifted gradually, by about 4 sec, towards longer periods during the interval. It was found that a spectrum of about a 2-h interval provided the frequency resolution necessary to show the equally spaced peaks of Fig. 2, but was not so long that the peaks were averaged out by the slow drift in period.

Fig. 4 presents pre-whitened spectra of micropulsations recorded simultaneously at Lebanon and Breezewood, Pennsylvania, 1,000–1,200 h local time, on a disturbed day when the *Kp* index was 4-. These stations are separated by 318 kilometres in an east-west direction. The spectra were computed using 2,400 data points at a 3.0-sec spacing. Two hundred and forty lags were used. The frequency resolution was 0.0007 c/s. At both stations the analogue records showed short period *Pc2* micro-

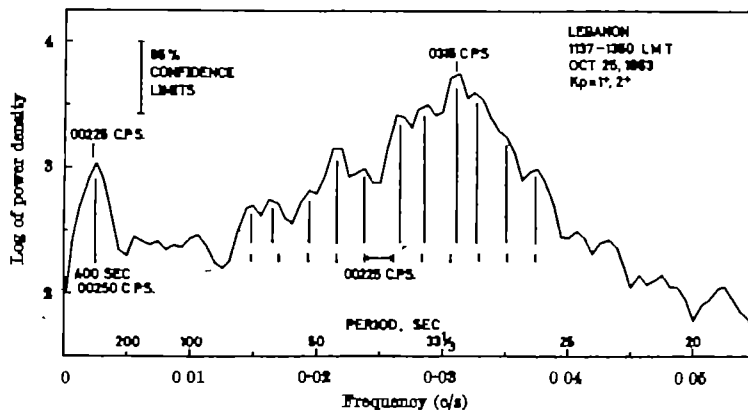


Fig. 2. Power spectrum c/s a 2-h and 13-min interval, including section of record in Fig. 1

pulsations superimposed on longer period variations. The close correspondence of peaks between stations is evident. Again the pattern of nearly equally spaced peaks is seen.

A phase modulated signal has a frequency spectrum with a peak at the carrier frequency plus an infinite number of sidebands separated from the carrier by integral multiples of the modulating frequency<sup>1</sup>. The phase modulated signal can be represented as:

$$a = A \sin(2\pi Ft + (\varphi_0 + \Delta\varphi \cos 2\pi\mu t))$$

where  $F$  is the carrier frequency,  $\mu$  the modulating frequency,  $\varphi_0$  the phase angle, and  $\Delta\varphi \cos 2\pi\mu t$  the phase deviation in radians. The sidebands of a phase modulation frequency spectrum diminish rapidly in magnitude when they are displaced from the carrier frequency by more than  $\mu\Delta\varphi$ , or equivalently, when the number of sidebands exceeds  $\Delta\varphi$ .

If the highest peak in Fig. 2 is assumed to be the carrier frequency, then  $F = 0.0315$  c/s. There are seven peaks on the low-frequency side of the carrier, extending to 0.0150 c/s and spaced an average of 0.00225 c/s apart. If 0.00225 c/s is taken as the modulating frequency  $\mu$ , then  $\mu\Delta\varphi$ , which must be the approximate half-width of the spectrum and which in this case is 0.0165, requires  $\Delta\varphi = 7.3$ .  $\Delta\varphi$  should not be much greater than the number of sidebands, which is so.

The most obvious spectral peak in Fig. 2 is the large one at 0.00250 c/s (400 sec period), which is very close to the assumed modulating frequency. It is suggested, then, that this peak represents the energy of a long-period magnetic variation which phase-modulated the shorter period signals.

In the Lebanon-Breezewood spectra of Fig. 4, the long-period peak has an average value on the two spectra of 0.0032 c/s (312 sec period), a higher modulating frequency than in Fig. 2. Though the spacing of the fine peaks is not entirely regular in Fig. 4, the average spacing

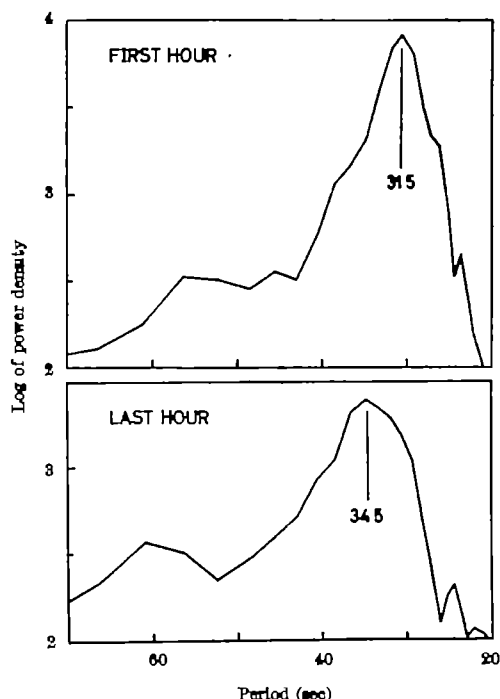


Fig. 3. 1-h power spectra of first and last hours of 4-h interval, 1,000-1,400 L.M.T., Oct. 25, 1963

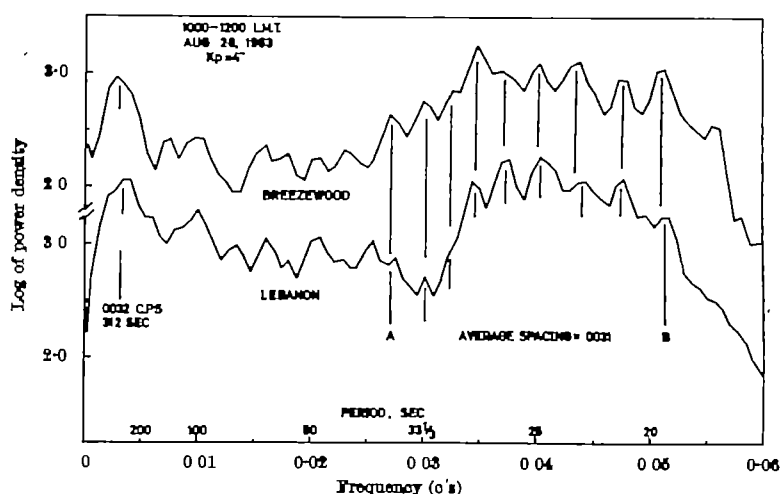


Fig. 4. Simultaneous power spectra at stations separated 818 km

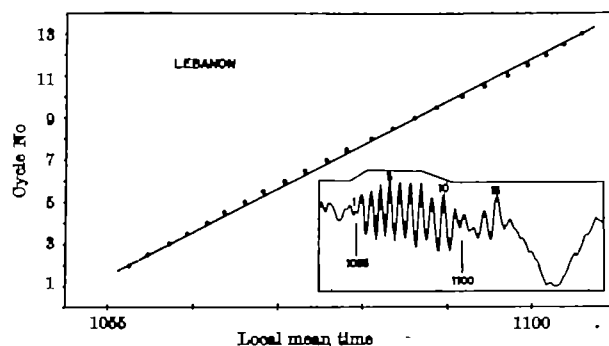


Fig. 5. Cycle-number versus time plot of wave-train shown in insert

between points A and B is 0.0031 c/s, a wider spacing corresponding to the higher modulating frequency.

A phase deviation of the size calculated (7.3 radians) should be easily visible on a cycle-number versus time plot of a continuous section of a micropulsation wave-train long enough to undergo a full cycle of modulation. Fig. 5 shows such a plot for a 320-sec long amplitude group which occurred during the 4-h interval already referred to. As was seen less well for several other sections of wave-train, the points lay about a straight line in a sinusoidal manner. The maximum phase deviation here is only about 1.5 radians, however. Fourier analysis of micropulsation groups, such as shown in Fig. 5, which occurred during intervals when the long period activity was present, also showed a frequency spectrum consisting of a carrier frequency accompanied by sidebands.

The equally spaced peaks are a very marginal feature on both the power spectra and the Fourier spectra. They can be detected only if very strong micropulsations occur superimposed on large amplitude, long period variations. No clear occurrence relationship was found between the two bands of activity. Twelve high-resolution spectra were computed. In six spectra, where strong Pc2 micropulsations were present, the long-period spectral peaks, which ranged from 312 to 400 sec period, were present and of high amplitude. Of these six, only the spectra shown in this report revealed the phase modulation effect clearly. In the other six spectra, instances were found where either one or the other of the period bands was present alone.

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<sup>2</sup> Goldman, S., *Frequency Analysis, Modulation and Noise* (McGraw-Hill Book Co., New York, 1948).

## ORIGIN OF CHONDRITES

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APPROXIMATELY 90 per cent of the meteorites that fall on the Earth are classed as chondrites. Chemical analyses of chondrites reveal some striking similarities and dissimilarities. The abundances of the major components are remarkably similar when considered in relation to the compositional variations of a comparable terrestrial petrologic class such as basalts<sup>1</sup>. This similarity is so characteristic that in the few cases where chondritic structures are lacking (for example, Type I carbonaceous chondrites) the chemical composition clearly serves to establish genetic affinity with the chondrite class. Superimposed on the general similarities in abundances of major components, there are some systematic differences of comparatively small magnitude which are characteristic of the different major classes of chondrites. One of the principal variations of this type is in the oxygen content. This exercises a major control over the mineralogy of chondrites, which is the basis of their classification<sup>1</sup>. Another is in the total iron content, which is also used for classification<sup>1</sup> (Fig. 1).

Contrasting with the relative uniformity of major element abundances, very large differences exist in the abundances of certain minor elements. For example, chlorine, iodine, tellurium, zinc, cadmium, germanium, lead, bismuth, thallium and mercury are depleted in ordinary chondrites compared with Type I carbonaceous chondrites by factors varying between 5 and 1,000 (ref. 3). Significant but smaller relative depletions exist for many other elements. Apart from mercury, the foregoing elements are only slightly depleted in enstatite chondrites compared with Type I carbonaceous chondrites. However, enstatite chondrites have suffered significant relative depletions (30–80 per cent) in a number of oxyphile elements, for example, calcium, barium, yttrium, rare earths, scandium, chromium, titanium, uranium, thorium<sup>2,4</sup>. The outstanding feature of the trace element abundance patterns is that many elements in all groups of chondrites are depleted compared with Type I carbonaceous chondrites. It would be possible to obtain the compositions of enstatite chondrites, ordinary chondrites and Types II and III carbonaceous chondrites solely by the removal of the appropriate amounts of trace and minor elements from Type I carbonaceous chondrites<sup>1–7</sup>.

The abundances of many elements, for example, lead, bismuth, tellurium, mercury, thallium, iodine, cadmium, zinc, germanium, uranium, thorium, in ordinary chondrites and to a lesser extent in enstatite chondrites depart widely from estimated 'cosmic' abundances, derived from nucleide systematics and theories of nucleosynthesis. On the other hand it is extremely significant that the Type I carbonaceous chondrite abundances are in reasonable agreement with the cosmic abundances for all but the most volatile elements<sup>1,3,8,9</sup>. This indicates that Type I carbonaceous chondrites have had a simpler chemical history than other classes of chondrites. This is supported by their highly oxidized state and by the high content of water, carbonaceous compounds and other volatiles which they contain. Letimer<sup>10</sup> and Urey<sup>11</sup> have pointed out that in the cold gas-dust cloud believed to be the immediate parent of the solar system, the dust particles would also be highly oxidized, volatile-rich, and composed of most elements in their primordial abundances. Accordingly Urey<sup>12</sup> suggested that the carbonaceous chondrites might be closely related to the primitive dust of the solar

nebula. This suggestion was developed in greater detail by Mason<sup>13</sup> and by me<sup>14</sup>. Although Type I carbonaceous chondrites appear to represent the closest approach to the primordial dust which we possess, it is probable that important differences exist. They appear to have had a mild thermal and metamorphic history and to have undergone slight differentiation. This may have occurred when the primordial dust accumulated into a small parent body.

From the relationships described here, it might be suggested as a working hypothesis that other groups of chondrites have evolved in some way from primitive material resembling the present Type I carbonaceous chondrites<sup>12,14,22</sup>.

## Oxidation-reduction Equilibria in Chondrites

From a critical study of the chemistry of chondrites Prior<sup>15</sup> concluded: "The less the amount of nickel-iron in chondritic stones the richer it is in nickel and the richer in iron are the magnesium silicates". This generalization has become known as Prior's rule, and is illustrated in Fig. 1 which is based on self-consistent chemical and mineralogical analyses. Fig. 1 shows that there can be no doubt about the validity of Prior's rule if it is taken as implying a general trend rather than a strictly quantitative relationship. As the amount of oxidized iron increases from 0 to 26 per cent, metallic iron falls from 30 to 0 per cent. A wide range in oxidation states (as defined by the  $\text{FeO}/\text{FeO} + \text{MgO}$  ratios of the silicates) is found. However, the range is not continuous. There is a large hiatus between enstatite and ordinary *H* chondrites<sup>14</sup>. More extensive investigations<sup>16,17</sup> indicate the presence of a small but distinct hiatus in  $\text{FeO}/\text{FeO} + \text{MgO}$  ratios between *H* and *L* groups. Keil and Fredriksson<sup>17</sup> concluded that Prior's rule is best used to describe and interpret redox relations between groups of chondrites rather than within individual groups. They make a strong case for this view when applied to the ordinary *H* chondrites. The metal contents within this group are not related closely to the  $\text{FeO}/\text{FeO} + \text{MgO}$  ratios of the silicates. It appears that the metal has become slightly fractionated independently of the silicates as in enstatite chondrites (Fig. 1). The situation with regard to the *L* group is not so clear. There has evidently been an analogous slight independent fractionation of metal for oxidized iron contents between 9 and 12 per cent. However, a distinct correlation of metal content with oxidized iron is present over the entire 9–16 per cent interval.

Urey and Craig<sup>8</sup> observed that the ordinary chondrites may be divided into two groups, *H* and *L*, according to their total iron content. The *L* group contains about 5 per cent less iron than the *H* group. This is shown clearly in Fig. 1, which suggests that the *L* group has been formed by the removal of about 5 per cent of metallic iron from the *H* group. It is therefore clear, as emphasized by Urey and Craig<sup>8</sup>, that the relationships between chondrites cannot be explained by a rigorous application of Prior's rule. In addition to variable redox states at constant total iron composition, some process for independent fractionation of metal must have operated<sup>14</sup>. This is also seen to have occurred in the enstatite chondrites (Fig. 1). Nevertheless, this complexity should not be permitted to divert attention from the importance of Prior's rule viewed as a general trend rather than as a rigorous relationship.

In the previous section it was concluded that the parental material of the chondrites was highly oxidized. It follows from the relationships described by Prior, and already discussed here, that the oxidized primitive material was subjected to widely varying degrees of chemical reduction. The two most abundant reducing agents are hydrogen and carbon and it is of vital importance to establish which of these dominated in the chondrite reduction processes.

Decisive evidence is supplied by the enstatite chondrites. The metal phases of these objects contain several per cent of elemental silicon in solid solution<sup>18</sup> and the FeO content of the silicates is of the order of 0.1 per cent<sup>19</sup>. These observations establish that enstatite chondrites formed under extremely reducing conditions. If they formed by a process of hydrogen reduction or by condensation of a hydrogen-rich gas phase, a very high hydrogen-to-oxygen ratio is required. Thermodynamic calculations indicate required minimum H/O ratios at appropriate temperatures varying between 300 and 2,000 (refs. 20, 21 and 22). For comparison, the H/O ratio of the solar atmosphere is about 1,000.

It is very difficult to understand the occurrence of sulphur, zinc, cadmium and carbon in enstatite chondrites if they formed under these conditions. Graphite is a significant constituent of most enstatite chondrites. However, it is completely unstable in the presence of excess hydrogen and/or oxygen at elevated temperatures. With the solar H—O—C abundances, carbon would occur as CO or CH<sub>4</sub> dependent on pressure. Enstatite chondrites also contain on the average about 3 per cent of sulphur occurring mainly in FeS. At 1,500° C the equilibrium  $\text{FeS} + \text{H}_2 = \text{Fe} + \text{H}_2\text{S}$  proceeds to the right when the H/S ratio exceeds about 70. The solar H/S ratio is 50,000 which is 700 times higher than will permit the stability of FeS or any other sulphide phase with equivalent sulphur activity. Analogous arguments apply to zinc and cadmium which are present in some enstatite chondrites in the same proportions as in Type I carbonaceous chondrites, that is, in their 'cosmic' abundances. It is impossible to condense or retain these volatile elements

in the presence of solar H—O—S abundances at temperatures above 1,000° C (ref. 7).

On the other hand, the retention of carbon, sulphur, zinc and cadmium is explicable if the redox state of enstatite chondrites was established through reduction by carbon rather than hydrogen. Minerals occurring in enstatite chondrites are schreibersite, (FeNi)<sub>3</sub>P; daubréelite, FeCr<sub>2</sub>S<sub>4</sub>; oldhamite, CaS; troilite, FeS; osbornite, TiN; graphite and iron silicide. All these minerals are characteristic of blast-furnace assemblages produced when iron ores are strongly reduced by carbon. The common occurrence of this group of minerals in both environments strongly suggests that the chemical processes which produced enstatite chondrites and blast-furnace irons have been similar, that is, that the iron in meteorites has been produced by reduction in a condensed system in the presence of carbon<sup>14,23</sup>. It may be noted that in such a system, in the presence of an appreciable pressure of sulphur, but at low hydrogen pressure, zinc and cadmium are stable at high temperatures as sulphides.

It is concluded that the mineral assemblage and chemical composition of enstatite chondrites cannot be accounted for either by reduction of primitive material by hydrogen or by condensation from a hydrogen-rich gas phase. On the other hand, formation by carbon-reduction of primitive oxidized material in a condensed system at high temperatures is consistent with the observations. In view of the clear genetic relationships between enstatite and ordinary chondrites, it is probable that the latter have been produced by a similar process. Further direct evidence for the production of nickel-iron and silicon-iron in the Kaba Type III carbonaceous chondrite and the Grady ordinary chondrite by carbon reduction has been described by Sztrókay *et al.*<sup>24</sup> and Ramdohr<sup>25</sup>.

### Origin

The foregoing arguments lead to the basic hypothesis that ordinary and enstatite chondrites have formed by autoreduction from primitive material similar to the Type I carbonaceous chondrites<sup>8,13,14,26</sup>. It must be admitted, however, that the physical and chemical conditions under which this autoreduction may have occurred are very poorly known and consequently the subject of much controversy. One of the principal unsolved problems concerns the mechanism by which the fractionations of elements between the principal groups occurred. Another basic problem concerns the identification of the parent bodies of the chondrites. Most evidence suggests that they are formed by collisions in the asteroidal belt<sup>8</sup>. However, from the short cosmic-ray exposure ages of chondrites, Urey<sup>27,28</sup> has argued in favour of an origin on the lunar surface. Evidence at present available does not permit a clear choice between these sources. Accordingly, it is appropriate to consider alternative models of origin.

(a) *Asteroidal Model.* The following model is a modification of an earlier hypothesis of mine<sup>8,14,29</sup>. Aspects of this hypothesis were criticized by Mueller<sup>30</sup> and Anders<sup>9</sup>, and the modifications have been introduced to meet these objections.

It is assumed that chondrites evolved on parent bodies in the asteroidal belt which formed by direct accretion from the primitive oxidized dust phase of the parental solar nebula. The composition of these bodies was similar to Type I carbonaceous chondrites. They were heated internally by short-lived radioactivities<sup>31</sup>, resulting in the reduction of oxidized iron by carbonaceous compounds, to produce a metal phase *in situ*, and in equilibrium with the surrounding silicates. Most of the reduction proceeded under sub-solidus conditions, and the gaseous reduction products were able to migrate to the surface of the parent body, where they escaped. In the early stages of reduction they consisted principally of CO, and H<sub>2</sub>O, while in the later stages, CO and H<sub>2</sub> predominated. In the case of the ordinary chondrites, reduction

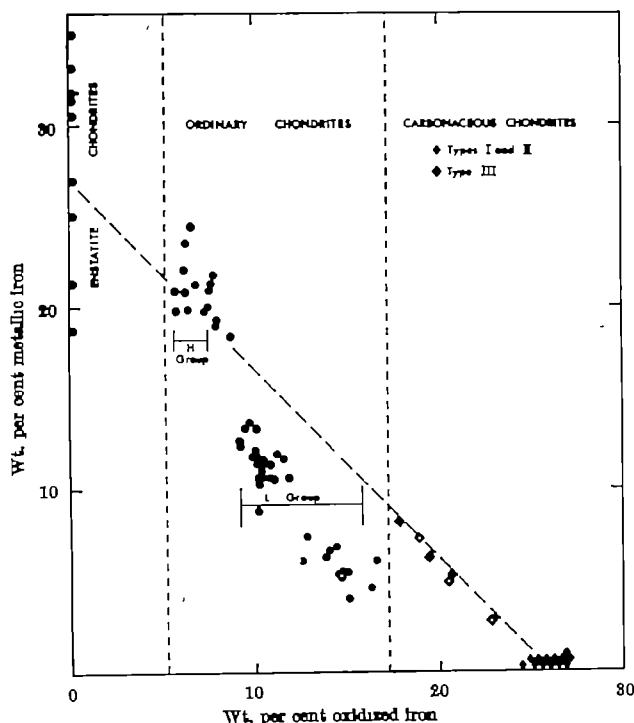


Fig. 1. Relationship between oxidized iron and metallic iron for chondrites characterized by self-consistent chemical and mineralogical FeO/FeO + MgO determinations. Adapted from ref. 14



proceeded until almost all the carbonaceous material had been consumed. The varying oxidation states of the ordinary chondrites are thus explained in terms of varying initial quantities of hydrocarbons trapped in the accreting dust. In the case of enstatite chondrites, carbonaceous compounds were in excess. Accordingly, reduction of iron to metal was complete and reduction proceeded sufficiently far to produce some elemental silicon. The enstatite chondrites contain graphite in accordance with this model.

With further heating, extensive melting occurred in the deep interior of the parent body or bodies. Turbulence generally prevented settling of unmelted metal particles and silicate crystals. However, in the parent body of the *L* group, turbulence was not sufficient to prevent a limited amount of fractionation caused by sinking and segregation of metal particles in the magma, and resulting in the depleted metal content characteristic of this group. The magma rose to the surface regions of the parent body. Decrease of confining pressure in the surface regions permitted reaction of residual carbon to proceed to completion. Rapid escape of gaseous reduction products (mainly CO and H<sub>2</sub>) from the magma at this stage caused disruption and resulted in a type of volcanism, leading to the formation of chondrules and the development of typical chondritic structures.

The conversion of a Type I carbonaceous chondrite into an ordinary chondrite by the above process involves the loss of about 30 per cent of the initial mass as volatile components—principally H<sub>2</sub>O, CO, H<sub>2</sub>S, CO and H<sub>2</sub>. These are lost continuously as dense, supercritical fluids, during heating and reduction. The composition of these fluids varies according to the stage of reduction. It is possible that the migrating fluids functioned as powerful selective solvents for many minor elements and that extensive chemical fractionations were thereby caused. Thus, loss of chalcophile elements from ordinary chondrites may have been caused by their solubility as complex hydrosulphide ions in H<sub>2</sub>O—H<sub>2</sub>S—H<sub>2</sub>S solutions<sup>1</sup>. A lower hydrogen fugacity in the parent body of enstatite chondrites may have prevented the formation of these soluble complex ions and accordingly the chalcophile elements were not lost<sup>1</sup>. Fractionation of oxyphile elements, for example alkaline earths and rare earths, may have been caused by solubility in H<sub>2</sub>O—CO<sub>2</sub> solutions given off at an early stage of reduction<sup>1,7</sup>.

The processes postulated as explanations of the fractionation patterns in chondrites are poorly understood and incomplete. One thing, however, is certain. The loss of 30 per cent of volatile components during auto-reduction of Type I carbonaceous chondrite material at elevated temperatures and pressures must inevitably cause extensive selective fractionations of many elements. Whether or not these fractionations correspond with those observed in ordinary and enstatite chondrites remains to be determined.

(b) *Lunar model.* The case for a lunar origin of chondrites rests largely on interpretation of their short cosmic ray exposure ages<sup>11,17</sup>. I have suggested elsewhere that the Moon contains substantial amounts of material similar in composition to Type I carbonaceous chondrites<sup>22,26,31</sup>. It might therefore be suggested that this material has evolved into ordinary and enstatite chondrites by the operation of processes analogous to those advocated above for the asteroidal model. It would be necessary to assume the presence of short-lived radioactivities in the Moon soon after its formation.

In this section I wish to propose an alternative model for the formation of chondrites on the Moon. It is suggested that ordinary and enstatite chondrites may have formed from planetesimals of Type I carbonaceous chondrite composition by impact phenomena when the latter fell on the Moon during its final stages of formation. The impacts would cause transient heating, leading to

shock melting. It is possible that carbonaceous material reacted with oxidized iron under these conditions leading to formation of a metal phase. The accompanying explosive loss of reduction products may have disintegrated the shock-melted chondrite, forming chondrules in the manner demonstrated by Fredriksson and Ringwood<sup>22</sup>. The mixture of chondrules and metallic particles then became consolidated into chondrites on the lunar surface. These events all occurred about 4.5 billion years ago. More recently, collisions of asteroids and/or comets with the Moon ejected substantial amounts of chondritic material which was afterwards swept up by the Earth<sup>22,27</sup>.

This mechanism appears to satisfy the major requirement discussed previously that ordinary and enstatite chondrites formed from primitive material by a process of carbon reduction in a condensed environment. The transformation of a carbonaceous chondrite into an ordinary chondrite requires about 300–400 cal/g. This may be compared with the free fall energy at the Moon's surface of approximately 700 cal/g. It is possible that the energy liberated by impact of the planetesimal with the Moon may have been substantially higher than this, depending on its previous orbit.

A serious difficulty of this hypothesis lies in explaining the fractionations of elements among the different chondrite groups. Previously it was suggested that these were caused by selective solubility of various elements in supercritical, dense fluids of H<sub>2</sub>O, H<sub>2</sub>S, CO<sub>2</sub>, CO and H<sub>2</sub>, which were expelled during heating in the parent body. An analogous explanation might be attempted in the present case. The volatile components mentioned here must also be liberated over a very short time-interval as a dense fluid during the impact and reduction, and there will be a partition of many other elements between the chondritic and the fluid phases. The nature of the partition equilibria which would occur under such conditions is quite unknown, but almost certainly they will lead to substantial chemical fractionations of some sort. Whether they are in the required direction can only be established by experiment.

The foregoing hypothesis is admittedly highly speculative. However, if the essential features could be supported by subsequent direct experiments, the hypothesis may prove to possess certain advantages over other explanations of the origin of chondrites, for example: (1) It is not necessary to assume the presence of short-lived radioactivity in the parent bodies of chondrites. (2) It provides a ready explanation for the common occurrence in meteorites of structures caused by shock waves. These structures would be produced by impacts on the Moon. It is doubtful whether collisions between asteroidal parent bodies could cause the observed phenomena, since the relative collision velocities of objects in asteroidal orbits are usually smaller than 1 km/sec<sup>28</sup>. (3) It is easier to understand the formation of polymict meteorites by collision phenomena on the lunar surface than between asteroids. Because of the small gravitational potential energy of the latter, collisions are more likely to lead to disruption and loss of material than thorough mixing and consolidation. (4) The Moon was originally much closer to the Earth than at present. It is possible that the Earth's magnetic field was stronger early in its history and that it possessed appreciable strength at the distance of the lunar orbit. The remnant magnetization displayed by chondrites<sup>29</sup> might then have been obtained in the terrestrial field. (5) Present explanations<sup>3,24</sup> of the occurrence of primordial gases in meteorites attribute it to a mixture of two components. One component resembles that possessed by carbonaceous chondrites and is thus readily explained on the present model. The other component is of unfractionated solar composition. Fredriksson *et al.*<sup>22</sup> have shown that rare gases can be incorporated in mineral grains by shock wave phenomena. The final stages of accretion of the Moon may have

occurred before the unfractionated gases of the solar nebula had been dissipated. Shock-wave phenomena accompanying the collisions of carbonaceous chondrite planetesimals with the Moon in the presence of unfractionated solar gases may have led to incorporation of the observed gases in chondrites (and other meteorites).

The foregoing discussion has been concerned with chondrites. It is also possible that other classes of meteorites have also been formed on the Moon under conditions which permitted complete melting and extensive differentiation of chondritic material<sup>10</sup>.

### Conclusion

Neither of the two hypotheses discussed here is complete or satisfactory in all respects. However, I believe that they are faced by less numerous and less serious objections than apply to the currently popular hypothesis that chondrites have formed from material which condensed directly from a hydrogen-rich gas phase during the passage of shock waves through the solar nebula<sup>11</sup>. A critical discussion of this latter hypothesis will be presented elsewhere<sup>12</sup>. Clearly it is of decisive importance to establish whether chondrites are derived from the Moon or from the asteroids. Until an answer to this question is forthcoming, great uncertainty surrounds all theories of origin, although many important boundary conditions have been revealed through recent chemical and physical investigations.

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## LATE WEICHSELIAN GLACIATION IN THE CHESHIRE-SHROPSHIRE BASIN

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SINCE Hull's<sup>1</sup> recognition of a tripartite sequence in the Pleistocene deposits of the Oldham area in 1864, the succession Upper Boulder Clay, Middle Sands, Lower Boulder Clay has been accepted by many subsequent workers in the Cheshire-Shropshire Basin and the adjoining parts of Lancashire. Dissenters from this view, such as Kendall<sup>2</sup> and Marsh<sup>3</sup>, regarded the succession as much more complex. T. I. Pocock<sup>4</sup> considered that a Lower Boulder Clay ice sheet found its limit to the north-west of the Warwickshire Avon, that the Middle Sands were laid down as outwash deposits during the retreat of this ice sheet, and that the Upper Boulder Clay represented a re-advance to a moraine stretching from Market Drayton to Ellesmere. Poole and Whiteman<sup>5</sup>, however, considered this moraine to be a Middle Sands moraine deposited during the retreat of the Lower Boulder Clay ice sheet, and afterwards overridden by the Upper Boulder Clay ice sheet which terminated in the Birmingham area.

It is now clear that in the northern part of the Cheshire-Shropshire Basin the sequence is often very complex. Sections quoted by McQuillin<sup>6</sup> between Ashley and Wrenbury, and further north near Aldford, show up to five clay bands separated by sands and gravels. Pocock<sup>4</sup>, in the Macclesfield area, found three boulder clays beneath Middle Sands, while examination of borehole data together with detailed mapping, between Bar Hill and Wrexham, shows sequences similar to those found by McQuillin. Many sections show Poole and Whiteman's<sup>5</sup> conception of Pocock's<sup>4</sup> Market Drayton—Ellesmere moraine as a Middle Sands moraine overlain by Upper Boulder Clay to be a considerable oversimplification.

The succession exposed in the sand quarries of the Chelford area has been described by Simpson and West<sup>7</sup> as a type example of the tripartite sequence. A detailed investigation of the quarry sections and borehole records reveals the situation shown in Fig. 1.

Data derived from trial borings show no evidence of a Lower Boulder Clay lying beneath the sands. A Boulder Clay-Sand Complex, consisting predominantly of lens-shaped masses of tough red-brown till in intimate association with red-yellow sands, overlies a series of strongly cross-stratified, grey-yellow, poorly sorted pebbly sands containing abundant coal fragments, the cross-bedding indicating a northerly derivation. These deposits lie unconformably on sands which are very distinctive, and for which the authors would suggest the term 'Chelford

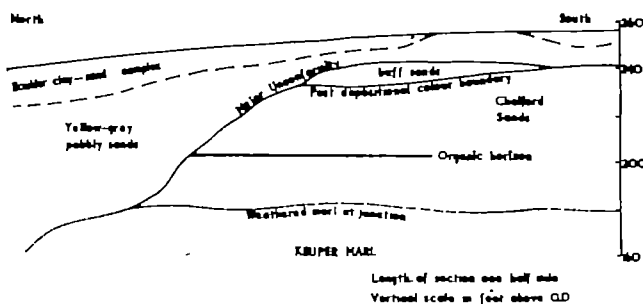


Fig. 1. Schematic diagram showing the sequence of drift deposits in the Chelford area.

Sands'. These Chelford Sands are characterized by a high degree of sorting (mechanical analyses on samples collected from single cross-strata show 95 per cent of the sample to lie between the 0.4-mm and 0.1-mm size grades) and high quartz content (87.4 per cent silica on chemical analysis). Low angle cross-stratification (up to 15°), tabular planar sets defined by plane beds, and minor distributory channels, indicate current flow from a source between north-east and south-east. Within the sands are many intraformational frost cracks up to 8 ft. in depth, and occasional dreikanter. The evidence suggests a depositional environment of low angle alluvial fans subject to occasional sheet flooding, in a cold arid climate. Locally the uppermost 10 ft. of the Chelford Sands are a buff colour due to iron staining; but this is progressively cut out by the unconformity. Several lens-shaped organic beds occur in the Chelford Sands, flat-lying tree trunks within them having a roughly north-east-south-west orientation. These beds, dated at 57,000 years B.P., give an upper age limit to the overlying glacial complex.

The examples quoted above, together with many other such complex sequences now known from the Cheshire-Shropshire Basin, throw doubt on the validity of the terms Upper Boulder Clay, Middle Sands, and Lower Boulder Clay as meaningful lithostratigraphic units. Boulder clays lying at surface on sands have in the past been automatically referred to the Upper Boulder Clay although, as McQuillin's<sup>6</sup> sections show, they may not be correlative. Similarly, the Chelford sections show the yellow-grey sands to be stratigraphically different from the underlying Chelford Sands, although both have been referred to as Middle Sands.

This often complex and thick sequence appears, however, to have a southerly limit in a ridge of high ground stretching in two arcs from Bar Hill to Whitechurch and Wrexham. Detailed mapping along the line of this ridge shows it to be a complex end-moraine.

It rises up to 150 ft. above the level of the surrounding country and is as much as 2 miles wide. At the eastern end, between Bar Hill and Adderley, large numbers of minor ridges are superimposed on the moraine and aligned in a north-east-south-west direction. These smaller ridges are often cut through, or flanked by, deep channels, and enclosed peat-filled kettles are prominent. Immediately east of Whitechurch the moraine is a very sharp ridge, and in the Ellesmere district it forms the southern limit to a broad area of hummocky 'stagnation' topography. The moraine is often broken through by valleys which are presumably melt water channels derived from the retreating ice, the form of the moraine indicating that the ice-front was bi-lobate in form, the ice being divided by the Mid-Cheshire Ridge (Fig. 2). Belts of hummocky terrain, running north-south along the eastern and western margins of the Basin, are continuous with the end-moraine, and it seems that these, together with deposits such as the Wrexham Delta Terrace<sup>6</sup>, were marginal or sub-marginal accumulations built up at an ice-front which lapped against the flanking hills. Indeed the deposits at Llay, described by Peake<sup>8</sup> as laid down by a further re-advance along the Welsh borders, appear to be an integral part of this marginal complex.

Many of the deposits lying north of the end-moraine appear to retain their original depositional forms. This is best seen in the hummocky 'stagnation' topography of Ellesmere, Padeswood, Wrinnehill and other areas. The present-day surface topography seems, over much of the area, to be similar to that which must have existed immediately subsequent to deglaciation, the different surface deposits in different places being due to the varying conditions of deposition within a single ice-sheet.

The boulder clays and related deposits to the south of the moraine are generally thinner and considerably more eroded than those to the north, often showing evidence of considerable solifluxion; depositional forms are rare. A similar contrast between the drifts north and south of the

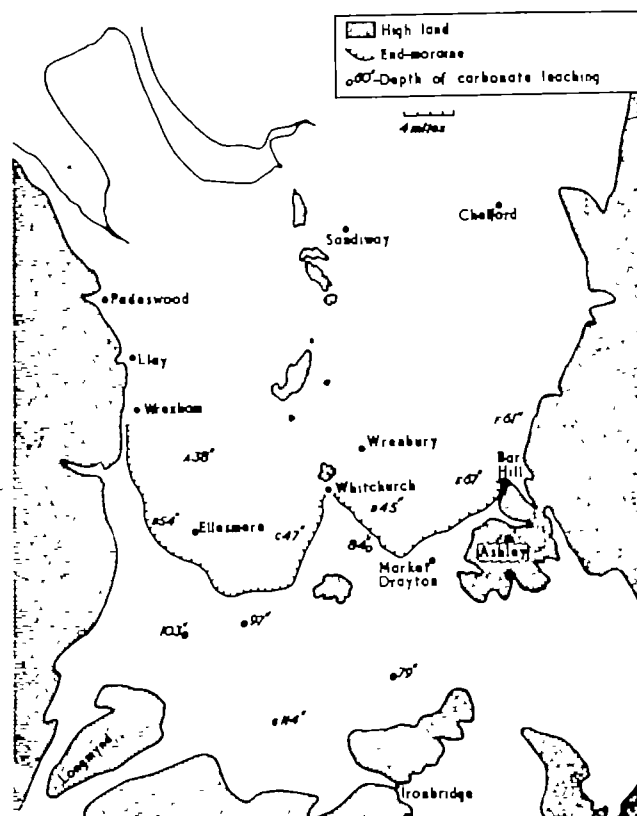


Fig. 2. The Bar Hill-Whitechurch-Wrexham moraine figures at localities A, B, C, D, E, F and G represent the mean of several leaching measurements taken from that general area. Figures marked O represent measurements taken from one locality only.

A, 4 results; B, 6 results; C, 11 results; D, 12 results; E, 7 results; F, 14 results; G, 5 results.

Brandenburg-Leenow moraines of the North European Plain is the basis of the Weichselian/Warthe distinction<sup>10</sup>.

The boulder clays which cap the end-moraine often appear to merge with those to the south. It may, however, be possible to distinguish between them by considering the depth of leaching in these deposits, which is a function of several variables—permeability, carbonate content, topography, climate and time. Depths of carbonate leaching in boulder clays of similar lithology at localities of similar topographic position, both north and south of the Bar Hill-Whitechurch-Wrexham moraine, were calculated, a correction for variation in original carbonate content being made using the equation<sup>11</sup>:

$$k \text{ (original thickness)} = \frac{100 \times d \text{ (measured thickness of leached profile)}}{n \text{ (\% of non-carbonate in unleached specimen)}}$$

The results are marked on the map (Fig. 2). 54 measurements north of the moraine gave a mean depth of leaching of 53 in. As yet only a few measurements have been made south of the moraine but the depth of leaching appears to be significantly different, the mean of 9 measurements being 104 in. This suggests that the surficial drifts north and south of the moraine do not belong to the same drift sheet, but that the drifts to the south are considerably older than those to the north. It would, therefore, appear that the ice-sheet which was responsible for the deposition of an Upper Boulder Clay in the northern part of the area advanced to a line extending from Bar Hill to Whitechurch and Wrexham, in which position an end-moraine accumulated. The ice then retreated and did not afterwards override this moraine.

Although morphologically the Bar Hill-Whitechurch-Wrexham advance is a well-defined unit, it is extremely difficult to determine the vertical extent of the deposits belonging to it. The apparent complex sequence of

deposits in this area invalidates the facile assumption that boulder clay = glaciation, and that sands = retreat deposits. Indeed, where the tripartite succession appears to hold, as in the Stockport area<sup>10</sup>, there is no evidence of a break or a weathering horizon in the sequence to indicate a time gap between two major glaciations, and the fact that Upper Boulder Clay and Lower Boulder Clay are lithologically identical is an argument against a two-glaciation explanation. Moreover, Upper Boulder Clay is often seen to overlie sand in which there is no trace of disturbance. Thus, as yet there is no definite evidence of deposits of a glacial advance above the horizon of the Chelford Sands and below the deposits which appear to belong to the Bar Hill-Whitchurch-Wrexham advance, which it is now possible to date.

Abundant molluscan shells, presumably derived from the Irish Sea, are contained within the Cheshire drift sequence. Shells obtained from coarse sands at the base of a till/sand complex (which has been referred to the Upper Boulder Clay), exposed in a pit at Sandiway near Northwich, have been dated by the radiocarbon method (Isotopes Inc., New Jersey). An assay on two specimens of *Nucella lapillus* (L.) gave an age of 28,000 years B.P. +1800, -1500. The shells were complete, relatively unleached and each about 20 g in weight; much of the outer shell was leached away by acid treatment prior to the assay, to minimize the effect of contamination.

On the latest data available from Europe<sup>11</sup>, there appears to have been a widespread climatic amelioration between 30,000 and 25,000 years B.P., known in Europe

as the Paudorf interstadial. This climatic amelioration was followed, in Europe and North America, by the maximum of the last or Weichselian glaciation. The dated molluscs, whatever their means of derivation, underlie and therefore pre-date a till/sand complex which has been correlated with the Upper Boulder Clay which terminates at the Bar Hill-Whitchurch-Wrexham moraine. Zone II organic deposits have been found lying upon this till sheet at Hatchmere (S.J. 555720) (J. W. Franks, personal communication). The Bar Hill-Whitchurch-Wrexham advance is, therefore, younger than 28,000 years B.P., and older than 10,000 years B.P. The most extensive glacial phase which falls between these two dates is the Late Weichselian glaciation which reached a maximum along the Brandenburg-Leanow moraines of North Europe at about 20,000 years B.P., and it is to this stage that the Bar Hill-Whitchurch-Wrexham advance must belong.

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## LITHOFACIES AND CORRELATION WITHIN THE LOWER TORRIDONIAN

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A STRIKING association of sedimentary units (Table 1) within the lower Torridonian Diabaig Group can be traced from Stoer to Rubha Réidh, Wester Ross (Figs. 2 and 3). Correlation is facilitated by three marker horizons which persist for up to 50 km: a tilloid, a limestone horizon, and an extraordinary sandstone-boulder conglomerate. In this report units 1-8 correspond to those established by the Geological Survey in the Diabaig Group at Stoer<sup>1</sup>.

At Coigach, Gracie<sup>2</sup>, mapping the area of Cnoc Mor an Rudha Bhig, sub-divided the lower Torridonian as follows:

- V Enard Bay 'shoreline'
  - (a) coarse-grained facies
  - (b) fine-grained facies
- IV sedimentary breccio-conglomerate facies
- III laminated sandstone facies
- II Loch na Seana-Chreig 'shoreline'
  - (a) coarse-grained facies
  - (b) structureless sandstone facies
  - (c) fine-grained facies
- I 'fanglomerate' facies

He recognized an unconformity beneath the sedimentary breccio-conglomerate facies and the 'probably contemporaneous' Enard Bay 'shoreline' (NO 028147, NO 033148). He also suggested a second unconformity at the base of the laminated sandstone facies (NO 027147).

Throughout the area (Fig. 2) the lower Torridonian unconformably overlies the Lewisian Gneiss. The succession (Table 1) begins with breccias and conglomerates (dominated by fragments of locally derived gneiss) and associated well sorted flaggy to laminated sandstones

(units 1-3). Their restricted occurrence, lithological variation, thickness and preservation are controlled largely by the pre-Torridonian gneiss topography. Sandstones of unit 4 are well exposed at Stoer (NO 034286 to 038283 and 037279 to 036273) and Badluhrach (NG 983957). Elsewhere they are of local occurrence or absent. The coarser sandstones display penecontemporaneous deformation structures and trough cross-beds. At

Table 1. UNITS WITHIN THE LOWER TORRIDONIAN

Unit	Occurrence	Approx. max. thck. (metres)
10. Flaggy to laminated sandstone with occasional shales.	Rubha Réidh	60 (Rubha Réidh)
9. Sandstone-boulder conglomerate	Coigach (V, a, b)* Coigach (IV)*, Badluhrach, Rubha Réidh, traces at Coigach, Loch Kerneary?	70 (Rubha Réidh)
8. Well laminated, cross-bedded sandstones	Stoer, Coigach (III)*, Coigach, Badluhrach, Rubha Réidh	486 (Coigach)
7. Red mudstones	Stoer, Coigach (II)*	50 (Stoer)
6. Sandstones and shales with thin beds of limestone	Stoer, Coigach (II)*	50 (Stoer)
5. Tilloid	Stoer, Coigach (II)*, Coigach, Badluhrach	43 (Coigach)
4. Coarse, red, occasionally contorted sandstones with interbedded mudstones	Stoer, Coigach (II)*, Badluhrach	300 (Stoer)
3. Gneiss conglomerate and breccia	Well developed in Stoer, occur locally from Coigach (I)* to Rubha Réidh	200 (Stoer)
2. Red, flaggy sandstone		
1. Gneiss conglomerate and breccia		

\* Refers to Gracie's divisions at Cnoc Mor an Rudha Bhig, Coigach<sup>2</sup>.



Fig. 1. Lower portion of the sandstone-boulder conglomerate at Badluchrach. Height of cliff 14 m

Badluchrach the troughs exceed 15 m in breadth. Sun-cracks and rippled surfaces occur in the interbedded red mudstones.

The first unit of correlative significance, the tilloid (as defined by Crowell<sup>12</sup>, cf. Pettijohn<sup>13</sup>), has been described in the Northwest Highlands memoir<sup>1</sup> as "a hard rib of sandy, calcareous mudstone with fragments of igneous rock" at Stoer; as "a breccia made up of igneous fragments, principally of epidiorite, at Badluchrach; and as "a dark purple grit deeply stained with ferric oxide, and composed of angular fragments of felsite and grains of quartz and feldspar" at Scoraig. At Coigach (NO 082146) the tilloid forms the lower portion of Gracie's IIB division.

Throughout the area the tilloid is characterized by abundant angular fragments of green, fine-grained igneous rocks, ranging up to 5 cm in maximum dimension, set in a subordinate red, muddy to sandy matrix. Fragments derived from the underlying sandstones and siltstones occur throughout, together with rare pebbles or cobbles of Lewisian rocks. The sharp, clear-cut basal contact is an erosional surface, broadly planar but with occasional minor relief (up to 2.5 m). The thickness of the tilloid at Stoer is 25 m, at Coigach 43 m and at Badluchrach approximately 20 m.

A remarkable feature of the tilloid at Stoer is the way in which parts of it laterally intrude and contort the strata of unit 4. Blocks of sandstone up to 2 m long, together with smaller fragments of mudstone, have been detached, transported and further deformed within the tilloid. Available evidence suggests that the tilloid is a volcanic mudflow with breccia-beds, and that it passes laterally into volcanic sandstones. Comparable deposits described by Crandell<sup>2</sup> are attributed to mudflows of volcanic origin.

Well-rounded 'sedimentary pebbles' up to 1 cm across, with strongly hematitized rims, were described by Gracie in his IIB division at Coigach. These have been found in the upper part of the tilloid at Stoer, embedded in a sandy matrix. They may be accretionary lapilli, or pisolites derived from a relict soil profile of uncertain location.

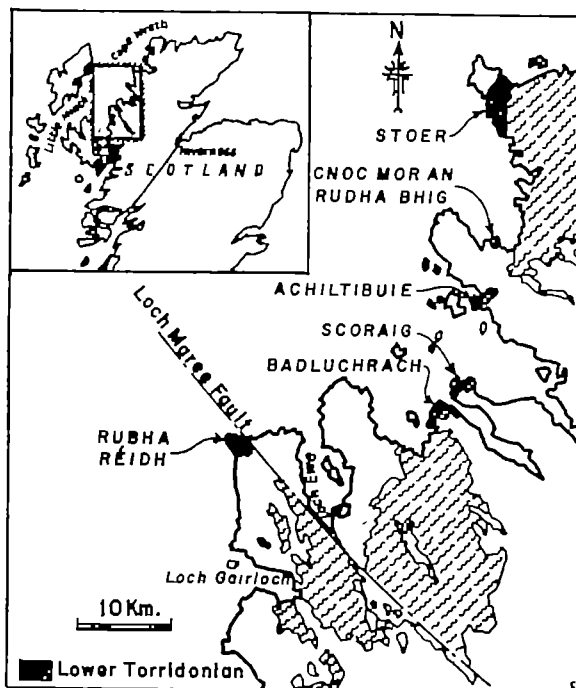


Fig. 2. Location of lower Torridonian outcrops referred to in the text

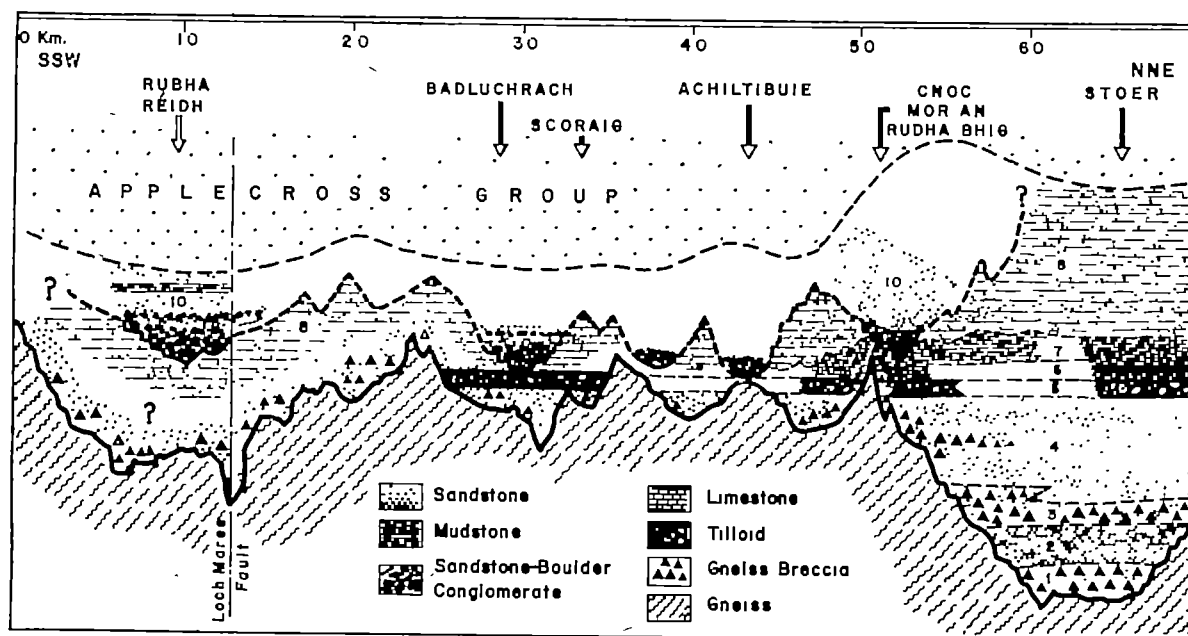


Fig. 3. Diagrammatic representation of present knowledge of distribution and relationship of units within the lower Torridonian. Vertical exaggeration  $\times 20$ . Loch Maree fault compensated for

Pisoliths of similar type commonly develop in present-day soils under alternating oxidizing and reducing conditions<sup>4</sup> and have been noted in piedmont soils developed from ferromagnesian-rich rocks<sup>5</sup>. This suggests an alternating wet-dry, warm climate at the time of deposition.

The limestone of unit 6 commonly comprises small (< 2 cm), grey, calcareous lentils and disrupted calcareous laminae within a red, silty matrix. At Coigach the limestone occurs in red, sandy mudstones (= the lower member of Gracie's IIc division) and laps on to the Lewisian-Torridonian unconformity, encrusting gneiss boulders. Microscopic textures suggesting algal activity have been found in the encrusting limestone by Miss E. R. Jamieson and these will be described elsewhere. At Stoer the base of unit 6 (lower 6 m) is mainly sandy, passing upwards into interbedded red sands and silts which form most of the unit. Two limestone beds totalling 104 cm in thickness occur approximately 12 m above the base, with grey siltstones and shales between, and for several metres above them. Graded beds occur at the top of the grey interval. Red mudstones interbedded with flaggy sandstones form the top of the unit, the flags showing sun cracks, ripple marks and pseudonodules. Above comes the uninterrupted sequence of red mudstones forming unit 7.

Well-developed thin stratification and frequent well-laminated cross-beds typify unit 8 wherever found. This new sedimentary style is initiated by a sharp erosional base at Stoer and Coigach. Lamination is regular and commonly planar or gently arcuate. Comparison with recent deposits<sup>6-8</sup> reveals a similarity to the lamination of intertidal and near-shore sandy sediments. In principle, however, a fluvial origin is also possible. Large-scale cross-bedding predominates in unit 8, with tabular and wedge-shaped sets of high or low dipping cross-laminae. Sets occur singly or in groups. The thickest known is 5.6 m (ref. 2); elsewhere maximum thicknesses are usually 1-2 m. Transport at Coigach appears to be from the east<sup>9</sup>, and at Badluhrach and Rubha Réidh from the north-east. These directions are contrary to those in the stratigraphically higher Applecross Group, which has been transported mainly from the west and north-west. Symmetrical and asymmetrical ripple marks are abundant at Rubha Réidh, their crests oriented predominantly north-east-south-west. These occur on beds between the cross-bedded units and are associated with sun cracks and some rainprints. At Stoer ripple marks are less frequent.

The sandstone-boulder conglomerate and spectacular unconformity beneath record a significant event in Torridonian depositional history hitherto recognized only in Coigach<sup>3</sup>. The Geological Survey reported the Badluhrach and Rubha Réidh unconformities, but assigned both conglomerates to the Trias<sup>10</sup>. The laminated sandstone (unit 8) and lower units are cut by a highly irregular steep erosion surface overlain by the conglomerate. At Rubha Réidh (NG 748922) a stepped contact cuts through 28 m of laminated sandstone in a horizontal distance of less than 40 m. With an average slope of 35° indented by steep steps with up to 5 m vertical relief, this exposure gives a convincing impression of an irregular cliffed shoreline buried beneath fallen debris of all shapes and sizes.

The lower unstratified portion of the conglomerate (up to 34 m thick) comprises a chaotic assemblage of sub-rounded to angular sandstone pebbles, cobbles, boulders and blocks (Fig. 1). The largest block measured 11 m × 4 m (ref. 2). The matrix (usually < 10 per cent of the rock by volume) consists of medium to coarse structureless sand enclosing occasional small fragments of vein quartz and granitic gneiss. No evidence was found of the 'Cambrian' pebbles previously reported at Rubha Réidh<sup>3</sup>. Pebbles of pale grey quartzite are found at Badluhrach but are not diagnostic of the Cambrian.

The sandstone fragments in the conglomerate are identical with the laminated sandstones beneath the unconformity. At Rubha Réidh isolated quartz veins and tension gashes are: (1) confined to the cobbles and boulders; (2) frequently oriented nearly perpendicular to the internal bedding. This implies sufficient time for the development of quartz veins during the interval between lithification of the laminated sandstone and subsequent erosion resulting in the unconformity. Predominantly vertical orientation of bedding in the large blocks and the quartz veining suggest that the conglomerate may have been derived from cliffs with strong vertical joints. Gracie<sup>3</sup> interpreted the conglomerate as a cliff rockslide deposit 'probably contemporaneous' with the sediments of his Enard Bay 'shoreline'.

In the upper portion of the conglomerate the fragments gradually decrease in size, a crude stratification appears, and the conglomerate becomes interbedded with sands progressively dominating the upper part of the sequence. These sands are well laminated, well sorted and display 'black bands' (predominantly iron ore minerals with traces of detrital epidote). The bands occur in zones 40-50 cm thick with individual concentrations ranging from 1 mm to 15 cm thick. The 15-cm bed at Rubha Réidh is exceptional and can be traced for more than 100 m. The black band zones occasionally show cross-beds and sag-and-drop structures (cf. Stewart<sup>11</sup>).

The sediments above the conglomerate are exposed only at Coigach and Rubha Réidh. At Coigach the conglomerate is transgressed by Enard Bay 'shoreline' sediments, separated from Applecross sandstone by an erosion surface. At Rubha Réidh the interbedded black band zones give way upwards to a considerable thickness of medium- to thin-bedded<sup>12</sup>, red (occasionally green) sandstones with cross-bedding, ripple marks and occasional thin concentrations of small (< 3 cm), well-rounded sandstone pebbles. These are overlain by 6-8 m of grey-green sandstones and sandy shales with large sun cracks and 'mud-with-lenticles lithology'<sup>13</sup>. Upwards the shales grade into red silts and sands by alternation. Coarse, pebbly, poorly sorted Applecross sandstones overlie the entire sequence with an irregular erosive contact.

The angular unconformity between the laminated sandstone and the Applecross east of Culkein Bay, Stoer (NG 042330), is marked by a sandstone-boulder conglomerate up to 1.3 m thick. Sub-rounded boulders of laminated sandstone (up to 1.2 m long) are set in a matrix of pebbly Applecross arkose<sup>14</sup>. Further work is necessary to determine the relationship of this conglomerate to the sandstone-boulder conglomerates of unit 9 to the south.

Definite changes in type of sedimentation at the bases of units 5, 8 and 9 separate the lower Torridonian into four major phases of deposition. The unconformity beneath the last phase (unit 9) may indicate a considerable lapse of time.

The assistance of Dr. A. J. Gracie and other colleagues in the Sedimentology Research Laboratory is gratefully acknowledged.

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# PRIMARY SEDIMENTARY STRUCTURES FROM THE SILURIAN AND LOWER DEVONIAN OF THE OSLO REGION, NORWAY

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DURING a detailed investigation of the Silurian and Lower Devonian of the central and southern Oslo region, Norway, with special emphasis on the Ringerike area, various primary sedimentary structures have been found, most of which have not been described previously from this region. This article records their character and uses the evidence which they provide to deduce palaeocurrent trends and general conditions of deposition. A fuller account will be submitted for publication in the *Norsk Geologisk Tidsskrift*. For the stratigraphy and facies variations of the region, see Kjørstam<sup>1,2</sup> and Henningsmoen<sup>3</sup>.

In the Silurian, 'stages' 6, 7, 8 and 9 (mainly marine), four lithologies predominate:

(1) *Calcareous siltstones*, especially in 'stages' 6c (Llandoveryan) and 8a (Wenlockian), showing the greatest variety of structures. These include ripple mark, ripple bedding, ripple-drift lamination, small-scale cross-lamination, convolution, ball-and-pillow structure, slumps, rapid thickness variations, channelling, groove and striation casts, bounce, prod and roll casts, current-oriented fossils, load casting, diapirism, and trace fossils. Grading is generally absent but laminated tops occur. There is one penecontemporaneous discontinuity (erosion) surface.

(2) *Limestones*, especially in 'stages' 7a and 7b (Llandoveryan), 8c (Wenlockian) and 9 (Ludlovian), often shelly, showing thickness variations, channelling, cross-bedding, slumping, several types of nodular development, 'nodule lineation', small-scale reef structures, algal surrounds to other fossils, partially filled brachiopods acting as 'geological spirit levels' and fossil orientation including elliptical stromatoporoids.

(3) *Shales*, present in 'stages' 6, 7, 8 and 9, showing distortion of bedding around more-resistant objects such as limestone nodules, compression below groove casts, and flame structures where the overlying beds are load casted. They show mud cracks as early as 8b (ref. 4) and again at several horizons in 'stage' 9, with reddening. There are no macroscopic primary structures which are of use in palaeocurrent analysis.

(4) *Calcareous sandstones*, present in 6b and 8b, massive, often cross-bedded and slumped, and sometimes with ball-and-pillow structure.

In the highest Silurian and lowest Devonian, 'stage' 10 (mainly non-marine 'red-beds'), a further three lithologies are found:

(5) *Conglomerates*, with clay or limestone pebbles in a sandy matrix, often showing trough cross-bedding.

(6) *Sandstones*, micaceous, massive-bedded to thinly laminated, with ripple mark, ripple-drift lamination, tabular and trough cross-bedding, primary current lineation, load casting, ball-and-pillow structure, slumps, channelling, trace fossils (especially eurypterid tracks), flute casts (two examples only), a single rill mark and several problematical top-surface and sole markings.

(7) *Shales and mudstones* (relatively poorly developed except at the base) showing mud cracks (a few with diapiric structures<sup>4</sup>) and flame structures underneath load casts.

Palaeocurrent indicators are well developed at certain horizons and several hundred azimuths have been measured. The different types of directional structures give consistent results for the marine Silurian: the non-marine 'red-beds' of the Lower Devonian show, as might be expected, a different pattern of sediment distribution.

Most abundant in the Silurian are groove casts, widespread areally over a limited thickness of strata in 6c, between 7 and 18 m below the first prominent limestones, rich in *Pentamerus borealis* in 7a. Throughout the Ringerike area, shales a few cm thick alternate with calcareous siltstones of similar thickness, and on the soles of some of the siltstones are well-developed groove casts about 8 cm wide and 4 cm deep. They are generally fairly symmetrical in cross-section (Fig. 1) but some are slightly deformed by load casting. Superimposed on many of the groove casts, and having the same bearing, are striation casts, bounce casts and prod casts. Many show trace fossils as convex hyporeliefs<sup>4</sup>. Current-oriented slender polyzoa, tentaculites and accumulations of brachiopods, notably *Plagiorhyncha* (*Camartoechia*) *decomplicata*, have been found in the bottoms of the grooves, which are usually filled with finely laminated calcareous siltstones with inclined laminae, although shelly limestones are also found. Occasionally, disconnected 'rods' of siltstone are seen resting on, and covered by, shale, as at the cliff sections at Stein and Ultvedtåsen. This type may be referred to as 'separated groove casts' by analogy with the 'separated ripples' of Pettijohn and Potter (Plate 90)<sup>5</sup>. They are due to an insufficiency of silty filling to produce a connected bed. Burrowing organisms were active but bioturbation is not well developed. The trends of 249 grooves in 6c at Ringerike give a very pronounced maximum between east-north-east and east or west-south-west and west (arithmetic mean of bearings is 083° or 263°, standard deviation  $\pm 18^\circ$ ).

Similar large groove casts with associated striation casts at the same horizon may be seen along the roadside near Avles station on the Oslo-Kolsås line. On the south-west shore of the island of Mahøya in Oslofjord, 35 km south-east of Ringerike, normal and separated groove casts are again displayed (Fig. 2). Within them, oriented brachiopods and polyzoa occur. Here, the groove casts are slightly earlier (6b) than those just described, and the facies is more shaly, occupying part of the Oslo-Scania depression. In the Skien area, grooves similar to those at Ringerike occur. In these three more southerly areas, current trends are approximately north-east or south-west.

Other palaeocurrent indicators, much less common, include very small-scale cross-lamination and ripple-drift lamination, either in cosets rarely exceeding a few



Fig. 1. Normal groove casts in cross-section 6c, Harum, Ringerike. (Hammer handle is 8 cm wide)





Fig. 2. Separated groove casts in cross-section. 6b, Malmøya, Oslofjord. (Hammer handle is 30 cm long. Beds rotated to horizontal for comparison with Fig. 1)

centimetres in thickness, or as small 'dunelets' on the tops of calcareous siltstone beds. Kuenen<sup>8,9</sup> first commented on the presence of cross-laminated tops to graded greywackes and explained them as having been formed during the declining stage of a single turbidity current. Bouma<sup>10</sup> developed this idea, placing them in his "interval of current ripple lamination, *Tc*". Instead of greywackes, turbidity currents can lay down calcareous siltstones with similar structures, as in Cummins's Montgomery Trough<sup>11,12</sup>, and it is possible that the Oslo region Llandovery siltstones could be similar turbidites. A point of interest is that the dip of the foresets is not in the direction of the currents which produced the grooves, but approximately at right angles to them, directed southwards, a feature noted by Prentice<sup>13,14</sup> and Kelling<sup>15</sup>. Kelling suggested that the nose of a turbidity current sets up a lateral surge-wave which may be reflected back from the marginal slopes of a trough, the time-lag being such that transverse depositional structures are produced at the top of the turbidite. Evidence for such marginal slopes in the Ringerike area is given by measurements of 143 slump axes. The mean trend is 80°, standard deviation  $\pm 28^\circ$ , indicating northward or southward inclined palaeoslopes, again at right angles to the eroding current directions. It must be emphasized that while the sediments of 6b and 6c show some features characteristic of turbidites, the general absence of grading, rarity of convolution, lack of flute casts, the well-preserved shelly fossils and evident shallow-water conditions (described later) makes them far from typical. Perhaps Kuenen's<sup>8</sup> "diving flash floods" were the cause, initiated on barren lands without land vegetation. These flash floods, on entering water, would become "turbidites of zero depth"<sup>16</sup>, picking up shells from the near-shore zone, and these turbidites, being heavily charged with sediment, would tend to produce groove casts rather than flutes, filled with non-graded beds as there would be insufficient time for velocity-differentiation of the sediments<sup>17</sup>.

At higher horizons, directional structures are less common and groove casts are rare. At Ringerike, in 7a and 7b, elliptical stromatoporoids and 'nodule lineation' indicate currents from just north of east or south of west. In the silty beds of 8a there are occasional striation, prod and bounce casts and some ripple-drift lamination. On the west coast of the island of Store, Ringerike, a well-defined roll cast, made by a longitudinally ribbed orthoconic nautiloid, is associated with other sole markings, indicating movement towards the east. In 8a, there are alternating limestones and shales with large numbers of the lengthy coral *Semaiophyllum*. One hundred and eighty-seven bearings on long axes were measured and fairly good current orientation is indicated (mean trend 83° or 283°, standard deviation  $\pm 33^\circ$ ).

For 'stages' 6, 7 and 8, the results of palaeocurrent measurements give a consistent pattern of water movement from west-south-west or east-north-east at Ringerike, while farther south, on much less data, south-west or north-east directions are suggested. As on other evidence a source area (the Telemark Land which extends northwards into the Jotunheim) is postulated to the west, north-west and north of the Oslo region, these currents would more probably have been moving sediment in a west to east sense, rather than the reverse. A remarkable early paper by Küster<sup>18</sup>, who anticipated the use of palinspastic maps, shows troughs and uplifted areas along north-east and south-west directions during 'stage' 5 (high Ordovician) in the Asker area, south-west of Oslo. In this southern part of the region, but not in the centre, later folding was along the same axes. In the north of the Oslo region (Hadeland and Feiring), Major<sup>19</sup> measured the cross-bedding in the more sandy facies of the Llandovery and deduced derivation from the west and north. Henningsmoen<sup>2</sup> reports considerable transport of terrigenous material from north and north-west in lower Wenlock times. Thus, currents seem to have been converging on an area just north-east of Oslo.

The non-directional structures in 'stages' 6, 7 and 8 are common in many different environments and are of little help in indicating the conditions of deposition, except for: (1) the shelly limestones of 7a and 7b with stromatoporoids and corals, many on end or overturned, indicating clear water with considerable currents, and the larger reef structures of 8c, suggesting very shallow, well-aerated water; (2) rapid thickness variations and the formation of irregular, steep-sided channels in the limestones and calcareous siltstones especially in 6c over a wide area, suggesting rapid lithification and erosion by fast-moving currents; (3) also in 6c, the occurrence at Limåstangen of a uniquely eroded top to a calcareous siltstone which is standing above the general level of the bed at this locality (Fig. 3). The clint-like erosion surface continues below the overlying Llandovery shale, showing that it was formed immediately after deposition: this particular structure suggests local brief emergence above sea-level.

The evidence from both kinds of primary structures thus points to fairly shallow or very shallow water with vigorous normal currents and, at times, atypical turbidity currents flowing over most of the Oslo region in the Llandovery and Wenlockian, with a somewhat deeper trough over Oslo itself. The term 'miogeosyncline', used by some authors, is best avoided: the description 'shelf conditions' is preferable and draws attention to some similarities with the British Silurian shelf facies.

Twice there were departures from the normal conditions of sedimentation at Ringerike: during 7c (Upper Llan-



Fig. 3. Chert-like erosion surface on bed of calcareous siltstone. 6c, Lunde, Ringerike. (10 cm of the scale is visible)

doverian) marine mudstones of greyish red colour (10E 4/2 on the *Rock Color Chart*) were laid down, the pigment possibly derived from erosion of lateritic surfaces of basalts, and soon afterwards, several bentonites were deposited, as in Jämtland, Västergötland, Gotland and Scania, again testifying to widespread volcanicity towards the end of the Llandoveryan.

'Stage' 9 (top Wenlockian-Ludlovian) is more calcareous and suggests shallower conditions of deposition than the preceding 'stages' and there are few directional structures: measurements of the orientation of specimens of *Armenoceras* on adjacent bedding planes near Skjervold farm, Ringerike, give inconclusive results, and it would appear that, as in Britain at this time, the depositional trough was filling up and currents were more variable in direction. Small reefs with *Favosites* and stromatoporoids, *Amplexopora* meadows and other polyzoa surrounded with algal growths bear witness to shallow, lime-rich waters. From 9c onwards, occasional loss of typical marine conditions is shown by thinly bedded green shales with a fauna of eurypterids, phyllocarids, ostracoderms, small brachiopods, ostracods and the alga, *Chaetocladus*<sup>2</sup>. Several horizons show reddening and mud cracks, indicating temporary exposure to the atmosphere, a prelude to the succeeding 'stage' 10.

In the thick sequence of non-marine 'red beds' (the Ringerike Sandstone, 'stage' 10) the best-developed and commonest directional structure is cross-bedding. Less common is ripple-drift lamination (type 1 of Walker<sup>11</sup>), ripple mark and primary current lineation, a depositional structure recently discussed by Allen<sup>12</sup>. The dip directions

of 111 foreset beds, including some ripple-drift foresets, have been measured and the mean is 169°, standard deviation  $\pm 52^\circ$ , indicating sediments derived broadly from the north-north-west. Fifty-two slump fold axes give a mean trend of 72°, standard deviation  $\pm 27^\circ$ , suggesting a palaeoslope directed towards the south-south-east. This is consistent with a marked thinning (from 1,000 m to 800 m) in the Skien area to the south<sup>4</sup>. The clay and shelly limestone pebbles in the conglomerates indicate derivation most probably from the Silurian which lies to the north, while a more distant source in the same direction, namely, the uplifted Jotun nappes of Central Norway, is suggested by the presence in the sandstones of fresh anti-perthitic feldspars characteristic of the jotunites. Ripple mark and primary current lineation measurements are rather variable and no general pattern emerges.

The non-directional primary structures, notably mud cracks and animal tracks, indicate the periodic desiccation of bodies of water in which fish and eurypterids flourished. There is a large collection of tracks in the Palaeontological Museum in Oslo, including the spectacular tracks of the giant *Microporus* and the delicate markings made by very small eurypterids.

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## HYDRATION OF PORTLAND CEMENT

IT was suggested earlier that the rate of hydration of individual minerals in Portland cement clinker depends on the position of Fermi-level in the individual minerals<sup>1</sup>. Furthermore, it has been shown that the reaction between Portland cement clinker and water is a semiconductor surface reaction, probably 'n' type reaction on 'p' type semiconductor<sup>2</sup>. Hydration of clinker minerals is, therefore, a chemisorption process occurring on the surface of clinker crystals (which may be semiconductors). Thus hydration (and similar reactions), in which Portland cement clinker takes part, should be controlled by the instantaneous position of Fermi-level in the minerals (see also ref. 3). It will be shown in this article that, as in the case of lime<sup>4</sup>, the lower the temperature at which thermionic emission starts from clinker minerals, the higher is its reactivity with water. This means that the

higher the position of the Fermi-level in clinker crystals the greater is the reactivity of the clinker with water. Thus the position of Fermi-level in clinker minerals controls the rate of hydration of Portland cement. This is in agreement with the theories put forward earlier<sup>1</sup>.

Experiments were carried out with the same cement clinker used in earlier experiments<sup>5</sup>. The clinker was powdered and the fraction passing a B.S. 300 mesh sieve was used once it had been freed of lime by repeated washing with absolute alcohol. Specimens used were (a) clinker, (b) clinker heated at 1,000° C for 4 h, (c) clinker heated with admixture of 1 per cent by weight, individually, of the following oxides: zinc oxide, chromic sesquioxide, oxides of molybdenum and manganese and lime and nickel oxide (the molybdenum oxide was obtained by heating ammonium molybdate, and the manganese

Table 1. GAIN IN WEIGHT DUE TO HYDRATION AND THE TEMPERATURES OF INFLEXION

	Original clinker	Clinker heated alone	Clinker heated with					
			ZnO	Cr <sub>2</sub> O <sub>3</sub>	MoO(↑)	MnO(↑)	CoO	NiO
Gain in weight due to hydra- tion (g/g)	0.0604 0.0668 0.0690 0.00153	0.0628 0.0661 0.0618 0.00140	0.0777 0.0744 0.0755 0.00173	0.0615 0.0613 0.0663 0.00160	0.0509 0.0697 0.0504 0.00130	0.0523 0.0716 0.0504 0.00130	0.0615 0.0737 0.0614 0.00144	0.0692 0.0708 0.0673 0.00143
Anhydrous material (1/T T (°K))	658 714	658 714	578 725	625 714	769 714	769 714	694 714	704 714
Hydrated material (1/T T (°K))	0.00139 719	0.00139 719	0.00138 725	0.00140 714	0.00140 714	0.00140 714	0.00140 714	0.00140 714

oxide by heating manganese acetate). Both heated and unheated materials were prepared in tablet form under pressure and the variation of electrical resistance of these tablets with temperature (in steps of 25° to 950° C) was measured, during heating and cooling, according to a method given elsewhere<sup>1</sup>.

Tablets were hydrated in water vapour for 3 days and then immersed in water for 7 days. Results of hydration have already been published<sup>2</sup> and these are made use of in this article (Table 1). Variation of resistance with temperature for the hydrated tablets, during heating and cooling, was measured in a similar manner.

Because the tablets cracked to different extents on heating and because the springs used to hold the samples quite often failed at high temperature, the resistance values obtained during cooling do not represent true values for the clinkers or of their hydrated products. Hence resistances of both anhydrous and hydrated materials only during heating are considered. In Fig. 1, only resistance-temperature ( $\log_{10} R \sim 10^4/T$ , where  $R$  is in ohms and  $T$  in degrees Kelvin) curves for anhydrous clinker and its hydrated product are shown. The curves for other materials are similar except that some of the kinks in the curves, which are attributable to phase changes, are either absent or suppressed to some degree in the heated samples.

In the resistance-temperature curve of each material there is an inflexion point, at which there is a sudden change in the slope (point A in Fig. 1). This change cannot be attributed to any phase change, as any such change does not occur at the temperatures corresponding to the inflexion point temperatures. The change in the slope of the curve is in the direction in which there is an increase in the electrical conductivity of the material. The temperature corresponding to this inflexion point is taken, on the basis of the arguments given in the earlier article dealing with lime<sup>4</sup>, to be the temperature at which thermionic emission starts from the material. Temperatures corresponding to inflexion points are given in Table 1. The points B and C (Fig. 1) may be due to phase changes

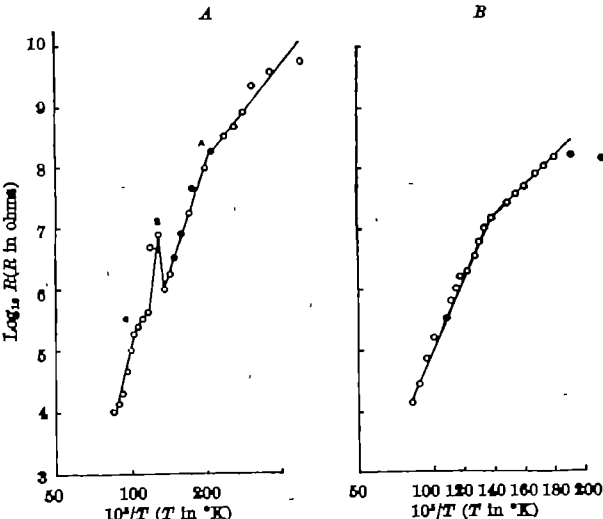


Fig. 1.  $\log_{10} R \sim 10^4/T$  plots (during heating). A and B are the plots for anhydrous and hydrated clinker (untreated) respectively

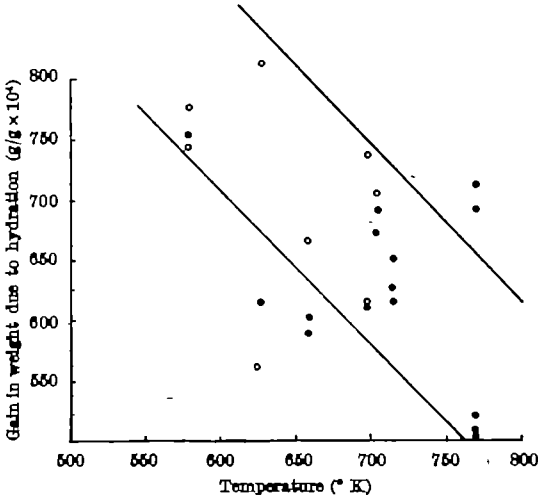


Fig. 2. Gain in weight of clinker samples due to hydration versus corresponding temperature of inflexion

occurring at these temperatures possibly in dicalcium silicate.

It can be seen (Table 1) that the temperatures at which the inflexions occur in the resistance-temperature curves are higher (except for ZnO and Cr<sub>2</sub>O<sub>3</sub>) than in the curve for the original clinker. The higher the inflexion-point temperature, that is, the higher the temperature at which thermionic emission starts, the lower is the Fermi-level. Thus with the clinker minerals (devoid of CaO<sup>1</sup>), the rate of hydration should decrease when heated alone or when heated with oxides of molybdenum, manganese, cobalt and nickel.

The temperature at which the inflexion occurs for the hydrated material (which had only partially hydrated) in all cases remains practically the same. It is higher than the inflexion point temperature obtained for corresponding anhydrous material except for the oxides of molybdenum and manganese. A rise in the inflexion point temperature (the rise in the temperature at which thermionic emission commences) may mean that the hydrated material is more of a 'p' type semiconductor than the anhydrous clinker. In the samples with the oxides of molybdenum and manganese it is the reverse, and the hydrated material appears to have become comparatively more of an 'n' type semiconductor.

The gain in weight of the different clinker samples due to hydration in the given time (three days vapour hydration, seven days water hydration) is plotted in Fig. 2 against corresponding temperatures of inflexion given in Table 1. The points are widely scattered, which is to be expected when one considers the inhomogeneous state of the samples, and that the effect of any of the oxides may not be identical for all the constituent minerals of the clinker. Nevertheless, there is a distinct tendency for the points to be in a band in which the gain in weight increases as the temperature of inflexion decreases. That is, as the temperature at which thermionic emission commences for the minerals is lowered, the gain in weight due to hydration in a given time—that is, the rate of hydration—increases. Any lowering of the temperature at which

thermionic emission starts means that the Fermi-level is raised (it comes nearer to the level just outside the crystal). It is therefore concluded that the higher the position of the Fermi-level in the clinker crystals the greater is their rate of hydration. Thus it is the position of Fermi-level in the Portland cement clinker minerals which controls their rate of hydration. The reason for this, in a mixture of minerals such as Portland cement, is now being investigated.

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- <sup>1</sup> Chatterji, A. K., and Phatak, T. C., *Nature*, **197**, 656 (1963).  
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IN this article we shall consider the relationships controlling the hydration of Portland cement.

The amount of hydration of Portland cement in a given time was determined from the non-evaporable water content in the hydrated cement or from the water absorbed during hydration. The rate of hydration has been taken to be synonymous with the rate of growth of the hydrated products. From the results obtained and from the data given in the literature it is concluded that (a) during the early period of hydration (0.5–5 h) the relation between the quantity of water taken up by cement (water of hydration) and the time of hydration cannot be unequivocally assigned to any particular law—some data can be made to fit directly to a logarithmic law, others to an inverse logarithmic law and others to an inverse mass law; (b) thereafter, to a period of 28 days (the period over which observations were taken), hydration follows directly according to a logarithmic law. It has also been found that minor additions of cuprous oxide, zinc oxide and alumina to Portland cement enhance the rate of hydration, while traces of ferric oxide, chromic sesquioxide, nickel oxide and oxide of manganese retard it. The direct logarithmic law is, however, adhered to in all these cases. The oxidation rate of metals also obeys (among others) this direct logarithmic law. It should be pointed out that the reason for a similar law controlling the rate of hydration of Portland cement and rate of oxidation of metals may be due to the similarity in the basic mechanisms of the two phenomena. It is further suggested that the ions liberated during hydration of cement and the degree of stability of the water structure may also be factors influencing the rate of hydration of cement.

The results are arranged according to the following time schedule: (a) First 0.5–5 h of hydration (our own data); (b) 4–31 h of hydration (data taken from Fulton<sup>1</sup>); (c) 1–7 days of hydration (data taken from Fulton<sup>1</sup>); (d) 1–28 days of hydration, with or without admixture of oxides (our own data).

(a) *Period 1.5–5 h.* These experiments were carried out in two sets. In one, normal Portland cement was used as such, and in the other the same cement was heated for 18 h at 110° C and then used for hydration. 2 g cement was used in each experiment and this was mixed with distilled water (water cement ratio 0.40 by weight). Mixing was by hand for 3 min—this period being included in the hydration time. The mixtures were placed over water in a closed vessel for the periods

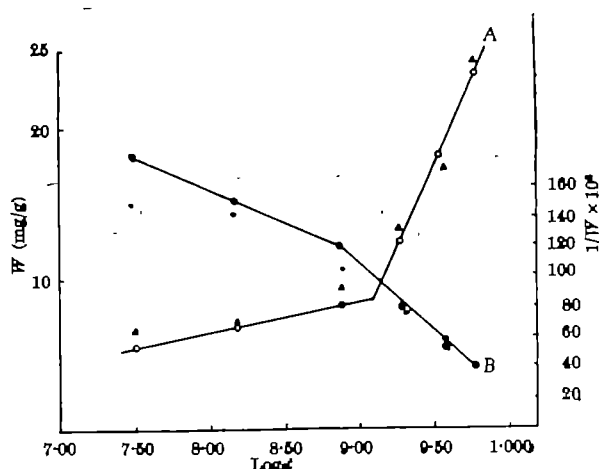


Fig. 1. Non-evaporable water content  $W \sim \log t$  ( $t$  in sec) for period 0.5–5 h. A  $W \sim \log t$ ; B,  $1/W \sim \log t$ .  $\circ$ ,  $\circ$ , unheated;  $\bullet$ ,  $\Delta$ , heated

27, 57, 117, 177, 237 and 297 min. Operations were carried out in a constant temperature room ( $\sim 78^\circ$  F). Materials were then placed in an oven and dried at 110° C to constant weight and the difference in weight of the dried sample and the original cement was taken as the non-evaporable water<sup>1</sup>—representing amount of water taken in hydration. Results are given in Table 1 and Fig. 1.

Gain in weight due to hydration per g of cement during a given time ( $W$  in mg/g) and its reciprocal are plotted against  $\log t$  ( $t$  in sec) and are shown as curves A and B, Fig. 1. A linear relation is obtained and a change in the slope of the curves is obtained after about 2 h hydration. The curve for  $1/W$  versus time is not shown, but it can easily be seen that a linear relation holds in this case also. Thus some data fit into a direct logarithmic relationship (curve A, Fig. 1), others an inverse logarithmic relationship (curve B, Fig. 1), and others inverse mass relationship. Hydration in this period (or in any of the subsequent periods) does not follow parabolic or cubic laws such as are found to hold good in oxidation of metals.

(b) *Period 4–31 h.* Data for water absorbed (mg/g) by cement when allowed to hydrate over this period, as given in Fulton<sup>1</sup>, are plotted against  $\log t$  ( $t$  in sec) (Fig. 2). Cements from 6 different sources were used in these experiments ( $H_1$ ,  $K_1$ ,  $L_1$ ,  $F_1$ ,  $M_1$  and  $N_1$ ). Data obtained with cements  $M_1$ ,  $H_1$  and  $F_1$  fall on a straight line. Inverse logarithmic and inverse mass relations were not obtained for this period. From Fig. 2 it can be seen that a sharp increase in the rate of hydration in these cements occurs after approximately 5.5–7 h. After this period the hydration continues at the same rate, for a given cement, for a period up to 31 h.

(c) *Period 1–7 days.* Treating Fulton's data for this period<sup>1</sup> in a similar way produced a linear relationship (Fig. 3) for plots of non-evaporable water content (mg/g) of hydrated cement against  $\log t$  ( $t$  in sec). (Cement had been taken from a number of sources, designated as  $R_1$ ,  $G_1$ ,  $O_1$  and  $F_1$ , and hydrated for periods 1–7 days.) Other relations do not hold good during this period.

(d) *Period 1–28 days.* Experiments were carried out using ordinary Portland cement as well as Portland cement mixed with 0.01 per cent by weight of cuprous oxide, zinc oxide, alumina and nickel oxide, and with 0.1 per cent by weight of ferric oxide, chromic sesquioxide

Table 1

Time (h)	0.5		1		2		3		4		5	
	W mg/g	1/W	W mg/g	1/W	W mg/g	1/W	W mg/g	1/W	W mg/g	1/W	W mg/g	1/W
Pure cement	5.50	0.182	6.60	0.152	8.10	0.124	12.30	0.081	18.06	0.056	23.4	0.043
Cement heated	6.70	0.149	7.10	0.141	9.40	0.106	13.20	0.076	17.00	0.059	24.2	0.041
Log t (sec)	7.4956		8.1887		8.8819		9.2873		9.5749		9.7981	

and oxide of manganese (obtained by heating manganese acetate) (0.1 per cent was used as additions of 0.01 per cent of the latter oxides to the cement did not produce any significant changes in its rate of hydration).

Cement paste was allowed to hydrate in saturated lime water and the decrease in volume of lime water was noted at intervals of 24 h for 28 days. The decrease in volume represents the amount of water absorbed by the paste, and it was taken to be directly proportioned to the non-evaporable water content of the paste at any particular stage of hydration<sup>1</sup>. The experimental method was the same as that used by Fulton<sup>1</sup>, except that an ordinary glass tube was used instead of a burette and 'Mobil' oil was used as the indicator. Change in volume was calculated from the change in level of oil in the glass tube and from the known average diameter of the tube. Change in volume due to any interaction between oil and lime water was corrected for, at any stage of hydration, by noting the volume changes in a blank with only lime water and oil, under identical setting. All the experiments were simultaneously conducted in a constant temperature room ( $\sim 78^\circ \text{F}$ ).

Results are shown in Fig. 4. The decrease in volume per g of cement at any stage of hydration has been taken proportional to the non-evaporable water content of the hydrated cement at the corresponding stage of hydration. The decrease in volume has been plotted against

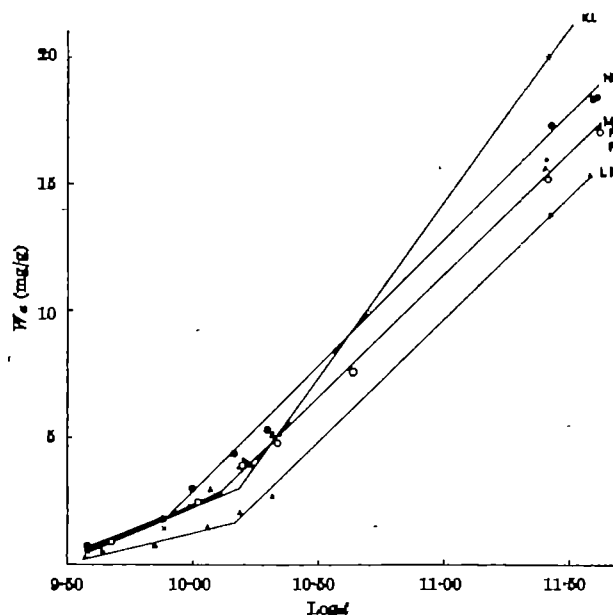


Fig. 2. Water absorbed ( $W_a$ )  $\sim \log t$  ( $t$  in sec) for period 4-31 h

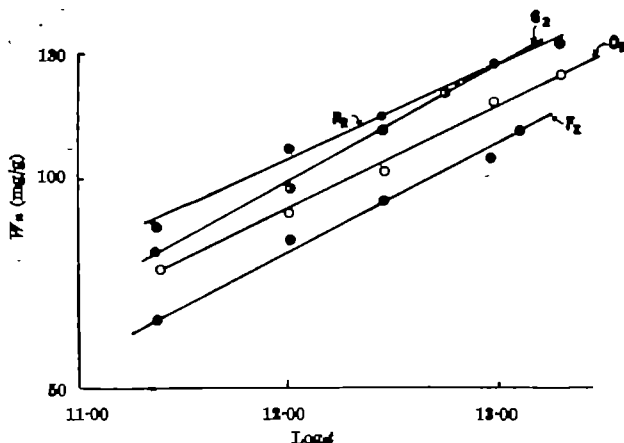


Fig. 3. Non-evaporable water content ( $W_n$ )  $\sim \log t$  ( $t$  in sec) for period 1-7 days

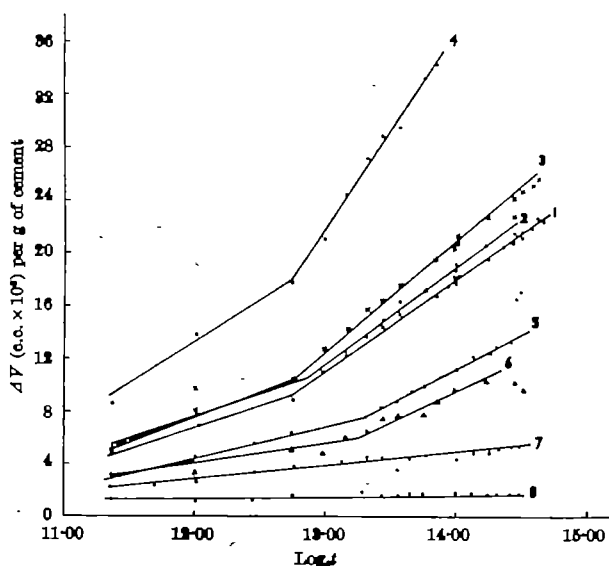


Fig. 4. Change in volume,  $\Delta V$ , during setting of cement  $\sim \log t$  ( $t$  in sec); period 1-28 days. Curve (1) is for neat cement; curves 2, 3, 4, 5, 6, 7, 8 are for cement mixed with  $\text{Cu}_2\text{O}$ ,  $\text{ZnO}$ ,  $\text{Al}_2\text{O}_3$ ,  $\text{MnO}_2$  (7),  $\text{NiO}$ ,  $\text{Cr}_2\text{O}_3$  and  $\text{Fe}_2\text{O}_3$  respectively

$\log t$  (time in sec) (Fig. 4). A linear relationship is obtained. (Other relations, such as inverse logarithmic or inverse mass, do not hold good.) There is a sharp increase in the rate of hydration of neat cement and cement mixed with cuprous oxide, zinc oxide and alumina on the fourth day of hydration. When nickel oxide and oxide of manganese are added to cement the change in the rate of hydration is delayed to the sixth day. With additions of oxide of chromium and iron the hydration rates, which are lowest, remain uniform.

It is seen that during the period 1-28 days, compared with 'pure' Portland cement the non-evaporable water content of cement mixed with cuprous oxide, zinc oxide and alumina is higher at any stage of hydration of Portland cement. That is, the rate of hydration of Portland cement is enhanced by admixtures of these three oxides in traces, but the reverse occurs, during the same period, on addition of chromic sesquioxide, ferric oxide, nickel oxide and oxide of manganese in similar small quantities. Thus the oxides of copper ( $\text{Cu}_2\text{O}$ ), zinc and aluminium act as accelerators and the oxides of chromium ( $\text{Cr}_2\text{O}_3$ ), iron ( $\text{Fe}_2\text{O}_3$ ), nickel and manganese act as retarders—when present in traces. Thus it might be possible to obtain adequate retardation in the hydration of Portland cement clinker by adding traces of these oxides in quantities relatively smaller than gypsum. This may afford a means of eliminating the use of gypsum in Portland cement.

We thus determined that: (1) The rate of hydration follows a direct logarithmic law (we are neglecting the other laws which might be operating during the first few hours of hydration). (2) Sudden changes in the rate of hydration occur at different periods during hydration. (3) The change in hydration rate which occurs at the fourth day may be delayed or eliminated by the additions of oxides containing cations of valency higher than that of silicon. (4) The rate of hydration of cement is enhanced by addition of traces of oxides to cement with cations of valency lower than that of silicon, while the reverse occurs when oxides having cations of valency higher than that of silicon are added (the choice of silicon as the standard is arbitrary at this stage of our discussion).

Logarithmic relationships have also been observed to hold good (among others) in oxidation of metals. These relationships have been deduced by Mott<sup>2</sup>, Cabrera and Mott<sup>3</sup>, Uhlig<sup>4</sup> and Evans<sup>5</sup>. It will be seen that, of these, the basic considerations of Uhlig (leading to a direct logarithmic law) are applicable to hydration of cements. According to Uhlig the rate of oxidation is controlled by

the rate of flow of electrons from metal to oxide—which in its turn is controlled by the work function of metals. It was shown earlier that the hydration of Portland cement clinker takes place by the transfer of electrons from the clinker minerals\*, and that the rate of hydration is controlled by the position of Fermi-level in these minerals and consequently by the thermionic work function (preceding article). It may be that because of the same basic mechanism operating in oxidation of metals and hydration of Portland cement both the processes obey the same rate laws.

Apart from these, it appears that Uhlig's approach to the problem of oxidation of metals is also capable of explaining another unusual finding concerning the hydration of Portland cement. Portland cement is a mixture of minerals. When it reacts with water it would be expected that its constituent minerals would react with water at their individual reaction rates—which vary from mineral to mineral. This, however, does not happen. These constituent minerals, when made into a mixture to form Portland cement, react with water at equal fractional rates (Copeland and Bragg†). This aspect will not be discussed here.

The effects on the rate of oxidation of metals of adding traces of oxides as impurities are as follows: when the oxide scale is an 'n' type semiconducting oxide, minor additions of the cations of valency lower than that of the metal (being oxidized) to the oxide scale raises the oxidation rate, while the reverse occurs when cations of higher valency are added. In 'p' type semiconducting oxides the situation is opposite. In both these cases diffusion of (metal) cation through the oxide layer controls the rate of oxidation. Diffusion is primarily controlled by the defect structure of the oxide.

Correspondingly, in hydration of cements it is known that the hydrated products have defect structures; the exact nature of the defects is, however, uncertain. Moreover, the nature of the diffusing ion in hydrated products and the nature of semiconduction (if any) in them are also unknown. However, there is some indication that the hydrated products may be 'p' type semiconductors (preceding communication). On this basis, hydration ought to take place by anion (OH) diffusion through the hydrated layers.

Acceleration and retardation of the rate of hydration will now be considered. Calcium ions are produced during hydration of calcium silicates. These ions may be absorbed by the clinker minerals or may remain in the body of the hydrated product. In either case, the reaction rate should be enhanced as the adsorption of calcium ions by clinker crystals may raise the Fermi-level of the crystals concerned; this may enhance the reaction rate as

explained elsewhere\* and also for the reason that the valency of calcium is less than that of silicon. The same may hold good for the hydration of tricalcium aluminate where alumina is liberated during hydration. Thus the process of hydration itself may provide for its own enhancement. It may be that the rate constant is controlled by the concentration of the ions liberated during hydration and may have different values for different ranges of concentration of ions.

The amount of hydration in a given time will also depend on the availability of hydroxyl ions which may diffuse through the hydrated layers. These hydroxyl ions would be provided by the water. If the water structure were disturbed, say, by the addition of chlorine ions (from calcium chloride, which is added to cement to accelerate hydration) a greater number of hydroxyl groups would be available for diffusion through the layers. Hence the hydration rate of Portland cement should increase both for calcium and chlorine ions when calcium chloride is added to it. Again the hydration rate is retarded on addition of gypsum to Portland cement clinker. Stabilisation of water structure by the (SO<sub>4</sub>)—tetrahedron fitting into the 'vacancies' in water structure (and thus stabilizing it) may be responsible for fewer hydroxyl ions being available for diffusion through the hydrated layer—and thus leading to a retardation of the hydration process. Thus the extent to which the water structure is stable may be a contributory factor in controlling the rate of hydration of cement. This, however, is to be considered as supplementary to, rather than replacement of, the chemical reasons behind the process of acceleration or retardation of the hydration of Portland cement.

Ions liberated during the hydration of cement should by themselves enhance the rate of hydration. The state of stability of the water lattice may also be a factor in controlling the rate of hydration of Portland cement. The greater the instability, the higher should be the rate of hydration.

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## NITROUS OXIDE – ACETYLENE FLAME IN ATOMIC ABSORPTION SPECTROSCOPY

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SINCE its introduction some ten years ago the technique of atomic absorption spectroscopy<sup>1</sup> has become a valuable method for the determination of trace concentrations of a wide range of metals in solution, and some 35 metals can be satisfactorily converted to the atomic form by spraying solutions of their salts into a low-temperature, pre-mixed flame such as air-coal gas, air-propane, or air-acetylene.

Metals such as the alkaline earths, chromium, molybdenum, and tin are only partially atomized, however, and chemical interferences tend to occur, while a number of metals such as aluminium, beryllium, silicon, titanium,

tungsten, and vanadium are not appreciably atomized at all in such flames.

The difficulty of completely atomizing the metal to be determined has long been recognized as the principal limitation of the atomic absorption technique in chemical analysis, and this article describes the use of a new type of flame which enables effective atomization of a wide range of metals and overcomes many of the chemical interferences found with conventional flames.

Some success in the atomization of the group of metals already mentioned (aluminium, beryllium, silicon, titanium, tungsten and vanadium) has been achieved by direct

injection of their solutions, usually in organic solvents, into turbulent oxy-acetylene flames<sup>2-4</sup>. Such flames, however, as well as being unpleasantly noisy, are of limited value for absorption measurements as they normally provide only a short light-path.

Amos and Thomas have successfully atomized aluminium<sup>5</sup> and other metals<sup>6</sup> by spraying solutions of their salts into a pre-mixed flame of acetylene and enriched air (50 per cent oxygen), but the high flame speed of this mixture severely limits the size and shape of the orifice at which it can be burned and there is a danger of flash-back if the flow of acetylene is reduced too far while adjusting or extinguishing the flame. The need for supplies of the special oxygen-nitrogen mixture may also prove a drawback.

Clearly it would be desirable to have flames giving the same temperature as that of enriched air-acetylene but with lower burning velocity, and the flames of acetylene burning in oxides of nitrogen suggest themselves. Table 1 shows the characteristics of such flames, together with those of the conventional flames normally used in atomic absorption work.

At the third Australian Spectroscopy Conference (Sydney, 1961)<sup>7</sup>, Mr. J. E. Allan reported that he had experimented with the nitrous oxide-acetylene flame, but these experiments, which were made with a burner having a 4.75 in.  $\times$  0.010 in. slot, were abandoned because of the poor sensitivities obtained<sup>1</sup>.

Since supplies of nitric oxide and nitrogen dioxide are not readily available I have confined my attention so far to nitrous oxide, which is widely distributed in liquid form for anaesthetic purposes. I have found that nitrous oxide-acetylene mixtures can be safely burned at a slot as large as 4 in.  $\times$  0.015 in. in a burner made of  $\frac{1}{8}$ -in. thick stainless steel, and that either gas may be turned off first without danger of flash-back. Water cooling is not necessary, and the burner may be fitted directly to the spray-chamber of an ordinary atomic absorption instrument.

Using a Techtron AA-3 single-beam spectrophotometer and replacing the normal tubular burner with one based on the design of Amos and Thomas<sup>5</sup> and having a 2 in.  $\times$  0.018 in. slot in a  $\frac{1}{8}$ -in. stainless-steel block I have obtained the sensitivities shown in Table 2, which are very similar

Table 1. CHARACTERISTICS OF FLAMES SUITABLE FOR ATOMIC ABSORPTION SPECTROSCOPY

Gas mixture	Maximum flame speed (cm sec <sup>-1</sup> )	Maximum temperature (°C)
Air-propane	82	1,925
Air-acetylene <sup>8</sup>	160	2,800
50% oxygen+50% nitrogen-acetylene	640	2,815
Oxygen-acetylene <sup>9</sup>	1,180	2,080
Nitrous oxide-acetylene <sup>5</sup>	180	2,065
Nitric oxide-acetylene <sup>5</sup>	90	2,060
Nitrogen dioxide-acetylene <sup>5</sup>	160	-

Table 2. SENSITIVITIES FOR METALS IN AQUEOUS SOLUTION USING THE NITROUS OXIDE-ACETYLENE FLAME

Metal	Spectral line (Å)	Concentration in µg/ml. giving 1% absorption
Al	3,098	1
Be	2,349	0.05
Bi	2,516	5
Ti	2,648	4
W	2,551	5
V	3,184	1.5

to those obtained<sup>1,6</sup> with the enriched air-acetylene flame. Sensitivities obtained with a burner having a 4 in.  $\times$  0.015 in. slot are usually much poorer.

Metals such as the alkaline earths, chromium, molybdenum, and tin show enhanced sensitivities with respect to those found in the air-acetylene flame. A particularly pleasing feature of the new flame is the disappearance of chemical interferences: calcium, for example, is not influenced by the presence of 100 times its concentration of phosphorus, nor is magnesium by 1,000 times its concentration of aluminium.

I thank Mr. M. D. Amos for discussion of his results before publication and for the loan of an experimental burner, and Atomic Spectral Lamps Pty., Ltd., for the loan of hollow-cathode tubes.

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## OXYGEN ISOTOPIC COMPOSITION OF ORTHOPHOSPHATE FROM SHELLS OF LIVING MARINE ORGANISMS

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UREY *et al.*<sup>1</sup> and Epstein *et al.*<sup>2,3</sup> succeeded in establishing a temperature scale based on the measurement of the isotopic composition of oxygen in the carbonate shells of marine animals and in the environmental waters. In the case of fossils the <sup>18</sup>O/<sup>16</sup>O ratio of the environmental water cannot be measured and palaeotemperatures are generally calculated assuming this ratio as equal to that of modern mean ocean water. This could lead to large errors. The uncertainty could be removed by establishing a second relationship between growth temperature and isotopic abundance of oxygen, for example, in the phosphate of shells. In such a way, measuring the oxygen isotopic composition of carbonate and phosphate from the same shell, and inserting these values into the Epstein equation<sup>3</sup> and in a second one of the same type determined for phosphates, the oxygen-18 content of ancient waters could be obtained.

In 1963 I started a palaeotemperature project with the purpose of establishing a relationship between

the growth temperature and the oxygen isotopic composition of the phosphate of living shells.

The technique used for the purification of the phosphate of shells and the removal of the oxygen by fluorination with BrF<sub>3</sub> is the same as described by Tudge<sup>4</sup>. The conversion of the oxygen to carbon dioxide for spectrometric measurements was made following the technique described by Clayton and Mayeda<sup>5</sup>. The method is summarized as follows.

The phosphatic shells were mechanically cleaned, then washed and air dried, crushed in a steel mortar and ground in an agate mortar. The powder was dissolved slowly with 10 M nitric acid and the organic matter oxidized by addition of 0.3 M KMnO<sub>4</sub> solution. The PO<sub>4</sub><sup>3-</sup> was precipitated twice as (NH<sub>4</sub>)<sub>2</sub> PO<sub>4</sub>·12 MoO<sub>3</sub>·2HNO<sub>3</sub>·H<sub>2</sub>O and then as MgNH<sub>4</sub>PO<sub>4</sub>·6H<sub>2</sub>O. The latter precipitate was dissolved again with 5.4 M nitric acid, then added to bismuth reagent solution (0.2 M Bi(NO<sub>3</sub>)<sub>3</sub>; 2.0 M HNO<sub>3</sub>). The precipitated BiPO<sub>4</sub> was filtered,



washed and dried for 12 h at 90° C then dried again in a vacuum furnace at 130° C for more than 3 h. After that the sample was ready to be introduced into the fluorination tube. To obtain a constant temperature in the vacuum furnace this was heated by vapours of boiling xylene. Xylene was frequently removed to avoid a lowering in the boiling-point (144.4° C), due to possible alteration. The details of the chemical procedure are described in Tudge's paper and have been followed closely with good results. The fluorination apparatus was made using nickel and fluorothane together with 'Hoke 413 K', monel, vacuum tight valves. In these valves 'Teflon' gaskets were used instead of 'Kel-F' gaskets. All metal connexions were silver-soldered. The fluorination reaction was made with samples of 20-30 mg and quantities of BrF<sub>3</sub> between 5 and 10 times the stoichiometric requirements. The reaction tube was pre-fluorinated before each measurement, evacuated for about 1 h, then high-purity dry nitrogen, which was dried further by passing it through a 5-m 'Pyrex'-tube trap at liquid nitrogen temperature, was admitted at a pressure of about 820 mm. After the introduction of the BiPO<sub>4</sub> sample, the reactor was evacuated for 4 or 5 h, then the BrF<sub>3</sub> was introduced, and the reactor was heated at about 100° C for 30 min, although the reaction seemed complete within a few minutes. The oxygen was then held for 3-4 min in each trap of the line at liquid nitrogen temperature, to separate the oxygen from volatile impurities; it was then 'toeplered' into a calibrated volume to calculate the yield of the reaction. The oxygen was then circulated by means of a Toepler pump over a hollow cylinder of carbon made from a spectrographic electrode, heated inductively at about 800°-850° C<sup>4</sup>, while contemporaneously the carbon dioxide was frozen out in a liquid nitrogen cooled trap. To catalyse the conversion to carbon dioxide of the carbon monoxide eventually formed, platinum wire was wound round the carbon cylinder.

Oxygen was run in three different portions of about 98, 3.8 and 0.2 per cent. Each portion was run to completion, then the successive portion was added. In this way<sup>4</sup> one can avoid large fractionation effects due to incomplete conversion to carbon dioxide of the carbon monoxide eventually formed. The reaction yield was then measured with a manometer and the <sup>18</sup>O/<sup>16</sup>O ratio of the sample was measured by means of an 'Atlas M88' double collector mass spectrometer. The results are given in the  $\delta$  terminology, as deviations in per mil of the <sup>18</sup>O/<sup>16</sup>O ratio from that in standard:

$$\delta = 1,000 \left( \frac{{}^{18}\text{O}/{}^{16}\text{O} \text{ sample} - {}^{18}\text{O}/{}^{16}\text{O} \text{ standard}}{{}^{18}\text{O}/{}^{16}\text{O} \text{ standard}} \right)$$

In order to avoid isotopic contamination all the conditions outlined by Tudge were fulfilled.

To check the reproducibility of the conversion of oxygen to carbon dioxide, and of the whole procedure, several measurements were made. Thirteen samples of purified tank oxygen were converted to carbon dioxide during different days and the samples of carbon dioxide analysed with two different 'Atlas M88' mass spectrometers. The working standard was carbon dioxide from a very pure white Carrara marble the <sup>18</sup>O/<sup>16</sup>O ratio of which is -1.80 parts per thousand compared with PDB-1 Chicago standard. This working standard was preferred for phosphate measurements because of its reproducibility, which has been well tested for many years. (A limestone, the  $\delta$  value of which is about -18.0 against PDB-1, is being tested for reproducibility for use as a working standard for further phosphate measurements.) The results obtained are listed in Table 1. The standard deviation calculated is  $\pm 0.12$  parts per thousand.

The reproducibility of the complete procedure was checked using four different samples obtained by mixing chemically pure reagent Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> with a powdered calcium carbonate shell. Each sample was purified using the standard chemical procedure and then divided into

Table 1. ISOTOPIIC COMPOSITION OF TANK OXYGEN CONVERTED TO CARBON DIOXIDE

Date	No. of samples	$\delta^{18}\text{O}$ vs. PDB-1*	Dev. from average (-11.26)
11.5.1964	F4	-11.32	-0.06
	F5	-11.34	-0.08
12.5.1964	F6	-11.53	-0.27
	F7	-11.19	+0.07
	F8	-11.20	-0.06
	F9	-11.29	-0.03
13.5.1964	F10	-11.08	+0.18
	F11	-11.31	+0.05
15.5.1964	F12	-11.43	-0.17
	F13	-11.00	+0.17
	F14	-11.18	+0.08
	F15	-11.10	+0.16
	F16	-11.32	-0.06

$\sigma = \pm 0.12$

\* This is versus CO<sub>2</sub> liberated from PDB-1 by reaction with H<sub>3</sub>PO<sub>4</sub>.

Table 2. OXYGEN ISOTOPIIC COMPOSITION OF CHEMICAL REAGENT Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>

Date	No. of samples	$\delta^{18}\text{O}$ (PO <sub>4</sub> ) <sup>3-</sup> vs. PDB-1*	Dev. from average (-29.61)
27.5.1964	F50a	-29.99	-0.38
29.5.1964	F50b	-29.98	-0.37
1.6.1964	F50c	-29.74	-0.15
3.6.1964	F51a	-29.60	+0.01
18.6.1964	F51b	-29.28	+0.33
18.6.1964	F52a	-29.21	+0.40
19.6.1964	F52b	-29.31	+0.30
25.6.1964	F53a	-29.88	-0.27
2.7.1964	F53b	-29.54	+0.07
17.7.1964	F53c	-29.60	+0.01

$\sigma = \pm 0.28$

\* As in Table 1.

two or three portions which were fluorinated separately. A total of ten measurements was made using two different mass spectrometers. The results are given in Table 2. The standard deviation is  $\pm 0.28$  parts per thousand. Considering the large difference between the standard and the samples and the use of different mass spectrometers this deviation is to be considered as an upper limit.

The choice of the living species which could be used was made at first on the basis of the results reported in Vinogradov's review<sup>7</sup> on the chemical composition of marine organisms. The purpose was the selection of the largest number of marine organisms with measurable amounts of phosphate in their shells or skeletons for utilization for an isotopic phosphate-water temperature scale, and for measurements on fossils. Different species and different specimens of the same species were analysed for their phosphate content. At present about one hundred species have been examined. These results will be published later. Owing to the small number of species containing appreciable amounts of phosphate and to the difficulty of obtaining samples, a first group of ten species was selected, containing both carbonate and phosphate. The oxygen isotopic composition of phosphate was measured as already described and that of carbonate was measured using the standard technique<sup>8</sup>. The main purpose of these first measurements was to obtain information on the range of the oxygen isotopic composition of phosphate in marine animals and on the possibility of a relationship with the growth temperature.

Because of the small content of phosphate, generally several specimens from the same fauna were processed together. The species measured, their location and the results obtained are listed in Table 3. The analytical results are reported against two different standards: carbon dioxide produced from PDB-1 by reaction with 100 per cent phosphoric acid, and standard mean ocean water (SMOW) as defined by Craig<sup>9</sup>. It must be pointed out that in the case of carbonate the difference of the oxygen isotopic composition between the carbon dioxide from the sample and the carbon dioxide from the PDB-1 standard is equal to that between carbonates. It is known that the <sup>18</sup>O/<sup>16</sup>O ratio in the carbon dioxide liberated from a carbonate by reaction with phosphoric acid is higher by about 10 parts per thousand than that in the carbonate itself<sup>8</sup>:

$$\alpha = \frac{({}^{18}\text{O}/{}^{16}\text{O})\text{CO}_2}{({}^{18}\text{O}/{}^{16}\text{O})\text{CaCO}_3} = 1.01$$

Table 3

No. of sample	Species	No. of specimens	Location	Depth (m)	$\delta^{18}\text{O}(\text{PO}_4^-)$ vs. $\text{CO}_2$ , PDB-1	$\delta^{18}\text{O}(\text{PO}_4^-)$ vs. SMOW	$\delta^{18}\text{O}(\text{CO}_3^{2-})$ vs. PDB-1	$\delta^{18}\text{O}(\text{CO}_3^{2-})$ vs. SMOW
1	<i>Syrinx solidissima</i> (1)	1	44° 11' N 40° 25' W	50	-19.50	+20.6	+2.45	+33.1
2	<i>Syrinx solidissima</i> (2)	1	44° 11' N 40° 25' W	50	-20.80	+19.8	+1.85	+32.5
3	<i>Chlamys islandica</i>	5	45° 02' N 51° 33' W	80	-19.86	+20.2	+2.24	+33.0
4	<i>Balanus</i> sp.	25	45° 02' N 51° 33' W	80	-20.72	+19.3	+2.73	+33.4
5	<i>Chinocrathus chatham</i>	5	45° 02' N 51° 33' W	80	-21.37	+18.7	+1.78	+32.4
6	<i>Mytilus galloprovincialis</i>	12	45° 33' N 10° 17' E	5	-21.61	+18.4	+0.12	+30.7
7	<i>Sepia officinalis</i>	5	Tyrrhenian Sea	Surface	-22.50	+17.5	-0.10	+20.5
8	<i>Ostrea edulis</i> (1)	1	40° 23' N 17° 14' E	2	-22.27	+17.7	-0.66	+29.9
9	<i>Ostrea edulis</i> (2)	1	40° 23' N 17° 14' E	2	-22.91	+17.0	-0.61	+30.0
10	<i>Terebratula vitrea</i>	80	45° 37' N 8° 41' E	150	-21.09	+18.9	+2.03	+32.7
11	<i>Brighia spinifrons</i> (1)	1	45° 33' N 10° 18' E	~1	-20.24	+19.8	-1.89	+29.2
12	<i>Brighia spinifrons</i> (2)	1	45° 33' N 10° 18' E	~1	-19.70	+20.4	-1.19	+29.4
13	<i>Portunus</i> sp. (1)	1	45° 33' N 10° 17' E	~2	-22.40	+17.6	-2.26	+28.3
14	<i>Portunus</i> sp. (2)	1	45° 33' N 10° 17' E	~2	-19.71	+20.4	-1.60	+28.9

In the case of carbonates the chemical preparation of the sample is the same as that of the standard. The same fractionation effects are determined, thus preserving the value of the difference of the oxygen isotopic composition between the carbonates.

This is not the case with phosphates. The phosphate oxygen is extracted quantitatively by a chemical reaction involving no isotopic fractionation, and then converted to carbon dioxide. The difference between the oxygen isotopic composition of this carbon dioxide and of the carbon dioxide liberated from PDB-1 by reaction with phosphoric acid is not equal to that between phosphate and PDB-1 carbonate.

The  $\delta$  values of carbonates and phosphates are given also against SMOW. They were calculated by the equation:

$\delta_{(x-st.2)} = \delta_{(x-st.1)} + \delta_{(st.1-st.2)} + 10^{-3} \delta_{(x-st.1)} \delta_{(st.1-st.2)}$  (1) by which an isotopic analysis of a sample  $x$  reported relative to a standard (st.1) may be converted to a  $\delta$  value relative to another standard (st.2),  $\delta_{(st.1-st.2)}$  being the difference of isotopic composition between standard 1 and standard 2 expressed in per mil.

From the equation given by Craig<sup>19</sup> the following value is assumed:

$$\delta_{\text{PDB-SMOW}} = +30.6$$

Taking into account the fractionation in the extraction of the carbon dioxide by reaction with phosphoric acid one gets:

$$\delta_{\text{CO}_2, \text{PDB-SMOW}} = +40.9$$

From these values and from equation (1) the isotopic compositions relative to SMOW are easily obtained.

The results are shown graphically in Fig. 1 and are corrected for the effect of the isotopic composition of the environmental water. The values used for this correction are +1.0 parts per thousand for Mediterranean species and -1.0 parts per thousand for species from Newfoundland Grand Bank. These values are only approximate, and have been deduced from some measurements of the Tyrrhenian surface sea-water in the collection area (Table 4) and on three measurements made on the Grand Bank bottom water. From these three measurements, and taking into consideration the variability during the year of the influx of water from the Labrador current, the average value of -1 parts per thousand relative to SMOW seems to be a reasonable one. The oxygen isotopic composition of phosphate is given against the growth temperature, as calculated from Epstein equation. The calculated temperatures are very close to the values which can be theoretically predicted on the basis of biological and physical considerations. The only exception is that of

crabs *Brighia spinifrons* and *Portunus* sp., which are supposed to form their shell along the coast of Tuscany at a temperature of about  $19^\circ \pm 4^\circ \text{C}$ . In this case a biological fractionation effect is probable either in carbonates or in phosphates. A sting ray from the Pacific Ocean and a *Squilla mantis* shell from the Tyrrhenian Sea were also measured ( $\delta$  values versus carbon dioxide from PDB-1):

Sting ray:  $\delta^{18}\text{O}(\text{PO}_4^-)$ -21.90;  $\delta^{18}\text{O}(\text{CO}_3^{2-})$ -6.20.

*Squilla mantis*:  $\delta^{18}\text{O}(\text{PO}_4^-)$ -17.50;  $\delta^{18}\text{O}(\text{CO}_3^{2-})$ -65.5 but the large fractionation effects shown by their calcium carbonate do not permit one to draw conclusions on the validity of the results.

Unfortunately these results cannot be compared with those of Tudge, which were given against a working standard of unknown oxygen isotopic composition.

The following conclusions can be drawn from this first group of measurements.

(a) The range of the oxygen isotopic composition of the phosphate precipitated by *Palaemonetes* and other marine animals is rather far from that of the calcium carbonate. Relating both measurements to the PDB-1 Chicago standard, and bearing in mind the fractionation effect determined by the chemical reaction of  $\text{CaCO}_3$  with phosphoric acid, the difference between the  $^{18}\text{O}/^{16}\text{O}$  ratios is about 12 parts per thousand.

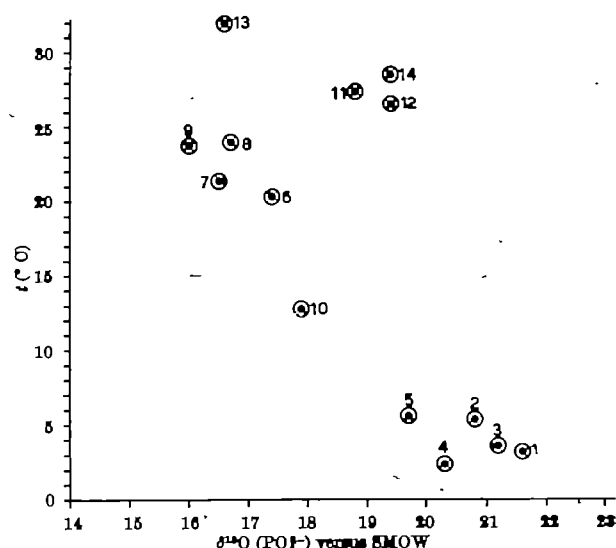


Fig. 1. Oxygen isotopic composition of phosphate from shells of living marine organisms against growth temperature. The  $\delta$ 's are corrected for the effect of the isotopic composition of the environmental water.

Table 4. OXYGEN ISOTOPIC COMPOSITION OF SEA-WATER FROM THE COLLECTION AREAS OF PHOSPHATIC SAMPLES

Samples and their location	Average $\delta^{18}\text{O}$ (SMOW)
8 surface samples 5 miles off La Spezia (August)	+1.10
6 coastal samples near Livorno (September–October)	+0.85
2 surface samples 3 miles off Calvi (Como) (December)	+1.15
2 surface samples Gulf of Genoa (July)	+1.05
2 bottom samples (80 m.) 44° 11' N (end of May) 45° 25' W	-1.20
1 bottom sample (80 m.) 45° 02' N 51° 35' W (July 1)	-1.45

(b) There exists a relationship between temperature and oxygen isotopic composition of phosphate. From the fact that there is a good agreement in the results obtained from different marine animals (Lamelli-branchiata, Cirripedia, Cephalopoda and Brachiopoda) one can assume that the precipitation of phosphate takes place in these cases under isotopic equilibrium conditions. If this is so, crabs seem to precipitate the calcium phosphate of their shell under non-equilibrium conditions. This probably happens also to other crustaceans. To check if there is a constancy of the biological fractionation effect, some crabs are being grown under controlled temperature conditions.

(c) Owing to the small number of samples and to the fact that specimens were not grown under controlled temperature conditions an equation is not given. However, the slope of a straight line calculated by the least-squares method is about -4.1. This value of the slope is very near to that calculated for carbonates (-4.3). Because of that it seems rather difficult, at least on the

basis of these first results, to state a phosphate-carbonate temperature scale. Phosphatic brachiopods will be grown under controlled-temperature conditions. It is possible to obtain from them measurable phosphate samples, a definite equation will be given from which it will be possible to prove the foregoing assumption or to check the possibility of deducing correct values of the oxygen isotopic composition of the sea-water from the carbonate and the phosphate equations.

(d) From the results obtained the fractionation factors can be calculated as follows:  $t = 25^\circ \text{C}$ ,  $\alpha = 1.0156$ ,  $t = 0^\circ \text{C}$ ,  $\alpha = 1.0218$ . These  $\alpha$  values are bigger than those calculated theoretically by Urey *et al.*<sup>1</sup>, which were:  $t = 25^\circ \text{C}$ ,  $\alpha = 1.0087$ ;  $t = 0^\circ \text{C}$ ,  $\alpha = 1.0104$ .

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## CRYSTAL-GROWTH POISONING OF *n*-PARAFFIN WAX BY POLYMERIC ADDITIVES AND ITS RELEVANCE TO POLYMER CRYSTALLIZATION MECHANISMS

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IN recent years much attention has been directed to crystal growth mechanisms in polymers. The growth of polyethylene crystals from solution has been studied particularly intensively. The earlier work showed that long-chain *n*-paraffins and polyethylene crystallized from solution as lozenge-shaped plates<sup>1</sup>. In addition it has been shown that a dendritic type of growth is also common in polyethylene, and that by varying the conditions of crystallization a gradual transition between regular lozenges and dendrites could be obtained<sup>1</sup>.

We wish to report that during studies of *n*-paraffin wax crystallization from middle distillate fuel oils it has been found that if several hundred parts per million of certain polymers are present in the oils, the crystal growth habit of the wax is changed from lozenge-shaped plates to dendrites and other forms. The findings, although of considerable general interest in their own right as examples of crystal-growth poisoning, are of particular value because of the similarities between the crystal habits of the wax and those reported in the literature for polyethylenes and other polymers<sup>2</sup>. Since the dendritic growth which we observed in *n*-paraffinic wax is caused by the presence of a polymer which interferes with the normal course of crystallization one can speculate, for example, that the dendrites reported for polyethylene may have been produced as a result of crystal modifiers which occur naturally in the polyethylenes.

The photographs of the wax crystals were taken with a Leica 35-mm camera fitted to a Leitz 'Laborlux' microscope using phase contrast transmitted light illumination.

Early in the investigations it was found that the cooling rate and the environment could have a marked influence on the size and shape of the wax crystals. Accordingly, all the photomicrographs presented in the paper were obtained by cooling the solutions in bulk (that is, minimum sample size 25 g) in a cold room using carefully controlled cooling rates in the range 0.2–2° F/h. When the wax crystals appeared, a few drops of each solution were transferred from the bulk containers on to a microscope slide, using a spatula, and then photographed. Where not stated otherwise, the polymeric additive used in the work described here was an ester-type copolymer which is available commercially as a 50 per cent solution in kerosene under the trade name 'Paradyne 20' (ref. 3). However, it has been found that similar photographs can be obtained whether the ester-type copolymer or a purely hydrocarbon-type is used as the additive<sup>4</sup>.

In the absence of any copolymer, the wax precipitated as overlapping and intergrowing lozenge-shaped crystals. In the presence of dissolved copolymer a considerable variety of crystal shapes and sizes was observed depending on the polymer concentration and the growth conditions.

The effect of 500 parts per million of dissolved copolymer on the wax crystallization is illustrated in Figs. 1, 2 and 3.

Fig. 1 shows a typical wax crystal produced on cooling a light catalytically cracked oil. The photograph shows an excellent example of dendritic growth and is very similar to many photographs of polymer crystals published in the literature<sup>3</sup>. Detailed investigations of the different types of dendrites which can be formed by polyethylene have been carried out by Wunderlich<sup>4</sup>. Corresponding types have also been observed with wax by ourselves. Fig. 2, for example, shows a twin dendrite which was obtained by cooling the same oil referred to in Fig. 1 at 2° F/h, instead of 1° F/h. In contrast, Fig. 3 shows a dendritic type unlike any yet published for polyethylenes. However, it should be mentioned that it is very doubtful whether any correlation between the type of dendrite and the crystallization conditions can be established since a number of structures can usually

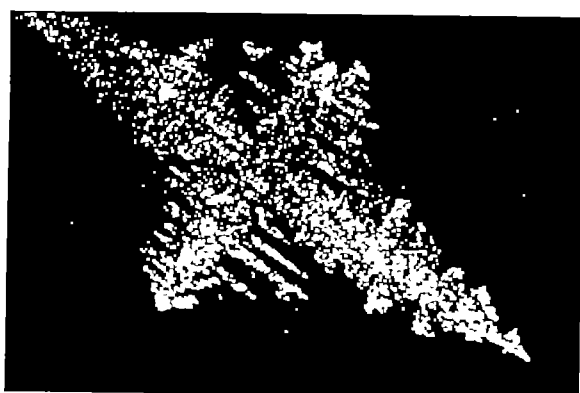


Fig. 1. *n*-Paraffin dendrite produced by cooling (at 1° F/h) light catalytically cracked oil containing 500 p.p.m. copolymer

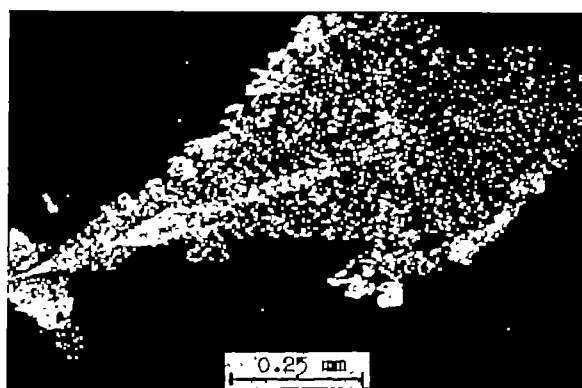


Fig. 2. Twin dendrite produced by cooling (at 2° F/h) light catalytically cracked oil containing 500 p.p.m. copolymer

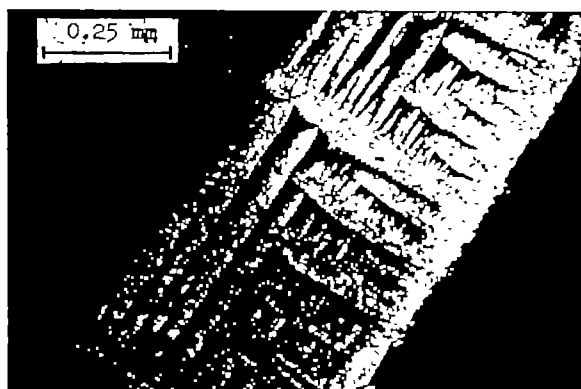


Fig. 3. Dendrite produced by cooling (at 0.2° F/h) light catalytically cracked oil containing 135 p.p.m. laboratory-synthesized ester copolymer

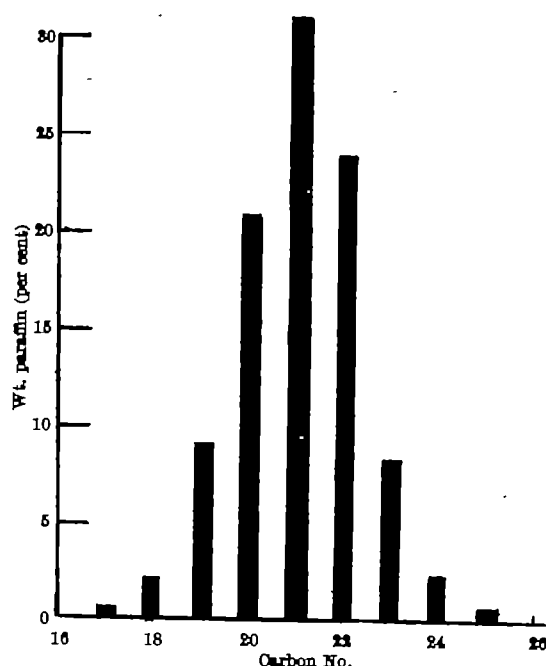


Fig. 4. Typical composition of the dendritic *n*-paraffin wax crystals

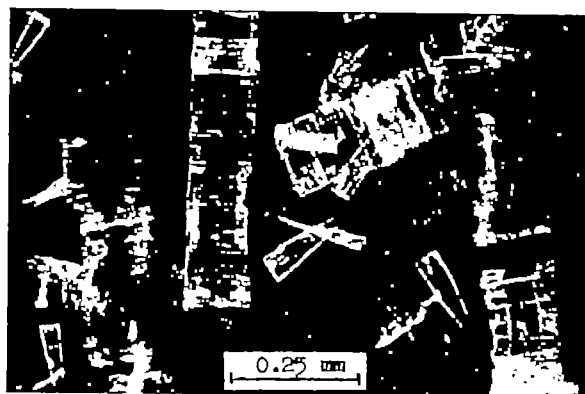


Fig. 5. Block-like and pyramidal *n*-paraffin crystals produced by cooling (at 1° F/h) a 50/50 blend of virgin and cracked oils containing 1,000 p.p.m. hydrocarbon copolymer

be distinguished in any given solution. Dendrites have been found in a wide variety of oils which have been treated with the additive. A gas chromatographic analysis on dendritic wax filtered from a typical oil is presented in Fig. 4. The wax consisted of C<sub>17</sub>-C<sub>24</sub> *n*-paraffins in solid solution accompanied by trace amounts of isoparaffins. The polymeric additive composition is important, but not critical, provided certain structural requirements are met.

Very often block-like crystals are produced at relatively high concentrations of copolymer. This effect is illustrated by the photograph shown in Fig. 5. These block-like crystals appear to be formed from would-be pyramidal crystals which have not completed their growth independently.

So far, true dendritic crystals have been observed only when mixtures of *n*-paraffins are crystallized in the presence of copolymer. Attempts to obtain true dendrites by: (a) crystallizing pure single *n*-paraffins from solution in the presence of copolymer, and (b) by crystallizing selected mixtures of *n*-paraffins in the absence of copolymer have been unsuccessful.

The mechanism by which dendritic growth occurs has been discussed in detail by Saratovkin<sup>5</sup> with reference to alloys and inorganic crystals. Essentially the same basic mechanism can be used to explain the way in which the

copolymers change the habits of the wax crystals<sup>4</sup>. We therefore propose that the dendritic wax crystals are obtained because the copolymers modify the crystal habit of the *n*-paraffin wax by selectively poisoning the growth of the wax crystals in the 011 (or *XY*) crystallographic plane.

The copolymer molecules are made up of runs of methylene groups which are broken periodically by branching. The poisoning action probably occurs because the copolymer molecules are sufficiently similar to the *n*-paraffins to incorporate themselves at the wax crystal growth step (perhaps by chain folding) and yet sufficiently dissimilar (by virtue of their branches) to obstruct the further addition of *n*-paraffin molecules to the crystal. When crystal growth is retarded in the 001 plane the crystal will grow relatively faster along its *Z* axis. Consequently 'thicker', that is more isometric, wax crystals are produced when copolymer is present. At high polymer concentrations, growth in the 001 plane is evidently restricted to such an extent that even dendrites cannot form; instead much of the crystal growth takes place along the *Z* axis and block-like or pyramidal crystals result, as is illustrated in Fig. 5.

The remarkable changes in the crystal growth habits of *n*-paraffin wax produced by low concentrations of polymers

are probably idealized examples of effects which are operating in many other systems. In particular, we think that similar mechanisms may well apply to polymer crystallizations themselves, and also to enzyme and protein crystallizations reported in the literature, for example, refs. 7 and 8. In these systems the crystals consist of mixtures of complex molecules. The crystal poisons would be present either as impurities or, more likely, occur naturally as components of the polymers themselves. In either event, the results clearly emphasize the desirability that morphological investigations of the crystallization of complex molecules should preferably be carried out under carefully controlled conditions and using only material which has been carefully purified.

A more detailed report on the mechanism of the poisoning will be made in a later publication<sup>5</sup>.

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## DISCONTINUITIES IN LIQUID FLUIDIZED BEDS

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IN an earlier communication<sup>1</sup> attention was directed to hitherto unsuspected discontinuities in liquid fluidized beds. Attention is now directed to a parallel existence of discontinuities in sedimentation. When aqueous dispersions of a variety of materials are settled, small mounds and craters are frequently observed on the surface of the

final sediment. This has been noticed in the case of natural products such as clays as well as chemical precipitates, and constitutes evidence of some sort of channeling effect in the sediment.

It is known that fine particles often settle as flocs, an individual floc comprising an aggregate of particles

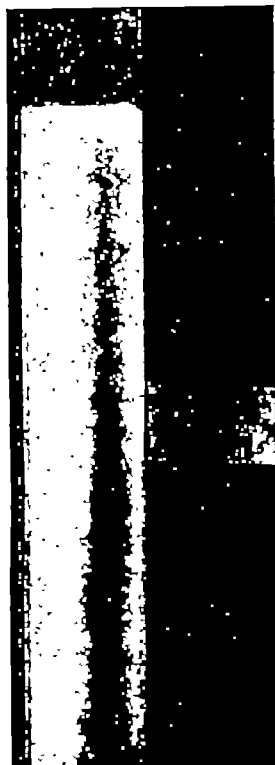


Fig. 1



Fig. 2



Fig. 3

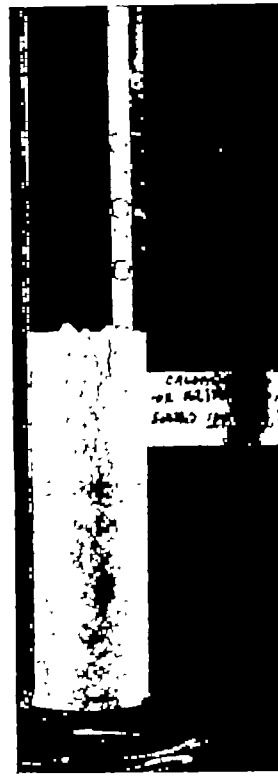


Fig. 4

embracing a core of liquid, with the result that the sediment initially had a low solids content. A natural inference is that a subsequent slow collapsing of the flocs releases liquid which, in percolating upwards through the sediment, creates preferential channels of flow, with possible formation of mounds by either of two mechanisms. Closer observation, however, has shown that these channels are initiated at a relatively early stage of settling, and in the disperse phase.

Figs. 1-4 show stages in the sedimentation of an aqueous dispersion of a precipitated chalk, initially at uniform



Fig. 5



Fig. 6

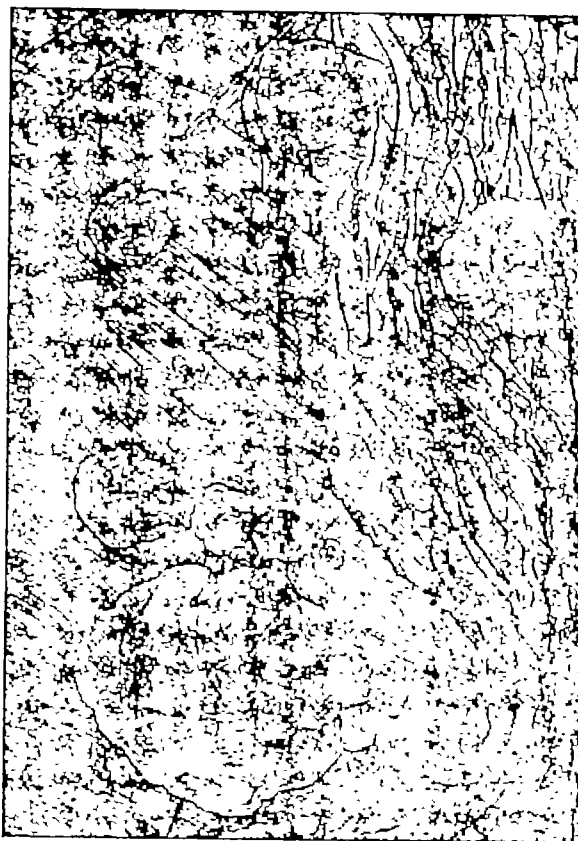


Fig. 7

concentration 0.02 v/v, in a glass measuring cylinder of 4 cm internal diameter. Within 3 min of commencement of settling, in which time the upper level had fallen less than 5 mm of its original height of 230 mm, the suspension showed signs of curdling and slight scores could be seen at the cylinder wall. As time progressed the scores developed into sensibly vertical or upwards-inclined channels of some 1-2 mm wide, wherein liquid streamed upwards carrying fine particles seemingly as individuals rather than as flocs, at a linear velocity of some 5 mm/sec. In reaching the surface they formed miniature volcanoes as seen clearly sectioned at the wall in Fig. 4.

That this is not merely a wall effect is shown by Figs. 5 and 6, taken at the surface of another sediment, where the eruptions chanced to be located randomly away from the wall. Such eruptions are often apparent at the surface about 10-15 min after the beginning of settling, in the case of suspensions at volume dilutions of between 15 and 50 : 1. The suspensions are quite fluid at the time of formation and for a considerable period thereafter, as demonstrated by the response to any slight movement of the cylinder. Despite the fluidity of the suspension, the channels are most persistent in their location, although random making and breaking can be observed as might be expected. The random nature of channel formation indeed could be largely responsible not only for the anomalous nature of many batch-settling curves, but also for the variations and differences noted in repeat batch-settling experiments.

It is interesting to observe that where suspensions of initial concentration greater than 0.06 did not give visual evidence of curdling or channelling at the wall, small mounds were nevertheless formed more centrally at the surface. It could be inferred as a general conclusion that channels initiate in accord with flow patterns in a suspension, which are set up by the prior shaking to obtain a uniform suspension, and persist for some time after commencement of settling.

Fig. 7 shows part of the surface of a sediment in a tank of some 40 cm  $\times$  60 cm cross-section. Here the eruptions have spread over wider areas, the larger spreads having a diameter of the order of 10 cm, and now another phenomenon is observed. This is a lateral shrinking of the sediment to form a series of vertical fissures. The horizontal scores which can be seen extending mainly over the non-fissured area are also of interest. That they are not extraneous seems evident since they do not extend across the eruption-spread areas.

The existence of channels explains certain anomalous measurements made in continuous thickening experiments in a tank of some 100 cm diameter and 200 cm deep, where hydrostatic head measurements at a series of depth intervals indicating quantity of suspended matter are

made to an accuracy of 0.01 mm water. Random fluctuations with an amplitude of up to 0.60 mm can now be explained in terms of such channelling.

Finally, the similarity of Fig. 5 to the appearance of a gentle bubbling gas fluidized bed is noted, and the possibility that dispersions of non-flocculated particles should settle with formation of parvoids cannot be excluded.

I thank Mr. J. Gascoine, of the Loughborough College Photo-printing Unit, for the photography, and the Department of Scientific and Industrial Research for a grant covering a wider investigation, enabling the engagement of junior research assistants. One of these, R. Buxton, assisted in the setting up of the experiments.

<sup>1</sup> Hammett, N. J., *Nature*, 189, 997 (1961).

## HYBRIDIZATION OF LACTIC DEHYDROGENASE *in vivo* AND *in vitro*

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ON the basis of a variety of observations it is generally accepted that lactic dehydrogenase (LDH) is composed of four sub-units, and that two basic types of sub-unit are present in tissues of most species<sup>1,2</sup>. Muscle or *M* type is the principal form in skeletal muscle, and heart or *H* type predominates in heart<sup>3,4</sup>. The various forms of LDH are represented schematically in Fig. 1. In contrast to mammals and birds, tissue extracts of a number of vertebrates do not show the 'normal' five bands of LDH activity on starch-gels. Several species have more than five bands in complex patterns which may be due to 2 *M* or 2 *H* types<sup>5</sup>. In several species as few as two electrophoretic bands are found. Implicit in the interpretation of electrophoretic patterns is the idea that, within a given cell type, random combination of LDH sub-units occurs, and that all possible combinations are stable under the experimental conditions; otherwise interpretation is complicated, if not impossible.

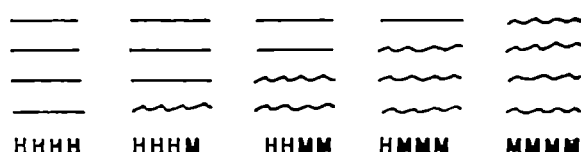


Fig. 1. Nature of the multiple molecular forms of lactic dehydrogenase

The development of methods that permit the formation of inter- as well as intra-specific hybrids<sup>6,7</sup> makes possible the investigation of fundamental questions concerning the degree of structural similarity among various LDH's and the interpretation of electrophoretic patterns.

The experiments of Singer and Itano<sup>8</sup> and subsequent investigations have shown that a very wide variety of haemoglobins can hybridize *in vitro* by treatment at low pH (refs. 9 and 10). It has also been shown that the structural features required for the formation of functional haemoglobin tetramers have been largely conserved during evolution<sup>11</sup>, even though the amino-acid sequences, oxygen-binding capacities, Bohr effects, and immunological properties of these proteins are different<sup>12-14</sup>.

More recently Huehns *et al.*<sup>15</sup> have observed hybridization of haemoglobins *in vitro* at neutral pH at 38° C. Their technique permits measurement of relative rates of hybridization, and in the course of their investigations they observed large differences in rates of interaction

between certain types of sub-units; it was suggested that differences in rates of interaction between haemoglobin sub-units also occur *in vivo*<sup>16</sup>.

In some respects our studies on LDH parallel investigations on haemoglobin. Both proteins are tetramers and have four 'active sites', and X-ray crystallographic studies have shown that at least one LDH has properties of symmetry comparable with those of haemoglobin<sup>17</sup>. Within a given species the *H* and *M* sub-units are catalytically and immunologically distinct and have different amino-acid sequences, but form functional hybrids. Therefore it was of interest to determine whether haemoglobin was unique in its ability to form functional interspecies hybrids, and to compare rates of hybrid formation between different types of LDH sub-units.

### Hybridization *in vitro*

Most of our experiments have been carried out with highly purified crystalline enzymes, but since hybridization also occurs in crude extracts, the degree of purity of the enzymes apparently is not critical. Purified enzymes or crude extracts were dialysed overnight against 1.0 M sodium chloride + 0.1 M sodium phosphate (pH 7.0), mixed, frozen in a dry-ice-methanol bath, and allowed to thaw at room temperature. Samples were then dialysed for 3-12 h against 0.1 M sodium phosphate (pH 7.0) to reduce the salt concentration, and subjected to starch-gel electrophoresis<sup>4</sup>. Table 1 summarizes the combinations which we have subjected to hybridizing conditions, and indicates whether or not hybrids were formed, and whether binomial electrophoretic patterns were produced.

Most of the enzyme combinations hybridized readily, showing that those structural features which are required for tetramer formation have been largely conserved during evolution. These results are in agreement with the preliminary observations of Markert<sup>17</sup>.

A number of interesting exceptions with respect to hybridization were observed. The *L* lactic acid-specific LDH from *Lactobacillus orabimosis* did not hybridize with dogfish *M* LDH, and when combinations of dogfish *M* LDH were frozen and thawed with lobster muscle LDH a faint, smeared band, staining for both enzyme activity and protein, and with electrophoretic mobility intermediate between the two pure types, was observed. Apparently hybrids were formed but were somewhat unstable.



Table 1. INTERSPECIFIC HYBRIDIZATION OF LACTIC DEHYDROGENASES

Enzyme sources	Hybridization	Binomial pattern
Chicken muscle + chicken heart	+	+
Chicken muscle + rabbit heart	+	+
Chicken heart + rabbit heart	+	+
Chicken heart + beef heart	+	+
Dogfish muscle + chicken heart	+	+
Dogfish muscle + beef heart	+	+
Dogfish muscle + <i>Lactobacillus arabinosus</i>	-	-
Dogfish muscle + ostrich heart	+	+
Hallbut muscle + rabbit heart	+	+
Sturgeon muscle + beef heart	+	+
Beef heart + catman muscle	+	+
Beef heart + alligator muscle	+	+
Human heart + human muscle	+	+
Chicken heart + rhes heart	+	+
Cod muscle + cod heart	-	-
Cod muscle + rabbit heart	+	-
Cod heart + dogfish muscle	-	-
Cod heart + chicken heart	-	-
Cod muscle + haddock muscle	+	-
Haddock muscle + haddock heart	-	-
Haddock muscle + rabbit heart	+	+
Haddock heart + chicken heart	+	-
Haddock heart + dogfish muscle	+	-
Hallbut muscle + rabbit heart	+	+
Hagfish muscle + hagfish heart	-	-
Lobster muscle + dogfish muscle	+	-
Bullfrog muscle + bullfrog heart	-	-
Bullfrog muscle + beef heart	+	+
Bullfrog heart + chicken heart	+	+
Congo eel muscle + congo eel heart	-	-
Congo eel muscle + beef heart	+	+
Congo eel heart + chicken heart	+	-
Congo eel heart + leopard frog heart	-	-
Congo eel heart + leopard frog muscle	+	+
Congo eel muscle + chicken heart	+	+
Leopard frog heart + leopard frog muscle	-	-
Leopard frog heart + pickerel frog heart	-	-
Leopard frog muscle + beef heart	+	-
Bullfrog heart + <i>Amygdalus</i> muscle	-	-
Bullfrog heart + leopard frog heart	+	-

Dr. A. O. Wilson of this Laboratory observed that tissue extracts from several species did not contain hybrids between *H* type and *M* type LDH's; they included the hagfish (*Myxostoma stouti*), the bullfrog (*Rana catesbeiana*), the congo eel (*Amphiuma tridactyla*), the codfish (*Gadus calarias*) and the haddock (*Melanogrammus aeglefinus*). In these species no tissues were found that showed hybrids, even though the two pure types were present in some. In the bullfrog a single faint band with a mobility approximately equidistant from the *H* and *M* bands was observed. Failure of the *H* and *M* enzymes of these species to hybridize *in vivo* is not neces-

sarily due to synthesis in separate cell types, because when extracts of these species were subjected to hybridizing conditions (by the freeze-thaw technique<sup>6</sup>) hybrids were not formed, and the undefined intermediate band in bullfrog tissue was not intensified (Fig. 2).

Failure to form hybrids *in vivo* under a given set of conditions may be due either to failure to dissociate into sub-units or to much lower rates of combination of sub-units into hybrids, as compared with reformation of the pure types. In previous reports we have presented evidence that, by varying the ionic strength, the nature of the ions, and coenzyme analogue concentrations, different LDH's will show quite different rates at which their sub-units will hybridize<sup>7,10</sup>. In order to test these two alternative hypotheses attempts were made to hybridize the *H* type and *M* type LDH's of the species that failed to hybridize *in vivo* as well as *in vitro*, with *H* type and *M* type enzymes of other species. Although the *M* type enzymes of codfish, haddock, congo eel, and bullfrog do not hybridize *in vivo* or *in vitro* with their corresponding *H* type LDH's, they do hybridize *in vitro* to give binomial patterns of multiple forms with *H* type enzymes from other species. This demonstrates that these *M* type enzymes have structural features sufficiently similar to other LDH's so that functional tetramers can be formed. When similar experiments were done with heart extracts from cod, haddock, bullfrog and congo eel, it was found that the *H* type enzymes differed markedly from their specific *M* types in their ability to hybridize under these conditions with other LDH's. A schematic representation of the kinds of patterns that were observed is shown in Fig. 2.

These results suggest that absence of hybrids *in vivo* is not necessarily owing to involvement of different cell types, and failure to hybridize *in vitro* is not necessarily owing to complete absence of dissociation. It is possible that when mixtures of *H* type and *M* type extracts from cod, haddock, bullfrog and congo eel are frozen and thawed, at least partial dissociation occurs but that the parent types are reformed in preference to hybrids. The results shown with chicken *H*<sub>4</sub> and bullfrog *H*<sub>4</sub> indicate that, although some hybridization occurs, the parent forms are still kinetically favoured. Since roughly one-third of the activity is lost under the freeze-thaw conditions, an alternative hypothesis is that failure to observe hybrids may be due to their instability.

We have found, for example, that the *H* type enzymes of different populations of bullfrogs have different stabilities at low pH. *H* type LDH from Louisiana bullfrogs shows no loss of activity at pH 5.6, whereas the same enzyme from Wisconsin bullfrogs loses half its activity after an hour at this pH. This is reflected in starch-gel, where there is a pH gradient from anodal to cathodal ends, in an absence of the heart band from Wisconsin populations but not from Louisiana populations. Differential heat stabilities result in similar distortions of starch-gel patterns, since there is also an anodal-cathodal temperature gradient along the gels.

#### Hybridization *in vivo*

We have carried out experiments which demonstrated a remarkable effect of environmental conditions on sub-unit association. The *H* type LDH's of the leopard frog, *Rana pipiens*, and of the pickerel frog, *Rana palustris*, do not hybridize *in vitro* with each other or their corresponding muscle types. However, binomial patterns of hybrid *H* types were formed *in vivo* in hybrid tadpoles between these species. Eggs were obtained from female *Rana pipiens* by the pituitary implant method<sup>11</sup>, and fertilized with a sperm suspension from ground testes of male *Rana palustris*. The tadpoles were reared until large enough for the hearts to be conveniently removed. Fig. 3 shows the electrophoretic patterns of the LDH's in crude extracts of hearts both of

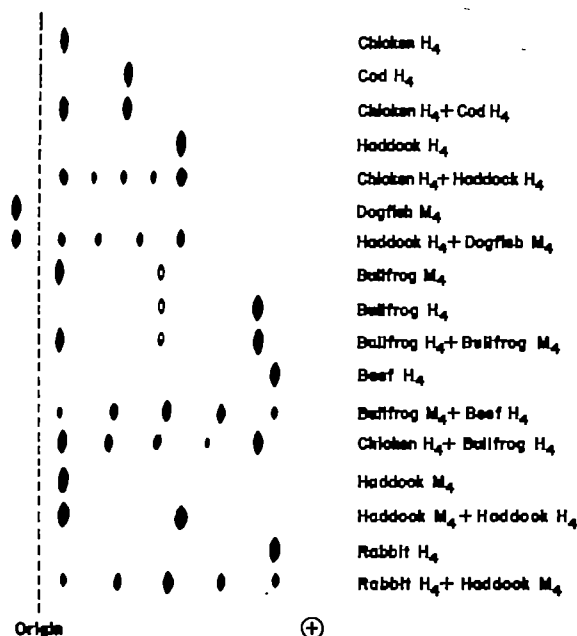


Fig. 2. Sketch of starch-gel electrophoretic patterns comparing hybridization between LDH's from heart and muscle of various species

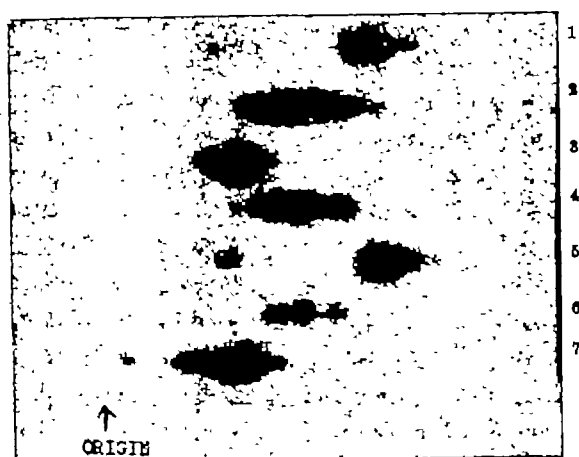


Fig. 3. Pattern of heart LDH of parental and hybrid frog hearts. 1 and 5, *Rana pipiens*; 2, 4 and 6, *Rana pipiens* x *Rana palustris* run at three different concentrations of enzyme activity in order to bring out different characteristics of the pattern; 3 and 7, *Rana palustris*. Electrophoresis was done on starch gel with citrate phosphate buffer at pH 7, 30 h. The gel was made up in half-strength buffer to increase the voltage, thereby increasing the separation of the sub-bands.

the parental species and of the hybrids. It is apparent that the products of the homologous genes of these species can combine to form biologically active hybrid molecules. Our success in hybridizing these two enzymes *in vivo* indicates that there may be *in vitro* conditions under which the various refractory heart LDH's will dissociate and reassociate, forming stable hybrid enzymes. These conditions have not yet been discovered, and the sub-units of this group of *H* type LDH's remain peculiar in this respect. In this case we have clearly shown that failure to produce hybrids *in vitro* is not due to hybrid instability because the *H*-type hybrids of *Rana pipiens* and *Rana palustris* produced *in vivo* are quite stable.

From our work with embryos we have observed that in *Rana pipiens*, *Rana palustris* and *Rana sylvatica*, the ova as they are deposited contain primarily the *H* type LDH, so far as can be determined by starch-gel electrophoresis. There is no observable change in electrophoretic pattern until about the time of hatching (Shumway stage 19), when the *M* band begins to become more intense. At this time also, in the hybrid embryos, the paternal *H* type LDH makes its first appearance on starch-gels, together with the hybrid LDH's composed of both paternal and maternal sub-units (Fig. 4). This figure indicates the presence of two faint intermediate bands between the *M* and *H* types. These bands appear to be similar to the single intermediate band in the bullfrog (Fig. 1). Like that band, they cannot be intensified by freezing and thawing muscle and heart extracts together. For this reason we feel that they may not be hybrids of *H* and *M* sub-units. Hybrids between the *M* sub-units of the two species cannot be seen on gels because they have identical mobilities. We have found that the *M* type LDH's of these two species are identical by every criteria that we have applied. They do not differ immunologically, catalytically, in temperature stability, or in electrophoretic mobility. The *H* LDH's of these two species, however, differ in all these respects.

It is not clear from electrophoresis alone at what point in development the *M* type LDH and paternal *H* LDH are first produced. They may be present at any stage before the one when they are first seen on starch gels, but in such small amounts, or concentrated in so few cells, as compared with the whole embryo, that they are below the limits of resolution of this method. The first appear-

ance of paternal *H* type and the first intensification of the *M* type coincide with the rise in enzyme activity per embryo reported to begin at around the hatching stage by Wallace<sup>10</sup>. We have confirmed Wallace's observation that before this stage there is no change in the level of LDH activity. It is possible that until this stage the enzyme activity in the embryo is caused wholly by the LDH already present in the ovum, and that the embryo does not begin to produce its own LDH until the hatching stage. Since both the initial intensification of the *M* type and the first appearance of the paternal *H* type occurs at the same stage, the structural genes responsible for their production appear to begin functioning at the same time.

On the basis of their tendencies to form hybrids under various conditions, LDH's can be divided into three broad groups. First, there are those that hybridize *in vitro* in an apparently random fashion with no types favoured kinetically. This group includes the majority of the LDH's that have been studied. Secondly, there are those *H* type LDH's (for example, bullfrog *H* type, congo eel *H* type, haddock *H* type) that will not hybridize with certain LDH's but will partially hybridize with others. In these cases dissociation apparently occurs, but the pure types are kinetically favoured. Thirdly, there are those *H* type LDH's (*Rana palustris H* and *Rana pipiens H*) which do not hybridize *in vitro* with any other LDH's under the conditions employed. Since no enzymes have been found that will hybridize with these types *in vitro* we cannot say whether these enzymes do not dissociate under these conditions; but *in vivo* conditions are such that random combination of sub-units does occur. However, the *H* types of *Rana pipiens* and *Rana palustris* do not appear to hybridize with their corresponding muscle types *in vivo*. By way of comparison with other species that have been studied, it does not seem reasonable that this phenomenon should be explained simply on the basis of synthesis in separate cell types. It is possible that the mechanism of synthesis within a given cell type controls which sub-units are put together. There is the alternative possibility that when the two *H* LDH's are synthesized in the same cell there is no preference of one sub-unit for another (Fig. 3), but when *H* and *M* sub-units are synthesized in the same cell the pure types are kinetically favoured.

Thus far the only sources of LDH's that will not form random hybrids *in vitro* with the usual techniques are the hearts of cold-blooded lower vertebrates, these being some frogs and salamanders as well as some teleost fishes. Since the sharks (dogfish) and birds and mammals do show normal hybrids between the heart and muscle sub-units, their absence in the groups indicated appears to have been an independent evolutionary acquisition in each of these three groups. This is further indicated by the fact that

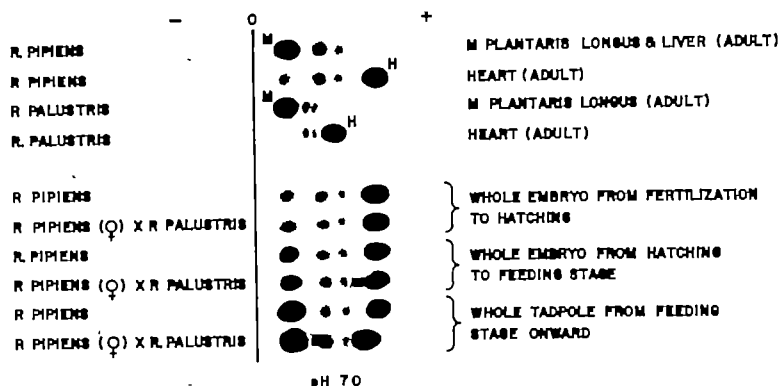


Fig. 4. Starch-gel electrophoretic patterns showing changes during development of hybrid and control embryos. Electrophoresis was done on starch gel with citrate phosphate buffer, at pH 7, 15 h. Under these conditions the hybrid *H* types do not separate, but the single band representing them is intermediate in mobility between the maternal and paternal *H* types.

in species of frogs and salamanders considered to be primitive on gross morphological grounds, the hynobiid salamanders, pipid frogs, and some discoglossid frogs, the usual three hybrids plus the pure *M* and *H* enzymes do occur (Salthe, unpublished). Since the muscle sub-units of all these species will hybridize *in vitro* with other kinds of LDH sub-units, this peculiar evolutionary change appears, from the data so far available, to have occurred in the heart sub-units only.

It would seem from these observations that the structural genes responsible for the production of *H* and *M* type sub-units have been evolving at different rates in some groups of vertebrates, the *H* sub-units undergoing greater modifications in some lower vertebrates than the *M* sub-units.

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## DDT IN HUMAN MILK

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IN 1960, Laug *et al.* investigated the DDT excretion-level in human milk in the United States<sup>1</sup>. These workers found DDT in 30 of 32 samples (94 per cent) of milk from women in Washington, D.C., with no recognized exposure to this insecticide other than that of the general population. The average concentration of DDT in these samples was 0.13 p.p.m. with a range of zero to 0.77 p.p.m. In 1962, tests on six individual samples and one pooled sample of human milk in California showed DDT-levels ranging from zero to 0.12 p.p.m. and DDE contents from none to 0.25 p.p.m.<sup>2</sup>. The highest total of DDT and DDE was 0.37 p.p.m. In Hungary, the milk of ten health nursing mothers was found to contain from 0.13–0.26 p.p.m. of DDT in whole milk or 3.3–6.6 p.p.m. in the lipid fraction<sup>3</sup>.

This article reports the excretion of DDT in human milk as measured in individual and pooled samples collected in several cities in the United States during 1960 and 1961. The work is a component part of a more comprehensive study. The complete project involves determination of DDT and DDE storage-levels in body fat and DDA excretion rates in urine for the general population and for specific environmental and occupational groups as well as measurement of DDT and DDE content of complete prepared meals. These surveys have been correlated by obtaining as many of the samples as possible from the same individuals. For example, a number of the subjects in the work recorded here also contributed meals from their households for study of DDT and DDE ingestion rates. The results of the other segments of the complete DDT study will be published separately<sup>4–6</sup>.

A total of 10 individual and 4 pooled samples of human milk were analysed. The individual samples were collected in Chicago, Illinois (4 donors), Wenatchee, Washington (5 donors), and Phoenix, Arizona (1 donor). Multiple samples representing time-periods varying from 2 to 7 months were collected from three of the donors in Chicago

to determine the temporal variation in the concentration of DDT and DDE. The pooled samples were collected by the Fitzsimmons Army Hospital in Denver, Colorado. These samples were pooled from many (13 to 44) donors who were dependants of military personnel from various parts of the United States.

The samples from Chicago and Denver were obtained as the precipitate formed by the treatment of 1–2 l. of formalized milk using the method of Murthy *et al.*<sup>7</sup>. The filtrates had been used for determination of strontium-90 levels. These precipitates were provided through the courtesy of the Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio. The samples from Wenatchee and Phoenix were frozen as whole milk in plastic bottles until analysis. The sample from Phoenix had been formalinized prior to freezing.

Complete histories of exposure to pesticides were obtained from the donors of the individual milk samples. Three of the donors from Wenatchee lived in houses immediately adjacent to orchards that had been periodically treated with DDT for many years. However, no DDT was applied to these orchards during the period of milk collection. None of the other donors had any known exposures to DDT other than those characteristic of the general population (that is, consumption of small amounts of residue in the diet and occasional household use of sprays or aerosols containing DDT). It was not practicable to obtain the histories of exposure to pesticides from the women who contributed to the four pooled samples.

Aliquots (usually 100 ml. of milk) were analysed by a modification of the Schechter-Haller spectrophotometric method<sup>8</sup>. Even though the limited accuracy of this method for quantities of DDT and DDE less than 5 µg was realized, all samples which produced recognizable Schechter-Haller colours were accepted at their calculated values. In all but three of the samples analysed, visible blue, red, or intermediate colour was produced in the Schechter-Haller reaction.

\* Present address: Community Pesticide Study Project, State of Washington Department of Health, Wenatchee, Washington.

Table 1. CONCENTRATION OF DDT AND DDE IN HUMAN MILK IN THE UNITED STATES

Type of sample	Location	Donor No.	Whole milk				Lipid fraction			DDE (% of total)
			Lipid content (%)	DDT (p.p.m.)	DDE as DDT (p.p.m.)	Total as DDT (p.p.m.)	DDT (p.p.m.)	DDE (p.p.m.)	Total as DDT (p.p.m.)	
Individual	Chicago, Illinois	1	3.63	0.09	0.09	0.18	2.5	2.2	4.9	50
		2	2.85	0.13	0.04	0.17	4.6	1.4	6.1	25
		3	2.83	0.09	0.02	0.11	3.3	0.7	4.0	20
		4	2.68	0.08	0.03	0.16	3.0	2.7	6.0	49
		Mean	2.99	0.10	0.06	0.16	3.3	1.7	5.3	36
Individual	Wenatchee, Washington	5	1.80	<0.01	<0.01	<0.02	<0.6	<0.6	<1.3	—
		6	5.81	0.07	0.01	0.08	1.2	0.2	1.4	14
		7	4.09	<0.01	<0.01	<0.02	<0.3	<0.3	<0.6	—
		8	3.29	<0.01	<0.01	<0.02	<0.3	<0.3	<0.6	—
		9	2.87	0.23	0.14	0.36	7.7	4.5	12.7	40
		Mean	3.57	0.06	0.03	0.09	2.0	1.2	3.3	39
Individual	Phoenix, Arizona	10	8.26	0.06	0.03	0.09	1.8	0.9	2.9	36
Individual	Mean for all individual samples		3.81	0.06	0.04	0.12	2.5	1.4	4.0	38
Pooled	Denver, Colorado, and other cities	Pool A	1.56	0.06	0.07	0.12	3.2	3.9	7.5	57
		Pool B	1.83	0.07	0.12	0.19	3.8	6.0	10.5	64
		Pool C	2.14	0.08	0.11	0.19	3.7	4.7	8.9	58
		Pool D	1.79	0.06	0.06	0.15	3.3	4.5	8.2	61
Pooled	Mean for all pooled samples		1.83	0.07	0.10	0.17	3.5	4.8	8.8	60

All the values given in this article for total DDT-derived material (DDT plus DDE) have been expressed in terms of DDT equivalent. Numerically this is accomplished by multiplying DDE concentration by the factor 1.11. Values for DDE expressed as a fraction of total DDT-derived material were determined by use of the following formula:

$$\text{DDE (\% of total)} = \frac{\text{DDE (p.p.m. as DDT equiv.)}}{\text{DDE (p.p.m. as DDT equiv.)} + \text{DDT (p.p.m.)}}$$

Recent investigations have indicated that gas chromatographic techniques are able to detect smaller quantities of DDT and DDE and to distinguish more quantitatively the various isomers and ratios of isomers than is the colorimetric procedure<sup>8</sup>. Nevertheless, the Schechter-Haller method has been used for so long that it is important for purposes of comparison with earlier investigations for some additional data to be collected in this way, at least until there is more information based on the newer techniques<sup>9</sup>.

The concentrations of DDT and DDE in these samples of human milk are shown in Table 1. Whole milk contained an average of 0.08 p.p.m. DDT and 0.04 p.p.m. DDE based on the individual samples and 0.07 p.p.m. DDT and 0.10 p.p.m. DDE based on the pooled samples. The lipid fraction contained an average of 2.5 p.p.m. DDT and 1.4 p.p.m. DDE based on the individual samples and 3.5 p.p.m. DDT and 4.8 p.p.m. DDE based on the pooled samples. There is good correspondence between the DDT levels for these two types of samples; however, the DDE levels correlate less closely.

Data collected in the survey recorded here on the variation in the excretory level of DDT and DDE in

human milk over a period of time is shown in Table 2. It can be seen that there was little change in the excretory levels of DDT and DDE for each individual subject over the 2-7-month periods investigated.

The trend in the concentration of DDT in human milk in the United States between 1950 and 1960-61 is illustrated in Table 3. The mean DDT concentration was 0.13 in 1950 as compared with 0.07-0.08 p.p.m. in 1960-61. A comparison of the frequency distribution shows that there were fewer samples at the higher concentration levels in the later survey. Results of the earlier survey were not reported on the basis of lipid fraction nor was the DDE content of the milk determined. The range of values in the work reported here for both DDT (<0.01-0.22 p.p.m.) and DDE (<0.01-0.14 p.p.m.) is comparable with that for samples collected in California in 1962 (DDT, zero to 0.12 p.p.m.; DDE, zero to 0.25 p.p.m.)<sup>11</sup>.

Table 3. TREND IN THE CONCENTRATION OF DDT IN HUMAN MILK IN THE UNITED STATES

Range of values (p.p.m. of DDT)	Percentage of samples		
	1950 (Laug et al.)	1960-61 (this paper)	
		Individual	Pooled
<0.01	6	30	0
0.01-0.05	9	0	25
0.06-0.10	25	50	75
0.11-0.15	41	10	0
0.16-0.20	6	0	0
0.21-0.30	6	10	0
0.31-0.40	3	0	0
0.41-0.50	0	0	0
>0.50	3	0	0
Total No. of samples	33	10	4
Mean concentration (p.p.m.)	0.13	0.08	0.07

It is of interest to compare the DDT and DDE levels in the fat moiety of human milk samples with the storage levels of these compounds in human body fat. In a recent investigation using analytical techniques comparable with those used in the work recorded here, storage levels of 6.8 p.p.m. DDT and 13.5 p.p.m. DDE in the extractable lipid fraction of human body fat taken from persons in the general population of the United States were found<sup>8</sup>. Thus, although concentrations in both tissues are of the same order of magnitude, DDT, and particularly DDE, levels appear to be somewhat lower in the fat moiety of human milk than in the extractable lipid fraction of human body fat. In the body fat, DDE represented 68 per cent of the total DDT-derived material, while in the milk DDE constituted 38 per cent (based on individual samples) or

Table 2. TEMPORAL VARIATION IN CONCENTRATION OF DDT AND DDE IN HUMAN MILK

	Donor I		Donor II		Donor III	
	DDT (p.p.m.)	DDE (p.p.m.)	DDT (p.p.m.)	DDE (p.p.m.)	DDT (p.p.m.)	DDE (p.p.m.)
Monthly replicates	0.08	0.07	0.09	0.03	0.13	0.04
	0.07	0.06	0.10	0.03	0.14	0.03
	0.04	0.03	0.10	0.04		
	0.05	0.06	0.09	0.04		
	0.06	0.06	0.12	0.16		
	0.03	0.04	0.12	0.07		
			0.12	0.09		
Mean	0.06	0.06	0.11	0.07	0.14	0.04

60 per cent (based on pooled samples) of the total. The DDT levels in human milk from Hungary (3.3–6.8 p.p.m. in the lipid fraction)<sup>3</sup> are associated with an average DDT storage level in human body fat in that country of 5.7 p.p.m.<sup>10</sup>

Apparently, Hungary is the only country other than the United States for which values for DDT excretion in human milk have been reported. The values from Hungary for DDT in human milk (0.13–0.26 p.p.m. in whole milk) appear to be somewhat higher than the levels reported here or those reported by West<sup>11</sup> for the United States. In comparing DDT storage levels in human body fat from various countries, Quinby *et al.*<sup>3</sup> concluded that the level in Hungary equalled or exceeded that in the United States, while the concentrations were lower for other countries (Canada, Britain, France, and Western Germany) for which data were available.

The pharmacodynamic relationships of DDT intake, storage and excretion are incompletely understood, even in physiological situations in which the complicating factors involved in lactation do not occur. However, it can be calculated that a daily output of 700 g of milk containing 0.07 p.p.m. of DDT would account for a total daily excretion of about 0.05 mg of DDT by this route. This amount would account for about 125 per cent of the estimated daily dietary intake of DDT for the general population of this country (0.04 mg/person/day)<sup>4</sup>. Another fraction of the intake is excreted in the urine as DDA. The finding that the daily DDT excretion for lactating women may be slightly greater than the average daily DDT intake of the general population may, at least partly, be accounted for by the fact that during lactation women may eat greater than average amounts of food and/or may mobilize stored body fat. The constancy of the DDT excretion-level in milk for individual donors over the period of investigation may indicate that the relatively large amount of DDT stored in body fat serves as a 'buffer' to prevent large daily changes in excretion associated with possible daily changes in DDT intake.

Cows' milk also contains DDT; and here, too, as in the case of human milk, the average concentration apparently has decreased over the years. Comparable values for surveys of market milk showed that 67 per cent of samples were positive for one or more pesticides in 1955<sup>12</sup>, but only 33 per cent were positive in 1958<sup>13</sup>. Zweig *et al.*<sup>14</sup> have reported that cattle fed a diet containing 0.5 p.p.m. of DDT excreted less than 0.01 p.p.m. of the insecticide in their milk. However, at levels of 1, 2, 3, and 5 p.p.m. of added DDT, detectable residues of DDT, proportional to the level of contamination in the feed, were found in the milk of all animals. The cows fed DDT at the lowest level (0.5 p.p.m.) ate an average of 20 kg feed/day. Thus, their daily dose of DDT was 10 mg/day. Assuming that the average weight of these animals was 400 kg, it may be calculated that the dosage was 0.025 mg/kg/day. This dosage resulted in a DDT concentration of <0.01 p.p.m. in the milk of the cows. Even if these cows averaged 0.01 p.p.m. and excreted 15 l. of milk a day, the total DDT excretion in the milk would be 0.15 mg/day or about 1.5 per cent of the dose. Calculations of this sort made for a number of other investigations in which cows have been fed DDT-contaminated diets give results of the same order of magnitude, excretion rates ranging from 1.8 to 29.8 per cent of the daily dose<sup>15</sup>. There seems to be a tendency for a larger fraction of DDT intake to be excreted in the milk at higher dosage rates.

It is interesting to compare these values for the cow with those already given here for women. At a DDT dosage level of about 0.025 mg/kg/day, the cow excretes DDT in milk at a concentration of less than 0.01 p.p.m. and eliminates only about 1.5 per cent of the daily DDT intake in this way. However, at a DDT dosage level of about 0.0005 mg/kg/day, lactating women excrete DDT in milk at a mean concentration of 0.07–0.08 p.p.m. and eliminate about 125 per cent of the estimated average daily DDT

intake in this way. Thus, although the calculations for the cow are based on a higher daily intake of DDT, the cow excreted less DDT in the milk both on a concentration basis and calculated as a fraction of daily intake than did lactating women.

Turning now to the question of safety, it appears, from study of available cases of accidental or intentional ingestion of the compound, that the single oral dose of DDT that will cause poisoning in man is about 10 mg/kg<sup>16</sup>. The repeated dose of DDT that is harmful to man is unknown, but studies in volunteers<sup>17,18</sup> and in men with massive occupational exposure<sup>19</sup> show that adults can withstand without detectable injury 200 times the average dietary level of 0.18 mg/man/day that prevailed in 1953<sup>20</sup>. Furthermore, the general dietary level of DDT has now decreased to about 0.03\* (ref. 21) or 0.04 mg/man/day<sup>4</sup>. This amount of DDT corresponds to a mean concentration of about 0.02 p.p.m. DDT in the total wet diet of adults in the United States<sup>4</sup>.

The results presented here indicate that babies having human milk as their sole food source are exposed to a somewhat higher dietary concentration of DDT (0.07–0.08 p.p.m.) than is the general population (about 0.02 p.p.m.). Furthermore, the food consumption per unit of body-weight is higher for infants than for adults. If a 5 kg (11 lb.) infant consumed 0.7 l. of milk daily containing DDT at an average concentration of 0.08 p.p.m., the resulting dosage would be 0.0112 mg/kg/day. This value may be compared with the average adult dosage of 0.0005 mg/kg. Also, there is a considerable amount of evidence that young animals, including foetal ones, are somewhat more susceptible than adults to many chemicals including DDT<sup>22</sup>; however, the difference is only a few-fold. On the other hand, there is one report that a single oral dose of DDT was less toxic to young (2.4 h old) than to adult rats<sup>23</sup>.

Thus, the information at present available for man indicates that there is, for adults, a large safety factor associated with the use of DDT. For infants, the safety factor appears to be smaller. Continued research and surveillance of the exposed population should be carried out with particular attention being given to the health of infants to ensure that undetected toxic effects do not follow long-continued, low-level exposure to DDT and other persistent pesticides.

\* This value was not given in Mills's paper but can be calculated from his data.

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# PROTEIN PRODUCTION BY LYMPH NODE CELLS OF RATS STIMULATED WITH PERTUSSIS VACCINE

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RECENT investigations have emphasized that lymphocytes are of major importance in the initiation of the primary immune response to certain antigens<sup>1,2</sup>. Although it has been reported that small lymphocytes enlarge after antigenic stimulation<sup>3,4</sup>, and that larger lymphocytes in germinal centres proliferate and produce certain immunoglobulins<sup>4,5</sup>, little is known of the protein metabolism of the various sizes of lymphocytes during such responses.

A group of eight adult male Lewis rats was given  $3.2 \times 10^6$  (0.05 c.c.) inactivated pertussis cells (Lilly, V-1035 pertussis vaccine, fluid) subcutaneously in each hind footpad. Eight control animals received 0.05 c.c. of normal saline. Six hours after the footpad injection, both experimental and control animals received 6  $\mu$ c./g body-weight of tritiated methionine (sp. act. 191 mc./mole) intravenously. Experimental and corresponding control rats were killed at 1, 6, 12, 18, 24, 36, 60 and 80 h after injection of tritiated methionine. One popliteal lymph node was minced in rat serum for smears while the contralateral popliteal node was fixed in 10 per cent formalin for 3 $\mu$  paraffin sections. Autoradiographs of both smears and sections were made by techniques previously described<sup>6</sup> and were exposed for 2, 5 and 8 weeks.

A second group of adult male Lewis rats was antigenically stimulated and killed as described for the aforementioned animals. Both popliteal nodes were colorimetrically analysed for deoxyribonucleic acid (DNA)<sup>7</sup> and ribonucleic acid (RNA)<sup>8</sup>.

Following stimulation with pertussis vaccine, the popliteal nodes rapidly increased in weight. Quantification of DNA (Fig. 1) in the enlarged nodes indicated that this increase in weight was due to cell proliferation. Differential cell counts on smear preparations showed that this proliferation occurred principally in the lymphocytic series. There was also a slight rise in the number of reticular cells present in the popliteal nodes, but no appreciable change was observed in the plasma cell series.

Protein metabolism was analysed in each cell series and category by studying autoradiographs of node smears. Both the percentage of labelled cells and the grain density were determined. The most active cell type in metabolizing radioactive amino-acid was the large lymphocyte

(Table 1, Fig. 2). The stimulated large lymphocytes were much more heavily labelled per unit area than were the controls. Part of the labelling of stimulated large lymphocytes no doubt reflected only the normally occurring increases in cytoplasmic constituents prior to division. However, since control large lymphocytes are also known to be dividing<sup>9</sup>, the difference in labelling between stimulated and control cells suggested increased protein production and turnover in the stimulated cells. Further evidence of increased turnover of protein was seen from the decrease in label with reference to time (Fig. 2), which was greater in the stimulated large lymphocytes than in the corresponding controls. Many of the heavily labelled large lymphocytes in the stimulated animals appeared more basophilic than the large lymphocytes in control animals. This histochemical reaction also suggested increased protein metabolism. While the present investigation alone does not distinguish between a non-specific increase in protein metabolism in large lymphocytes and the production of specific immune substances, the work of others<sup>4,6</sup> has implicated this cell type in the production of immunoglobulins.

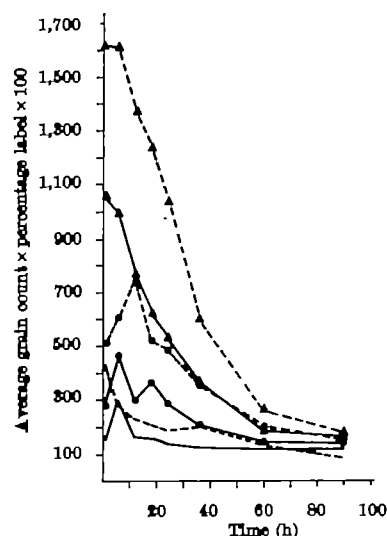


Fig. 2. The data in Table 1 are combined to show labelling patterns of pertussis-stimulated and control lymphocytes at intervals after a single injection of tritiated-methionine. Small lymphocytes: —, control; ---, experimental. Medium: ●—, control; ●---, experimental. Large: ▲—, control; ▲---, experimental.

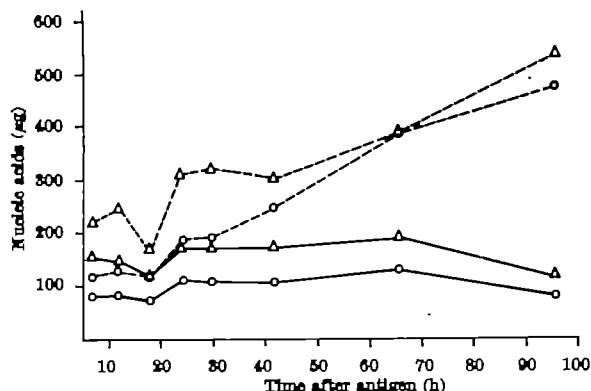


Fig. 1. Colorimetric methods were used to show the quantity of DNA and RNA in popliteal lymph nodes at intervals after a single footpad injection of pertussis vaccine (experimental) or saline (control). Δ, DNA; ○, RNA; ---, experimental; —, control.

The second highest labelling intensities with tritiated-methionine were seen in the medium-sized lymphocytes (Table 1). Again it was noted that the stimulated cells exhibited more activity than the controls. Like the large lymphocytes, the greater labelling intensity in the stimulated medium lymphocytes may have been due in part to the synthesis of cellular constituents prior to division. However, previous investigations in this laboratory have shown that even in non-stimulated rats medium lymphocytes turn over protein more rapidly than may be expected from mitosis alone<sup>11</sup>, and since immune substances have been shown to be produced in stimulated medium cells<sup>4</sup>, it seems probable that the increased labelling

Table 1. LABELLING PATTERNS OF LYMPHOCYTES IN THE POPLITEAL NODE

Hours post H <sup>3</sup> -Meth.	Large lymphocytes		Experimental Medium lymphocytes		Small lymphocytes	
	Label %	Average grains	Label %	Average grains	Label %	Average grains
1	100	16.2	94	5.4	77	5.8
6	99	16.3	94	6.4	80	2.6
12	87	16.0	88	8.8	64	2.6
18	96	12.9	89	5.9	57	3.7
24	87	12.3	83	6.0	54	3.5
36	69	8.7	76	4.7	58	3.5
60	59	4.4	50	4.1	38	3.4
90	51	3.5	43	3.5	30	2.8
Controls						
1	90	11.7	78	3.9	53	2.9
6	92	10.8	85	5.5	74	4.0
12	77	10.0	70	4.3	53	3.1
18	74	8.4	78	4.7	47	3.4
24	75	6.6	73	4.0	46	3.0
36	67	5.4	58	3.5	39	3.1
60	38	4.9	37	4.0	40	2.9

The percentage labelled and average grain counts of all sizes of lymphocytes in stimulated and non-stimulated popliteal nodes are shown. Injections of pertussis vaccine (experimentals) or saline (controls) were made into the footpads 6 h before isotope administration. Other experiments have shown that 100 per cent of control lymphocytes may be labelled 1 h after isotope if a sufficient quantity of radioactivity is given.

reflected such a synthesis. The decrease in radioactivity was greater in stimulated medium lymphocytes than in the controls (Fig. 2), and this too suggested a turnover and/or secretion of proteinaceous substances early in the immune response.

The small lymphocytes labelled with the least intensity of all lymphocytic cells. However, as in the large and medium lymphocytes, the stimulated small lymphocytes labelled with greater intensity than the non-stimulated. Fig. 2 shows that after the first 12 h, non-stimulated small lymphocytes maintain a relatively constant labelling intensity. This is most probably related to the fact that non-stimulated small lymphocytes do not divide<sup>8</sup>. The slight rise in labelling seen in the first 6 h may be the result of heavily labelled medium and large lymphocytes dividing to form small cells. Fig. 2 also shows that, unlike control small lymphocytes, stimulated small cells decreased in labelling intensity during the first 18 h. This may have been the result of a decrease in the number of labelled small lymphocytes as these stimulated cells enlarged to form medium lymphocytes<sup>1-3,12</sup>.

Fig. 2 further demonstrates that, concurrent with the drop in the labelling of small lymphocytes, there was an increase in the labelling intensity of medium cells. This probably was due both to a division of labelled large cells to form medium-sized lymphocytes, and also to the enlargement of some heavily labelled small cells.

Nucleic acid analysis supported the autoradiographic evidence for increased protein metabolism in stimulated lymph node cells. Fig. 1 shows increases in both the amount of nodal RNA and the RNA/DNA ratio per cell.

The results of the labelling patterns of the reticular cells are shown in Fig. 3. The reticular cell series of the stimulated nodes showed only a slight increase in number and a moderate increase in protein metabolism. The decrease

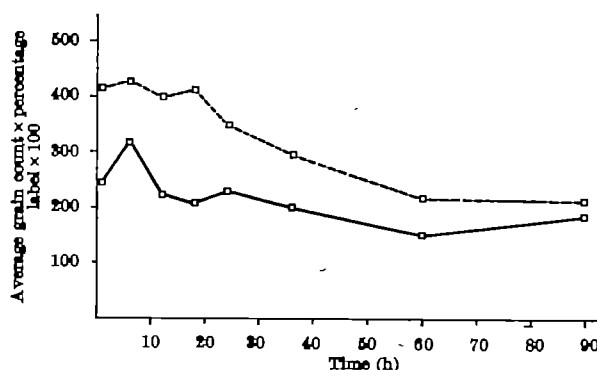


Fig. 2. Labelling intensity of reticular cells in experimental and control popliteal lymph nodes is shown at intervals following the administration of tritiated-methionine. —, Control; ---, experimental

in some label throughout the experiment occurred in both experimental and control nodes. The moderate turnover of label as compared with the marked turnover in lymphoid cells suggests that reticular cells do not exhibit markedly increased metabolism following antigenic stimulation. This is not intended to minimize the role of the reticular cell in the immune response but does support the evidence that they do not function as stem cells<sup>10</sup> and also argues against these cells being primary producers of immune substances.

It is concluded that rat lymph nodes stimulated with pertussis vaccine exhibited a lymphocytosis without a concurrent plasmacytosis during the initiation of the primary immune response. During the lymphocytosis the stimulated lymphocytes increased in both RNA and protein synthesis. Because reticular cells show only a slight increase in number and a moderate increase in protein metabolism during the initial immune response, they do not appear to behave as stem cells or as producers of immunoproteins.

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## ACTIONS OF SOME COAGULANT SNAKE VENOMS ON BLOOD PLATELETS

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RECENTLY, Nahas, Denson and Macfarlane<sup>1</sup> reported details of the coagulant actions of eight viper venoms. We have examined effects of six of these on washed human blood platelets. These actions on platelets were compared with those of thrombin, which is believed to be a principal agent in the formation and consolidation of platelet haemostatic plugs, and of thrombi, *in vivo*<sup>2</sup>.

The venoms used, and their coagulant properties, are listed in Table 1. The Russell viper venom was a commer-

cial preparation ('Stypven', Burroughs Wellcome). Other venoms were the crude preparations used in the coagulation studies<sup>1</sup>. Stock solutions of 1–2 mg/ml. were made in physiological saline solution buffered with tris (tris-hydroxymethyl-aminomethane) to pH 7.4; these were stored at –20° C and thawed and diluted as required. We also obtained a purified coagulant fraction of *A. rhodostoma* venom from Dr. M. P. Esnouf, as a solution of 120 µg/ml. in saline. This substance has a thrombin-like action on



Table 1. VENOMS STUDIED AND COAGULANT ACTIVITY

Venom	Factor X activation*	Thrombin- like activity*	Acceleration by platelets†
Russell's viper	++++	0	+++
<i>Bothrops atrox</i>	+++	+	+++
<i>Bothrops jararaca</i>	++	++	++
<i>Echis colorata</i>	++	0	+
<i>Anelastrolon rhodostoma</i>	+	++++	+
<i>Trimeresurus purpuromaculata</i>	0	+	±

\* From reference 1.

† Of clotting of cell-free plasma.

fibrinogen, splitting off fibrinopeptides A and B, as well as a number of peptides not produced by thrombin<sup>3</sup>. Of the other venoms, that of the Russell viper contains an activator of factor X (ref. 4); that of *Bothrops jararaca* has been found to clot fibrinogen directly, but to remove only fibrinopeptide A in doing so<sup>5</sup>.

We determined fibrinogen clotting times using purified human fibrinogen, 98–99 per cent clottable<sup>6</sup>, 25 mg/ml. in saline, plus 0.025 M calcium chloride solution (0.1 ml. of each) and enough *tris*-saline to give a final volume of 0.5 ml. after addition of the venom solution tested and any other additions. The mixtures were incubated at 37° C, and coagulation times recorded. Plasma clotting times were determined by substituting pooled normal human plasma (which had been stored for some months at -20° C) for fibrinogen in this test system. Calcium was sometimes omitted. Inhibitors tested included 'Hirudex' (crude extract of *Hirudo medicinalis*; Laboratoires ANA, Paris) and heparin ('Liquemine', Hoffmann-La Roche, 5,000 units/ml.). Platelets to be added in coagulation tests were first washed as follows, and suspended in *tris*-saline buffer to a concentration of  $1-5 \times 10^8$  per ml.; 0.1-ml. aliquots of such suspensions were used.

Platelets were obtained as previously described<sup>7</sup> and washed three times after isolation from plasma: twice with 0.9 per cent sodium chloride (9 parts) plus 3.6 per cent trisodium citrate (1 part); once with *tris*-saline buffer containing 0.5 per cent glucose. They were resuspended in a minimum volume of *tris*-saline-glucose, in concentrations of 80–100 mg of protein ( $4-5 \times 10^8$  platelets) per ml.: protein was determined by the biuret method<sup>8</sup>.

For venom investigations, 0.4-ml. aliquots of such suspensions were placed in glass tubes; unless otherwise stated 5  $\mu$ moles of calcium chloride solution were added, followed by the substance to be tested, with sufficient *tris*-saline to bring the final volume to 1 ml. Inhibitors, when tested, were included in this volume. Tubes were incubated for 15 min at 37° C and the extent of macroscopic platelet aggregation, and of contraction of the formed aggregates, were recorded. Then 0.2 ml. of a solution of EDTA (disodium ethylenediamine tetraacetate, 0.077 M) was added, the tubes were placed in crushed ice, and their contents diluted to about 10 ml. with *tris*-saline. After centrifugation at 1,500g for 20 min in the cold the supernatants were separated, the platelets resuspended in a further 8 ml. of *tris*-saline, and both fractions extracted with 2 per cent (final concentration) perchloric acid in the cold. After further centrifugation the supernatants were neutralized with potassium hydroxide, and their ultra-violet absorptions determined at 260 m $\mu$  in a Beckman DU spectrophotometer. The proportion of the total absorbing material in each supernatant fraction was calculated.

In experiments in which it was desired to exclude ionized calcium from the incubation mixtures, the addition of calcium chloride was omitted. In its place were added 2  $\mu$ moles of the calcium-chelator EGTA (ethyleneglycol diaminoethyl tetraacetate, Geigy; 0.1 M solution of the sodium salt, pH 7.4).

The results of coagulation tests performed with the venom were compatible with the patterns of their coagulant activities shown in Table 1. The degree of acceleration of plasma clotting by the addition of platelets was

proportional to the amount of factor X activation produced by the venom tested.

In control platelet experiments, platelets incubated with calcium and buffer showed no macroscopic changes but lost some 20–30 per cent of their ultra-violet-absorbing contents to the supernatant. Thrombin (Hoffmann-La Roche; 63 N.I.H. units/mg), at a final concentration of 10 units/ml. caused aggregation within 1 min; contraction of aggregates followed within 2 min and was complete within 5. 60–70 per cent of total ultra-violet-absorbing material was in the supernatant. Spectroscopic investigations have established that this material is mainly adenine nucleotides<sup>9</sup>.

Table 2 shows the effects of the venoms studied at various concentrations. That of *T. purpuromaculata* was the only one the action of which was comparable to that of thrombin in all respects (venom concentrations of 20  $\mu$ g/ml. and above). The two *Bothrops* venoms caused platelet aggregation and nucleotide release without contraction, up to 80  $\mu$ g/ml. *E. colorata* and *A. rhodostoma* venoms caused slight aggregation and release only at 200  $\mu$ g/ml. The pure *A. rhodostoma* coagulant was without effect up to 24  $\mu$ g/ml. (equivalent to approximately 250  $\mu$ g crude venom<sup>3</sup>).

Russell's viper venom caused platelet aggregation only at the lower concentrations tested (5–20  $\mu$ g/ml.), but nucleotide release was observed with all concentrations tested. At 200  $\mu$ g/ml. its release effect was equivalent to that of thrombin. Unaggregated platelets which had been treated with Russell's viper venom were aggregated within 20 sec of the addition to them of thrombin (20 units/ml.) or of a saline extract of platelet protein (12 mg protein/ml.): this extract had no demonstrable coagulant activity. Such platelets were also aggregated, but less rapidly, by the addition of serum or fibrinogen, or of plasma in the presence of 'Hirudex'.

Calcium dependence of three venom actions was tested by replacing calcium in the incubation mixtures with EGTA. This inhibits platelet aggregation by thrombin almost totally, but nucleotide release only partially. The effects of *T. purpuromaculata* venom were similarly altered, but all actions of the *Bothrops* venoms were inhibited in the presence of the chelator (Table 3).

Table 2. ACTIONS OF VENOMS ON PLATELETS

Venom	Concentration $\mu$ g/ml.	Aggregation	Contraction of aggregates	% Nucleotide released
Russell's viper	5	++	0	49.9
	20	++	0	56.7
	100	0	0	57.3
	200	0	0	58.4
<i>B. atrox</i>	40	++	0	57.2
	80	+++	±	59.9
<i>B. jararaca</i>	40	+	0	49.4
	80	+++	0	48.6
<i>E. colorata</i>	40	0	0	29.4
	200	±	0	37.7
<i>A. rhodostoma</i>	40	0	0	28.7
	200	+	0	50.0
<i>A. rhodostoma</i> Pure coagulant	12	0	0	29.6
	24	0	0	29.3
<i>T. purpuromaculata</i>	10	+++	+	43.7
	20	++++	+++	53.3
	40	++++	++++	62.9
	80	++++	++++	73.5
Thrombin	10 units/ml.	++++	++++	60.3–69.5
Buffer	—	0	0	21.2–29.7

Table 3. CALCIUM AND VENOM ACTIONS

Venom	Platelet effect (80 $\mu$ g/ml.)			Plasma clotting, (sec) (20 $\mu$ g/ml.)	
	Aggregation	Contraction	% Nucleotide release	With CaCl <sub>2</sub>	No CaCl <sub>2</sub>
<i>B. atrox</i> + CaCl <sub>2</sub>	+++	±	59.9	41.3	—
+ EGTA	0	0	29.8	—	64.0
<i>B. jararaca</i> + CaCl <sub>2</sub>	+++	0	43.6	35.0	—
+ EGTA	0	0	25.0	—	65
<i>T. purpuromaculata</i>	+++	+++	73.5	63.4	—
+ CaCl <sub>2</sub>	+	0	60.8	—	93.2
+ EGTA	+	0	60.8	—	93.2
Thrombin 10 units/ml.	+++	+++	60.3–69.5	—	—
+ CaCl <sub>2</sub>	+++	+++	60.3–69.5	—	—
+ EGTA	±	0	43.8–57.0	—	—

Table 4. ACTIVITY OF *T. purpureomaculata* VENOM

Venom preparation (originally 400 µg/ml.)	Amount (ml.)	Platelet effects		% nucleotide release	Fibrinogen- clotting (sec)
		Aggre- gation	Contra- ction		
Intact	0.1	++++	++++	60.2	167
Dialysed	0.1	++++	++	48.9	165
	0.2	++++	++++	57.1	—
Dialysis buffer	0.2	0	0	20.8	>1,000 *
	0.6	0	0	26.6	—
Heated 56° C, 30 min	0.1	+++	+	58.7	162
	0.2	++++	++	62.9	—
Heated 100° C, 10 min	0.1	0	0	31.6	230
	0.2	+	0	39.5	—
+ 'Hirudex' 0.1 ml.	0.1	0	0	46.3	>1,000 *
+ Heparin 500 units	0.1	+	0	49.2	>1,000 †
Thrombin 10 units		++++	+++	60.3-69.5	<10
+ 'Hirudex' 0.1 ml.		0	0	35.3	>1,000
+ Heparin 500 units		0	0	23.1	>1,000

\* Mixture then clotted by 10 units thrombin: &lt;10 sec.

† Mixture then clotted by 10 units thrombin, 60 sec.

Further experiments were performed to characterize the active substance in *T. purpureomaculata* venom (Table 4). It proved to be non-dialysable (48 h against 100 volumes of Tris buffer, pH 7.4; or against 2 vol. of buffer when the buffer was tested for activity). It was not inactivated by heating solutions to 56° C for 30 min, but most activity was lost after 10 min at 100° C. Its activity has been found quite stable during storage of solutions for at least 2 months at -20° C and at least one month at 4° C. Its action was inhibited, but to a lesser extent than that of thrombin, by 'Hirudex' (0.1 ml./40 µg venom) and heparin (500 units/40 µg). The clotting activities of the variously treated venom solutions were similar to their activities towards platelets. Absence of residual thrombin-inhibiting activity in the venom-'Hirudex' mixture suggests that the venom, like thrombin, reacts with hirudin to form an inactive product.

We conclude, tentatively, that the coagulant and platelet-altering substance in *T. purpureomaculata* venom are the same. This factor differs from thrombin in the rate of its action on fibrinogen and its greater heat-stability. It is, on the other hand, similarly calcium-dependent in its action on platelets and is similarly inhibited by hirudin and by heparin.

In these studies the venom coagulant (*A. rhodostoma*) which we knew reproduced the effect of thrombin on fibrinogen failed to alter platelets. Venoms with a partial proteolytic effect on fibrinogen (*B. jararaca*; ? *B. atrox*) only partially reproduced thrombin's action on platelets. In contrast, *T. purpureomaculata* venom had a platelet effect quite disproportionate to the rate of its coagulant activity. Now, this venom may prove to have the same proteolytic actions on fibrinogen as does thrombin, but these must proceed so much less rapidly that other explanations need to be sought for the similarity of thrombin and venom actions on platelets. Thrombin is known to release some amino-acids and a few small peptides from fibrinogen, as well as the well-defined fibrin-peptides A and B (ref. 10). Other potential substrates of thrombin include the coagulation factors V (ref. 11), VIII (ref. 12) and XIII (ref. 13). Of these,

factors V (ref. 14) and XIII (ref. 15), as well as fibrinogen, are closely associated with platelets. One of these or a similar protein may prove to be the substrate of similar actions of thrombin and this venom coagulant.

Certainly, these observations make it difficult to entertain any longer the concept that thrombin acts on the fibrinogen of platelets just as it acts on plasma fibrinogen to induce platelet metamorphosis<sup>16</sup>. Other evidence against this concept has recently been brought forward<sup>17,18</sup>.

The action of Russell's viper venom in platelets deserves further mention. In this case, the release of platelet nucleotides is dissociated from platelet aggregation, even in the presence of calcium, when high concentrations of the venom are used. It has been shown that aggregation of platelets by thrombin is mediated by the adenosine diphosphate (ADP) it releases<sup>19</sup>; but aggregation by ADP alone requires a plasma co-factor<sup>20</sup>, whereas aggregation by thrombin does not, as these experiments illustrate. Platelets which have been exposed to Russell's viper venom retain their ability to aggregate when thrombin is added to them. The venom therefore does not destroy any substance essential for aggregation. A possible conclusion is that thrombin releases ADP and also a substance which is an essential co-factor for aggregation; while Russell viper venom releases nucleotide alone. Our experiments suggest that such a co-factor is present in the soluble fraction of platelet proteins, and in small amounts in plasma, in serum and in preparations of fibrinogen. It has not yet been identified.

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## ENVIRONMENTAL INDUCTION OF HERITABLE CHANGES IN *Nicotiana rustica*

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EVER since Johannsen<sup>1</sup> carried out his work with dwarf beans (*Phaseolus vulgaris*) it has been realized that the phenotype expressed by any given individual results from the joint action of its genotype and its environment. Normally the external environment has no permanent effect on the phenotypic expression of a lineage.

but there are two exceptions to this general rule, namely induced mutation and conditioning. Both of these are phenotypic alterations brought about by a specific set of environmental conditions and persisting even after the removal of these conditions. Induced chromosomal mutation occurs at random, however, whereas conditioning,

like most induced extrachromosomal mutations, is a non-random process which has been compared by Mather<sup>2</sup> to the phenomenon of paramutation found in maize by Brink<sup>3</sup>.

Since this article will be concerned primarily with conditioning, it is necessary to define this term at the outset. Conditioning can be regarded as the directed alteration in the normal development of the phenotype of an individual resulting from the action of external agents. Such an alteration may be temporary, that is, a phenocopy lasting only for the lifetime of the individual concerned, or it may be more permanent, the effect being transmitted from parent to offspring. Such a more permanent effect may nevertheless show a gradual diminution in its intensity over successive generations such as occurs, for example, with dauermodifications<sup>4</sup>. It may, on the other hand, remain undiminished even after several cycles of sexual reproduction. The effects observed by Highkin<sup>5</sup> when he grew inbred lines of peas under differing temperature régimes come into this last category, as do those recorded by Durrant<sup>6</sup> and Durrant and Tyson<sup>7</sup>, who used all possible

fertilizers for at least the preceding ten years. The experiment was laid out in a randomized block design with six replicates, and supplementary light was provided. Rosette diameter of the plants was measured at fortnightly intervals, while flowering time in days from an arbitrary date and final height in inches were also recorded. Analysis of the data showed that overall the most significant feature was the effect of potassium in increasing rosette diameter. Potassium also tended to promote flowering, but this effect just failed to reach significance at the 5 per cent level of probability, no doubt because of the high block interactions which were used as the estimate of experimental error. No significant differences occurred between the treatment groups in respect of final height. Plants grown on these different fertilizer treatments will be referred to collectively as the  $C_0$  generation. For each variety and within each treatment line three plants were chosen at random and selfed. The seed from these plants was harvested and used to grow out the  $C_1$  generation (that is, one generation removed from the original treatment) during the summer of 1963 (Fig. 1).

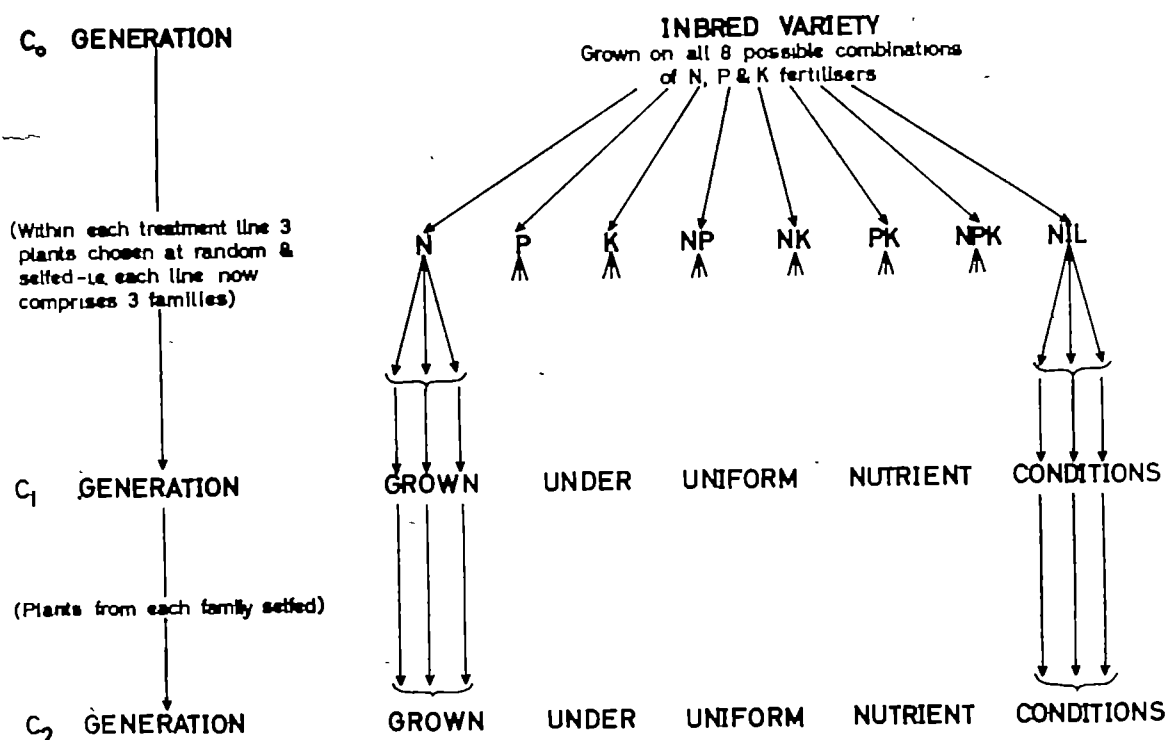


Fig. 1. Diagrammatic representation of the experimental procedure

combinations of nitrogen, phosphorus and potassium fertilizers to induce heritable changes in an inbred line of flax. Durrant was the first to emphasize the genetical significance of this phenomenon.

Following an earlier unsuccessful attempt to condition *Arabidopsis thaliana*<sup>8</sup>, an experiment was conducted to see whether heritable changes could be induced in inbred varieties of *Nicotiana rustica* using different combinations of fertilizer treatments. The varieties chosen were inbred when they were first obtained by Prof. Mather in the early 1940's<sup>9</sup>, and since that time they have been maintained by strict selfing for approximately twenty further generations. There can therefore be no doubt as to the inbred nature of the material involved. The first experiment was conducted in a glasshouse during the winter of 1962-63 using three varieties of *N. rustica*. These varieties were grown on soil taken from a set of lined plots which had been treated with the eight different combinations of nitrogen, phosphorus and potassium

The  $C_1$  generation was grown on an experimental area which had received a uniform dressing of complete fertilizer applied at standard agricultural rates (about 15 cwt./acre). Basically the experiment was arranged in plots within a randomized block design. Each plot contained an equal number of plants from each of the originally treated lines, the varieties being allocated at random to the plots and plants at random to positions within each plot. Only two varieties were grown in this experiment because the seed of the remaining variety failed to germinate satisfactorily. Seed from the various lines was sown in appropriately numbered paper pots containing John Innes No. 1 compost mixture. Two seeds were sown in each pot and after approximately a fortnight the seedlings were thinned out, leaving only the centre plant in each pot. This procedure was adopted in order to minimize the risk of conscious selection. Afterwards the plants were planted in the experimental field, still in their paper pots, in rows set 27 in. apart with 12 in.

between plants within rows. Again the characters recorded were plant diameter with time, flowering time and final height.

It became apparent from this experiment that one of the varieties had not been permanently conditioned by the original glasshouse treatment, for the differences seen in the  $O_2$  generation were no longer present in  $O_1$ . However, on analysis the remaining variety (V.16) did show highly significant differences between the treated lines for both flowering time and final height (Table 1). These proved to be significant even when tested against the differences between families within lines, that is, differences between the progeny of individual  $O_2$  plants accumulated over all 8 treatments. An orthogonal breakdown of the sum of squares between lines reveals potassium to have the most significant effect in promoting flowering. For height, on the other hand, practically all the comparisons are significant. If the treatments applied in this variety we would further expect a high correlation between the line means of the  $O_2$  and  $O_1$  generations for flowering time. The correlation is in fact 0.86 while the regression sum of squares of  $O_1$  on  $O_2$  is highly significant irrespective of which item in the analysis it is tested against. A similar expectation does not hold for height because the differences produced in the  $O_2$  generation were not significant at the 5 per cent level of probability.

Table 1. ANALYSIS OF  $O_2$  EXPERIMENT FOR V.16

Character Item	Flowering time D.F.	M.S.	Final height D.F.	M.S.
Between lines	7	1,175.00†	7	981.87*
Between families within lines	16	242.91†	16	284.99†
Error	634	17.35	632	12.90

\*  $P = 0.01-0.001$ .

†  $P = < 0.001$ .

At the end of 1963 individual plants within each of the eight original lines of V.16 were again selfed. The experimental design for the 1964 test of the  $O_2$  generation was essentially similar to that of the previous year except that the blocks were not divided into plots, the individual plants being randomized within the blocks. Again, the experiment was grown over a uniformly treated area, and as before flowering time and final height were recorded.

Analysis of the 1964 flowering time data indicates the presence of significant differences between lines and between families within lines when tested against the experimental error (Table 2). That the mean square between lines is not significantly greater than the mean square within lines is due solely to the erratic germination of one of the families within the phosphorus line. On removal of this family the between-lines item becomes highly significant when tested against the within-lines item. An orthogonal breakdown of the sum of squares between lines reveals a pattern similar to that which emerged from the  $O_1$  experiment. For flowering time the effect of potassium is the most striking, whereas for height practically every comparison proves to be significant. This similarity is borne out by the correlation between the line means of the  $O_2$  and  $O_1$  experiments, which for flowering time is 0.86 and for height is 0.92. The regression sum of squares for both characters is significant against whatever item in the appropriate analysis it is tested. Table 3 gives the line means for all the various generations and the ranking of the different treatment lines in each experiment. The marked reduction in the range between the earliest and latest flowering lines from the  $O_2$  to the  $O_1$  generation is not to be attributed to a diminution of the conditioning effect but rather to the very warm summer of 1964 which compressed the range of flowering time in all the many experiments carried out with *N. rustica* in this department. Height, which is less affected by temperature than flowering time, does not show a similar decrease; in fact, if anything, the difference between the tallest and shortest lines is greater in the  $O_2$  generation.

Table 2. ANALYSIS OF  $O_2$  EXPERIMENT FOR V.16

Character Item	Flowering time D.F.	M.S.	Final height D.F.	M.S.
Between lines	7	89.61	7	838.00*
Between families within lines	16	53.51*	16	144.76*
Error	878	3.22	880	8.29

\*  $P = < 0.001$ .

Table 3. MEANS OF TREATED LINES

(Figures in parentheses refer to relative order)

Line	$O_2$	$O_1$	$O_2$	$O_1$	$O_2$	$O_1$
N	20.67 (6)	12.14 (5)	4.91 (5)	43.74 (6)	38.65 (6)	
P	16.67 (3)	14.43 (6)	5.13 (8)	41.90 (8)	34.87 (8)	
K	17.67 (5)	10.18 (2)	9.63 (2)	44.89 (5)	40.67 (4)	
NP	24.23 (7)	16.74 (7)	5.90 (6)	45.88 (4)	39.43 (5)	
PK	11.67 (1)	9.86 (1)	8.83 (3)	43.66 (2)	46.15 (1)	
PK	17.00 (4)	10.68 (4)	2.65 (1)	43.35 (7)	37.80 (7)	
NPK	13.33 (2)	10.48 (3)	4.20 (4)	46.06 (3)	41.67 (3)	
NIL	27.00 (8)	17.77 (8)	5.90 (6)	49.73 (1)	44.60 (2)	

No. of plants on which mean is based

6

120

60

120

60

Further experiments are being carried out, in particular  $F_1$  and  $F_2$  diallel crosses between the eight lines, to examine the within family variances, but this résumé of the results to date indicates that the changes produced by the application of different fertilizer treatments in an inbred variety of *N. rustica* must be heritable since they have now been transmitted through two generations without any further treatments being applied. The apparent diminution in the range of mean flowering times is, as we have seen, almost certainly due to meteorological differences between the two years. For height the changes have been maintained without any apparent diminution even though there were no significant differences between the original treated plants—doubtless because these were grown in the glasshouse where they barely attained one-quarter of their normal stature.

The causes of conditioning remain a matter of conjecture for the time being. From Durrant's work on flax some of the more obvious explanations can be eliminated, but evidence of a more positive nature is not yet forthcoming. One thing is clear, however. Heritable changes have not so far been induced in material or for characters which have been subject to deliberate and intense selection. Such attempts have always failed, whereas both flax and *Nicotiana*—which have proved conditionable—have undergone relatively little conscious selection. Presumably material which has been heavily selected has also been grown in the most favourable environments and any conditioning which might have taken place will have gone hand in hand with selection. Once induced changes of this type have occurred they may well be irreversible under the normal range of environmental conditions experienced by the plant. Thus one is forced to seek out as material suitable for conditioning those plant species which have not been the objects of artificial selection, while experimental convenience requires that they be either obligate inbreeders or else readily made to self-pollinate so that the nature of any induced changes may be demonstrated.

The precise form of any practical applications which conditioning may have for crop improvement cannot be foreseen at present. Its most likely practical use would be if some hitherto commercially unimportant species were to assume agronomic importance. But, in such circumstances, one presumes that conditioning would automatically occur as selection was practised on the material.

I thank Prof. K. Mather and Dr. J. L. Jinks for their advice.

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# LETTERS TO THE EDITOR

## ASTRONOMY

### Evidence that the Dark Areas on Mars are Elevated Mountain Ranges

SINCE the time it was definitely concluded that the dark areas on Mars were not large, open bodies of water, much speculation has occurred concerning the question as to whether these areas are elevated above or depressed below the bright desert regions.

Apart from seasonal polar rifts (for example, the Mountains of Mitchel) and brightening of certain areas (Hellas), which indicate the possibility of snow deposits on isolated peaks and high plateaux, there has been no direct physical evidence for the existence of extensive mountain ranges on Mars.

The planet is so far from Earth that even with the largest telescope under the best seeing conditions elevation differences could not be resolved easily, unless one knew exactly where to look. Lowell<sup>1</sup> long ago calculated that no mountains in excess of 2,500 ft. in height could have escaped detection. However, he seems to have assumed lunar type mountains, that is, having steep slopes and being large in extent, a type of mountain which would cast a well-defined and easily detectable shadow.

If we consider the long dark strip Sabaeus Sinus, which is about 400 km wide, to be a symmetric mountain ridge 3,000 m in height, it would only have slopes of about 1°. It is difficult to believe that a mountain ridge on Mars with a 1° slope could cast a shadow detectable from Earth. In any event, Sabaeus Sinus is aligned in an east-west direction and is bordered by dark areas to the east and to the west so that any shadows cast would fall on areas already dark in contrast.

It is therefore not unreasonable to conclude that the dark areas on Mars could be mountainous ranges of considerable height, but with gentle slopes.

In 1964 I began a statistical survey of localized white and yellow cloud occurrences to look for possible relations with topographical features. The formation of localized clouds on Mars is indeed a rare occurrence, and the literature survey therefore has been extended over observations of the past century in order to gain some reasonably meaningful information.

One of the most interesting results of this analysis is the fact that many white clouds are observed to form over bright desert regions bordering along dark areas and to remain stationary in these positions for some time.

Notable areas of these instances have been recorded by J. Focas and A. Dollfus<sup>2</sup> for the regions of *Edom* ( $\lambda=355^\circ$ ,  $\varphi=-10^\circ$ ) to the north of Sabaeus Sinus and for Deucalionis to the south of Sabaeus Sinus, although similar occurrences in other regions are not unknown. (Other instances of these occurrences have been observed in Libya, Isidis Regio, the borders of Margaritifer Sinus, over the central part of Mare Acidalium in the region of Achillis Pons, and in Zephyria as reported in a personal communication by J. Focas.) These white clouds form over the bright regions just mentioned but align themselves lengthwise adjacent to the dark area Sabaeus Sinus.

From polarization investigations Dollfus<sup>2</sup> has interpreted the curves for white clouds to be "... identical with that observed for ice crystal clouds". Therefore they are convective phenomena and are subject to a thermodynamic analysis.

In invoking an explanation for these phenomena, the theory of the formation of standing waves to the lee side

of mountain ridges developed principally by R. S. Scorer<sup>3</sup> has been used.

If a pressure gradient is aligned with an east-west mountain ridge, the air currents blowing perpendicular to the upwind side of the mountain will cause standing sinusoidal waves to form on the lee side. These winds are generally referred to as Föhn winds and have been extensively investigated in the mountainous Alpine ranges of Europe<sup>4</sup>.

A Fourier representation of the mountain profile (assumed symmetric) takes the form :

$$\zeta = \frac{a^2b}{a^2 + x^2} = ab \int_0^\infty e^{ikx} - kx \, dk \quad (1)$$

where  $b$  is the height of the mountain,  $a$  is the width of the profile at half-maximum measured in a direction  $x$  from the centre of the mountain, and  $k$  is the wave number. The mountain profile acts as a disturbance to the otherwise laminar flow which propagates itself upwards through the airstream.

The disturbance at some height  $z$  of the airstream away from its undisturbed state then is given by:

$$\zeta(z) = ab \int_0^\infty \frac{\zeta_{z,0}}{\zeta_{1,0}} e^{ikx} - kx \, dk \quad (2)$$

where the function  $\frac{\zeta_{z,0}}{\zeta_{1,0}}$  is derived from the vertical wind equation:

$$w'' + (l^2 - k^2)w = 0 \quad (3)$$

and is explained in detail in the works by Scorer<sup>3,7</sup> and summarized by Colby<sup>8</sup>.

If a parcel of air containing water vapour is carried along one of these airstreams and is raised along one of the sinusoidal disturbances to a height above the level of condensation, it will make itself visible as a cloud having the lens-shape of the sine wave at that point. These so-called lenticularis clouds are stationary and always form to the lee of mountains since that is where the sinusoidal pattern is strongly concentrated (Fig. 1).

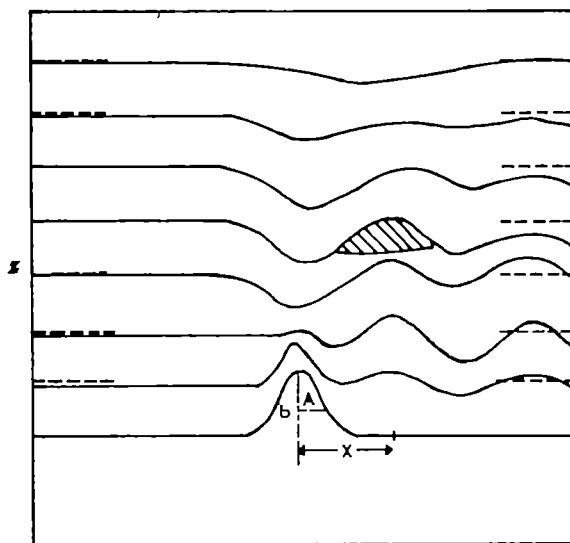


Fig 1

With this as the explanation for the peculiar white cloud formations described on Mars, we must *a fortiori* believe that the dark areas are extended mountain ranges causing lee-waves to form whenever meteorological conditions are suitable.

The required horizontal pressure gradients are known to exist on Mars as manifested in Hess's now familiar isobaric map of the planet<sup>8</sup>. In addition, mapping of the tropical wind patterns by McLaughlin<sup>10</sup> reveals the possibility of Föhn winds in these regions.

Theoretically, then, equation (2) should be solvable for the altitudes of these ranges. However, it should be noted that deriving meteorological data for the planet Mars is an extremely difficult task and must of necessity be extended over many years of observations from which only average numerical values are obtained.

Treatment of equation (2) is being carried out in detail by me and results will be published in a paper at a later date. At least some idea of the magnitude of mountain heights on Mars is hoped to be obtained by this procedure in spite of the precariousness in using the numerical values derived from meteorological observations.

Nevertheless, it should be safe to accept these white cloud formations as physical evidence of the presence of mountain ranges on Mars, and it will be extremely interesting to see if this theory is corroborated by the photographs of Mars transmitted back to Earth by the *Mariner IV* space craft.

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## ASTROPHYSICS

### Limits on the Density of Intergalactic Ionized Hydrogen

OBSERVATIONS of background radiation at radio frequencies can be utilized to place limits on the amount of ionized hydrogen which exists in intergalactic space. For this purpose, I have calculated the intensity to be expected at low frequencies from cosmic sources in an Einstein-de Sitter universe. Intergalactic matter is assumed to possess the following properties:

- (1) The electron kinetic temperature is independent of time, as suggested by the form of the cosmic energy equation given by Layzer<sup>1</sup>.
- (2) Intergalactic matter is pure hydrogen (that is, no other source contributes electrons).
- (3) The electron density has attained its equilibrium value for specified temperature and density.
- (4) The electron temperature  $T > 10^4$  °K (that is, hydrogen is ionized).
- (5) The electron temperature is much greater than the temperature appropriate to the radiation density.
- (6) The density of intergalactic hydrogen is a constant fraction,  $\chi$ , of the mean density,  $\bar{\rho}$ , that is:

$$\rho_H = \chi \bar{\rho} = \frac{\chi}{8\pi G k^2} \quad (1)$$

where  $\chi$  is  $\leq 1$  and independent of time.

The intensity is computed by means of the relation<sup>2</sup>:

$$I_\nu = c \int_0^t j(\nu, t') \left[ \frac{R(t')}{R(t)} \right]^3 e^{-[\tau(\nu, t) - \tau(\nu, t')] } dt' \quad (2)$$

where:

$$\nu' = \nu \frac{R(t)}{R(t')} \quad (3)$$

and  $j(\nu, t)$  is the emission coefficient per unit volume;  $R(t)$  is the expansion scale factor, proportional to  $t^{1/3}$ , for early stages of all relativistic evolving universes and at all stages of an Einstein-de Sitter universe;  $\tau$  is a dimensionless time variable analogous to optical depths:

$$\tau(\nu, t) - \tau(\nu', t') = c \int_{t'}^t K \left[ \frac{R(t)}{R(t')} \nu' \right] dt' \quad (4)$$

So long as the frequency  $\nu'$  corresponding to  $t'$  is so small that bound-free absorption is negligible and  $h\nu'/kT < 0.1$ , the optical depth can be represented by the analytic expression:

$$\tau(t) - \tau(t') = 1.589 \times 10^{11} T^{-1} \nu'^{-2} t'^{-2} \left( \frac{\alpha}{S} + 1 \right)^{-1} \chi^2 W$$

where:

$$W = \left[ \left( \frac{t}{t'} \right)^3 - 1 \right] \left[ \frac{5}{4} \ln \left( 1.545 \times 10^{11} \frac{T^2}{\nu'^2} + 1 \right) - \frac{5}{3} \left( \frac{t}{t'} \right)^3 \ln \left( \frac{t}{t'} \right) \right] \quad (5)$$

$\alpha/S$  is the ratio of the collisional-radiative recombination coefficient to the collisional-radiative ionization coefficient<sup>3</sup>. If one chooses values of  $T$  and  $\chi$  that produce the greatest possible optical depth for given frequency and red-shift, one finds from equation (5) that effects of intergalactic free-free absorption on discrete source spectra at frequencies greater than 10 Mc/s will not be noticeable at red-shifts less than about 80. A red-shift of 80 is significantly greater than the present largest known red-shift,  $z = 2.01$  (ref. 4). (Further details on the calculation of optical depth and intensity will be published elsewhere.)

We consider two types of emission: (a) free-free emission from ionized intergalactic hydrogen, and (b) emission from discrete, non-thermal extragalactic radio sources. The latter is represented by an empirical model of the evolution of radio-source luminosity obtained by Davidson and Davies<sup>5</sup> by fitting radio source counts. The following changes were made in the Davidson and Davies model: dispersion in source power at any given epoch was neglected, and no cut-off was introduced for the emission coefficient. (Davidson and Davies set the emission coefficient equal to zero for red-shifts greater than  $\delta^*$ ;  $\delta^* = 4.44$  in an Einstein-de Sitter universe.)

The intensity resulting from the combination of free-free emission and emission from non-thermal discrete sources is plotted in Fig. 1. Observed points are taken from the summary by Wielebinski and Yates<sup>6</sup> for  $\nu = 1-404$  Mc/s and from observations by Alexander and Stone<sup>7</sup> below 5 Mc/s. Wielebinski and Yates find that the observed background intensity can be fitted with a spectral index  $\alpha = -0.65 \pm 0.15$  throughout the range 18.3-404 Mc/s. A measurement of brightness temperature  $T_B = 3.5^\circ$  K at 4,080 Mc/s by Penzias and Wilson<sup>8</sup> indicates that, somewhere between 404 and 4,080 Mc/s,  $\alpha$  must become positive. Now, in general, the combined emission from discrete sources and from intergalactic  $H^+$  would yield a spectral index that is negative at low frequencies and positive at high frequencies, since the intensity due to discrete sources has a negative spectral index, while the intensity due to  $H^+$  has a positive spectral index  $\alpha = 1.1$ . One notices in Fig. 1 that if  $\chi = 1$  and  $T \geq 2 \times 10^4$  °K, the spectral index becomes positive at too low a frequency and the calculated intensity is too large to agree with observations at  $\nu = 100-400$  Mc/s. Also, it is evident

Table 1. THEORETICAL BRIGHTNESS TEMPERATURE AT 4,000 Mc/s

$T$ °K	Intergalactic contribution		Discrete source contribution $T_s$ with $\chi=0.1$ °K
	$T_s$ with $\chi=1$ °K	$T_s$ with $\chi=0.1$ °K	
$10^4$	6.3		
$2 \times 10^4$	9.3	1.6	0.065
$6 \times 10^4$	16.0	2.9	0.075
$2 \times 10^5$	28.2	5.3	0.087
$6 \times 10^5$	46.5	7.4	

from Table 1 that for  $T \geq 6 \times 10^4$  °K and  $\chi = 1$ , the predicted background brightness temperature is significantly greater than 3.5° K at 4,000 Mc/s. We therefore eliminate an Einstein-de Sitter universe with  $\chi = 1$  and  $T \geq 2 \times 10^4$  °K.

Both galactic and extragalactic sources may contribute significantly to the observed background intensity. There is some confusion at present about the existence and size of the galactic contribution. If we assume that the background is primarily the result of cosmic sources, we can place more stringent restrictions on the density of intergalactic  $H^+$ . If  $\chi = 1$ , the calculations for temperatures less than  $2 \times 10^4$  °K yield too little radiation at frequencies below 30 Mc/s. The best fit to the observed intensity in the range  $\nu = 5-100$  Mc/s is obtained for a value of  $\chi$  between 0.1 and 0.01. Over the frequency interval 1-100 Mc/s, the models corresponding to  $\chi=0.1$  yield spectra with indexes  $\alpha = -0.50$  to  $-0.57$ , slightly flatter than the observed spectrum (the theoretical spectra would be steeper if the time dependence of the discrete source luminosity were weaker than that derived by Davidson and Davies), and the contribution to the intensity from non-thermal discrete emission dominates the contribution from intergalactic emission.

The discrepancy between calculated and observed intensities below 3 Mc/s does not invalidate the general conclusion that the observed background at low frequencies can be attributed to the integrated effect of non-thermal discrete sources. This discrepancy may reflect either the crudeness of our model for discrete source emission or local galactic absorption. We assumed that the spectral index of a discrete source is independent of time and frequency, whereas it is very likely that discrete source spectra turn over at low frequencies due to internal processes such as synchrotron self-absorption and thermal absorption.

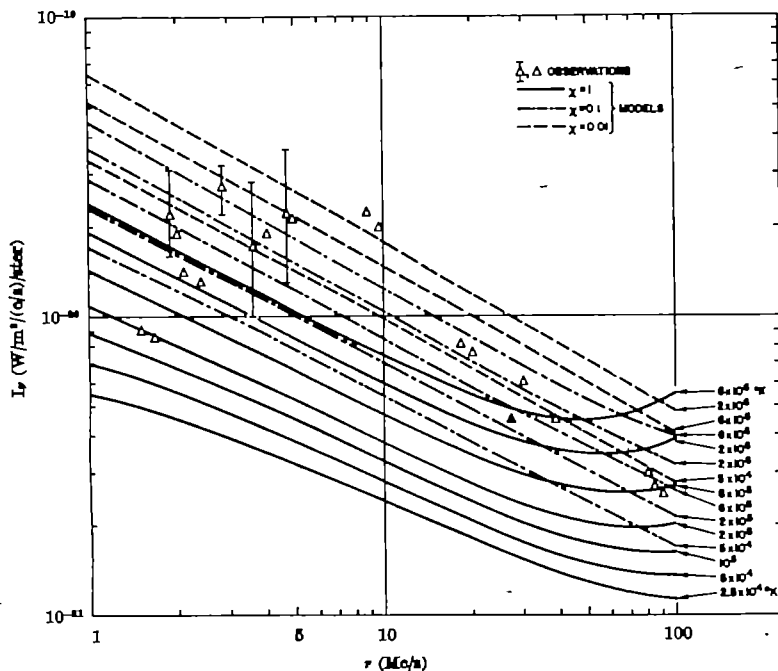


Fig. 1. Intensity versus frequency

As one can see from Table 1, agreement with the observed background intensity near 4,000 Mc/s is quite good for  $\chi = 0.1$ . Unlike the intensity at low frequencies, the theoretical intensity near 4,000 Mc/s results mainly from intergalactic emission.

Thus, with the same value of intergalactic electron temperature and density, we obtain general agreement with observation both near 4,000 Mc/s and in the range 4-100 Mc/s. We conclude that the density of intergalactic  $H^+$  is probably at least an order of magnitude smaller than the mean density of an Einstein-de Sitter universe, and that the spectrum of background radiation at radio frequencies may be the product of extragalactic sources.

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### Contribution of Characteristic X-rays to the Radiation of Solar Flares

ATOMS bombarded by energetic electrons may experience ionization through the loss of a K-shell electron. Part of the subsequent rearrangement of the ion to its ground-state involves the movement of an outer (usually an L- or M-shell) electron to fill the K-shell vacancy. The energy released in this transition appears in one of two forms. Either a photon is emitted (characteristic radiation) or one or more outer electrons are expelled from the ion (the Auger process). The purpose of this communication is to point out that characteristic X-rays emitted as a result of such inner-electron transitions may make an important contribution to the X-ray emission of solar flares. It is not yet known with certainty whether the radiation from flares in the  $\lambda\lambda$  1-3 Å region is predominantly of thermal<sup>1,2</sup> or non-thermal<sup>3</sup> origin. The detection of the characteristic radiation of iron atoms in the X-radiation from flares would indicate that non-thermal processes are important in the production of X-rays in this wavelength range.

Equation (1) gives the emission per unit volume due to collisionally excited characteristic radiation in the K-series lines of any atomic constituent of a gas:

$$\epsilon_{ch}(Z) = N_Z W_Z \hbar \nu_Z \int_{E_{min}}^{\infty} N_e(E) v_e(E) Q_Z(E) dE \quad (1)$$

dE erg cm<sup>-3</sup> sec<sup>-1</sup>

where:  $N_Z$  = number density of Z atoms;  $N_e(E)dE$  = number density of electrons of energy  $E$ ,  $E + dE$ ;  $v_e(E)$  = velocity of electrons of energy  $E$ ;  $Q_Z(E)$  = K-shell ionization cross-section;  $W_Z$  = K-fluorescence yield (fraction of K-shell ionizations which produce K-series photons);  $\hbar \nu_Z$  = energy of K-series photon;  $E_{min}$  = electron energy required to ionize K-shell. Most of the energy of these K-series



transitions goes into the  $K\alpha_1$  ( $K - L_2$ ) transition with lesser amounts going into  $K\alpha_2$  ( $K - L_3$ ) and higher order transitions. These lines are closely grouped, however, and in computing the total energy in the  $K$ -line they may be considered together, using the  $K$ -fluorescence yield for all transitions to the  $K$ -shell.

Little is known about the emission of characteristic radiation by partially ionized atoms. However, it seems unlikely that the loss of some, less than half, say, of the outer electrons of an atom would markedly affect the  $K$ -shell ionization cross-section,  $Q_K$ , or the  $K$ -fluorescence yield,  $W_K$ . This is because the energies involved in the inner-shell transitions are much larger than the energies involved in the removal of the outer electrons. In these computations the  $K$ -shell ionization cross-section formula of Arthurs and Moseiwitch<sup>4</sup> have been used. The cross-section is maximum for incident electrons with energies of three times the  $K$ -shell ionization potential.

If sizeable fluxes of non-thermal electrons in the 10–30-keV energy-range are produced in solar flares, as is indirectly indicated by the scintillation counter observations of Anderson and Winkler<sup>5</sup>, then these electrons will excite the inner-shell transitions which produce characteristic X-rays. The most interesting element for investigation is iron. It is relatively more abundant than other heavy elements ( $N_{Fe} \approx 8 \times 10^{-4} N_H$ ) and its characteristic radiation falls in the wave-length range ( $\lambda$  1–2.5 Å) responsible for the sudden disturbances of the lower ionosphere which accompany some flares<sup>6,7</sup>.

In order to evaluate the relative contribution of the characteristic radiation excited by non-thermal electrons it is convenient to compare this emission to the bremsstrahlung emitted by the same electrons at the same wave-length. Using equation (1) with  $W_{Fe} = 0.35$ ,  $h\nu_{Fe} = 1.1 \times 10^{-8}$  erg/photon, and neglecting the variation with electron energy of the parameters under the integral, we estimate the emission in the characteristic lines of iron (with an uncertainty of roughly a factor of three) to be:

$$\epsilon_{ch}(Fe) \approx 2 \times 10^{-10} N_e N_{Fe} \text{ erg cm}^{-2} \text{ sec}^{-1} \quad (2)$$

It is acceptable in this case to neglect the integration over electron energy since this dependence is not strong throughout the 10–30 keV region. Electrons of energy greater than 30 keV appear to be relatively few in number<sup>8</sup> and so contribute little to the excitation of characteristic radiation. For comparison with the bremsstrahlung emission from these same electrons we re-write equation (2) in terms of the hydrogen atom density,  $N_H$ :

$$\epsilon_{ch}(Fe) \sim 16 \times 10^{-10} N_e N_H \text{ erg cm}^{-2} \text{ sec}^{-1} \quad (3)$$

The standard formulae are used to calculate the intensity of the bremsstrahlung emission per Å from these electrons at the wave-length of the iron  $K$ -line assuming a gas consisting of 87 per cent hydrogen and 13 per cent helium. Again the result is not strongly dependent on electron energy, within the 10–30 keV energy range. The bremsstrahlung intensity is:

$$\epsilon_{brem}(\lambda = 1.9 \text{ Å}) \approx 35 \times 10^{-10} N_e N_H \text{ erg cm}^{-2} \text{ sec}^{-1} \text{ Å}^{-1} \quad (4)$$

We see from this comparison that if sizeable numbers of energetic electrons are created by a flare then the characteristic radiation from neutral or weakly ionized iron may make a substantial contribution to the X-ray emission of the flare in the wave-length interval around 2 Å. Furthermore, the iron  $K$ -line multiplet is only about 0.03 Å broad so the characteristic line should be distinctly observable above the bremsstrahlung continuum with a spectrographic resolving power of roughly 0.2 Å. Self-absorption of the characteristic radiation is negligible. The optical depth of the line at the level of the photosphere is of the order of 0.001.

Since the X-ray line emission in this spectral region provides a diagnostic tool to measure the relative roles of thermal and non-thermal processes in flares it is useful

to compare the features of the line spectra of the highly ionized atom FeXXV and the characteristic radiation of neutral or weakly ionized iron. FeXXV would be formed and excited in thermal regions with a very high ( $T_e > 10^7$  °K) electron temperature whereas the characteristic radiation of neutral or weakly ionized iron would evidence the injection of energetic non-thermal electrons into a relatively much cooler gas.

FeXXV is a helium-like atom and its resonance transition appears as a doublet ( $1s^2 1S_g - 1s2p^1P_1$  and  $1s^2 1S_g - 1s2p^3P_1$ ) at  $\lambda$  1.87 Å with a separation of 0.006 Å between the two components. Transitions at  $\lambda$  1.69 Å ( $1s^2 1S_g - 1s3p^1P_1$ ) and  $\lambda$  1.51 Å ( $1s^2 1S_g - 1s4p^1P_1$ ), as well as others of higher order, may also appear.

The characteristic spectrum of neutral iron is well known. The strongest component is  $K\alpha_1$  at  $\lambda$  1.932 Å with  $K\alpha_2$  appearing one-half as intense at  $\lambda$  1.936 Å.  $K\beta_1$  appears at  $\lambda$  1.906 Å with about 18 per cent of the intensity of  $K\alpha_1$ . Other (satellite) lines may appear with much fainter intensity depending on the local conditions of ionization.

I thank Dr. Paul Kelly, of Lockheed Missiles and Space Co., who performed the Hartree-Fock calculations yielding the energy-levels of FeXXV. Dr. R. G. Athay made the original suggestion that characteristic radiation might be of importance in solar flares. This work was begun at the High Altitude Observatory, Boulder, Colorado, under the support of contract NONr-393(07) from the Advanced Research Projects Agency through the Office of Naval Research; and was completed under the Lockheed Independent Research Program.

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## RADIO ASTRONOMY

### A Model of the Quasi-stellar Radio Variable CTA 102

ACCORDING to Sholomitsky<sup>1</sup>, the quasi-stellar<sup>2</sup> radio source CTA 102 (ref. 3) has a variable flux density at 32.5 cm, the period being about 100 days. Sholomitsky takes this to mean that the source cannot be larger than  $\sim 0.1$  parsec, which is the distance light travels in one period. Since its angular diameter is not less than about  $0.01 \text{ sec}^{\circ}$ , he concludes that it must be closer than 2 Mpc, and is possibly inside our own Galaxy. However, Schmidt<sup>4</sup> has recently announced that the optical object identified with CTA 102 has a red shift  $z = \delta\lambda/\lambda$  of 1.037, and so is probably at a distance comparable with the radius of the universe ( $\sim 3,000$  Mpc).

Although it is by no means certain that the observed variations originate in the source itself, we wish to propose a model which assumes this, and is consistent with the red shift observations.

The model is illustrated in Fig. 1. The radio emission is produced in a spheroidal shell the axis of symmetry of which is approximately along the line of sight. (Shell models for radio sources have been discussed by several authors<sup>5</sup>, and spheroidal shells in particular by Layzer<sup>6</sup>.) The main part of the emission comes from the region ADB, and its spectrum is taken to have a peak at about 300 Mc/s (Fig. 2). The variable part is assumed to come from the disk-like region AOB, which is pulsating (perhaps as

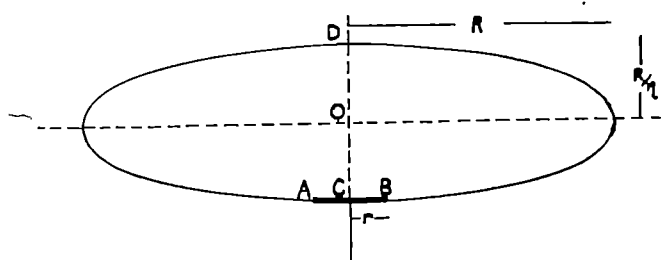


Fig. 1. Proposed model of OTA 102

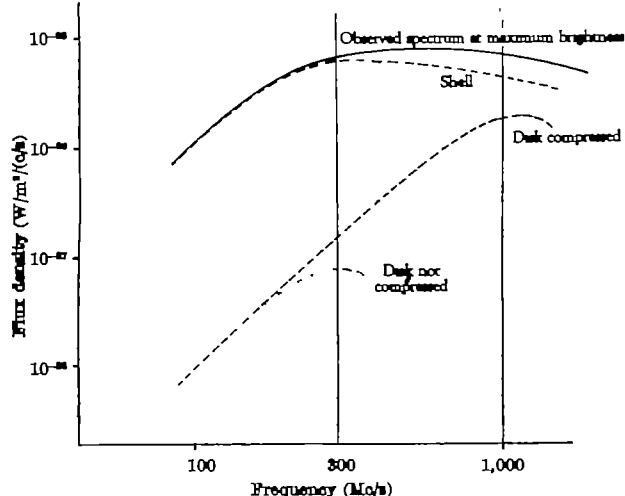


Fig. 2

a result of an explosion occurring at O, the effect of which may reach all parts of the disk at about the same time). When this region is compressed, the magnetic field strength will rise, the individual electrons will be accelerated by the betatron mechanism, and the radiated power will be greatly enhanced. When radiating at its maximum, it is required to emit  $\sim 25$  per cent of the total flux observed at 1,000 Mc/s in order to account for the observed variations (that is, its flux density must be  $\sim 2 \times 10^{-10}$  W/m<sup>2</sup>(c/s)). Its spectrum is taken to be as shown in Fig. 2. The total spectrum then agrees with the observed spectrum of OTA 102 (ref. 9).

The disk will have its minimum size consistent with the required flux if it is opaque at (proper) frequencies up to  $\sim 1,000 (1+z)$  Mc/s when compressed. Furthermore, if its emission is to vary with a (proper) period of  $100/(1+z)$  days, its thickness cannot exceed  $3 \times 10^{17}/(1+z)$  cm. The electrons which radiate at frequencies around 1,000  $(1+z)$  Mc/s have energies of  $\sim 2 \times 10^{-4} (1+z)^{1/2} H^{-1/2}$  ergs, each electron producing  $\sim 2.16 \times 10^{-23} H$  ergs/sec/(c/s) (where  $H$  is in gauss). If synchrotron self-absorption is occurring, the power radiated from the surface of the disk at this frequency is  $\sim 3 \times 10^{-4} (1+z)^{1/2} H^{-1/2}$  ergs/sec/(c/s)/cm<sup>2</sup>. The number density of these electrons is therefore  $\sim 5 \times 10^{-4} (1+z)^{1/2} H^{-1/2}$  per c.c., and their energy density  $\sim 10^{-3} (1+z)^{1/2} H^{-1/2}$  ergs/c.c. Allowing for the fact that they only contribute a few per cent of the particle energy density, and assuming that the total particle energy is comparable with the magnetic energy, we conclude that the magnetic field when the disk is compressed is  $\sim 5 \times 10^{-3} (1+z)$  gauss. A threefold increase in the field strength will probably be sufficient to produce the required increase of about 20 in luminosity at 1,000  $(1+z)$  Mc/s (though the exact factor depends on the energy spectrum of the electrons). The pulsations will be sufficiently rapid if the Alfvén speed  $\sim c$ , and this will be true if the particle density of the ambient gas does not exceed  $\sim 1$  per c.c.

The angular diameter of the disk when the magnetic field has the foregoing value is  $\sim 3 \times 10^{-3} (1+z)^{1/2}$  sec. (This assumes that the electrons are radiating incoherently.

If they were coherent the angular diameter of the source could be much smaller, which might permit its linear diameter to be  $\sim 0.1$  parsec, consistently with a large red shift.) It follows that:

$$r \sim 22.5 \frac{z}{(1+z)^{1/2}} \text{ parsecs}$$

in the steady state cosmology. (In the Einstein-de Sitter model this value must be decreased by a factor  $\sim 2$  if  $z \sim 1$ .) Estimating the average magnetic field over the whole shell as  $10^{-3} (1+z)$  gauss, we deduce from the occurrence of self-absorption below 300 Mc/s that, in the steady state model:

$$R \sim 225 \frac{z}{(1+z)^{1/2}} \text{ parsecs}$$

This estimate of  $R$  enables the parameter  $\eta$  to be determined, for since the disk must not deviate from the tangent plane at O by more than  $\sim 0.1/(1+z)$  parsec, it follows from the geometry that  $R \sim 2,500z^2/\eta$  and so  $\eta \sim 11z(1+z)^{1/2}$ .

If  $z \sim 1$  the dimensions are:  $R \sim 160$  parsecs,  $r \sim 16$  parsecs, and  $\eta \sim 15$ . The lifetime of the electrons is 50–100 years in the fluctuating region, and rather longer in the rest of the source. The total energy of the source is at least  $\sim 10^{47}$  ergs, of which  $\sim 10^{44}$  ergs is in the disk. The probability that the shell should be oriented so that the disk points towards us is about  $3 \times 10^{-4}$ , which implies that very few radio sources can conform to our model. This model enables us to make the following predictions:

(1) The amplitude of the variations depends on the frequency  $\nu$  of observation as follows:

$$\begin{aligned} & \sim 0 & 0 < \nu < 300 \\ & \sim 0.013 \left\{ \left( \frac{\nu}{300} \right)^{3.1} - 1 \right\} & 300 < \nu < 1,000 \\ & \sim 0.25 & \nu > 1,000 \end{aligned}$$

where  $\nu$  is in megacycles. Thus the observations of Caswell and Wills<sup>10</sup>, who found no variations at 178 Mc/s, are not necessarily inconsistent with Sholomitsky's observations.

(2) For  $300 < \nu < 1,000$  the intensity reaches a maximum in a time  $t(\nu)$  days, say, ( $t < 50$ ), and then remains constant for a time  $(100-2t)$  days (that is, while the disk is opaque to frequencies between  $\nu$  and 1,000 Mc/s). The rise-time  $t(\nu)$  is an increasing function of  $\nu$ , as illustrated in Fig. 3.

(3) The times at which the intensity is a minimum should be the same at all frequencies  $\nu > 300$  Mc/s, unless there is appreciable dispersion.

However, as regards (3), even if there is negligible dispersion in the source, there may be appreciable dispersion produced by ionized intergalactic gas<sup>11</sup>. At 400 Mc/s, for example, the resulting delay may be as large as 2 h. At this frequency our model predicts an increase in flux of more than 1 per cent in about 10 days following the minimum, so that an intergalactic delay might be detectable. Moreover, there may be, superposed on the main variation, an additional small amplitude variation of much shorter time-scale than 50 days, which might then be detectable when the main variation is at a minimum. It would therefore appear to be worth while to develop the sophisticated techniques necessary to detect the possible dispersion, and so to test the hypothesis that there is a significant ionized gas in intergalactic

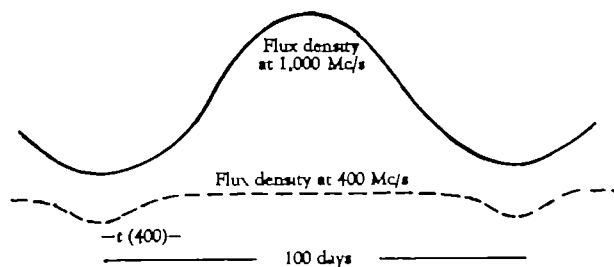


Fig. 3. Time variation of flux density

space, and perhaps even to determine the scale-factor of the universe if other radio variables are discovered<sup>11</sup>.

We thank Profs. F. T. Haddock, A. Sandage and M. Schmidt for their advice. This work was begun while one of us (D. W. S.) was visiting the Department of Physics and Astronomy, University of Maryland, under National Aeronautic and Space Administration grant NAG 5860. He is grateful to Profs. H. Lester and G. Westerhout for their hospitality.

*Note added in proof.* It has been reported by W. A. Dent (*Science*, 148, 1458; 1965) that the quasi-stellar source 3C 273 (and possibly 3C 279 and 3C 345 as well) is variable at 8,000 Mc/s, and that again there is a discrepancy between light-size, red shift and angular diameter. However, the discrepancy is much less than for OTA 102, and a special geometry of the type considered here might be a possible explanation without leading to such an unfavourable probability factor.

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## RADIOPHYSICS

### Spectral Variations of the Structure of the Radio Sources Cassiopeia A and Cygnus A

In a recent article, Ryle, Elmore and Neville<sup>1</sup> gave a beautiful synthesis of the brightness distributions associated with the radiation at 1,400 Mc/s from the radio sources Cygnus A and Cassiopeia A. No discussion was given in their article of the relevance of earlier observations at other frequencies. A comparison of the results of Ryle *et al.* with the original low frequency measurements highlights some interesting features of both sources and stresses the need for further measurements at even lower frequencies.

**Cassiopeia A.** The approximate circular symmetry of the Cassiopeia A source was determined by Jennison and Das Gupta<sup>2</sup> at 125 Mc/s. Later phase-sensitive measurements by Jennison and Latham<sup>3,4</sup> at 127 Mc/s showed that the diameter of the source in position angle 90° was 4.1 min arc, that the distribution was limb brightened more than the radiation from a uniformly illuminated disk (and therefore considerably more than that from a volume emissive sphere) and that there was a faint spur to the east which extended for 4 min beyond the limb when projected into position angle 90°. Concurrent optical observations by Minkowski<sup>5</sup> confirmed the optical diameter as 4.1 min and also showed a faint spur extending from the main body of the nebula in position angle 70°. The independent agreement on the existence of this spur lends considerable support to its reality, but though it may be marginally detectable in observations made by Conway<sup>6</sup> at 500 Mc/s in 1955, it has not been observed in later measurements at higher frequencies. The enigma has recently been discussed elsewhere<sup>7</sup>, wherein it was pointed out that if it resulted from material or shock waves propagated from the original explosion normal to the line of sight, the velocity of propagation would have to be in excess of 15,000 km/sec. It seems unlikely that the spur was a background feature, independent of the supernova remnant, or that the radiation could be so transient that

it faded before the measurements were made at higher frequencies. It appears to be more probable that the feature has a steep spectral index.

The Cambridge super-synthesis of Cassiopeia A at 1,400 Mc/s shows a break in the shell and a general reduction of brightness from the centre through the gap near the position angle 75°. Not only is there this diminution of brightness at this position angle within the nebula, but also a faint spur extending from the gap is marginally detectable in Fig. 4 of Ryle's paper. This latter feature would probably not be significant were it not for the earlier measurements. The optical filament to the extreme east of the nebula does not lie within the main contours of the nebula but appears to define the southerly limit of part of the flare. The breakaway of material in this sector might result from less opposition to the expansion by the ambient gas clouds, but this seems unlikely, for the general expansion of the main body of the nebula appears to be proceeding with remarkable symmetry, although, as Ryle points out, the intense peak to the west is diametrically opposite to the gap. A preferential ejection in the initial explosion could give rise to the spur, but it is not easy to see why the gap has remained open and why the optical and low-frequency radio emissions appear to be distributed along the spur. A later secondary explosion or flare is a possibility, but the ejection velocity must then be considerably in excess of 15,000 km/sec. Layzer<sup>8</sup> has suggested that axial expansions may be expected in many radio sources, but the spectrum may indicate that the electrons radiating in the Cassiopeia flare are less energetic than those in the shell.

The results of Ryle *et al.* support by inference the existence of the Cassiopeia flare, and the interpretation of the reason for its existence is clearly of considerable interest. Detailed measurements at low frequencies are very desirable to confirm the structure, polarization and spectral index of the flare, and, indeed, to see if the feature still exists.

**Cygnus A.** The resolution of the Cygnus A source into two major components by Jennison and Das Gupta<sup>2,3</sup> in 1952 showed that at a frequency of 125 Mc/s the separation between the two major components was approximately 85 sec arc along the major axis, the position angle of which was slightly in excess of 90°. Jennison and Latham<sup>4,5</sup> using improved techniques at 127 Mc/s then determined the apparent separation between the centres of emission as 83 sec arc along the major axis in position angle 97° and showed that the  $\alpha$  source, leading in R.A., was slightly the stronger. Later measurements (Rowson; Twiss, Carter and Little; Lequeux; Bracewell; Maltby, Moffet and others) were confined to high frequencies. In all these later measurements the major binary distribution was confirmed, with some disagreement over the brighter of the two components and a suggestion of a central component of different spectral index.

The measurements by Ryle, Elmore and Neville are in remarkable agreement with those of Rowson at 3,000 Mc/s<sup>6,10</sup>. The convolution of their detailed distribution with the angular distribution corresponding to a lower limit for the resolving power, as used by Rowson, smooths the weaker inner source into the profile of the adjacent major component and reduces the apparent spacing between the major components to a value closer to that obtained at 3,000 Mc/s. The position angle of the major axis in the Cambridge synthesis is identical to that found by Rowson.

The more interesting comparison is that with the one-dimensional synthesis and major axis determinations made by Jennison and Latham at 127 Mc/s. If the tertiary component has a steep spectral index the spacing may be reduced, but the position of the source at lower frequencies will no longer agree with that determined by Smith<sup>11</sup> unless a similar spectrally sensitive component appears to the east. There is a change of slope in the Cambridge profile at approximately 19h 57m 46s which may constitute

such a component. It is possible that both these inner components are enhanced at lower frequencies relative to the outer parts of the distribution. Ryle's synthesis shows a negligible central contribution at 1,400 Mc/s and underlines the need for detailed measurements with a high resolving power at frequencies below 125 Mc/s.

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## PHYSICS

### Nature of the Spearpoints observed during the Combustion of Zirconium Droplets

PHOTOGRAPHIC time exposures of rapidly moving metal droplets which are burning luminously in a gaseous oxidizer often exhibit curious spearpoints. These streak images: (1) become less intense and gradually narrower; (2) suddenly intensify and broaden; (3) finally narrow again as they gradually extinguish altogether. The overall photographic trace resembles a pointed spear, hence the name.

Spearpoints are frequently observed among the sparks formed when metals are ground against an abrasive wheel<sup>1-4</sup>. Similar traces occur when particles of zirconium are ignited by flash heating<sup>5</sup>.

The work recorded here shows that the spearpoints associated with the combustion of zirconium droplets result from the solidification of molten spheres of slightly sub-stoichiometric zirconium dioxide. It follows that the sudden intensification of the photographic image is caused by an increase in the emissivity of the droplet of oxide as it solidifies.

Freely falling droplets of zirconium were formed and ignited by an intense pulse of light from a capacitor discharge lamp; the apparatus and technique, except for slight improvements, have been described previously<sup>6</sup>. The burning droplets were photographed by their own incandescence with a camera the shutter of which was opened electrically for 0.5 sec after the scattered light from the heating flash had extinguished. A typical spearpoint is shown in Fig. 1a, which was recorded with a droplet 525  $\mu$  in diameter burning in flowing oxygen at a pressure of 625 torr.

The characteristics of the spearpoints were examined by interposing an inclined glass plate in the droplets' path of fall. This is shown in Figs. 1b and c. Whenever it was placed above the level at which the image abruptly widened and intensified, the droplets adhered to the plate and continued to burn, as shown in Fig. 1b. But when the plate was inserted in the path below this level the glowing droplet bounced elastically from the plate and dropped into the bottom of the apparatus where it bounced several times again before extinguishing. This is shown in Fig. 1c. When the plate was placed very close to the shoulder of the spearpoint, within about 1 mm, semi-elastic deflexions occurred.

The most reasonable explanation of these phenomena is that the trace intensifies as the droplet solidifies. The partially elastic deflexions probably result from a droplet with a solid exterior and a molten interior.

When droplets burning, as in Fig. 1, were quenched in liquid argon 2-3 mm above the shoulder of the spearpoint,

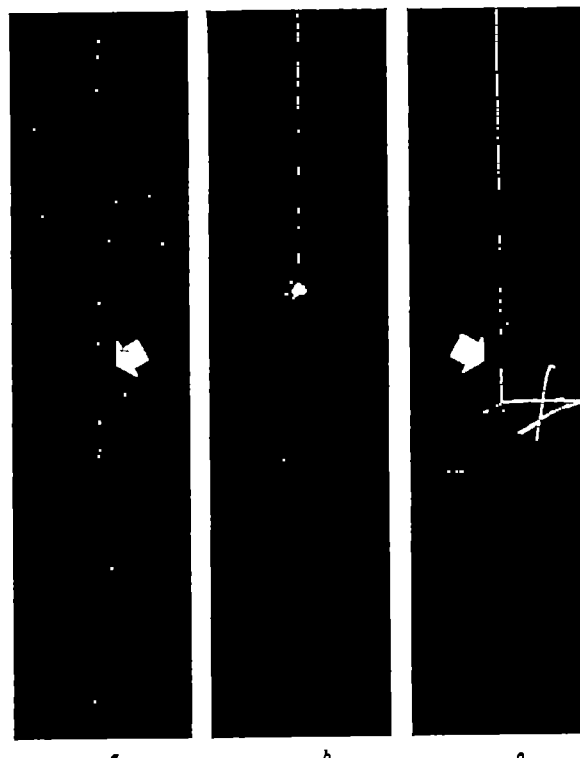


Fig. 1. Time exposures of freely falling zirconium droplets burning in flowing oxygen at 625 torr, photographed by their own incandescence. Shoulders of spearpoints are indicated by arrows. Droplet diameter was 525  $\mu$ . Width of each photograph corresponds to approximately 7 cm. In a, the droplet falls freely, showing complete spearpoint. In b and c, glass plate has been inserted in droplet's path of fall above and below shoulder of spearpoint, respectively. Adherence to plate in b indicates molten droplet; elastic deflexion in c indicates solidified droplet.

their weights had increased 34-35 per cent. This corresponds to the composition range  $ZrO_{1.04-1.05}$ .

The composition is consistent with the colour of the quenched droplets, which were dark grey or black with faint grey striations. Droplets which were quenched 2-3 cm above and below the shoulder of the spearpoint were black and white, respectively. Zirconium oxide of the compositions  $ZrO_{1.03}$ ,  $ZrO_{1.04}$  and  $ZrO_{1.05}$  is black<sup>7,8</sup>,  $ZrO_{1.07}$  is dark grey<sup>9</sup>, and  $ZrO_{1.08}$  is white<sup>8</sup>.

It is concluded that the sudden intensification of the photographic traces is caused by the transition  $ZrO_{x-s}$  (liquid)  $\rightarrow$   $ZrO_{x-s}$  (solid), where  $x$  is of the order 0.03. This process should occur very close to the melting point of  $ZrO_2$ , which is 2,700°-2,800° C (ref. 10). It is unlikely that the chemical composition of the droplets will change significantly in the short time required to form the shoulder of the spearpoint. Likewise, the temperature of the droplets cannot change during a phase transition. Therefore the intensification of the photographic image must be caused by an increase in emissivity as the molten sub-stoichiometric oxide solidifies.

A similar explanation may apply to the spearpoints observed during the combustion of droplets of metals other than zirconium<sup>4</sup>.

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### Nucleation of Freezing by Cavitation in Sub-cooled Bismuth and Gallium

In the article entitled "Nucleation of Freezing by Cavity Collapse and its Relation to Cavitation Damage"<sup>1</sup>, it was stated that there was experimental evidence to support the suggestion that freezing in substances such as bismuth, germanium, gallium and silicon (the freezing points of which diminish with pressure up to relatively high compressions) may not be nucleated by cavitation. The complete basis for this comment was not provided and it is clear that an additional explanation is necessary. To my knowledge, no experiments involving the use of ultrasonically generated cavitation have been performed on these substances in their sub-cooled liquid states. The statement was based instead on information regarding other forms of treatment that normally induce dynamic nucleation. Apparently both sub-cooled gallium<sup>2</sup> and bismuth<sup>3</sup> are known to be surprisingly immune to such treatment. I believe that all dynamic nucleation is due to cavitation. The behaviour of gallium and bismuth is taken, therefore, to indicate that they are immune to the nucleating effects of cavitation, no matter how it is generated. Such a conclusion is obviously open to debate. The real test would seem to lie in the ultrasonic cavitation experiments.

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### Viscosity of Emulsions

An iterative process, using Vand's formula<sup>1</sup>, appears almost always to be successful in obtaining a linear plot of the results of measurements of the viscosity of emulsions at varying contents of the disperse phase. The process can be carried out conveniently by means of a high-speed computer, and results are given for three dispersions, of which two are bituminous.

There is a need for a linear plot of the results of the measurements of the viscosity of emulsions, using the concentration of the disperse phase, or the equivalent of this, as the independent variable. Vand's formula gives a relation between the viscosity of a suspension and the volume occupied by the continuous phase. If the reciprocal of the logarithm of the relative viscosity  $\eta_R$  is plotted against the volume concentration of the disperse phase, a

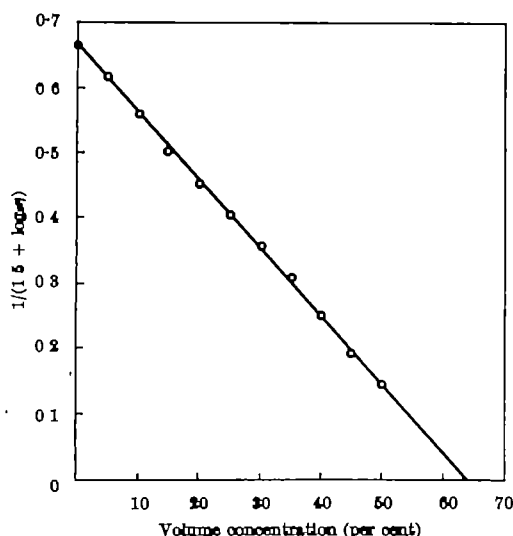


Fig. 1. Plot of  $1/(1.5 + \log \eta)$  against volume concentration—results from Vand (ref. 2).

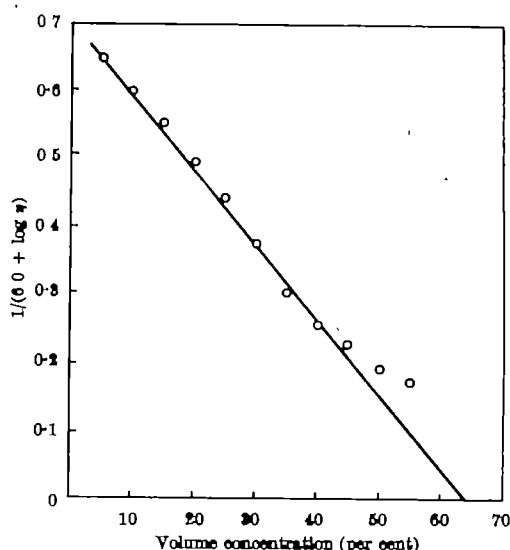


Fig. 2. Plot of  $1/(0.0 + \log \eta)$  against volume concentration of the disperse phase—results from Black (ref. 3).

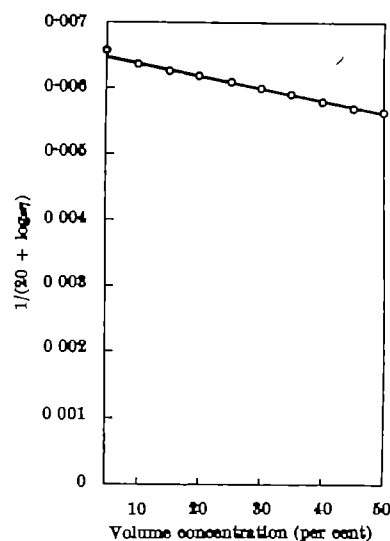


Fig. 3. Plot of  $1/(20 + \log \eta)$  against volume concentration of the disperse phase—results from Black (ref. 3).

plot can be obtained which is linear up to about 50 per cent concentration. If the disperse phase consists of spherical particles of nearly uniform size, it appears that the best straight line through the experimental points so obtained intersects the concentration axis at about 60 or 70 per cent volume concentration. The ordinate of the accompanying figures is:

$$\frac{1}{0 + \log \eta}$$

where  $\eta$  is the measured viscosity of the suspension in poises, and  $O$  is the quantity to be determined by iteration using some linearity criterion for the final plotted points.

The results of Vand<sup>1</sup>, which were obtained using a suspension of glass spheres in a solution of potassium iodide, are shown in Fig. 1, and indicate that a plot can be obtained which is closely linear. Fig. 2 shows results which were obtained from measurements of the viscosity of an anionic emulsion of bitumen particles with diameters varying from 3 to 6 microns<sup>2</sup>. This emulsion shows a variation in viscosity similar to that observed in the case of the suspension of glass spheres, except that the points diverge from a straight line at a lower concentration than those obtained from measurements on the suspension.

Measurements of the viscosity at varying concentrations of the disperse phase<sup>3</sup> were made on an anionic emulsion

which had a wide range of particle sizes (1 micron to 10 microns in diameter). The results are shown in Fig. 3. Emulsions with volume concentrations higher than those shown were not stable and measurements could not be made on them. The behaviour of this emulsion as shown by the results is totally different from that of the previous one. Only some of the particles appear to take part in resisting the applied shear stress, otherwise the straight line would point towards the 70 per cent point on the concentration axis. It is reasonable to suppose that the large particles are the ones which do so, and that the small particles occupy the interstices.

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### Electron Transfer in Spherical Vacuum Chambers

In an effort to explain the breakdown of proportionality between ionization current and gas pressure in cavity ionization chambers, Greening<sup>1</sup> postulated the transfer of slow electrons between the chamber electrodes. Using an approximate formula for the energy distribution of these electrons, he analysed the behaviour of 'vacuum' chambers with planar, spherical and cylindrical geometries, and thereby accounted for the asymmetrical current-voltage characteristic reported by Taylor<sup>2</sup>. Greening noted that the characteristic is in general dependent on the directional distribution of the emitted electrons; but stated that the expressions derived for the case of spherical electrodes should be independent of this angular function. Burlin and Husain have recently adopted a spherical vacuum chamber in their experiments on electron transfer, and have compared their results with those calculated on the basis of Greening's semi-empirical theory.

Neither Greening nor Burlin and Husain<sup>3</sup> have taken into account the effect of the surface-potential barrier on the slow electrons (~ few eV) and the consequent nature of the angular distribution function. Haehenberg and Brauer<sup>4</sup> give a description of this in their review article on secondary electron emission. Electrons having energies very much greater than the barrier height will be affected to a negligible extent and their energy-angular distribution will remain unchanged. In this case one would expect an angular distribution symmetrical about the axis of irradiation, and Greening's theory would hold. However, low-energy electrons suffer a refraction at the medium/vacuum interface and the angular distribution will be symmetrical about the normal to the surface. The following abridged analysis illustrates the effect of this process on the characteristic of a spherical vacuum chamber, and also demonstrates the consequences of non-uniform radiation fields.

Let  $N(E, \omega) dE d\Omega$  equal the number of electrons per unit area per rad emerging from an irradiated electrode, which have energies between  $E$  and  $E + dE$  and directions in the element of solid angle  $d\Omega$  around the unit vector  $\omega$ . In the case of azimuthal symmetry, we can write:

$$N(E, \omega) dE d\Omega = \frac{2\pi \sin \theta}{4\pi} N(E, \theta) dE d\theta \quad (1)$$

where  $\theta = 0$  is the surface normal. Consider Fig. 1, which depicts two concentric spherical shells. In general the absorbed dose at any surface is a function of  $\varphi$ , that is,  $D_A(\varphi)$  and  $D_B(\varphi)$ , which take on known values according to the nature of the radiation field. The total number of electrons emitted from the outer sphere which are collected by the inner sphere in the absence of a potential difference is given by:

$$Q_{BA} = \int_0^\pi 2\pi b^2 \sin \varphi D_B(\varphi) d\varphi \int_0^\pi \int_0^{2\pi} \frac{1}{2} \sin \theta N(E, \theta) d\theta dE \quad (2)$$

Similarly, the transfer from inner to outer sphere is:

$$Q_{AB} = \int_0^\pi 2\pi a^2 \sin \varphi D_A(\varphi) d\varphi \int_0^\pi \int_0^{2\pi} \frac{1}{2} \sin \theta N(E, \theta) d\theta dE \quad (3)$$

The net loss of electrons by the inner sphere in the absence of a bias voltage is given by:

$$Q = Q_{AB} - Q_{BA} \quad (4)$$

The foregoing equations show that in general the characteristic of the spherical vacuum chamber does not intersect the origin. In the special case of a uniform radiation field and an energy-angular function separable into the form  $N(E) \cdot \cos \theta$ , the current will be zero at zero bias voltage. Hence the zero-voltage current intercept found by Burlin and Husain is a consequence of one or both of the following factors: (1) A non-uniform radiation field due to inverse square and exponential attenuation in the chamber walls. (2) A distribution function  $N(E, \theta)$  which cannot be separated into independent energy and angular functions of the type  $N(E) \cdot \cos \theta$ .

When the outer sphere is held at a positive potential with respect to the inner, equation (3) still holds. The analogous equation for  $Q_{BA}$  can be obtained by the method used by Greening. Referring to Fig. 1, a diameter such as  $X'OY'$  can always be drawn parallel to any direction of emission, and a parameter  $r$  thereby defined. By considerations of energy and angular momentum conservation, electrons from  $B$  will be captured by  $A$  when  $r \leq a\sqrt{1 - V/E}$ . Since  $r = b \sin \theta$ , this condition determines the upper limit of the integration over  $\theta$ .

Thus:

$$Q_{BA}(V) = \int_0^\pi 2\pi b^2 \sin \varphi D_B(\varphi) d\varphi \int_0^\pi \int_0^{\sin^{-1} \frac{1}{2} \sqrt{1 - V/E}} \frac{1}{2} \sin \theta N(E, \theta) d\theta dE \quad (5)$$

and:

$$Q(V) = Q_{AB} - Q_{BA}(V) \quad (6)$$

The integral equation (6) can only be solved for an energy distribution  $N(E)$  which is independent of the angle of emission, that is, a function of the form:

$$N(E, \theta) = N(E) \times f(\theta) \quad (7)$$

where  $N(E)$  and  $f(\theta)$  are functions of only  $E$  and  $\theta$  respectively. Furthermore, the form of  $f(\theta)$  must be known.

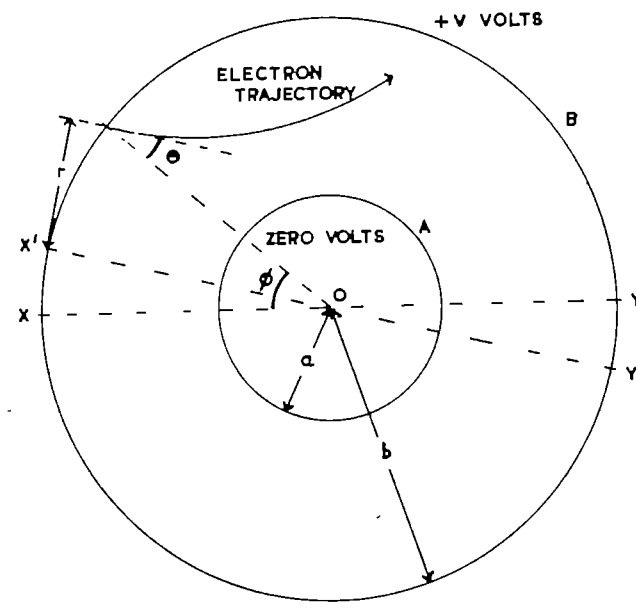


Fig. 1

In this special and rather unlikely instance an analysis of  $Q(V)$  will yield the function  $N(E)$ . In experiments on secondary electron emission, the source of secondary electrons is usually situated at the centre of a collecting sphere, and all electrons are emitted radially and experience the same retarding potential. The energy distribution measured in such experiments is the quantity  $\int_0^\infty N(E, \sim) d\Omega$ . Such an energy distribution cannot be

found by analysing the characteristic of the spherical vacuum chamber, and the advantages of such a chamber over a parallel plate arrangement are illusory.

The analysis given here is a corollary of my investigations<sup>1</sup> into the behaviour of a parallel plate vacuum chamber. I thank Dr. J. R. Greening for his advice, and the Faculty of Medicine of the University of Edinburgh for supporting the investigations.

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### Response of Time-dependent Materials to Oscillatory Motion

A CONSIDERABLE amount of attention has been directed in the past to the rheological behaviour of suspensions of deformable particles when subjected to oscillatory motion, with the result that there is now a reasonable body of theory and experimental data relating to the gross behaviour. However, there appears to be little information available for suspensions of undeformable particles such as the clay-water system reported here, although they may exhibit very interesting behaviour.

As an illustration we consider an equation of state of the form:

$$P'_{ij} = 2\mu(t)\epsilon_{ij} \quad (i, j = 1, 2, 3) \quad (1)$$

where the total stress is given by:

$$P_{ij} = P'_{ij} + g_{ij}P \quad (2)$$

and  $P$  is an arbitrary isotropic stress,  $\epsilon_{ij}$  is the strain rate tensor.

In laminar shearing the time-dependent viscosity may take the form:

$$\mu(t) = \mu_0 + \int_{-\infty}^t \frac{df}{dI}(I[t'])M(t-t')dt' \quad (3)$$

$\mu_0$  is the initial viscosity,  $f(I)$  is a suitable function of the quadratic strain rate invariant, for example:

$$f(I) = 1 - \exp\left(-\frac{1}{2}\alpha\epsilon_{ij}\epsilon_{ij}\right) \quad (4)$$

and  $M(t-t')$  is a memory function defined by,

$$M(t-t') = \int_0^\infty \frac{B(\Pi)}{\Pi} \exp\left(-\left(\frac{t-t'}{\Pi}\right)\Pi d\Pi\right) \quad (5)$$

In equations (3) and (5)  $B(\Pi) d\Pi$  is the contribution to the viscosity deficit at the current strain rate  $\epsilon_{ij}(t)$  after an indefinitely long period at that strain rate by all the elements of flow with relaxation times between  $\Pi$  and  $\Pi + d\Pi$ .

It may be shown that if the memory of the material is short so that higher moments of the relaxation spectrum ( $Bn$ ) than the first are negligible where:

$$Bn = \int_0^\infty \Pi^n B(\Pi) d\Pi \quad (6)$$

then equation (3) takes on the simpler form:

$$\mu(t) = \mu_0 - R_1 f(I) + R_1 \frac{df}{dI}(I) \quad (7)$$

where:

$$\frac{d}{dt} = \frac{\partial}{\partial t} + u^i \frac{\partial}{\partial x^i} \quad (8)$$

It is noted that the relaxation spectrum need not be either wholly positive or negative.

Restricting attention now to oscillatory shearing motion in which the shear rate  $\dot{\gamma} = \dot{\gamma}_0 \cos \omega t$  and retaining only the first term in  $\dot{\gamma}$  in (4) then the shear stress  $\tau$  is given by:

$$\tau = \left[ \mu_0 - \frac{R_1 \sigma}{2} \dot{\gamma}_0^2 - R \sin(2\omega t + \phi) \right] \dot{\gamma}_0 \cos \omega t \quad (9)$$

It is clear from (9) that the shear stress may contain odd harmonics of the shear rate frequency. The viscous shear stress may be zero either when the shear rate is zero or when the viscosity is zero, that is:

$$\mu_0 - R_1 f(I) + R_1 \frac{df}{dI}(I) - \dots = 0 \quad (10)$$

An exactly similar argument applied to a time-dependent yield criterion shows that the shear stress may contain even harmonics from this source and the yield stress is zero under an analogous criterion to equation (10).

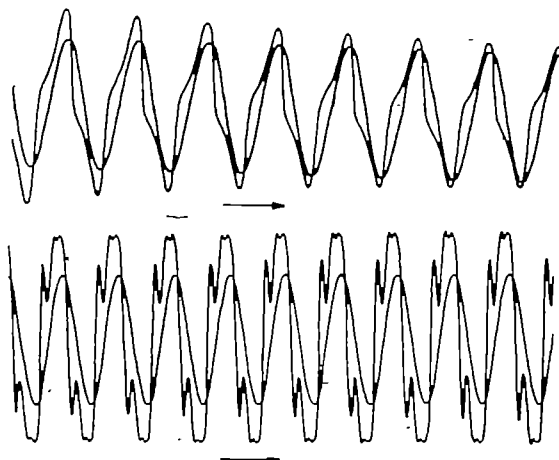


Fig. 1. Barol clay in distilled water (30 g/20 ml.). Shear amplitude, 0.141. (a) 0.0792 c/s, stress amplitude diminishes with time, single peak; (b) 7.21 c/s amplitudes of stress increase with time, multiple peaked. Apparatus, Weissenberg rheogoniometer; natural frequency of torsion head, 42.4 c/s.

Examples of the response of a clay-water system are given in Figs. 1 a and b. Horizontal displacement of the shear stress relative to the strain rate may be due to elasticity, or time-dependency of the viscosity or yield stress. It is interesting to note that at the low frequency the stress amplitude diminishes with time whereas at the high frequency the stress amplitudes increase with time, suggesting that the associated relaxation spectra may not be entirely positive.

A more comprehensive account of these phenomena will be published elsewhere.

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# GEOPHYSICS

## Occurrences of Sinoite, $\text{Si}_2\text{N}_2\text{O}$ , in Meteorites

THE recent discovery of the new mineral sinoite,  $\text{Si}_2\text{N}_2\text{O}$ , in the Jajh deh Kot Lalu enstatite chondrite<sup>1-3</sup> prompted a systematic search for this compound in the other fourteen enstatite chondrites at present known to exist in the world's meteorite collections (Abee, Adhi-Kot, Atlanta, Bethune, Blithfield, Daniel's Kuil, Hvittis, Indarch, Khairpur, Kota-Kota, Pillistfer, St. Marks, Saint-Sauveur, Ufana). Of these fourteen enstatite chondrites, three were found to contain sinoite. These three are the Hvittis, Ufana and Pillistfer stones. The appearance of



Fig. 1. Several crystals of sinoite (centre, light grey) from the Pillistfer enstatite chondrite. Grey main mass is enstatite, white is metallic nickel-iron. Reflected light

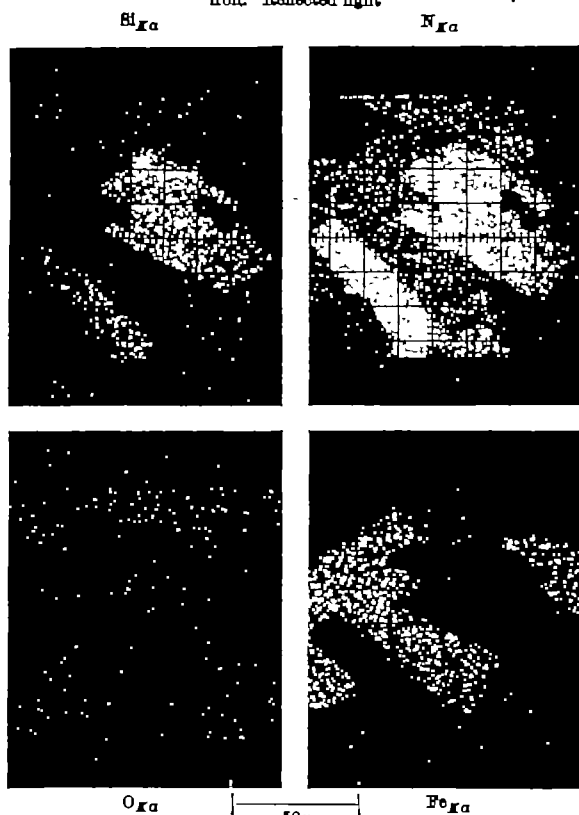


Fig. 2. Electron beam scanning pictures of sinoite,  $\text{Si}_2\text{N}_2\text{O}$ . These pictures are obtained by scanning a microscopically selected area of the sample with the electron beam and recording the characteristic X-rays from the spectrometers on an oscilloscope screen. Sinoite has high silicon and nitrogen and moderate oxygen contents. O-rich areas are enstatite ( $\text{MgSiO}_3$ ); Fe-rich areas are metallic nickel-iron. The apparent N $\alpha$  intensities on enstatite and nickel-iron are due to high background values at this wave-length

Table 1. COMPOSITION OF SINOITE,  $\text{Si}_2\text{N}_2\text{O}$ , FROM THE HVITTIS, PILLISTFER, UFANA AND JAJH DEH KOT LALU ENSTATITE CHONDRITES (Weight per cent)

Element	Hvittis	Pillistfer	Ufana	Jajh deh Kot Lalu
Silicon	56.7	56.8	57.1	56.6
Nitrogen	31.7	31.4	31.9	31.6
Oxygen	13.0	13.0	—	13.1
Total	101.4	101.2	—	101.2

the mineral in these meteorites is very similar to its appearance in the Jajh deh Kot Lalu enstatite chondrite. In reflected light, the crystals are distinctly lighter than the surrounding enstatite and are up to about  $200\mu$  in length (Fig. 1). Under electron bombardment in the electron microprobe, sinoite is easily recognized by its bright greenish luminescence. In each meteorite, ten different grains of the mineral were analysed qualitatively (Fig. 2) and quantitatively for silicon, nitrogen and oxygen by means of electron microprobe techniques using methods previously described<sup>4</sup>. The quantitative analyses were carried out using sinoite from Jajh deh Kot Lalu as a reference standard. The results of these analyses are summarized in Table 1. The chemical identity of the compound in the four meteorites is apparent.

At our request, Dr. B. Mason kindly investigated thin sections of the Hvittis and Pillistfer meteorites available at the American Museum of Natural History, New York. Dr. Mason found the optical properties of the mineral in Hvittis and Pillistfer to be identical with those of sinoite previously described from Jajh deh Kot Lalu<sup>5</sup>. Dr. Mason also pointed out that Lacroix<sup>6</sup> described the optical properties of an unknown compound found in 1905 in the Hvittis and Pillistfer stones. Lacroix, however, was unable to identify the mineral. From Lacroix's description of the optical properties, it appears that this compound and sinoite are identical.

We thank Dr. B. Mason for his advice and for supplying most of the meteorite samples. We thank Dr. J. Larsen-Badse and J. Erlichman for assistance in the microprobe work.

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## Attenuation of Telesismic Body Waves

AT the Royal Society discussion on 'Recent Advances in the Technique of Seismic Recording and Analysis' (January 28-29, 1965), de Noyer directed attention to the difficulty of observing short-period (about 1-sec period) shear waves at angular distances in the range  $25^\circ < \Delta < 100^\circ$ . The following analysis suggests a possible reason.

Consider a solid possessing a complex rigidity modulus  $\mu$  but real bulk modulus  $k$  and density  $\rho$ . Such a material exhibits absorption of both compressional,  $P$ , waves and shear,  $S$ , waves, and we denote the corresponding specific attenuation factors<sup>1</sup> by  $Q_P$  and  $Q_S$  and the corresponding wave velocities by  $\alpha$  and  $\beta$ , respectively. Then the  $P$  and  $S$  parameters are related by the Poisson's ratio,  $\sigma$ , of the material, by the following equations:

$$\alpha^2 (1 - 2\sigma) = 2\beta^2 (1 - \sigma) \quad (1)$$

$$(Q_S/Q_P) = (4/3)(\beta/\alpha)^2 = (2/3)(1 - 2\sigma)/(1 - \sigma) \quad (2)$$

In the Earth, both  $\alpha$  and  $\beta$  are functions of the radius, and their ratio is not constant, that is,  $\sigma$  is also a function

of radius. However, for propagation in the mantle, which for  $\Delta < 100^\circ$  is the case in point,  $\sigma$  only varies between 0.25 and 0.30, so that for order of magnitude calculations it can be taken as constant and equal to 0.28.

The subsequent analysis is now considerably simplified because, accepting this model, the  $P$  and  $S$  waves from a seismic source both travel identical paths to the receiver. Our object now is to consider their relative attenuation.

For each element of path,  $ds$ , within the Earth the amplitude of the  $P$  wave of angular frequency  $\omega$  will be reduced by an amount  $\exp(-\omega ds/2Q_P\alpha)$ . To evaluate the effect over the whole path we need the detailed variation of both  $Q_P$  and  $\alpha$  with radius. However, in the absence of such data we can write the result of integrating the expression over the whole travel path as  $\exp(-\omega T_P/2Q_P^*)$ , where  $T_P$  is the travel time and  $Q_P^*$  the effective mean  $Q_P$  over the whole path. Similarly, for the  $S$  wave the attenuation can be written  $\exp(-\omega T_S/2Q_S^*)$ . But, since we have assumed a constant Poisson's ratio, both the  $P$  and  $S$  waves follow exactly the same path, so that:

$$\beta T_S = \alpha T_P$$

and therefore, from equation (2):

$$(T_S/Q_S^*) = (T_P/Q_P^*) (3\alpha^2/4\beta^2)$$

For the specific case  $\sigma = 0.28$  we have:

$$(T_S/Q_S^*) \approx 4.4 (T_P/Q_P^*)$$

At the Royal Society discussion, one of us (E. W. C.) presented results consistent with a value of  $T_P/Q_P^* \approx 1$  for signals recorded at teleseismic distances (from shallow events). Flinn and Archaambeau have computed  $T_P/Q_P^*$  as a function of distance, using a Gutenberg-Birch velocity structure and a  $Q_P$  structure derived, using equation (2), from the model  $F$  for  $Q_S$  given by Anderson and Archaambeau<sup>3</sup>. On the direct branch of  $P$ , beyond  $20^\circ$ , Flinn and Archaambeau's results lie close to  $T_P = 0.8 Q_P^*$ , in satisfactory agreement with Carpenter's results.

Using such values, it can be shown that at periods of 1-sec,  $S$  wave amplitudes are attenuated by a factor of  $10^4$  more than  $P$  wave amplitudes. At 6-sec period, where  $S$  waves are, in fact, often observed, the corresponding factor is only 5. The model thus seems adequate to explain the observations.

It seems clear from Anderson and Archaambeau's results<sup>3</sup> that the majority of the attenuation occurs in the low-velocity layer at depths of the order of 100 km.

It would thus appear that examination of  $P$  and  $S$  wave spectra from suitably chosen seismic events can prove of great significance in determining the lateral variability of the low-velocity layer.

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## METALLURGY

### Evidence for a Ternary Phase in the Aluminium-Magnesium-Silver System

Polmear and Sargent<sup>1</sup> and Polmear<sup>2</sup> have recently reported that small silver additions will influence the age-hardening characteristics of aluminium-magnesium alloys. They show, for example<sup>1</sup>, that the precipitate in a wrought Al-7 wt. per cent Mg alloy is finer and more uniformly dispersed with resultant increase in hardening, on the addition of 0.5 wt. per cent silver, and they suggest

that there may be an interaction between the magnesium and silver atoms.

Recently, this effect has been investigated in this laboratory where a 0.3 wt. per cent silver addition has been made to a commercial-purity based Al-5 wt. per cent Mg alloy (99.5 per cent Al, 99.93 per cent Mg). Ageing curves were obtained at 150°, 175° and 200° C, and these closely followed those obtained by Polmear<sup>2</sup> (at 150° and 200° C) for an alloy of the same composition. At 200° C, the maximum hardness occurred between 1 and 10 days.

Transmission electron microscopy revealed that not only the size and distribution of the precipitate were altered, as had been shown by Polmear<sup>1</sup>, but also that the morphology of the precipitates was quite different. Fig. 1 shows an Al-5 wt. per cent Mg alloy aged 7 days at 175° C, and Fig. 2 the equivalent material containing 0.3 wt. per cent Ag. The areas shown are grain boundary regions, the boundary in both cases being on the right of the micrograph. The latter alloy was then aged for 32 days at 200° C or 3 days at 350° C to coarsen the precipitate particles. These gave diffraction patterns which could be indexed according to a body-centred cubic unit cell of approximately 14.4 Å. This cell closely resembles those of the ternary phases, Al<sub>3</sub>CuMg<sub>2</sub> and Mg<sub>11</sub>(Al,Zn)<sub>12</sub>, which both have body-centred cubic unit cells of 14.28 and 14.16 Å respectively<sup>3</sup>. It seems possible, therefore, that a similar phase may be present in the Al-Mg-Ag system based on an empirical formula Al<sub>10</sub>AgMg<sub>12</sub> (44.1 wt. per cent Al, 29.4 wt. per cent Ag, 26.5 wt. per cent Mg). A melt of this composition was prepared from super-purity materials and chill cast. The resulting alloy was very brittle and could be ground to powder. The X-ray powder diffraction pattern obtained confirmed the existence of a phase with a body-centred cubic unit cell of  $14.50 \pm 0.05$  Å. Electron probe microanalysis of a polished section of the alloy showed it was almost single

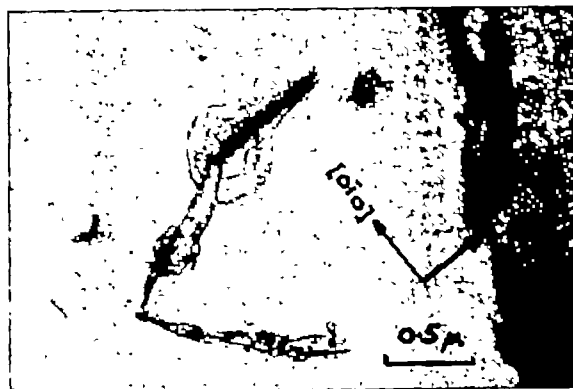


Fig. 1. Wrought Al-5 wt. per cent Mg alloy aged 7 days at 175° C [101] zone

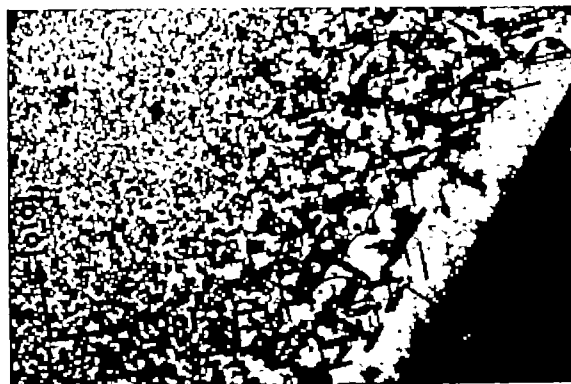


Fig. 2. Wrought Al-5 wt. per cent Mg - 0.3 wt. per cent Ag alloy aged 7 days at 175° C [001] zone

phase with very little segregation. The composition of the main constituent was 43 wt. per cent Al, 29 wt. per cent Ag, 28 wt. per cent Mg.

The foregoing results establish that there is a phase in the Al-Mg-Ag system similar to the  $\text{Al}_2\text{CuMg}_4$  and  $\text{Mg}_{11}(\text{Al,Zn})_4$  ternary phases and it follows that this is the phase which was present in the Al-Mg-Ag alloy aged for 32 days at 200° C. This ageing treatment produces a slightly over-aged material but it seems probable that the precipitates which are present at peak hardness must be closely related to the same Al-Mg-Ag ternary phase. Thus it would seem that aluminium-magnesium alloys containing small additions of silver should really be regarded as ternary precipitation-hardening alloys.

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### Oxide Films on Mild Steel

It is sometimes assumed that the oxide film formed on iron at room temperatures is uniform in thickness across a specimen, and that where several oxides exist together they are present as layers stacked neatly one above the other. In an electron microscope investigation of the oxidation of mild steel using oxide films stripped by the iodine/methanol technique<sup>1</sup> the only oxide that consistently conforms with this idea is magnetite,  $\text{Fe}_3\text{O}_4$ . Fig. 1 shows magnetite with its diffraction pattern on a film stripped from mild steel. The metal specimen was electropolished in a 5 per cent perchloric acid/95 per cent acetic acid mixture, washed in methanol and given no additional air exposure before the surface layer was stripped. It is interesting to note that these stripped oxide films remain quite stable if kept in a desiccator for a year and still give the same diffraction results after this length of time. Magnetite typically appears as an almost featureless layer, though

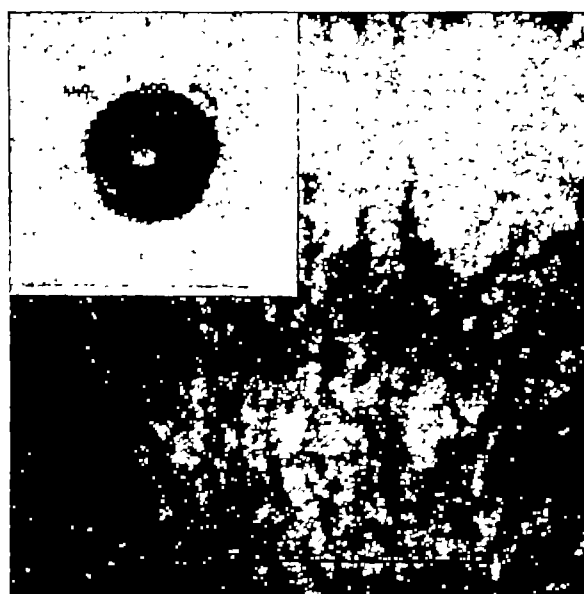


Fig. 1. Featureless iron oxide with diffraction pattern. Scale, 1  $\mu$ .

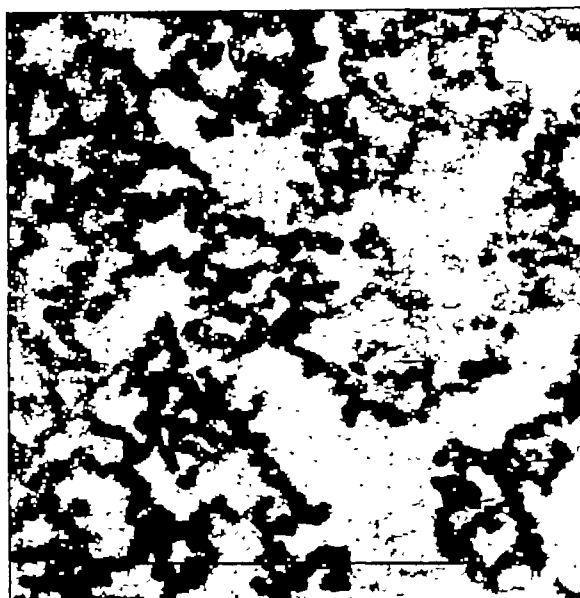


Fig. 2. Non-diffracting iron oxide. Scale, 1  $\mu$ .

with experience its presence may be recognized without examination of the very characteristic orientated arced diffraction patterns it produces. The (001) projection, as in Fig. 1, occurs most frequently although other orientations have been observed on the surface layers produced on thin foil corrosion specimens and there is evidence that the orientation changes from grain to grain. The usual difficulty in distinguishing between  $\text{Fe}_3\text{O}_4$  and  $\gamma\text{-Fe}_2\text{O}_3$  was experienced in this work<sup>2</sup> since these oxides have the same crystal structure and lattice parameters. It is noticeable that the (200) reflexions are absent and that no mixed index reflexions appear—these facts point to  $\text{Fe}_3\text{O}_4$ . There are, however, eight (311)<sup>3</sup> reflexions that would not be expected and these consistently appear with this type of pattern on foils as well as on stripped oxide films. They suggest that the film is of the order of one unit cell in thickness, approximately 10 Å, and that the reflexions could arise by streaking<sup>4</sup>.

After short air exposures, oxide often appears superimposed on the surface of the featureless magnetite in a form looking like a string of spherical beads (Fig. 2) each approximately 500 Å in diameter. This material frequently occurs in small discrete areas rather than a continuous layer. It gives no sharp diffracting spots of its own, only a diffuse pattern added to the magnetite arcs.

This oxide may be truly amorphous, or the lack of identifiable diffraction spots may be due to radial broadening because each bead is composed of smaller spherical particles. After prolonged exposure of electropolished and etched mild steel to air at room temperature without special precautions to exclude moisture (Fig. 3, three days' exposure), oxide growth consisting of large numbers of small crystallites approximately 100 Å in diameter is the commonest form encountered. It is usually possible to identify the oxide type from the ring patterns produced by these crystallites. Goethite,  $\alpha\text{-Fe}_2\text{O}_3\cdot\text{H}_2\text{O}$ , as shown here, is the one most frequently encountered in corrosion specimens although its physical form can on occasions be very different from the small crystals shown.  $\gamma\text{-Fe}_2\text{O}_3$  also occurs as small crystallites of similar size to those shown in Fig. 3 and gives a ring diffraction pattern. Even after three days of air exposure, however, the oxide layer does not appear to be of uniform thickness nor is the specimen surface completely covered with crystallites. There are still areas similar to Fig. 2 showing discrete patches of the non-diffracting oxide, featureless magnetite or no

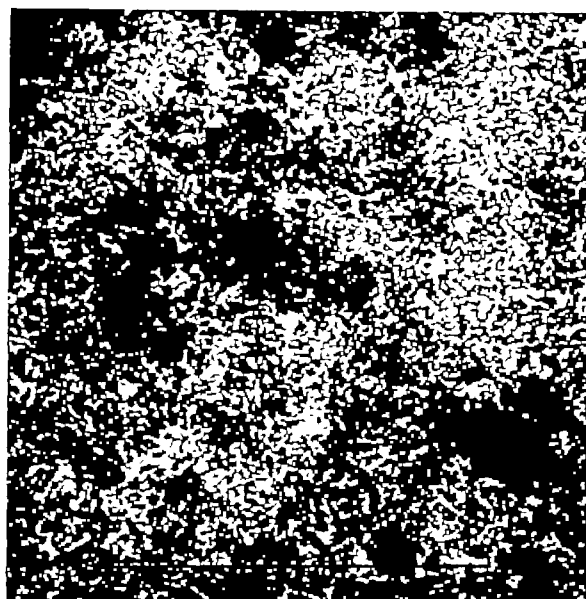


Fig. 3. Crystalline iron oxide.  
Scale, 1 $\mu$

apparent oxide at all. It is considered that the detailed structure of the oxide film is of importance in deciding where corrosion will start and how it will continue when the metal is exposed to air or water.

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### Failure of Stainless Steel by Intergranular Decohesion during Creep

In an experiment where some stainless-steel tubes had been ruptured by internal gas-pressure, failure was observed to have occurred by the nucleation, growth and impingement of intergranular cavities. An attempt has been made to relate this failure-mechanism with the observed dispersion in life-to-rupture. The tubes were of double vacuum-melted 20Cr-25Ni-niobium-stabilized stainless steel. Their nominal dimensions were 0.40 in. bore diam.  $\times$  0.015 in. wall thickness: they were made by extrusion and cold-drawing followed by external grinding. About 120 cap-ended lengths of the tubing were internally pressurized with 1,750 lb./in.<sup>2</sup> argon ( $\sigma_e = 2\sigma_s$ ) at 650°C in an atmosphere of carbon dioxide + 10 per cent carbon monoxide and their lives-to-rupture measured. Optical metallography revealed that intergranular decohesion had been nucleated at many sites and had spread until rupture was complete. A process-rate model of decohesion was constructed and this led to the following equation:

$$F = 1 - \exp \left[ \frac{-Kt^{m+1}}{m+1} \right] \quad (1)$$

where  $F$  is the fractional area of non-coherent grain-boundary at time  $t$ , and  $m$  should be 1.33.

Equation (1) is a special solution of the differential equation:

$$\frac{dF}{dt} = Kt^m(1-F)^n \quad (2)$$

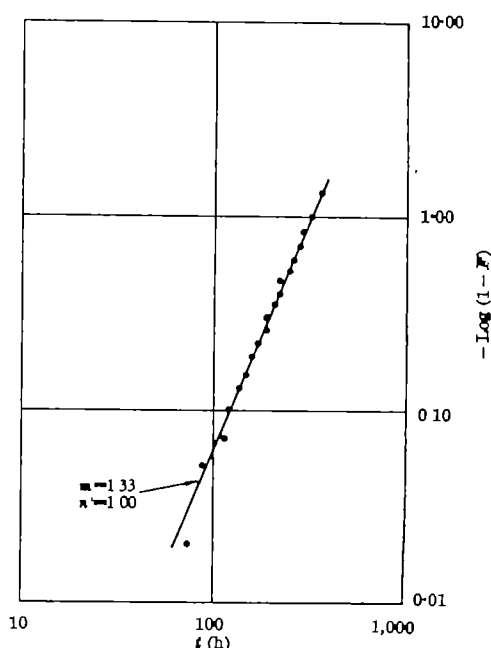


Fig. 1. Experimental points and the theoretical line

For  $n > 1$ , equation (2) has the alternative solution:

$$F = 1 - \left[ \frac{K(n-1)t^{m+1}}{m+1} + 1 \right]^{1/(1-n)} \quad (3)$$

Taking  $F$  as equivalent to the fractional number of tubes which had failed up to a certain time in the experiment, the data were compared with equations (1) and (3): in the case of the latter values of  $K$ ,  $n$  and  $m$  were established by plotting  $\log(1-F)$  against  $\log t$  for large  $t$  and  $\log F$  against  $\log t$  for small  $t$ . These tests revealed that the data correspond closely to the values of  $m$  and  $n$  deduced from the process-rate model. Fig. 1 illustrates this: here the data points are plotted together with the theoretical line for  $m = 1.33$  and  $n = 1.0$ .

From a practical point of view the existence of a physically based distribution function is a valuable asset since it permits reasoned estimates of low failure-rates from necessarily limited experimental data. Arbitrary use, without theoretical justification, of a function (such as, for example, the log-normal distribution) which happens to fit the available data can produce serious errors of extrapolation.

The process-rate model which led to equation (1) also predicts the effects on  $F$  of variables such as stress, ambient pressure, temperature and grain-size. Preliminary comparisons of these predictions with experimental data have given encouraging results.

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## CHEMISTRY

### Preparation of Thiazyl Fluorides

THE action of silver difluoride on tetrasulphur tetranitride has been shown to yield the thiazyl fluorides  $\text{SNF}$  and  $\text{SNF}_2$ , but little is known about the action of other fluorinating agents on sulphur nitride. We have examined the action of several liquid and gaseous fluorides on sulphur nitride, and also the reaction of sulphur tetrafluoride with ammonia.

In glass, selenium tetrafluoride reacts with sulphur nitride at  $-10^\circ$  to give thiazyl fluoride, NSF (15 per cent yield), thionyl fluoride, silicon tetrafluoride, and selenium. The reaction is incomplete even when the selenium tetrafluoride is in large excess, and some  $S_2N_4$  can always be recovered from the solid residue. Iodine pentafluoride behaves in a similar manner towards sulphur nitride; iodine is liberated, and a white solid of composition  $S_2N_4(NSF)_4$  is formed, which decomposes at  $50^\circ$  to give thiazyl fluoride. In contrast, sulphur tetrafluoride reacts only slowly under pressure with sulphur nitride, at  $180^\circ$  in a steel bomb, yielding a little thiazyl fluoride, and much larger proportions of nitrogen and sulphur. Antimony pentafluoride combines slowly with sulphur nitride at  $25^\circ$ , and more rapidly at  $100^\circ$ , to give a green solid of composition  $S_2N_4(SbF_6)_4$ , a behaviour which recalls the similar reaction with sulphur<sup>2</sup>. At  $145^\circ$  this adduct melts with decomposition, to give thiazyl fluoride (10 per cent), together with sulphur dioxide and silicon tetrafluoride. Vanadium pentafluoride, which has been reported to have strong fluorinating properties<sup>3</sup>, reacts with sulphur nitride, but volatile products are not formed.

Elementary fluorine has been reported to react with  $S_2N_4$  to give sulphur fluorides and nitrogen<sup>4</sup>, but we find that under very mild conditions at  $-75^\circ$ , in an apparatus similar to that used by Brown and Robinson to synthesize sulphur tetrafluoride<sup>5</sup>, thiazyl fluoride and thiazyl trifluoride, NSF, are formed in good yield.

Sulphur tetrafluoride reacts with ammonia. At  $-95^\circ$ , with ammonia in excess, the principal product is tetrasulphur tetranitride (70 per cent yield); if the temperature of the reaction is raised the yield of sulphur nitride decreases. At  $25^\circ$ , with the sulphur tetrafluoride in excess, in a flow system, thiazyl fluoride (20 per cent yield) is formed.

Anhydrous hydrazine reacts with excess of disulphur decafluoride below  $25^\circ$ , but the only volatile product is nitrogen, and none of the possible compounds with nitrogen-nitrogen or sulphur-sulphur bonds is formed.

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### Separation of some Metals by Extraction-chromatography using New Neutral Phosphoro-organic Compounds

THE separation of metal cations by means of partition chromatography with reversed phases, known also as extraction-chromatography<sup>1</sup>, has recently been extensively applied in the radiochemical field. Studies of cation separation, using phosphoro-organic compounds by this method, have been carried out by Sikierski *et al.*<sup>2</sup>, O'Loughlin and Banks<sup>3</sup>, and others, besides ourselves<sup>4</sup>.

Recently the new neutral phosphoro-organic compounds like tetrabutylpyrophosphate (TBPP)<sup>5</sup> and a mixture of TBPP with tetrabutylhypophosphate (TBHP)<sup>6</sup> have been employed in the static and dynamic extraction of metals.

These compounds, synthesized by Michalski and Modro<sup>7</sup>, form complexes with metal salts similar to those of tri-*n*-butyl-phosphate (TBP). The partition coefficients of various metals using TBHP and TBPP as the complexing

agents depend, among other factors, on the concentration of mineral acids in the aqueous phase. This last dependence makes possible a separation of some cation mixtures.

0.5 g of the 1:1 mixture of TBHP and TBPP was adsorbed as a stationary phase on silica gel ('Hyflo Super Cell') doubly treated with dichlorodimethylsilane. The gel, of 0.08 mm grain diameter, filled a 120 mm column of glass tubing, 2.8 mm in diameter. The rate of drop formation (one drop of 0.03 ml in  $60 \pm 5$  sec) was controlled by pressure of the order of 0.1 kg/cm<sup>2</sup>. The measurements were performed by the radioactive tracer method. The concentrations of the solutions of salts labelled with radioisotopes used in the experiments are given in Table 1.

Table 1. CONCENTRATIONS OF THE SALTS

Salt	Concentration of metal-ion (M/L)	Radio-indicator
MnCl <sub>2</sub>	$2.5 \times 10^{-4}$	<sup>54</sup> Mn
FeCl <sub>3</sub>	$1 \times 10^{-4}$	<sup>59</sup> Fe
CoCl <sub>2</sub>	$1.2 \times 10^{-4}$	<sup>57</sup> Co
NiCl <sub>2</sub>	$2.1 \times 10^{-4}$	<sup>63</sup> Ni
CuCl <sub>2</sub>	$5 \times 10^{-4}$	<sup>64</sup> Cu
ZnCl <sub>2</sub>	$7 \times 10^{-4}$	<sup>65</sup> Zn
CdCl <sub>2</sub>	$2 \times 10^{-4}$	<sup>115</sup> mCd
Od(NO <sub>3</sub> ) <sub>3</sub>		
SbCl <sub>3</sub>	$4 \times 10^{-4}$	<sup>124</sup> Sb
TeCl <sub>4</sub>	$2.5 \times 10^{-4}$	<sup>127</sup> mTe
Tb(NO <sub>3</sub> ) <sub>3</sub>	$7 \times 10^{-4}$	<sup>160</sup> Tb
UO <sub>2</sub> (NO <sub>3</sub> ) <sub>2</sub>	$1.2 \times 10^{-4}$	<sup>235</sup> U

In order to determine the sorption conditions for the ions studied, 0.03–0.06-ml. samples of the solutions of salts of the investigated metals in HCl or HNO<sub>3</sub> of various concentrations were introduced in the column and then eluted with 9, 5, 1, 0.5, 0.1, and 0.01 N hydrochloric or nitric acid. The drops of eluate were collected on polystyrene foil and dried with an infra-red lamp. The activity of the initial solutions and the eluates was measured with a Geiger-Müller end-window counter 'AAH-55' type, measurements of the activity of radio-nickel were performed with a 2 $\pi$  flow counter, 'AET-60' type, and those of uranium with a scintillation counter, applying the method described previously<sup>8</sup>.

It has been shown in preliminary experiments that the divalent ions of transition metals are not adsorbed from the concentrated hydrochloric acid solutions and can be eluted in the first free volume of the column, whereas ions of trivalent iron become adsorbed and can be washed out with diluted HCl.

Fig. 1 shows a chromatogram of the separation of Mn (II), Co (II), Ni (II), Cu (II), Zn (II), and Cd (II) chlorides from Fe (III) ions, obtained on eluting the column first with 1.4 ml. of 9 N HCl and then with 0.9 ml. of 0.5 N HCl, which elutes the Fe (III) ions.

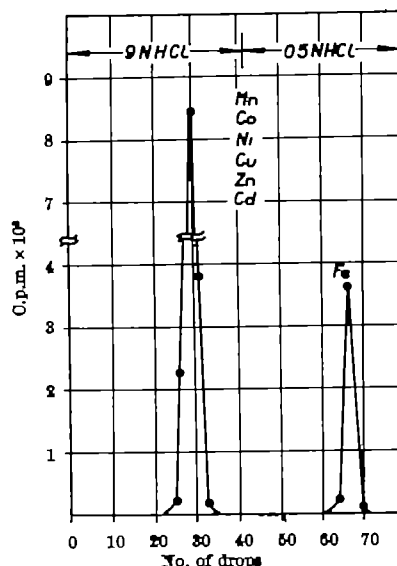


Fig. 1. Separation of (Mn, Co, Ni, Cu, Zn, Cd)—Fe.

In the case of Te (IV) and Sb (V) ions adsorption, it has been found that antimony is best adsorbed from 1 N hydrochloric acid, whereas tellurium ions are not adsorbed and can be eluted in the first free volume of the column. Fig. 2 gives the result of the separation of Te (IV) and Sb (V) ions after elution with 2 ml. of 1 N HCl, and then with 1.6 ml. of 0.1 N HCl, the latter desorbing the antimony ions.

Similarly, as in the case of hydrochloric acid, some metal ions are adsorbed from nitric acid solutions. Studies on metal ion separation using nitric acid as an eluant were carried out on sorption and desorption of cadmium, terbium, and uranyl nitrates. It has been proved that terbium, representing lanthanides, and cadmium are not adsorbed from 5 N HNO<sub>3</sub> solutions, whereas UO<sub>2</sub> (II) ions become adsorbed in these conditions.

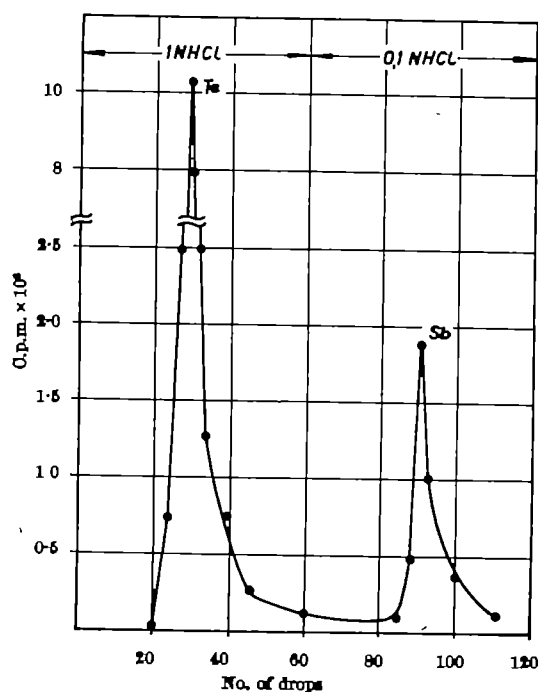


Fig. 2. Separation of Te-Sb

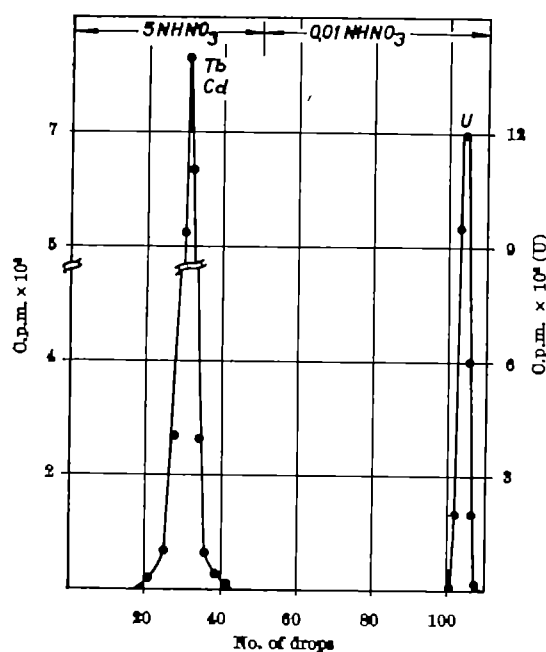


Fig. 3. Separation of (Tb, Cd)-U

Fig. 3 gives a chromatogram showing the separation of Cd (II) and Tb (III) ions from UO<sub>2</sub> (II) ions, this being the result of elution with 1.6 ml. of 5 N HNO<sub>3</sub> and 2 ml. of 0.01 N HNO<sub>3</sub>, the latter desorbing uranyl ions.

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### Polarographic Catalytic Properties of Cobalt-nitrohydroxylamine Complex

THE polarographic catalytic waves, observed by Brdička<sup>1</sup> in the complexes of cobalt or nickel solutions, are considered to be characteristic of organic molecules containing many active groups. It is accepted, in general, that the —SH group is the most specific for the catalytic process in a cobalt-containing medium, but there are also many other substances which do not contain sulphur in their molecular structure but which form catalytic waves: uric acid<sup>2,3</sup>, certain amino-acids<sup>4</sup> as histidine or lysine, glyceraldehyde, dihydroxyacetone<sup>4</sup> and also ethylenediamine<sup>5</sup>.

We have found that nitrohydroxylamine also forms catalytic waves in a cobalt-containing medium (Fig. 1). The formation of the catalytic wave is accompanied by a diminution of the wave due to the cobalt-nitrohydroxylamine complex. The concentration of this complex can be determined by the difference between the free cobalt wave and the wave formed on addition of the nitrohydroxylamine. The dependence of the catalytic wave of the cobalt-nitrohydroxylamine complex as a function of nitrohydroxylamine concentration is represented in Fig. 2 and shows the characteristic form of a catalytic wave. There is a marked analogy between the complex concentration and the catalytic wave-height. By plotting the

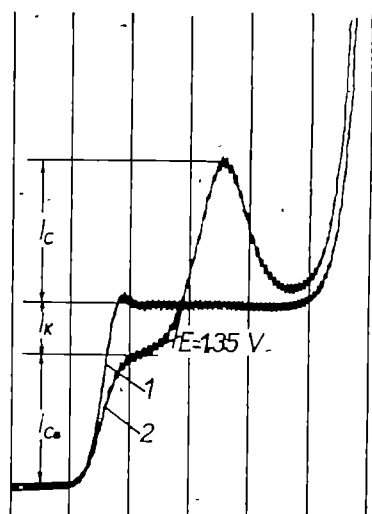


Fig. 1. Catalytic and complexing effect of nitrohydroxylamine;  $m = 2.75 \text{ mg s}^{-1}$ ,  $t = 5 \text{ s}$ ,  $A = 85 \text{ cm}$ , beginning at  $0.8 \text{ V}$ ,  $200 \text{ mV/sec}$ ,  $i_c$  = catalytic current,  $i_L$  = limiting current of active complex,  $i_{c_0}$  = the same current of inactive complex. (1)  $10^{-3} \text{ M Co}^{2+} + 0.025 \text{ M NH}_2\text{OH} + 0.05 \text{ M NH}_4\text{Cl} + \text{gelatine } 0.01 \text{ per cent}$ ; (2) the same solution  $+ 4 \cdot 10^{-4} \text{ M Na}_2\text{N}_2\text{O}_4$ .

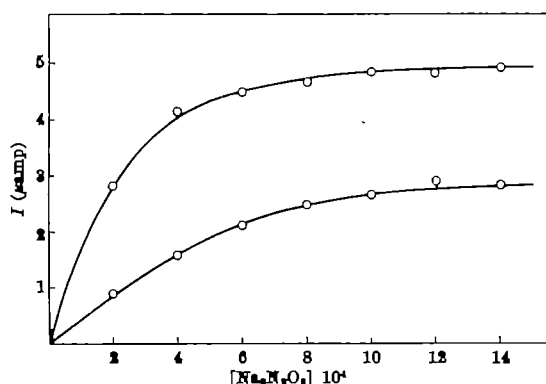
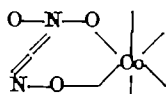


Fig. 2. Plot of the catalytic and complex  $[\text{Co}(\text{N}_2\text{O}_2)(\text{NH}_4)]^+$  waves as a function of nitrohydroxylamine concentration; the conditions and the solution are the same as in Fig. 1. (Upper curve, catalytic wave; lower curve, active complex wave)

ratio of currents  $i_0/i_{00}$  ( $i_{00}$  is the limiting current of cobalt complex in absence of, and  $i_0$  the same current of the complex in the presence of, nitrohydroxylamine) as a function of the ratio  $[\text{Na}_2\text{N}_2\text{O}_2]/[\text{Co}^{++}]$  it is found that the catalytically active complex contains only one molecule of nitrohydroxylamine. The complex may be formulated as  $[\text{Co}(\text{N}_2\text{O}_2)(\text{NH}_4)]^+$ . It has also been found that a catalytic wave is formed in the absence of ammonium or any other indifferent electrolyte; in this case the catalytically active complex has the formula  $[\text{Co}(\text{N}_2\text{O}_2)(\text{H}_2\text{O})_4]^0$ . The molecular active group is then the chelate portion of the complex:



The same complex is formed with nickel, but this only exhibits a double diffusion wave and no catalytic wave is observed.

From these experimental results we can deduce the mechanism of the polarographic catalytic evolution of hydrogen and explain the role of the cobalt ion.

In order that catalysis shall occur it is necessary for the cobalt mono-chelate complex to have a more negative discharge potential than for the catalytic evolution of hydrogen. The decrease in the concentration of the active complex at the electrode surface is accompanied by a decrease in the current in the form of characteristic maxima for a catalytic current.

The value of the catalytic current is determined by two factors: (1) the concentration of the active complex; (2) the cobalt discharge potential in this complex. In the case of the nitrohydroxylamine there is a high concentration of active complex, but the cobalt discharge occurs at positive potentials ( $E_{1/2} \approx -1.6$  V; Fig. 1). With cysteine there is only a small concentration of active complex (practically unimportant in comparison with the total amount of cobalt), but the discharge potential, determined by extrapolation of the curve in Brdička tampon solution with cysteine, is very negative ( $E_{1/2} \approx -1.9$  V). This explains the low value of nitrohydroxylamine catalytic wave in comparison with the cysteine wave. The fact that the discharge potential of the nickel-nitrohydroxylamine complex occurs at an even more positive potential ( $E_{1/2} \approx -1.1$  V) explains the absence of the catalytic wave (begins at  $-1.35$  V, Fig. 1).

In connexion with the molecular structure of substances which exhibit catalytic waves in the presence of cobalt we have observed that it is essential for the molecule to contain at least two functional groups which are capable of chelate formation. One of these groups is capable of being adsorbed at the mercury electrode ( $-\text{SH}$ , for example), hence fixing the complex at the surface; the other accomplishes the function of a proton accepting-

donating group ( $-\text{NH}_2$  or  $-\text{COOH}$ , for example). The proton of this group is neutralized through the mediation of the complex. Also in the case of nitrohydroxylamine the chelation is produced between one adsorbing group



and one other protonated group  $=\text{N}-\text{OH}$ .

In conclusion we can state that no single functional group can show catalytic properties; it is essential that at least two functional groups be present in the molecule before catalysis can occur.

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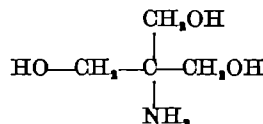
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## Radiation Dosimetry with Sodium Cacodylate and Tris

DURING work on the irradiation of bakers' yeast, *Saccharomyces cerevisiae*, it was found that irradiation of the buffers, sodium cacodylate (sodium dimethylarsinate),  $(\text{CH}_3)_2\text{AsOONa}$ , and of tris (hydroxymethyl) amino-methane ( $=\text{tris}-2\text{-amino}-2\text{-hydroxymethyl}-1, 3\text{-propane-diol}$ ),



yielded substances giving positive Fiske-SubbaRow<sup>1</sup> and ninhydrin<sup>2</sup> colorimetric tests, respectively. Further investigations have been carried out to assess the value of these systems in radiation dosimetry.

Seven-ml. aliquots of sodium cacodylate (0.05 M, pH 5.5) were irradiated in  $17 \times 100$  mm polyethylene or polypropylene test-tubes<sup>3</sup>. This concentration and pH gave optimal colour development in the Fiske-SubbaRow<sup>1</sup> test. The test-tubes, in a wooden block accommodating 92 test-tubes in 4 rows, were passed through the 1.0 megacurie cobalt-60 source so as to receive  $10^4$ – $10^6$  rads of  $\gamma$ -irradiation with each passage through the source<sup>4</sup>. Radiation doses were monitored by ferrous sulphate-copper sulphate dosimeters<sup>5</sup>. After irradiation, a 0.5 ml. sample of sodium cacodylate was diluted with 8.5 ml. of water, and 2.5 ml. of reagent A (2.5 per cent ammonium molybdate in 5 N sulphuric acid) and 0.5 ml. of reagent B (filtered solution of 0.75 g 1-amino-2-naphthol-4-sulphonic acid + 1.5 g  $\text{Na}_2\text{SO}_4$  in 150 ml. water mixed with a filtered solution of 45 g of  $\text{Na}_2\text{S}_2\text{O}_8$  in 150 ml. water) were added. Reagents A and B are stable indefinitely when stored in the dark. The mixture was incubated in a water bath at 60° C for 10 min. Irradiated sodium cacodylate yielded, in this modification of the Fiske-SubbaRow<sup>1</sup> test, a blue colour resembling the colour of the phosphomolybdate complex formed with phosphorus. The intensity of the blue colour, read in a Klett-Summerson photoelectric colorimeter with a 660 m $\mu$  filter (Fig. 1), was proportional to dose up to a dose of about  $5 \times 10^4$  rads. The colour was stable for at least 7 h. The method was reproducible within 4–8 per cent (Fig. 1 indicates standard deviations based on 8 replicates). Repeated experiments at intervals over a 6-



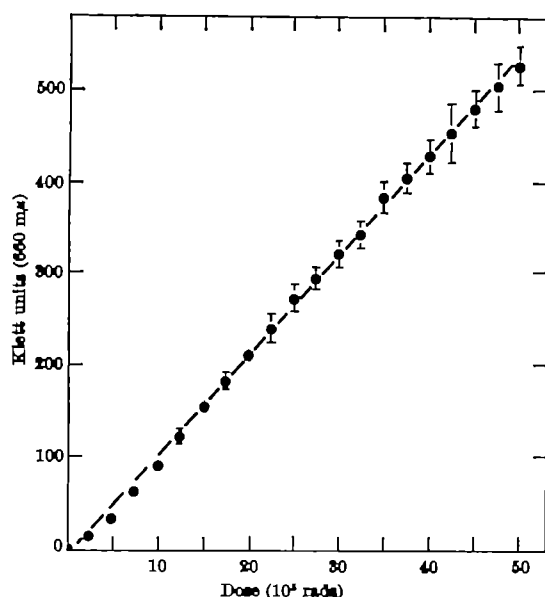


Fig. 1. Sodium dimethylarsinate (cacodylate) as a radiation dosimeter. Irradiated cacodylate, 0.05 M, pH 5.5, was tested (ref. 1) and intensity of the blue colour was read (Klett 560 mμ). Each point represents the mean of 8 replications. Radiation at  $2.5 \times 10^4$  rads per passage through cobalt-60 source. Standard deviation ( $\sigma$ ) from mean is indicated; where  $\sigma$  is not shown, it was no greater than the radius of the circle representing the mean.

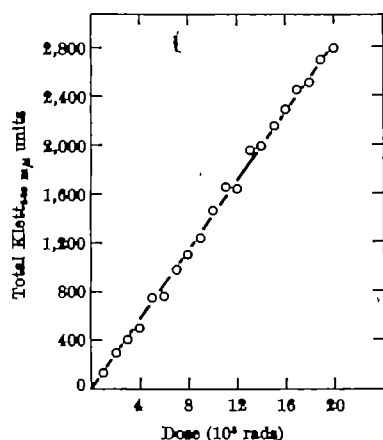


Fig. 2. Tris (2-amino-2-hydroxyethyl-1,3-propanediol) as a radiation dosimeter. Radiation at  $10^4$  rads per passage through a cobalt-60 source. Tris, 0.05 M, pH 7.0, was irradiated, and the ninhydrin-amino nitrogen colour (ref. 2) was read (Klett 560 mμ). Total Klett units = Klett reading  $\times$  sample dilution.

months' period gave Klett readings falling within one standard deviation ( $\sigma$ ) of the mean values shown in Fig. 1. The intensity of the blue colour depended only on total radiation dose and was independent of the dose per passage through the cobalt-60 source.  $\beta$ -irradiation\* of sodium cacodylate gave results which were similar to those obtained with  $\gamma$ -irradiation (same colour intensity for the same total dose). Colour intensity was independent of  $\beta$ -irradiation dose rate over a 4-fold range of dose rates.

The reaction mechanism for the conversion of sodium cacodylate to a compound giving a blue colour in the Fiske-SubbaRow is unknown. However, sodium arsenate gives a positive test (forming an arsenomolybdate complex) and the mechanism may involve oxidation (of the dimethylarsinate to arsenate) with accompanying demethylation, perhaps through the mediation of radiation-produced hydrogen peroxide. The postulated oxidation mechanism was given some credibility by the failure to obtain a positive (blue colour) test when sodium cacodylate was irradiated in the presence of a reducing agent such as ascorbic acid (ascorbic acid, added after irradiation,

does not interfere with development of the blue colour).

In experiments with tris (0.05 M, pH 7.0), 7.0 ml. samples were irradiated as already described here for sodium cacodylate. After irradiation, 0.2 ml. of sample, diluted so that the final Klett reading did not exceed 300, was mixed with 1.0 ml. of ninhydrin<sup>2</sup> reagent in a Klett tube, covered with a glass bead, and immersed in a boiling water bath for 20 min. After dilution with 5 ml. of 50 per cent *n*-propanol, the intensity of the purple colour determined at 560 mμ, and corrected for sample dilution, was proportional to the radiation dose (Fig. 2) up to at least 2.0 megarads.

The cacodylate and the tris systems appear to offer promise as simple radiation dosimeters. The range ( $5 \times 10^3$ – $5 \times 10^6$  rads), ease of preparation and of routine assay, low cost, and reproducibility of the cacodylate system make this a potentially useful system where megarad doses are common, as in food irradiation, or in cases where the increased ease of operation is more important than the greater precision of other chemical dosimeters.

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<sup>3</sup> Samples irradiated in 'High Temp' plastic culture tubes supplied by Falcon Plastics Co., Division of Becton, Dickinson and Co.

<sup>4</sup> To receive  $10^4$  rads, the conveyor travelled at about 5.5 ft./min through the 4.73 ft. of the source.

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<sup>6</sup>  $\beta$ -irradiation with the 2.0-MeV Van de Graaff Electron Accelerator at Electronized Chemicals Corp., Burlington, Mass.

### Whisker-like Crystals of Potassium Chloride

Porous glass<sup>1</sup> has pores of molecular dimensions (20–40 Å). During recent work on the use of porous glass in electrochemistry<sup>2</sup>, long, fibre-like crystals of potassium chloride were found growing from a dried-out porous glass-potassium chloride salt bridge. These crystals, of about 2 μ in diameter with lengths up to several centimetres, were weak but flexible. Among other compounds investigated sodium chloride, barium chloride, potassium nitrate, potassium chromate, copper sulphate and chrome alum showed the same phenomenon, but the resulting growth was slower than for potassium chloride, and the crystals were not so well defined. The rate of growth for potassium chloride crystals was about two centimetres per day. As there is interest in whisker crystals at present<sup>3</sup>, it was felt that this facile technique should be more widely known.

A plug of porous glass about 3 mm in diameter was sealed into 'Pyrex' tubing with 'Araldite' cement and



Fig. 1. Two weeks' growth from saturated potassium chloride solution.

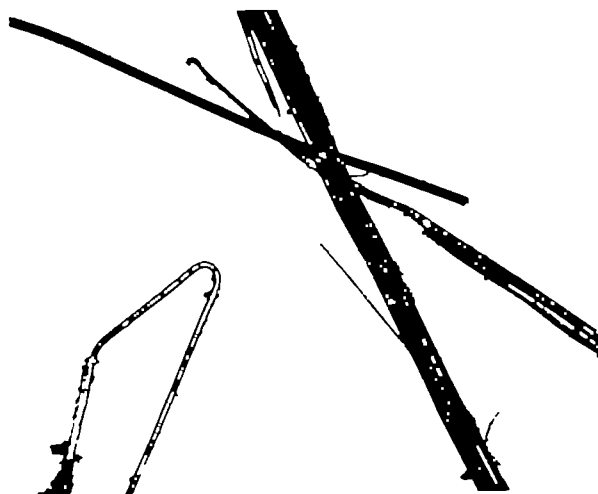


Fig. 2. Potassium chloride fibres suspended in bromoform ( $\times a. 350$ )



Fig. 3. Potassium chloride whiskers growing from porous glass

ground flat by hand with water and corundum. The progress of the saturated potassium chloride solution through the dried porous glass plug could be seen as a sharply defined dark area which took about an hour to traverse the plug. The fibres could be observed, first as a white bloom on the plug, and later as a tight bunch of silk-like fibres resembling glass wool. These fibres bent into random configurations, due, it was felt, to imperfections in the sealing of the plug, and local air currents during crystallization. The crystals were usually grown in a normal laboratory atmosphere, but if the tube was placed in a desiccator with phosphorus pentoxide at atmospheric pressure, the phenomenon was inhibited. Marking the fibres with dye confirmed the assumption, made from general observations, that the crystals grow from the surface of the porous plug. Preliminary X-ray measurements of the potassium chloride fibres show that they have the normal crystalline form of face-centred cubic and that crystal growth is mainly along one main axis.

We thank Dr. I. D. Chapman, of Corning Glass, who kindly supplied the porous glass, and CIBA (A.R.L.), Ltd., Cambridge, who provided the cement (AF111/

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## BIOCHEMISTRY

### Induction of Tyrosinase in Higher Fungi

TYROSINASE is a common enzyme in mycelia and fruit bodies of several fungi. In contrast to laccase it is a typical endoenzyme which is not excreted into the culture medium. In some fungi its occurrence is inconstant and dependent on the stage of development of the mycelium. Whereas in *Phellinus laevigatus*, *Inonotus cuticularis* and *I. obliquus* tyrosinase can be found in young mycelia, in *Phellinus ribis* and *Gloeoporus adustus* its activity increases with the ageing of the hyphae. In some fungi (*Fomes marginatus*, *Leninus lepidus*, *Piptoporus betulinus*) tyrosinase is only present under special cultural conditions, but it can be induced by several chemicals, which are not substrates for this enzyme.

*Fomes marginatus* (strain 16a Eberswalde) proved a suitable test-organism because its mycelia grown on malt solution contain almost no tyrosinase activity. Addition of phenol, catechol, *p*-oxibenzoic acid and protocatechuic acid in final concentrations of  $5 \times 10^{-3}$  M did not induce any enzyme formation or any significant change in the growth rate of this fungus. Catechol was decomposed by the mycelia without formation of tyrosinase. Tyrosine was also ineffective.

A strong tyrosinase induction could be obtained by the addition of some metabolic inhibitors to the culture fluid (Table 1). Lower concentrations were often more effective than higher ones. In some cases the dry weight of the mycelia decreased due to a toxic effect on the fungus, but enzyme induction was not correlated with mycelium autolysis. Strong toxicity due to high concentrations, or a combination of different inducing toxins, inhibited the formation of the enzyme. Besides that, induction of the enzymes could be brought about not only by compounds which had a stimulating effect on the mycelium growth (with arsenate dry weight reached 132 per cent of the controls) but also by many that did not.

To determine whether the increase in activity was due to a mere liberation of the enzyme in the cell or to synthesis of a new protein, the effect of pentachlorophenol (PCP)—a strong inductor—was combined with that of actinomycin D, a specific inhibitor of RNA metabolism (the antibiotic was kindly supplied by Prof. Horváth, Budapest).

Table 1. TYROSINASE ACTIVITY (COLOIMETRIC UNITS) OF *Fomes marginatus* AFTER APPLICATION OF DIFFERENT METABOLIC INHIBITORS. CONTROLS WITHOUT ACTIVITY. (LUTHARDT)

Chemicals	Conc. (M)	Days after application							
		1	2	3	5	7	9	12	
Arsonate	$2 \times 10^{-4}$	25	90	80	65	32			
Na-Azide	$1 \times 10^{-4}$	0	0	0	0	0	0	0	
2,4-Dinitrophenol	$2 \times 10^{-4}$	0	0	10	10	0			0
	$1 \times 10^{-4}$	100	100	90	60	20	10	0	
Monodiodoacetate	$2 \times 10^{-4}$	0	48	88	23	10	0	0	
	$1 \times 10^{-4}$	100	100	90	85	74	65	55	
Oxytetraacycline	$2 \times 10^{-4}$	0	10	16	10	10	0		
	$1 \times 10^{-4}$	10	16	35	32		30	12	
Pentachlorophenol	$1 \times 10^{-4}$	100	84	62	53	40	10		
$\gamma$ -Thujaphidine	$1 \times 10^{-7}$	77	50	50	34	40		35	
<i>p</i> -Chloromercuribenzoic acid	$2 \times 10^{-4}$	+++	+++	+++	++	—			
Potassium cyanide	$5 \times 10^{-4}$	—	—	—	—	—			
Fluoroacetic acid	$5 \times 10^{-3}$	—	+++	+++	+++	+			
Malonic acid	$5 \times 10^{-4}$	—	—	—	—	—			

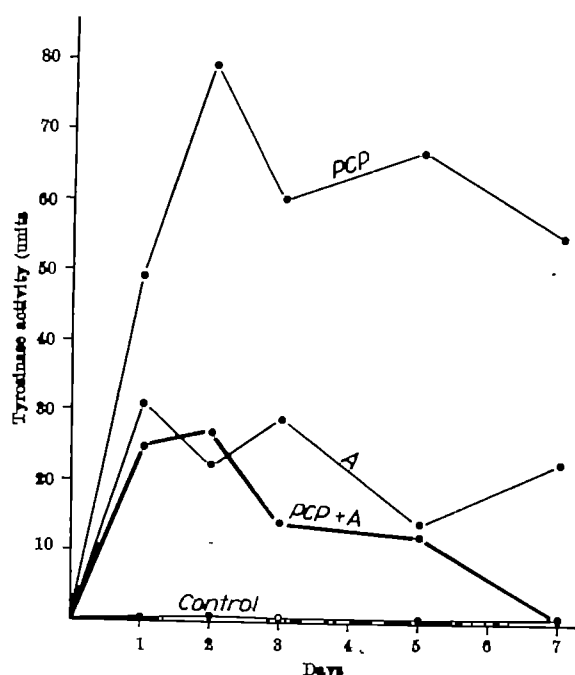


Fig. 1. The effect of actinomycin D ( $7.5 \times 10^{-5}$  M) on the induction of tyrosinase by pentachlorophenol (PCP) ( $1 \times 10^{-4}$  M) in *Fomes marginatus*.

The results are shown in Fig. 1: With PCP a strong tyrosinase induction could be obtained, but this decreased on addition of actinomycin D to that level, which was reached by addition of the antibiotic alone. The controls contained no tyrosinase activity. The results show that actinomycin D inhibits tyrosinase induction by PCP to a high degree. This process seems, therefore, to be a real enzyme induction bound to an active protein metabolism. That actinomycin alone provoked a tyrosinase activity may be due to a liberation of a bound or inactive form of the enzyme, which was demonstrated by Horowitz and San-Chiun Shen<sup>2</sup> and by Esser<sup>3</sup>. The weight of mycelia decreased on addition of PCP during the first two days but recovered thereafter. Actinomycin brought about no additional inhibition of growth so that an excessively strong toxic effect can be excluded.

As can be seen from Table 1, a wide spectrum of substances is able to induce the formation of tyrosinase in *F. marginatus*. It is difficult to find a common basis for an explanation of this effect. Most of the inducers are able to inhibit the oxidative phosphorylation or respiratory processes (Krebs cycle) in fungi (2,4-dinitrophenol, PCP,  $\gamma$ -thujaplicine, arsenate ( $\text{Lyr}^4$ )). Oxytetracycline seems to have a similar effect in higher doses, because it liberates organic bound phosphate in yeast ( $\text{Lyr}^4$ ). *p*-Chloromercuribenzoic acid inhibits several dehydrogenases of the Krebs cycle. The weak effect of Na-azide, a known uncoupling reagent, is due to its low stability. Potassium cyanide, malonate and chloramphenicol were also inactive (probably for the same reason), whereas fluoroacetate had a strong inducing effect. It would therefore seem that a disturbance of the oxidative metabolism, especially of the ATP-formation (provided it is not too severe), leads to an enzyme induction. At present it is difficult to say whether this depends on the liberation of an internal inducer (an increased content of tyrosine in the mycelium could not be demonstrated), or whether the tyrosinase induction is bound to the abolition of a metabolic repression. By this we mean that the synthesis of the enzyme commences when a number of factors in the general metabolism have been satisfied, and these may vary from species to species. This would explain the appearance of the enzyme in young or old mycelia and in fruit-bodies. A very similar process seems to be the induction of laccase ( $\text{Lyr}^4$ , Fah-

raus<sup>5</sup>). It is possible that the formation of these unspecific enzymes is not bound to the presence of a specific inducer, but is regulated by the situation of the metabolism. Probably this mode of induction of tyrosinase is not restricted to fungi, but may also occur in higher plants where tyrosinase activity often increases in the region of sublethal injuries as part of a defence mechanism. It is worth mentioning that the enzyme is able to detoxify some of the inducing poisons, for example, PCP, oxytetracycline, thujaplicines ( $\text{Lyr}^{4,6,7}$ ). Whether this mechanism operates *in vivo* and is of importance for the fungus is not yet quite clear.  $\beta$ -Thujaplicine, on the other hand, is one of the most effective inhibitors of tyrosinase activity (Luthardt<sup>8</sup>).

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### Selective Inhibition by Aldehydes of Streptokinase Activation of Plasminogen

Using synthetic substrate *p*-tosyl arginine methylester (TAME) and lysine methylester (LMe), Ablondi and Hagan<sup>1</sup> observed that plasminogen incubated in 50 per cent glycerol phosphate solution lost proactivator activity and gained spontaneous plasmin activity. Kline<sup>2</sup> examined pseudoglobulin (acid-extracted) plasminogen under the same conditions and found no decline in proactivator activity during the spontaneous activation process. We repeated these investigations with euglobulin (tricalcium phosphate-extracted) plasminogen and we examined the effects of lysine and glyceraldehyde on the system.

We incubated 10 mg/ml. of euglobulin (euglobulin plasminogen was obtained from Cutter Laboratories, Berkeley, California) or pseudoglobulin plasminogen in 50 per cent glycerol with 0.01 M phosphate buffer at pH 7.4, 37° C. The pseudoglobulin plasminogen gained spontaneous activity, without losing proactivator activity as assayed on TAME<sup>3</sup>, LMe<sup>4</sup> and casein<sup>5</sup> (Fig. 1). The euglobulin plasminogen also gained spontaneous activity, but lost proactivator activity. Three other properties of

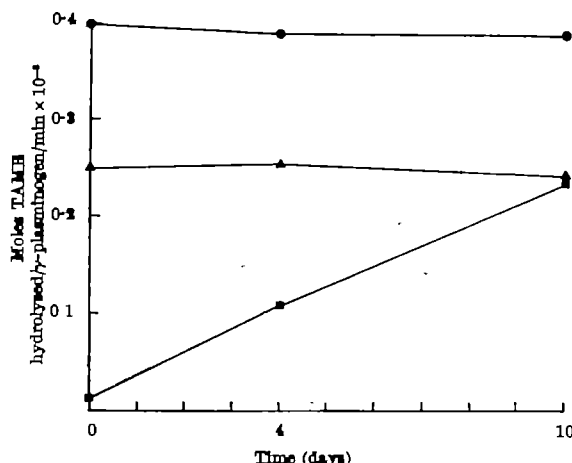


Fig. 1. Effect of incubation in 50 per cent glycerol-phosphate solution (pH 7.4, 37° C) on the spontaneous plasmin activity (■), 100-μ streptokinase-induced plasmin activity (▲), and activator activity (●) of acid-treated plasminogen.

euglobulin plasminogen changed: (a) the rate of migration on immunoelectrophoresis changed; migration was as an  $\alpha_1$ -globulin instead of an  $\alpha_2$ -globulin. (b) Precipitation by streptokinase was no longer possible. (c) Caseinolysis by activated plasminogen was no longer inhibited by excess amounts of streptokinase. We concluded that the physical chemical properties of acid-extracted plasminogen were more stable in 50 per cent glycerol than those of euglobulin plasminogen.

This loss of proactivator activity (and other properties of euglobulin plasminogen) might have been explained by either a change in conformation of the enzyme or a reaction of plasminogen with an impurity in glycerol. Physical measurements of conformation revealed no change in optical rotation or viscosity. Therefore, we concluded that conformational changes were not causing the loss.

Two different lots of glycerol<sup>8</sup> were examined for compounds with reactive aldehyde groups by both the Fehling and Schiff tests<sup>9</sup>. The tests were positive. Therefore, glyceraldehyde, 0.001–0.01 M, was added to the plasminogen-glycerol system, and the effects observed after 6–12 h in this system were identical with those observed after 7 days in glycerol alone (Fig. 2). Lysine inhibited the effects of glyceraldehyde (Fig. 3). We concluded that glyceraldehyde selectively inactivated proactivator by reacting with its amino-groups (for example, glucose amine  $\text{NH}_2$ ,  $pK$  6.5, lysine  $\text{NH}_2$ ,  $pK$  10.1) and that this phenomenon was inhibited by compounds such as lysine, the amino groups of which had a  $pK$  sufficiently low to compete with the protein for aldehyde groups at  $pH$  7.4. Because the loss of proactivator activity and changes in physical chemical properties were accelerated

in the glycerol-glyceraldehyde system and because of the presence of aldehydes in glycerol we concluded that this might be the mechanism by which euglobulin plasminogen lost proactivator activity during 'spontaneous' activation in glycerol. It should be emphasized that glyceraldehyde produces a bovine type of plasmin from a human (SK-reacting) type.

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### An Unstable Ribonucleic Acid in Normal Human Erythroblasts

It is now well established that the primary information required for protein synthesis is sited on DNA. The mechanism whereby, in animal cells, this information is conveyed and elaborated, so that protein synthesis may occur, is still uncertain.

The existence of a metabolically labile RNA in the nucleus of many animal cell types has been repeatedly observed by various techniques<sup>1,2</sup>. Polyribosomes have been shown to be directly responsible for protein synthesis<sup>3</sup>, and in this connexion an RNA fraction, similar to bacterial messenger RNA, because of its variable molecular size and high metabolic instability, has been isolated from the polyribosomes of rat liver<sup>4</sup>. Recently, another RNA messenger fraction has been isolated from the cytoplasm of rat hepatocytes<sup>5</sup>.

However, the results of some experimental observations are difficult to reconcile with the concept that the information required for protein synthesis in animal cells is obtained through a mechanism akin to that of bacteria, that is, through a continuous flux of labile RNA molecules, which are synthesized on the DNA model. It has been shown, for example, that the RNA present in reticulocytes, which are known to synthesize haemoglobin, is stable<sup>6</sup>. Furthermore, while the main site of protein synthesis appears to be located in the cytoplasm, no definite proof has yet been brought forward, as Harris has recently pointed out<sup>7</sup>, to show that transfer of RNA takes place from nucleus to cytoplasm. This author has been able to provide evidence that the greater part of the unstable RNA in the nuclei of some animal cells may be degraded without simultaneously affecting the rate of protein synthesis<sup>8</sup>.

The introduction of actinomycin D has provided a new and extremely useful tool for the study of RNA metabolism. This substance, by inhibiting the synthesis of all types of DNA-dependent RNA<sup>9</sup>, is ideally suited for assessing the rate of RNA degradation, especially in experiments at the cellular level, which are greatly influenced by the intracellular pool of metabolites and the re-utilization of turnover products.

A first series of experiments on the RNA metabolism of normal bone marrow cells, studied by means of actinomycin D, has already been reported elsewhere<sup>9</sup>. The purpose of the present communication is to describe the findings of some further experimental work on the RNA

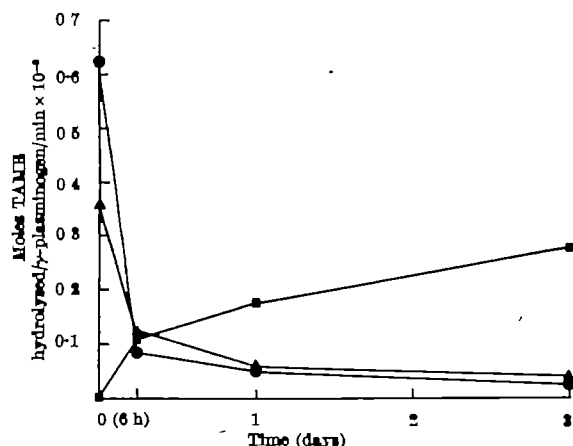


Fig. 2. Effect of incubation in 50 per cent glycerol-phosphate solution (pH 7.4, 37° C) on spontaneous plasmin (■), 100-μ streptokinase-induced plasmin (▲) and activator activity (●) of euglobulin plasminogen and euglobulin plasminogen with 0.02 M glyceraldehyde

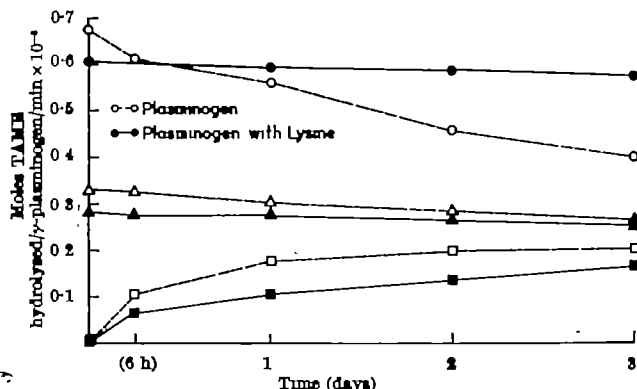


Fig. 3. Effect of incubation in 50 per cent glycerol-phosphate solution (pH 7.4, 37° C) on the spontaneous plasmin (■), 100-μ streptokinase-induced plasmin (▲) and activator activity (●) of euglobulin plasminogen and euglobulin plasminogen with 0.07 M lysine

and protein metabolism of normal human erythroblasts, using tritiated precursors for autoradiography, and actinomycin D. Our studies were undertaken to obtain additional information on the following points of the RNA and protein metabolism of erythroblasts: (a) the relationship between the rates of RNA and protein synthesis in the various stages of maturation of the red cell series; (b) the rate of labelled RNA degradation; (c) the effect of RNA inhibition on the rate of protein synthesis.

$^3\text{H}$ -uridine was used as an RNA precursor and  $^3\text{H}$ -leucine as a protein precursor (Radiochemical Centre, Amersham). The uptake of leucine may be taken to indicate the synthesis of a polypeptide chain<sup>10</sup>. So far as  $^3\text{H}$ -uridine uptake in nuclear RNA is concerned, its complete inhibition in erythroblasts, previously treated with actinomycin D<sup>9</sup>, seems to suggest that true RNA synthesis is taking place and not just a simple binding of terminal groups belonging to soluble RNA. Experiments were carried out on the bone marrow of three individuals with no haematological disorder.

To study the relationship between rates of RNA and protein synthesis, bone marrow cells were incubated for 1 h at 37° C with  $^3\text{H}$ -uridine and  $^3\text{H}$ -leucine at a concentration of 1  $\mu\text{C}/\text{ml}$ . and afterwards smeared on gel slides. For the purpose of differentiating between DNA and RNA labelling, cells before contact with the Kodak 'AR 10' films were treated for 2 h with a solution of DNase ('Sigma', 0.1 mg/ml.) in *tris* buffer<sup>11</sup> containing  $\text{MgSO}_4$ . After an appropriate period of contact, the autoradiographs were developed and fixed, and counterstained with May Grunwald-Giemsa stain. At least 40 cells for each different maturation stage were examined, and the standard error did not usually exceed 15 per cent. In Fig. 1 the rates of RNA and protein synthesis, indicated by the average grain counts, are shown in relation to the maturation stage of the red cell precursor. It is apparent from the diagram that the rate of RNA synthesis decreases as maturation progresses, but at a faster rate than that of protein synthesis.

The rate of RNA degradation was measured using the inhibition of the synthesis of all types of RNA induced by actinomycin D. The suspension of bone marrow cells was incubated for 1 h with  $^3\text{H}$ -uridine at a concentration of 1  $\mu\text{C}/\text{ml}$ . and a proportion of the cells was immediately smeared. Afterwards, in order to inhibit as rapidly as possible labelled RNA precursor uptake, not only actinomycin D was added to the cultural medium as in previous experiments<sup>9</sup>, but the cell suspension was centrifuged and the cells re-suspended in Gey's medium containing actino-

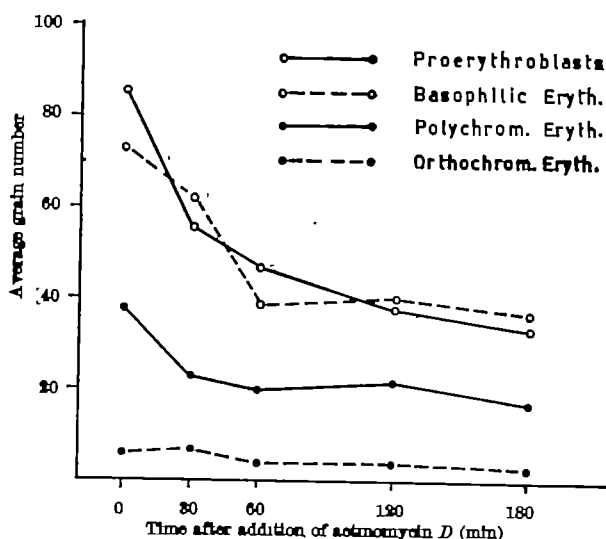


Fig. 2. Decrease of RNA radioactivity, after addition of actinomycin D to the medium in erythroblast precursors previously incubated for 1 h with tritiated uridine.

mycin D at a concentration of 10  $\mu\text{g}/\text{ml}$ . and non-labelled uridine  $10^{-3}$  M. Smears were made after 30, 60, 120 and 180 min, and autoradiographs were prepared after incubation with DNase.

The results are summarized in Fig. 2, which clearly shows that in proerythroblasts and in early and intermediate erythroblasts, at least two metabolically different types of RNA are synthesized, one relatively unstable which becomes rapidly degraded within an hour, and another considerably more stable. The decrease in labelling was in every way similar to that previously observed after the mere addition of actinomycin D to the culture medium<sup>9</sup>. It should be emphasized that the labelling remained exclusively nuclear for the whole duration of the experiment.

To study the influence of RNA synthesis inhibition on the protein synthesis of erythroblasts, actinomycin D (10  $\mu\text{g}/\text{ml}$ .) was added to a suspension of bone marrow cells. Afterwards, at intervals of 60, 120 and 180 min an aliquot of medium was aspirated and incubated for 30 min with  $^3\text{H}$ -leucine (1  $\mu\text{C}/\text{ml}$ .) and smears were prepared. Cells obtained from an actinomycin-free medium and incubated with  $^3\text{H}$ -leucine after the same intervals of time acted as controls. From Fig. 3 it is obvious that the rate of protein synthesis in erythroblasts treated with actinomycin remains similar, during the time of the experiment, to that of erythroblasts untouched by actinomycin.

The present results suggest that an RNA fraction, characterized by a high degree of metabolic activity, is synthesized in the more immature stages of the red cell series. This RNA fraction does not act as a template for the proteins which are synthesized simultaneously, since the inhibition of its synthesis, and its breakdown, do not cause a parallel decrease in the rate of protein synthesis.

The existence of an RNA fraction with such characteristics in the more immature stages of the red cell series is of interest. In fact, in the final stages of this cellular line, that is in reticulocytes, haemoglobin synthesis takes place in the presence of a stable RNA fraction<sup>6</sup>, while the synthesis of a DNA-dependent RNA must be excluded<sup>6,12</sup>. Consequently it seems probable that the information which is required for protein synthesis must have been prepared in earlier stages. On account of the presence in these early cells of an unstable RNA and because the metabolic instability observed appears to be characteristic of messenger RNA<sup>4</sup>, it seems not unreasonable to suppose that the elaboration of information is linked with the synthesis of a labile RNA messenger fraction in erythroblasts also.

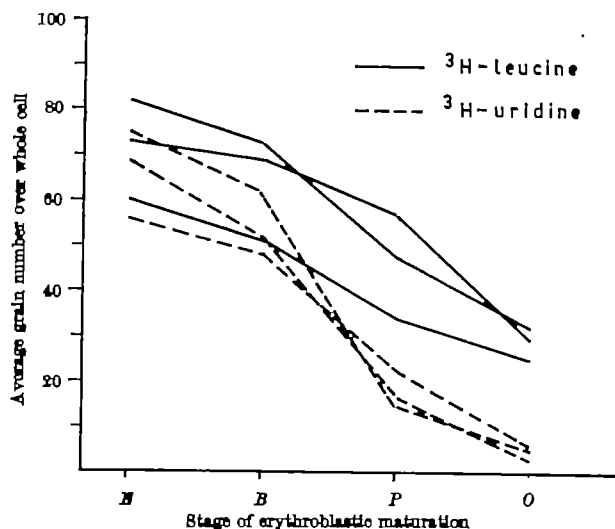


Fig. 1. Incorporation of tritiated uridine and leucine into erythroblasts at different stages of maturation. N, early erythroid precursors; B, basophilic erythroblasts; P, polychromatic erythroblasts; O, orthochromatic erythroblasts.

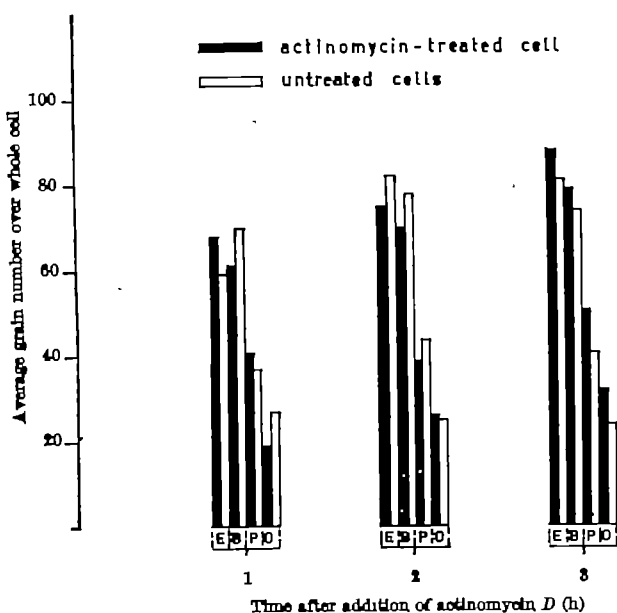


Fig. 3. Incorporation of tritiated leucine into erythroid precursors at different intervals after addition of actinomycin D to the medium

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## Salicylic Acid as a Metabolite of L-Tryptophan

The metabolites appearing in the urine after an oral load of L-tryptophan have been separated by paper, thin-layer and column chromatography. After ingestion of L-tryptophan (70 mg/kg body-wt.) the urine was collected at timed intervals over the next 24 h. Aliquots of the urine were examined by two-way paper chromatography (n-butanol-acetic acid-water, 12:3:5, followed by 20 per cent KCl in water), and by thin-layer chromatography on silica gel using chloroform, methanol, acetic acid (76:22:2 by volume). The metabolites were located with Ehrlich and Pauly reagents<sup>1</sup> and by fluorescence in ultra-violet light at two wave-lengths (253 and 366 mμ).

During the course of this investigation, 9 out of 23 normal individuals examined were found to excrete an unknown metabolite, which had an *R<sub>f</sub>* of 0.75 on thin-layer chromatography and which occupied a position to the right of acetyl-3-hydroxykynurenine<sup>2</sup> on two-way paper chromatography. The unknown compound could

also be separated by chromatography of the urine on a column (50 cm × 0.9 cm) of DEAE 'A-25 Sephadex' (Cl- form) which had been equilibrated with 0.2 N hydrochloric acid in 10 per cent isopropanol. The unknown substance was eluted after the passage of 40–45 ml. of the acid propanol mixture, and after acid hydrolysis was found to contain glycine. An authentic sample of salicylic acid showed identical behaviour under all these conditions.

It is well known that ingested aspirin is largely excreted as salicylic acid but apparently it has escaped notice so far that salicylic acid is excreted by many people after a load of L-tryptophan, and when the possibility of acetylsalicylic acid ingestion during the period of the experiment has been rigorously excluded.

We believe that the salicylic acid arises from L-tryptophan by bacterial action in the colon, is then absorbed, and after conjugation with glycine is excreted as salicylic acid. The colonic origin is suggested by the facts that: (1) the peak of excretion is 10–14 h after oral ingestion of the L-tryptophan, which is considerably later than the peak excretions of tryptophan metabolites of endogenous origin; (2) salicylic acid is not found in the urine of patients who are receiving neomycin; (3) the introduction of L-tryptophan directly into the colon causes a much greater excretion of salicylic acid, as compared with the oral dose.

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## Methods for Detection of Enzymatic Activity after Electrophoresis on Polyacrylamide Gel in *Drosophila* Species

Our laboratory is investigating several proteins within the genus *Drosophila*. In conjunction with this work, we have developed techniques for detecting enzymatic activity following electrophoresis on polyacrylamide gel. Several other methods for demonstrating specific enzyme activity in *Drosophila* have been reported<sup>1–3</sup>; these were applied after starch-gel electrophoresis.

The methods here described have given satisfactory results for malic dehydrogenase, α-glycerophosphate dehydrogenase and non-specific esterases when used to test a number of species. *D. virilis* Pasadena strain and *D. melanogaster* Oregon-R strain have served as controls.

Polyacrylamide gels are prepared, using a modification of the method of Hubby<sup>4</sup>. Before catalysts are added the 5 per cent monomer solution is heated to 45° C. The heating allows polymerization to occur within 20 min in the presence of only 0.8 per cent ammonium persulphate (AP) and 0.13 per cent dimethylaminopropionitrile (DMAPN). The reduction in amount of AP eliminates the necessity for an equilibration period before electrophoresis. The gels are cooled during electrophoresis by a Sargent water cooler, which maintains the temperature of the gel at 9° C.

Extracts are prepared of whole adult frozen flies, 1–2 days in age. The flies are ground in five parts of 5 per cent sucrose in 0.1 M phosphate buffer, pH 6.5, and centrifuged 20 min in a Servall model 'A' centrifuge at 6,780g. A 20-μl. aliquot of the supernatant is placed in each trough of the cooled gel.

Electrophoresis is usually carried out for 90 min at 28 V/cm. The current is about 75 m.amp. At the end

of this period the gels are removed and placed in the appropriate incubation medium.

The methods for the detection of the dehydrogenases are similar to one another. For malic dehydrogenase (MDH): 0.1 M neutralized sodium malate; 20 µg/ml. phenazine methosulphate (PMS); 0.3 mg/ml. nicotine adenine dinucleotide (NAD); 0.3 mg/ml. nitro blue tetrazolium (Dajac Laboratories); in 0.1 M phosphate buffer, pH 7.4. For  $\alpha$ -glycerophosphate dehydrogenase ( $\alpha$ -GDH): 0.1 M glycerophosphate ( $\alpha$ , $\beta$ -mixture; Cal. Biochem.); 16 µg/ml. PMS; 0.15 mg/ml. NAD; 0.3 mg/ml. Nitro BT; in 0.1 M *tris* HCl buffer, pH 8.3. Gels are incubated 3–4 h at 25° C.

For the esterase assay the gel is soaked in 0.5 M boric acid (pH 4.1) for 90 min at 4° C. This procedure lowers the pH of the gel itself from 8.9 to approximately 7.0, at which pH the reaction proceeds readily. The low temperature keeps diffusion of the protein within the gel at a minimum. The gel is then rinsed rapidly in two changes of distilled water and placed in a tray containing 1 ml. 2 per cent  $\alpha$ -naphthyl acetate in acetone and 50 mg fast red TRN (*p*-chloro-*o*-toluidine diazonium salt) in 100 ml./0.1 M phosphate buffer, pH 6.5. The gel is incubated with constant agitation 3–4 h at 25° C.

All systems were tested without substrate to determine the specificity of the reaction. Bands which appeared only in the presence of the complete system were considered to represent sites of activity of the enzyme in question. The number of bands observed after staining depends on the particular species tested as well as the particular enzyme (see Fig. 1).

In *D. virilis* Pasadena, MDH is represented by a single band, whereas there are two in *D. melanogaster* Oregon-R. Both have one band of  $\alpha$ -GDH; it has the same mobility in the two species. There is an additional band present nearer the origin when the gel has been stained for MDH or  $\alpha$ -GDH. This band is not substrate specific, but seems to be present in any NAD-linked dehydrogenase system. There are ten sites of esterase activity in *virilis*, used six to

seven in *melanogaster*. A few bands have the same mobility in both species.

The methods described here are being used to obtain information on evolutionary relationships in the genus *Drosophila* and to examine genetic variation in natural populations.

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### A Simple Method for measuring Ribonucleic Acid Content of Preparations of Deoxyribonucleic Acid

In recent years an increasing number of varied biological activities have been found associated with highly purified DNA. It is of some importance, in interpreting the effects of DNA on biological systems, to determine quantitatively the RNA content of such DNA preparations. Unfortunately, at the present time there is no generally accepted standard procedure to estimate small amounts of RNA in the presence of high concentrations of DNA. The orcinol method<sup>1,2</sup>, as is well known, gives falsely high RNA values because DNA itself reacts with the reagent to a limited extent. We have tested a variety of reported modifications of this orcinol procedure and have found them unsatisfactory. They have produced results indicating the presence of 5–10 per cent "RNA" in DNA preparations containing no demonstrable uracil after acid hydrolysis and paper chromatography (<3 per cent RNA). Therefore, a simple, rapid method, used successfully in this laboratory to estimate RNA content, appears to be worth reporting.

RNA is soluble but DNA is insoluble in 1 N cold perchloric acid. Ogur and Rosen<sup>3</sup> in 1950 suggested that this solubility difference could be used to extract differentially the nucleic acid from tissues. Following their methods, we incubated nucleic acid solutions for 18 h at 4° C in 1 N perchloric acid, and found all the RNA in solution. However, as much as 10 per cent of the DNA had also dissolved under these conditions of prolonged exposure of DNA to the acid. It was noted, in contrast to the earlier work with tissues, that the RNA in aqueous solution was immediately completely soluble in the cold perchloric acid and the DNA was insoluble. Therefore, prolonged incubation was unnecessary and the method was standardized as follows.

DNA is dissolved in distilled water to a concentration of approximately 0.5 mg/ml. and kept chilled at 0° C in an ice bath. Ice-cold concentrated perchloric acid is added to the DNA solution to a final concentration of 1 N. A white, turbid DNA precipitate forms and the suspension is immediately filtered three times repeatedly through the same filter paper. The tubes receiving the filtrates must be in the ice bath and the cycle of repeated filtrations should be complete within 20 min. To prevent any precipitated DNA from redissolving, it is essential that the filtrations be performed rapidly and in the cold. The final filtrate is crystal clear. The absorbance of the filtrate is determined in a Beckman DU spectrophotometer (room temperature) at 260 mµ against a blank of 1 N perchloric acid.

Yeast RNA (Schwartz Bioresearch) was analysed by this method at concentrations of 2–20 µg/ml. A

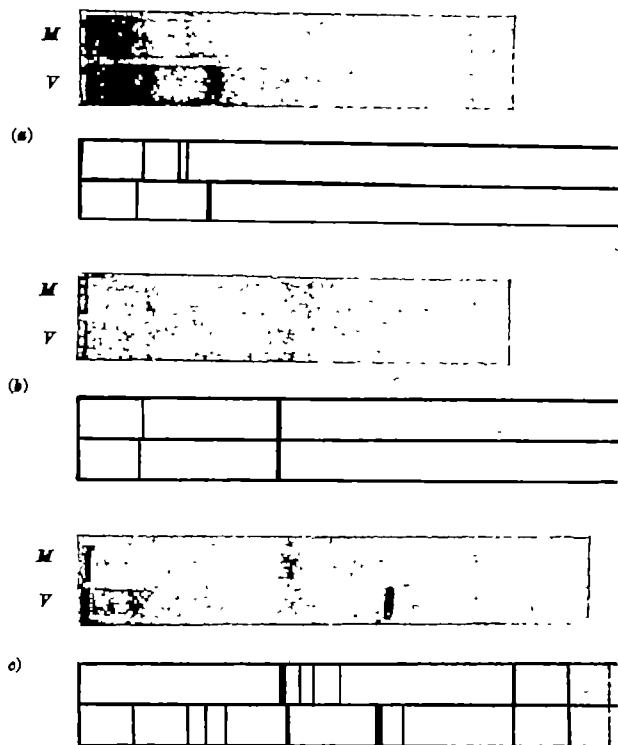


Fig. 1. Enzyme activity after polyacrylamide electrophoresis of *D. melanogaster* Oregon-R (M) and *D. virilis* Pasadena (V). (a) MDH, (b)  $\alpha$ -GDH, (c) non-specific esterase (dotted line indicates a band usually present but not shown in the photograph)



straight line running through the origin with a slope of 0.021 O.D./ $\mu$ g RNA represents our standard curve for quantitative determinations. Examination of highly purified, freshly prepared, mammalian DNA solutions<sup>4,5</sup> showed <1 per cent (0.5–0.8 per cent) RNA content by this method. Known quantities of RNA have been added to these DNA solutions and the mixtures analysed. The results were a simple summation of the expected RNA values plus the absorbances of the DNA solutions alone.

The small amount of perchloric acid-soluble material in the DNA solutions probably reflects the limited reaction of the DNA itself with the reagent rather than the true presence of RNA. This is suggested by our finding of a significant increase in the perchloric acid-soluble material when the DNA is exposed to the acid for longer periods at slightly higher temperatures. This reaction of DNA with the reagent is similar to the interference of DNA with other methods of measuring RNA. An advantage of this method, performed rapidly in the cold, is that the interference of the DNA is quantitatively so small (<1 per cent) that an accurate measurement of RNA is possible. Therefore, the finding of more than 1 per cent RNA in a DNA solution, by the procedures described here, indicates the presence of significant amounts of RNA.

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### D-Tyrosine Metabolism in *Sorghum vulgare* Seedlings

It has previously been reported that sorghum seedlings incorporate carbon-14 from DL-tyrosine-2-<sup>14</sup>C into the nitrile carbon of *p*-hydroxymandelonitrile- $\beta$ -glucose<sup>1</sup>. Furthermore, it was demonstrated that <sup>14</sup>C from D-tyrosine-2-<sup>14</sup>C was not incorporated into *p*-hydroxymandelonitrile- $\beta$ -glucose<sup>1</sup>. It was also shown that a radioactive material which migrated differently from either the glucoside or tyrosine could be extracted from seedlings allowed to metabolize either DL-tyrosine-2-<sup>14</sup>C or DL-tyrosine-3-<sup>14</sup>C. Similar experiments with L-tyrosine-<sup>14</sup>C did not yield appreciable radioactive material in this portion of the chromatogram. These preliminary experiments suggested that D-tyrosine metabolism in *Sorghum vulgare* seedlings may not be affected through either a deamination or racemization as either process would lead to precursors of *p*-hydroxymandelonitrile- $\beta$ -glucose<sup>1,2</sup>.

This report records results of experiments of the metabolism of D-tyrosine in sorghum seedlings. The data suggest that D-tyrosine is metabolized by some pathway other than oxidative deamination, transamination or decarboxylation. These processes would yield *p*-hydroxyphenyl pyruvate and tyramine, respectively.

Sorghum seeds were germinated as previously described<sup>1</sup>. Seedlings, 2–3 cm high, were transferred to 15 ml. of solution in 50-ml. beakers containing 10  $\mu$ moles of D-tyrosine and 2  $\mu$ o. of D-tyrosine labelled with <sup>14</sup>C in either the carboxyl,  $\alpha$ - or  $\beta$ -carbon atoms. An additional beaker containing similar quantities of L-tyrosine and L-tyrosine-<sup>14</sup>C was used as a control. Each beaker contained 15 seedlings. The solutions were aerated for 40 h at room temperature in the light. At the end of the aeration period the shoots were separated from seeds and

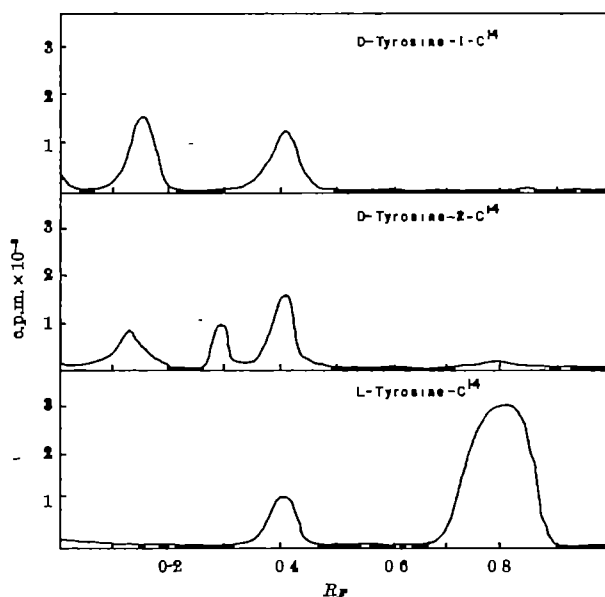


Fig. 1. Chromatographic separation of metabolites of D-tyrosine and L-tyrosine obtained from 80 per cent alcoholic extracts of *Sorghum vulgare* seedlings. Figures show that carboxyl or  $\alpha$ -labelled D-tyrosine yield alcohol soluble components that are unlike the major component (*p*-hydroxymandelonitrile- $\beta$ -glucose,  $R_f$  0.8 (1)) derived from L-tyrosine

roots. The shoots were macerated and extracted with hot 80 per cent ethanol. The alcoholic extracts were chromatographed in butanol, pyridine, water (6:4:3) and the chromatograms scanned with a 4-pi scanner.

Fig. 1 shows the separation of radioactive components. This figure shows that <sup>14</sup>C from both D-tyrosine-1-<sup>14</sup>C and D-tyrosine-2-<sup>14</sup>C was incorporated into material that migrated at  $R_f$  0.15. No radioactivity from L-tyrosine-<sup>14</sup>C was incorporated into this area of the chromatograms. Chromatography of extracts from seedlings that had metabolized D-tyrosine-3-<sup>14</sup>C (not shown) gave a pattern similar to D-tyrosine-2-<sup>14</sup>C. It should be noted that chromatograms of these extracts containing  $\alpha$ - or  $\beta$ -labelled D-tyrosine also contained a radioactive area at  $R_f$  0.3 which was not found in extracts of seedlings containing carboxyl-labelled D-tyrosine. Similar experiments with tyramine-8-<sup>14</sup>C did not yield <sup>14</sup>C in the  $R_f$  0.15 or 0.30 areas of the chromatograms. This figure and other results suggest that decarboxylation occurs in the metabolic process in which D-tyrosine is converted to the material migrating at  $R_f$  0.30. In addition, the materials migrating at  $R_f$  0.15 appear to contain the propanoid carbons of tyrosine. These experiments do not indicate whether the phenyl propanoid skeleton of tyrosine has remained intact.

Table 1 shows the rate of migration of some phenolic compounds when chromatographed in butanol, pyridine, water (6:4:3). It can be seen that only *p*-hydroxyphenyl serine and 3,4-dihydroxyphenyl alanine migrate more slowly than *p*-tyrosine. The unknown radioactive compound migrating at  $R_f$  0.3 is probably neither of these compounds because it has not retained the carboxyl carbon as shown by the lack of radioactivity derived from

Table 1. RATE OF MIGRATION OF SOME *p*-HYDROXYPHENYL COMPOUNDS IN BUTANOL, PYRIDINE, WATER

Compound	$R_f$
<i>p</i> -Hydroxyphenyl serine	0.80
3,4-Dihydroxyphenyl alanine	0.80
<i>p</i> -Tyrosine	0.40
<i>m</i> -Tyrosine	0.43
<i>o</i> -Tyrosine	0.44
<i>p</i> -Hydroxymandelate	0.40
<i>N</i> -Acetyl DL-tyrosine	0.50
<i>O,N</i> -Diacetyl DL-tyrosine	0.52
Tyramine	0.55
DL-tyrosine amide	0.55
<i>p</i> -Hydroxycinnamic acid	0.70
<i>p</i> -Hydroxymandelic acid	0.75
<i>p</i> -Hydroxyphenyl acetate	0.80
<i>p</i> -Hydroxybenzaldehyde	0.90

DL-tyrosine-1- $^{14}\text{C}$  in this area of the chromatogram. The material migrating at  $R_F$  0.3 is not the decarboxylation product of either *p*-tyrosine or *p*-hydroxyphenyl pyruvic acid which migrated at  $R_F$  0.55 and 0.80, respectively, in this solvent.

Experiments with cell-free systems were carried out on extracts of seedlings. The enzyme preparation was obtained by macerating the shoots of 4-day-old seedlings in 0.1 M acetate buffer, pH 5.5, followed by centrifugation at 10,000*g*. One  $\mu\text{mole}$  of L-tyrosine per ml. was added to the supernatant solution, the pH readjusted to 5.5 and the solution held at 60° C for 5 min. The solution was rapidly cooled to 5° C, followed by centrifugation at 5,000*g* for 10 min. The supernatant solution was dialysed overnight against 100 vol. distilled water followed by adjusting the dialysed solution to 0.6 saturation with solid ammonium sulphate. The precipitate formed was collected by centrifuging at 10,000*g* for 10 min and the residue taken up in 0.1 M acetate buffer, pH 5.5. Each assay contained the material isolated from 600 mg of plant material.

Fig. 2 shows the results of incubating DL-tyrosine-2- $^{14}\text{C}$  with this enzyme preparation in the presence or absence of a boiled extract from the sorghum tissue. In the presence of boiled extracts and DL-tyrosine-2- $^{14}\text{C}$  (Fig. 2c) radioactivity was incorporated into material that migrated at  $R_F$  0.1 in the solvent system previously described. Similar experiments with L-tyrosine- $^{14}\text{C}$  gave only one radioactive peak at  $R_F$  0.4 which corresponds to the rate of migration of tyrosine.

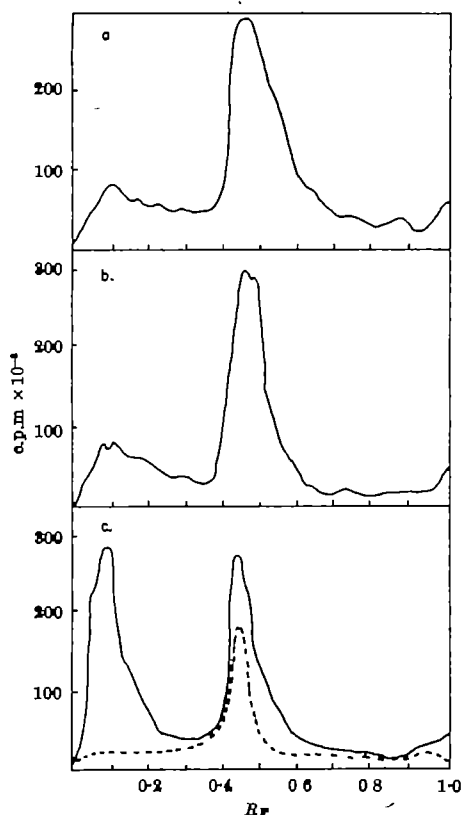


Fig. 2. Enzymatically catalysed formation of ' $R_F$  0.1' material from DL-tyrosine-2- $^{14}\text{C}$ . A partially purified enzyme from 600 mg of plant material was incubated for 1 h in 0.07 M sodium acetate buffer pH 5.5 with  $3 \times 10^{-4}$  M DL-tyrosine-2- $^{14}\text{C}$  in the presence (Fig. 2c) or absence (Fig. 2a) of a boiled extract from *Sorghum vulgare*. An aliquot of the extract was chromatographed on Whatman number 1 paper in butanol, pyridine, water (6:4:3), followed by scanning the chromatograms for radioactivity. Fig. 2a shows the zero time control. The reaction mixture was boiled prior to adding DL-tyrosine-2- $^{14}\text{C}$ . Fig. 2c shows that in the presence of DL-tyrosine-2- $^{14}\text{C}$  and boiled extract (solid line) material migrating at  $R_F$  0.1 is formed. When DL-tyrosine-2- $^{14}\text{C}$  was replaced by L-tyrosine- $^{14}\text{C}$  (dotted line) no radioactivity was observed in this portion of the chromatogram. Fig. 2b shows the effect of omitting the boiled extract.

These experiments suggest that sorghum seedlings metabolize D-tyrosine as well as L-tyrosine, however, by different pathways. *In vivo* experiments (not shown here) have shown that the conversion of D-tyrosine to materials migrating at  $R_F$  0.15 and 0.30 is blocked if  $\text{NaN}_3$ ,  $\text{NaHAsO}_4$ ,  $\text{NaF}$  or 2,4-dinitrophenol are added to the nutrient solution containing D-tyrosine. However, methylene blue did not block the formation of these materials.

These experiments give little information as to the chemical composition of the materials derived from D-tyrosine other than to eliminate a number of possible phenolic compounds including *p*-hydroxyphenyl pyruvic acid and tyramine. Chemical and chromatographic examination of the material(s) migrating at  $R_F$  0.15 indicate that it is unstable in dilute acid and gives rise to a number of  $^{14}\text{C}$ -labelled materials when derived from D-tyrosine-2- $^{14}\text{C}$ . The rate of migration in the slightly basic solvent suggests the materials to be either more acidic or of higher molecular weight than tyrosine.

The physiological importance of D-tyrosine metabolism in *Sorghum vulgare* is not known. There appears to be little or no reversible inter-conversion of L-tyrosine to D-tyrosine, while D-tyrosine appears not to be metabolized through the usual pathways for D-amino-acids, that is, deamination or decarboxylation.

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### Loss of Methyl Tritium from [ $^3\text{H}$ ] Acetate in Rumen Fluid

THE availability of both [ $^{14}\text{C}$ ]- and [ $^3\text{H}$ ]-labelled compounds, together with the development and refinement of scintillation counting procedures, has encouraged the use of radioisotopes in the investigation of metabolic processes. It has generally been assumed that with carbon-14 little or no isotope effect is produced and that the labelled substrate behaves in a similar way to the non-labelled compound. With tritium, however, considerable isotope effect may occur because of the large differences in atomic weights of tritium and hydrogen<sup>1</sup>.

Constant infusions of labelled substrates into animals have been widely used to measure entry rates of these compounds<sup>2</sup>. We have applied these techniques to the measurement of production rates of volatile fatty acids in the rumen of the sheep, and in the course of the work we have found that infusion of [ $^3\text{H}$ ] acetate gave considerably higher acetate production rates than infusion of [ $^{14}\text{C}$ ] acetate; this led us to investigate the possible lability of methyl tritium of [ $2\text{-}^3\text{H}$ ] acetate in rumen fluid.

Merino ewes (4-5 years old), fitted with a permanent rumen fistula, were used in these experiments. The sheep were housed indoors in single pens and were fed hourly (75 g lucerne chaff) between the hours of 8 a.m. and 7 p.m.

The animals consumed their ration in 15–20 min and were trained to eat during infusion experiments. Small quantities of a highly radioactive mixture of [ $1\text{-}^{14}\text{C}$ ] acetate and [ $2\text{-}^3\text{H}$ ] acetate were infused into the anterior end of the rumen through the rumen fistula. Rumen samples were obtained at intervals of 30 min over a period of 240 min and the acetate and butyrate isolated by column chromatography on silicic acid using hexane-butanol<sup>8</sup>. Aliquots (10 ml.) were collected off the column; 5 ml. was titrated and 5 ml. mixed with 5 ml. xylene containing 0.01 per cent 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) and 0.4 per cent 2,5-diphenyloxazole (PPO). These samples were counted in a Nuclear Chicago scintillation counting system 725 using the channels ratio method for dual isotopes<sup>4</sup>.

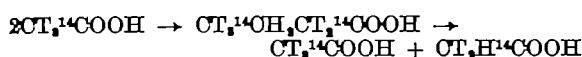
The ratio of [ $^3\text{H}$ ] to [ $^{14}\text{C}$ ] in acetate isolated from the rumen fell with time, indicating the loss of tritium from the acetate molecules (Table 1). Radioactivity appeared in the isolated butyrate and the ratio of  $^3\text{H}$  to  $^{14}\text{C}$  in this is also shown in Table 1. Infusions of [ $2\text{-}^{14}\text{C}$ ] and [ $2\text{-}^3\text{H}$ ] acetate gave essentially similar results to infusions of [ $1\text{-}^{14}\text{C}$ ] and [ $2\text{-}^3\text{H}$ ] acetate, demonstrating that the methyl group of acetate was not exchanged as a unit. Incubation of freshly obtained rumen fluid with the acetate (mixed label) at 39.5° C and in an atmosphere of 100 per cent  $\text{CO}_2$ , resulted in a similar loss of the tritium from labelled acetate (Table 1).

Table 1. THE RATIO OF  $^3\text{H}$  TO  $^{14}\text{C}$  IN RUMEN ACIDS (ACETIC AND BUTYRIC ACIDS) AND IN BLOOD ACETATE DURING AN INFUSION OF [ $^3\text{H}$ ] AND [ $^{14}\text{C}$ ] ACETATE. RESULTS FROM INCUBATION OF THE MIXED ISOTOPE WITH RUMEN FLUID *in vitro* ARE ALSO INCLUDED (EXPERIMENT 4).

Time (min)	Experiment 1 Rumen acetate $^3\text{H} : ^{14}\text{C}$	Experiment 2 Rumen butyrate $^3\text{H} : ^{14}\text{C}$	Experiment 3 Blood acetate $^3\text{H} : ^{14}\text{C}$	Experiment 4 Rumen fluid acetate $^3\text{H} : ^{14}\text{C}$
0	3.08	—	3.82	4.01
30	3.44	2.27	3.75	3.54
60	3.25	1.76	3.70	3.40
90	3.25	2.08	3.83	3.35
120	3.13	2.04	3.58	3.27
150	2.87	1.92	3.41	—
180	2.65	1.87	3.73	—
210	2.73	1.81	—	—
240	2.70	1.84	—	—

A mixture of [ $2\text{-}^3\text{H}$ ] acetate (approximately 2  $\mu\text{Ci}/\text{ml}$ ) and [ $1\text{-}^{14}\text{C}$ ] acetate (approximately 0.5  $\mu\text{Ci}/\text{ml}$ ) was infused intraruminally (1 ml./min) (experiments 1 and 2), or intravenously (0.33 ml./min) (experiment 3). The ratio of [ $^3\text{H}$ ] to [ $^{14}\text{C}$ ] was determined in acetate and butyrate isolated from rumen contents and in acetate isolated from blood. Samples were taken at 30-min intervals throughout the infusion period in all experiments. The ratio was also determined in acetate isolated from samples obtained from rumen fluid incubated *in vitro* with a mixture of [ $2\text{-}^3\text{H}$ ] and [ $1\text{-}^{14}\text{C}$ ] acetate at 39.5° under a gas phase of 100 per cent  $\text{CO}_2$  (experiment 4).

Acetate is lost from the rumen mainly through absorption into portal blood<sup>9</sup>, but little is known of the reactions of acetate in rumen fluid. It is established that there is incorporation of acetate carbon into butyrate and *vice versa*<sup>8</sup>, but the extent of this and the recycling of acetate molecules is not well known. In work now being prepared for publication we have shown that of the butyrate produced in the rumen approximately 25 per cent came from acetate and of the acetate produced 10 per cent arose from butyrate. The cycle, acetate to butyrate and back to acetate, may be responsible for some of the loss of tritium from acetate. If, however, the tritiated acetate underwent conversion to butyrate at a reduced rate this would tend to increase the ratio of  $^3\text{H}$  to  $^{14}\text{C}$  in acetate. The labelling in butyrate shows that an appreciable amount of tritium appears in isolated butyrate and the cleavage of this molecule to acetate would tend to reduce the ratio as indicated by the following equation, in which T is used to indicate tritium:



However, the recycling of acetate molecules through the above scheme must be small as indicated by the inter-conversion percentages.

From the results obtained in this investigation it is apparent that although production rates or entry rates of

acetate in the rumen may be measured using intraruminal infusions of [ $^{14}\text{C}$ ] acetate, acetate labelled with tritium cannot be used because of the apparent lability of the tritium. That [ $^3\text{H}$ ] acetate could be used for the measurement of entry rates in the whole animal was shown by intravenous infusions of [ $^{14}\text{C}$ ] and [ $^3\text{H}$ ] acetate. There was no change in the ratio of  $^3\text{H}/^{14}\text{C}$  in acetate isolated from blood over the period of infusion (Table 1).

If the lability of hydrogen in this position is truly represented by that of the tritium then acetate may supply readily mobilized hydrogen atoms for hydrogenation reactions. At present this seems highly unlikely, but the incorporation of tritium into unsaturated fatty acids in the rumen is under investigation. Since the tritium molecule is not lost from [ $2\text{-}^3\text{H}$ ] acetate in solution in water, the exchange of isotope may be a consequence of the strongly reducing conditions of the rumen. On the other hand, the exchange may be catalysed by elements or compounds in rumen fluid.

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## PHYSIOLOGY

### Effect of Glucose administered *in vivo* or *in vitro* on the Respiratory Quotient of Rat Liver Tissue after Partial Hepatectomy

AFTER partial hepatectomy, the respiratory quotient of liver tissue has been found to decrease to about 0.7, which suggests predominant utilization of lipids<sup>1</sup>. As early as in the first hours after surgery a decrease of glycogen and an increase of lipids can be observed<sup>2</sup>. In these circumstances, however, marked enhancement of lipids in liver tissue probably does not represent a manifestation of a degenerative process. The lipids increase mainly in the peripheral cells of the liver lobules, where the processes connected with liver regeneration are most conspicuous<sup>3,4</sup> and thus provide a substance which, after partial hepatectomy, is utilized by liver tissue as the main source of energy. Under normal conditions, the energy of the liver tissue in rats fed *ad libitum* with a mixed diet is obtained by combustion of glycids, which is shown by the respiratory quotient near 1 (ref. 5). We therefore wondered whether the liver tissue after partial hepatectomy, when its respiratory quotient has decreased, is able to utilize glucose for its energetic metabolism if glucose is offered in excess during the acute experiment.

The experiments were carried out using Wistar male rats aged 4–5 months, weighing 240–310 g, which were fed throughout, including the course of the experiment, *ad libitum* with the standard laboratory diet: 700 g bruised wheat, 100 g dry-milk powder, 150 g casein, 30 g crushed alfalfa hay, 15 g calcium chloride, 50 g fat (100 per cent), 7.5 g liver oil, 2.5 g sodium chloride and an amount of water sufficient to obtain a pasty consistency. The respective measurements were made in normal, non-operated rats 48 h after partial hepatectomy or simple laparotomy. Rats which had not undergone surgical intervention were included in the first experimental group;

Table 1. OXYGEN INTAKE, CARBON DIOXIDE RELEASE AND RESPIRATORY QUOTIENT OF THE LIVER TISSUE AFTER ADMINISTRATION OF GLUCOSE *in vivo* OR *in vitro* TO THE RATS WHICH HAVE UNDERGONE PARTIAL HEPATECTOMY OR SIMPLE LAPAROTOMY

Groups	Oxygen intake (mm <sup>3</sup> mg <sup>-1</sup> dry tissue per 60 min)			Carbon dioxide release (mm <sup>3</sup> mg <sup>-1</sup> dry tissue per 60 min)			Respiratory quotient		
	Glucose			Glucose			Glucose		
	without glucose	<i>in vivo</i>	<i>in vitro</i>	without glucose	<i>in vivo</i>	<i>in vitro</i>	without glucose	<i>in vivo</i>	<i>in vitro</i>
Non-operated rats	3.21 ± 0.20	—	3.80 ± 0.37	2.96 ± 0.29	—	3.44 ± 0.42	0.94 ± 0.028	—	0.89 ± 0.07
48 h after partial hepatectomy	4.83 ± 0.39	5.18 ± 0.23	4.75 ± 0.28	3.76 ± 0.61	3.83 ± 0.31	3.70 ± 0.38	0.75 ± 0.069	0.76 ± 0.06	0.79 ± 0.04
48 h after laparotomy	3.33 ± 0.25	4.09 ± 0.50	3.60 ± 0.24	2.86 ± 0.36	4.03 ± 0.06	3.52 ± 0.41	0.99 ± 0.063	1.00 ± 0.11	0.97 ± 0.11

the rats of the second group were given by gastric tube 1.25 ml. of a 40 per cent solution of glucose per 100 g body-weight 30 min before killing and withdrawal of samples; in the third experimental group glucose was added in excess to the incubation medium in the Warburg flasks to a concentration of 270 mg per cent. About 70 per cent of the liver tissue was removed, following the partial hepatectomy procedure of Higgins and Anderson<sup>4</sup>. The formation of carbon dioxide and intake of oxygen by the liver slices were measured by means of the direct method of Warburg and Negelein<sup>5,6</sup>, using standard flasks with side arms and an absorption cup according to Pardee<sup>7</sup> and Krebs<sup>8</sup>. This technique enabled us to maintain a constant pressure of gaseous carbon dioxide. The tissue was suspended in Krebs-Ringer's saline solution with lower content of sodium bicarbonate (0.35 per cent) following the method of Bellamy and Bartley<sup>9</sup>. The results were evaluated statistically by means of Student's *t*-test<sup>10</sup>.

The results are summarized in Table 1. In rats which had undergone simple laparotomy the values of oxygen intake and carbon dioxide formation by the tissue slices were found, 48 h after the surgical operation, to be approximately the same in normal rats. However, in those rats which had undergone partial hepatectomy a significantly higher oxygen intake ( $P < 0.01$ ) and only a slightly higher release of carbon dioxide were observed at the same time interval after the operation. The respiratory quotient of the liver of these rats decreased to values near 0.7 ( $P < 0.05$ ) when compared with intact rats, or those with simple laparotomy. After administration of glucose *in vivo* and after its addition in excess *in vitro*, the oxygen intake and the carbon dioxide formation by the liver slices (and thus also the values of its respiratory quotient) did not change in any of the experimental groups.

Clerici and Ciccarone<sup>11</sup> have reported a lowering of the 'Crabtree effect' in the partially 'resected' liver tissue. In normal circumstances, this phenomenon consists of inhibition of the oxygen intake in the tissue slices after the administration of glucose into an incubation medium. Clerici and Ciccarone failed, however, to explain the cause of the changes in the metabolic reactivity in the liver tissue after partial hepatectomy. Our experimental results offer a simple explanation. After partial hepatectomy the liver tissue—the metabolism of which is adapted to the predominant utilization of lipids—is not able to use glucose offered in excess *in vivo* or *in vitro* during the acute experiment. The lowering of the so-called 'Crabtree effect' would thus be associated with a decrease of the utilization of glucose. Derauche *et al.*<sup>12</sup> have directed attention to the decrease of glycolysis in regenerating liver tissue. It is not clear whether the rebuilding of metabolism in the liver tissue merely represents a manifestation of an adaptation to the lack of glucose and to the simultaneous increase of lipids in the liver tissue. Many of the facts, however, suggest that fat metabolism is of great importance for regenerating liver tissue. In our own experiments we had an opportunity to observe that retardation of fat infiltration in the liver tissue after partial hepatectomy pro-

voked by protracted infusion of glucose was associated with a decrease of the rate of regeneration<sup>13</sup>.

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### Crabtree Effect and the Anaerobic Glycolysis of the Regenerating Rat Liver

In the preceding communication Šimek and Sedláček state that the Crabtree effect observed by us<sup>1</sup> in the regenerating rat liver should be due to a supposed inability of the growing tissue, the metabolism of which is adapted to predominant utilization of lipids, to use glucose *in vitro*.

We believe that such an explanation is not fully acceptable. In fact, the Crabtree effect has been demonstrated only four days after partial hepatectomy—namely, when the fatty infiltration of the liver is definitely waning after having reached a peak 1–2 days following surgery, as has been shown by many research workers<sup>2–4</sup>, and recently confirmed by one of us<sup>5</sup>.

A preferential utilization of lipids instead of carbohydrates, which is verisimilar immediately after lobectomy<sup>6</sup>, seems less likely at later stages when the weight gain and the chemical composition of the liver are almost normal. However, taking for granted that four days after surgery the regenerating liver mainly relies, for its respiratory needs, on the endogenous lipids, it is nevertheless evident that it is still able to react to any addition of glucose by a lowering of the oxygen uptake (and not of the Crabtree effect, as stated by Šimek and Sedláček in the preceding communication).

On the other hand, the 'utilization' of glucose is not a determining factor in causing the Crabtree effect, since this is exerted by sugars which are substrates for hexokinase and also by 2-deoxyglucose, a carbohydrate phosphorylated by such enzyme but not further glycolysed. Glycolysis can even be blocked by iodoacetate or bromoacetate without interference with the Crabtree effect<sup>7</sup>.

So far as we know, there is no proof that the regenerating rat liver is unable to phosphorylate glucose.

From analogy with the regenerating liver, it is worth mentioning that the inhibition of respiration by addition of glucose has also been demonstrated in some neoplastic tissues which show an active endogenous oxidation of fatty acids<sup>8</sup>.

Simec and Sedláček cite a paper by Derache *et al.*<sup>9</sup> as proof that carbohydrate metabolism, or, to be exact, anaerobic glycolysis, is decreased in the regenerating rat liver. Since one of us has investigated the metabolism of the regenerating liver many times, we wish not only to emphasize that the behaviour of the anaerobic glycolysis in such experimental conditions is still highly controversial<sup>10-12</sup> but also to underline that, according to Derache *et al.*<sup>9</sup>, the anaerobic glycolysis is not decreased but increased 2 and 48 h after surgery and remains unaltered in between, as compared with controls. Furthermore, Derache *et al.*<sup>9</sup> do not specify whether the rats are fasted or fed; in such conditions it is not possible to appraise adequately the value of glycolysis, the rate of which depends on the feeding state of the animals<sup>11</sup> and, consequently, on the glycogen storage in the liver<sup>12</sup>.

Finally, the paper by Derache *et al.*<sup>9</sup> is difficult to apprehend clearly since the lactic acid was evaluated by a method<sup>13</sup> which is suited to measuring phosphates but not lactic acid; the results are also expressed in a rather odd way, as  $\mu\text{moles}$  of lactic acid phosphorus/mg proteins/h.

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## Increase of the Effect of Histamine by *E. coli* Endotoxin on the Smooth Muscle

ONE of the properties of the *Brucella abortus* and *B. melitensis* endotoxins is their ability to promote motility of the uterus muscle—this we demonstrated recently<sup>1,2</sup>. The *Brucella* endotoxins can induce a remarkable sensitivity in the isolated uterus against oxytocin<sup>1-4</sup> and histamine<sup>2,5</sup> which cannot be observed in the ileum<sup>6</sup>.

The *E. coli* endotoxin exhibits a similar effect on the smooth muscle of uterus and ileum. This communication describes the sensitizing effect of the *E. coli* endotoxin on the smooth muscle in respect of histamine.

The endotoxin of the *E. coli* strains 0 55 and 0 111 were prepared by Boivin's trichloroacetic acid method<sup>7-9</sup>.

In order to demonstrate the stimulation of the effect of histamine by *E. coli* endotoxin we began with different histamine concentrations in a scale beginning with a final concentration of  $0.5 \times 10^{-6}$  down to a concentration causing a weak contraction only. This amount of histamine was said to be 100 per cent. The contraction caused by this amount and a previous 5-min incubation of the endotoxin was compared with a histamine contraction of an equal amplitude of the histamine scale. In all

Table 1. THE INCREASE OF THE EFFECT OF HISTAMINE BY *E. coli* ENDOTOXIN ON THE ISOLATED GUINEA-PIG UTERUS

No.	% Increase	No.	% Increase	No.	% Increase
1	100	10	900	19	400
2	—	11	100	20	900
3	400	12	400	21	900
4	900	13	900	22	400
5	—	14	400	23	100
6	400	15	100	24	400
7	100	16	900	25	150
8	150	17	900		
9	900	18	400		

experiments the amount of *E. coli* endotoxin was 50 $\gamma$ . The incubation volume (Tyrode's solution) was 10 ml.

The effect of histamine on the isolated guinea-pig uterus is stimulated to the extent of 100-900 per cent after addition of *E. coli* endotoxin. Only in 2 of 25 cases was a histamine-promoting effect of the endotoxin missed (Table 1).

The *E. coli* endotoxin can be washed out from the guinea-pig uterus in the recipient with stationary contents. The histamine, having been stimulated by endotoxin, has thereafter an effect scarcely above its threshold, namely, the amplitude of the contraction becomes the same as it was previously. This is to be seen in Fig. 1. It should be pointed out that in some cases even smaller amounts of histamine causing no contraction are followed by a strong contraction after addition of endotoxin.

The *E. coli* endotoxin without any previously administered histamine has no effect on the guinea-pig uterus when applied in amounts of 50-1,000 $\gamma$  in a recipient with stationary contents. However, after we observed that rat uteri respond to a single application of *Brucella* endotoxins with a rise in amplitude and frequency and a lesser rise in tonus when using a perfused recipient<sup>3</sup>, we undertook to investigate the effect of *E. coli* endotoxin in a perfused recipient.

Independently from our investigations<sup>1,2</sup>, Wiedermann *et al.*<sup>10</sup> described a direct effect of *E. coli* endotoxin on the contractility of the guinea-pig uterus in a perfused recipient which we, as already stated, could not observe in a recipient with stationary contents. Goodno *et al.*<sup>11</sup> were unable to confirm this finding when they used rat uterus or strips of human uterus. However, Wiedermann<sup>10</sup> pointed out the importance of the method applied and of the hormonal conditions of the animals. We have shown<sup>3</sup> that uteri of rats pretreated with oestradiol react more strongly than untreated controls when examined with *Brucella*

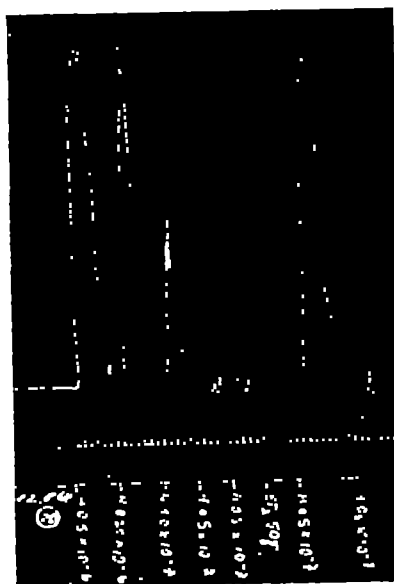


Fig. 1. Increasing effect of 50 $\gamma$  *E. coli* endotoxin during incubation for 5 min on the effect of an amount of histamine scarcely above its threshold

endotoxin in a perfused recipient. We have also emphasized that the hormonal condition is an important factor controlling the way the *Brucella* endotoxins<sup>1</sup> are acted on.

In 1961, one of us<sup>1</sup> propounded a new abortion theory in virtue of the effect of *Brucella* endotoxins on the isolated or pregnant uteri of animals (rat, guinea-pig, rabbit), and discussed the importance of different endotoxins, particularly of *E. coli* endotoxin, to the abortion and pointed to Thomas's observations<sup>12,13</sup>. Our experiments explain the abortions induced by injections of *E. coli* endotoxins with pregnant mice<sup>14</sup>.

The effect of *E. coli* endotoxin on the isolated uterus described here confirms the suggestions that abortions in parallel with increased bacterial breakdown are caused by the uterus-motility promoting effect of the endotoxins. It explains Kass's observations<sup>14,15</sup>; he demonstrated an increase of abortion rates with an increasing number of *E. coli* bacteria in the urinary bladders of pregnant women.

The effect of endotoxins on the blood vessels is well known<sup>16,17,18</sup>. In view of our results the question is raised of whether the increase of the histamine effect on the smooth uterine muscle by endotoxins is also responsible for the effect of endotoxins on the smooth blood-vessel muscles<sup>4</sup>. This might play a part in several pathophysiological phenomena, for example, in septic shock, in the localized Schwartzmann reaction, and in all phenomena in which histamine and endotoxin are involved. Hence, we have initiated suitable experiments which will be reported later. The preliminary results show that this increase of the histamine effect by endotoxins on the smooth muscle might be a general phenomenon.

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### Action of Angiotensin on Sympathetic Nerve Endings in Isolated Blood Vessels

THE recent experimental findings<sup>1,2</sup> of a diminished effect of administered-angiotensin on blood pressure after acute sympathectomy or pretreatment with reserpine have directed attention to a possible relationship between the effects of angiotensin and the sympathetic nerve system. In dogs, McCubbin and Page<sup>3</sup> described an effect of angiotensin on the sympathetic nervous system resulting in an enhanced response to agents and procedures which cause release of endogenous noradrenaline. Later, Lewis<sup>4</sup> ob-

served an angiotensin-induced release of catecholamines from the suprarenal medulla of cats. In connexion with this finding he tentatively discussed an action of angiotensin at the vascular wall by local liberation of catecholamines from the sympathetic nerve endings. The two kinds of experiments (a and b) presented here were undertaken to obtain further information on the relationship between angiotensin and the sympathetic vasomotor action.

(a) Quantitative determination of noradrenaline was carried out on large blood vessels (aorta and a. carotis) of pigs. Preliminary results had shown that the pattern of mechanical activity of these arteries was similar to that of rat aortae used in type (b) of our experiments. Each artery was cut lengthwise to yield two identical strips. One strip of each pair was then rinsed for 2 h at 37° C at a rate of 2 ml./min with Tyrode's solution, the other with the same solution containing angiotensin II ('Hypertensin', 10<sup>-6</sup> g/ml.) at the same rate and temperature. Afterwards the muscle strips were homogenized and the noradrenaline content determined by means of the fluorimetric method of Bertler, Carlsson and Rosengren<sup>5</sup>.

After exposure to angiotensin, the noradrenaline content of the strips was found to be  $0.4 \pm 0.1 \gamma/g$  ( $\pm S.E.$ ) wet weight as compared with  $0.7 \pm 0.2 \gamma/g$  in the controls.

(b) Aortae of anaesthetized adult albino rats were cut spirally. A muscle strip, 1.5 mm wide and 12 mm long, was mounted in a narrow 0.3-ml. bath and rinsed continuously at a rate of 2 ml./min. with Tyrode's solution of 37° C. For the isometric recording of contractions a variable inductive transducer (Philips PT 1200) connected to an inkwriter (Varian G 11 A) was used.

The maximum amplitude of angiotensin-induced contractions ('Hypertensin' 10<sup>-6</sup> g/ml.) declined exponentially to zero with each subsequent application. Rinsing for 10 min with noradrenaline ('Arterenol' 10<sup>-6</sup> g/ml.) gave a nearly maximum contraction which was followed by a complete relaxation after washing out the drug. Subsequent renewed application of angiotensin now produced a contraction of  $85 \pm 14$  per cent of the initial angiotensin effect. These observations suggest that the vasoconstrictor activity of angiotensin is caused by a release of noradrenaline from the stores which are known to exist at the sympathetic nerve endings in the vascular wall. Thus, angiotensin can exert no effect if the stores are depleted by repeated exposure to angiotensin to such a degree that noradrenaline can no longer be released in an amount sufficient to induce a contraction. After partial replenishment by exogenous noradrenaline, angiotensin regains its activity. We were able to show that addition of cocaine (10<sup>-3</sup> g/ml.) prevents this restoration normally observed after administration of noradrenaline while it does not influence the normal contraction caused by noradrenaline. This may conveniently be explained by the known inhibitory action of cocaine on the noradrenaline uptake by the stores<sup>6,7,8</sup>. However, cocaine does not seem to block only the uptake but seems also to inhibit to some extent the release of noradrenaline. Thus, the administration of cocaine during the initial phase of an experiment partially or completely suppressed the vasomotor reaction to angiotensin. Such an inhibitory effect of cocaine on the liberation of catecholamines has already been suggested by Bejrablya, Burn and Walker<sup>9</sup>.

Our preparations from normal animals under prolonged exposure to angiotensin began to relax about 3 min after the maximum of the contraction had been reached. In preparations from animals which were pretreated with reserpine (1 mg/kg 'Serpasil' intraperitoneally 48 and 24 h before the experiment) relaxation started about 1.5 min after reaching the maximum. The shortening of the angiotensin effect can be explained by the extensive depletion of the noradrenaline stores following reserpine pretreatment<sup>9</sup>.

In some experiments the effects of tyramine (10<sup>-6</sup> g/ml.), the indirect action of which on vascular smooth muscle is well established<sup>9</sup>, were investigated. The

responses to angiotensin and tyramine were found to be qualitatively similar although angiotensin proved to be the more effective.

It is concluded that the action of angiotensin on vascular smooth muscle is an indirect one mediated by liberation of noradrenaline from the sympathetic nerve endings in the vascular wall, but an additional direct action cannot yet be excluded. This action of angiotensin may be of importance for a better understanding of various forms of hypertension.

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## PHARMACOLOGY

### Comparison of T-Wave Changes in the Dog following Intravenous Administration of Chlorpromazine and Fluphenazine

As early as 1954, flattening of the T-wave following chlorpromazine administration to anaesthetized dogs was reported by Moyer *et al.*<sup>1</sup>. Marked T-wave changes were also noted by Melville<sup>2</sup> in 1958 following the administration of chlorpromazine to dogs under similar conditions. Other investigators have noted electrocardiographic alterations in man after parenteral administration of chlorpromazine. Undesirable effects on the electrocardiogram and ballistocardiogram by chlorpromazine were observed by Eliakim *et al.*<sup>3</sup>. Kupatz<sup>4</sup> recommended that chlorpromazine should not be used to tranquilize children prior to electrocardiogram determinations because the compound induced changes in the T-wave. Minor T-wave changes in the electrocardiograms of several patients treated with chlorpromazine were noted by Moyer *et al.*<sup>1</sup>.

In the phenothiazine series, electrocardiographic changes are not restricted to chlorpromazine. In man, Graupner and Murphree<sup>5</sup> found that 67 per cent of 55 adult males given daily oral doses of 150-900 mg thioridazine showed electrocardiographic changes. In the same report, these authors indicated that T-wave changes were produced in

4 of 11 dogs following 6 mg/kg oral doses of thioridazine. Kelly *et al.*<sup>6</sup> reported that oral doses of 200 mg or more of thioridazine altered ventricular repolarization (T-wave change) in 28 adults. Two patients, given 1,500 and 3,600 mg a day, died; their terminal electrocardiogram patterns were those of heart block, alternating with episodes of ventricular tachycardia.

We have found that fluphenazine has no greater depressive effect on the T-wave than does chlorpromazine when administered intravenously to dogs at the same mg/kg dose. However, the compounds are not used therapeutically at the same dosage. In a study of 400 acutely ill schizophrenic patients<sup>7</sup>, a daily oral dose of 2 mg of fluphenazine produced improvement similar to that afforded by a 200-mg daily oral dose of either chlorpromazine or thioridazine. The much lower clinical dose suggests a wider margin of safety for fluphenazine.

Investigations in our laboratory have demonstrated similar acute toxicities for chlorpromazine and fluphenazine in three animal species<sup>8</sup>. Both compounds showed corresponding cardiovascular effects in anaesthetized and unanaesthetized dogs at similar intravenous doses. Afterwards a direct comparison of the effects of the two compounds on the amplitude of the T-wave in dogs was completed.

For this investigation lead II electrocardiograms were recorded via surface electrodes from 12 unanaesthetized dogs of either sex. Chlorpromazine hydrochloride was then given intravenously in doses of 1, 3, and 10 mg/kg to each dog on different days and electrocardiograms were recorded hourly for 6 h. Eight of these dogs that showed a chlorpromazine-induced depression of the T-wave were selected for further investigation. On any given day two dogs were tested with chlorpromazine hydrochloride and two with fluphenazine hydrochloride at a dose of 3.0 mg/kg. A week later they were crossed-over as to drug and in subsequent weeks the experiment was repeated using 0.3 mg/kg doses.

The height of the T-wave at each hourly interval after dosing was compared with the pre-dose height and was expressed as a percentage increase or decrease. A no-drug control was obtained for each dog by comparing an initial T-wave height with heights measured at six-hourly intervals thereafter, without the administration of any substance. The six percentage values for changes in T-wave height obtained for each dog at each hour were averaged and are presented in Table 1. One dog was eliminated from the calculation of the mean change in T-wave height after each regimen because of aberrant values.

The data indicate that the mean percentage change after the 0.3 mg/kg dose of fluphenazine was similar to that after no drug had been given. On the other hand, this same dose of chlorpromazine and the 3 mg/kg doses of chlorpromazine and of fluphenazine caused definite reductions in the height of the T-wave.

These results demonstrate that at the same mg/kg intravenous dose fluphenazine has no more effect on the

Table 1. MEAN PERCENTAGE CHANGE IN T-WAVE HEIGHT FOLLOWING INTRAVENOUS ADMINISTRATION OF CHLORPROMAZINE HYDROCHLORIDE AND FLUPHENAZINE HYDROCHLORIDE TO UNANAESTHETIZED DOGS

Dog No.	Sex	Weight (kg)	No-drug control	3.0 mg/kg		Weight (kg)	No-drug control	0.3 mg/kg	
				Chlorpromazine	Fluphenazine			Chlorpromazine	Fluphenazine
2230	F	7.4	+58	-48	-56	7.2	+9	-10	-5
2203	F	9.3	-32	-70	-17	8.8	-22	-48	-18
2356	M	6.7	+43	-3	-33	6.6	(+121)†	(-76)†	(+147)†
2399	F	7.0	0	-32	-18	7.1	-27	-44	-7
2390	F	7.0	(+29)*	(+90)*	(-30)*	6.9	-10	+14	-9
2347	M	8.8	-32	-34	-51	8.5	-12	+1	-68
2347	M	8.2	-13	-53	-47	8.1	+20	-87	+76
2302	M	8.4	-13	-16	-2	8.2	-7	0	+2
Group mean				+12	-34	-32	-7	-25	-4

Each + or - figure is the mean of values obtained at each of six hourly intervals after dosing: + = increase in T-wave height; - = decrease in T-wave height.

\* Eliminated from calculation of the group means because of aberrant response to chlorpromazine.

† Eliminated from calculation of the group means because of aberrant response to fluphenazine and as no-drug control.



T-wave than does chlorpromazine. Using either the recommended starting daily doses of 30 mg for chlorpromazine and 1 mg for fluphenazine, or the 100-fold dose ratio found in the one clinical investigation, fewer undesirable cardiovascular and electrocardiographic side-effects would be expected when fluphenazine is used.

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### Inhibition of Adrenocortical Responsiveness to ACTH by Actinomycin D *In vivo*

It has been reported recently<sup>1-3</sup> that acute or chronic administration of ACTH to rats results in a marked increase in adrenal protein synthesis. This is evidenced by a rise in the DNA and RNA concentrations in the gland and an increased capacity for incorporation of <sup>14</sup>C-glycine into protein in cell-free preparations of rat adrenals. Furthermore, Farese<sup>4</sup> has suggested on the basis of studies of this type that these changes in adrenal protein synthesis are specifically concerned with the mechanisms whereby ACTH induces adrenal hypertrophy and that even the steroidogenic effect of ACTH may be mediated by an effect of this hormone on adrenal protein synthesis. In order to elucidate this problem it was decided to investigate the effect of actinomycin D on the production of corticosteroids by the adrenal cortex.

Approximately 40 male rats, hypophysectomized 24 h previously and weighing 80-100 g, were given a subcutaneous injection of normal saline (0.5 ml./100 g body-weight) or a saline solution of actinomycin D in a dose of 25 µg/0.5 ml. saline/100 g body weight. Control rats were killed 30 min later. At various time intervals the remaining animals were given an intravenous injection of ACTH (2 mu./100 g body-weight), and killed 15 min later. The adrenals were removed and weighed, and adrenal corticosterone concentrations were determined by a modification of the method of Guillemain *et al.*<sup>5</sup>. The results were expressed as µg corticosterone/100 mg adrenal tissue.

Fig. 1 shows the effect of actinomycin D on the changes in adrenal corticosterone concentration produced by the administration of ACTH. It can be seen that 24 h after actinomycin the adrenals of these animals did not respond to the injection of ACTH with an increase in the production of corticosterone ( $P < 0.05$ ) while those of the saline-injected controls responded in a normal fashion.

Actinomycin D appears to be one of the most specific and best characterized inhibitors of protein synthesis. It is said to produce its effects by blocking the DNA-directed RNA synthesis catalysed by RNA polymerase by binding at specific sites on the DNA primer<sup>6-8</sup>. The present data indicate that actinomycin can eliminate the increased synthesis of steroids in the adrenals of rats *in vivo* in response to ACTH. Ferguson<sup>9,10</sup> has shown a similar inhibition of adrenal steroid synthesis in response to ACTH or 3',5'-AMP *in vitro* using the antibiotic puromycin. This substance is believed to inhibit protein synthesis in mammalian tissue at the point of transfer of amino-acids from soluble RNA to ribosomal nucleoprotein<sup>11</sup>.

Both the *in vitro* work with puromycin and the present *in vivo* findings with actinomycin D are consistent with

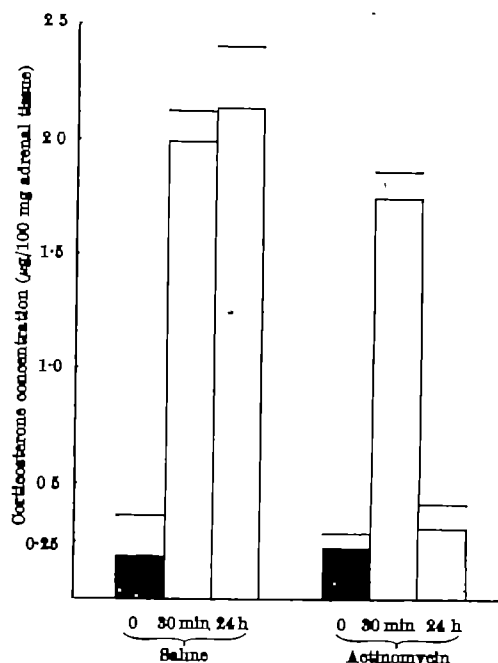


Fig. 1. The effect of actinomycin D on the changes in adrenal corticosterone concentration produced by the administration of ACTH to hypophysectomized rats. Horizontal lines represent the standard errors. ■, Controls; □, ACTH 2 mu./100 g.

the idea that protein synthesis is necessary for adrenal corticosteroid responsiveness to ACTH. It is interesting to note that, under similar conditions, actinomycin will also inhibit the decrease in adrenal ascorbic acid produced by ACTH<sup>12</sup>.

There have recently been several reports on the apparent increase in protein synthesis that accompanies the effects of estrogens<sup>13</sup>, androgens<sup>14</sup>, parathyroid hormone<sup>15</sup>, thyroxine<sup>16</sup>, and other hormones. Talwar and Segal<sup>17</sup> have proposed that a common feature in the mechanism of action of a variety of 'stimulatory hormones' is the triggering of the synthesis of messenger RNA, specific for bringing about biological changes characteristic of their respective target organs. It would appear that the mechanism of action of ACTH on the adrenal cortex adds further support to this thesis.

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# HAEMATOLOGY

## Haemoglobin J and E in a Thai Kindred

SINCE its discovery in 1956 by Thorup *et al.*<sup>1</sup> in an American Negro woman, haemoglobin J has been identified in other ethnic groups including Gujarati Indians<sup>2,3</sup>, Indonesians<sup>4,5</sup>, French-Canadian<sup>6</sup> and Swedish-American<sup>7</sup> Caucasians, Algerians<sup>8</sup>, Chinese in Singapore<sup>9</sup>, Hakkanese Chinese in Taiwan<sup>10</sup>, and Hawaiian-Chinese-Caucasians<sup>11</sup>. This communication concerns the occurrence of haemoglobin J in a Thai family; the family is of special interest because it has haemoglobin E in addition to J.

The presence of an electrophoretically fast-moving haemoglobin in addition to normal haemoglobin A was detected in one subject among 676 presumably healthy Thai soldiers stationed at Nakhornratchasima (Korat), Thailand. Results of the haemoglobin distribution<sup>12,13</sup> and haemoglobin E distribution<sup>13</sup> in that group of subjects were reported previously. Subsequent studies indicated that the electrophoretic mobilities of the haemoglobin under various pH conditions appeared to be identical to those of a fast haemoglobin found in members of a Hakkanese Chinese family in Taiwan<sup>10</sup>. Furthermore, both the Thai and the Chinese fast-moving haemoglobins appeared to have mobilities identical with that of a sample of haemoglobin J provided by Dr. Oscar Thorup from his original patient<sup>1</sup>. In addition, a sample of the Hakkanese Chinese haemoglobin was studied electrophoretically by Dr. H. Lehmann, who identified it as J. Pending completion of structure studies on the Thai haemoglobin it can be identified as J<sub>Korat</sub>.

Subsequent investigations on blood samples from 16 other members of the Thai family by Smithies's vertical starch-gel electrophoresis procedure<sup>14</sup> at pH 9.0, with the *tris*-EDTA-borate buffer system<sup>15</sup> as used by Goldberg<sup>16</sup>, disclosed the family to comprise a mixture of individuals possessing E, A + J, A + E and E + J type haemoglobin combinations. The distribution of the haemoglobin phenotypes in the kindred is shown in Fig. 1 and representative electrophoretic patterns of the haemoglobins are illustrated in Fig. 2. The E type haemoglobin components in the present subjects were identified by comparison of their electrophoretic mobilities with those of numerous other haemoglobin E and A + E samples found in the earlier studies on the 676 Thai blood samples. In those investigations an authentic Thai blood sample with haemoglobins A + E, provided by Dr. Prawase Wasi, Siriraj Hospital, Bangkok, was used for comparison. Numerous studies have reported the presence of haemoglobin E in South-east Asia since its occurrence in Thais<sup>17</sup> was initially recognized. Our work<sup>18</sup> among the 676 Thai subjects, for example, disclosed that 39, or 5.8 per cent, of the individuals were homozygous for the haemoglobin E gene and that 246, 36.4 per cent, had haemoglo-



Fig. 2. Results of starch-gel electrophoresis<sup>14-16</sup> of haemoglobins from Thai kindred. From top downward the subjects and haemoglobin types are: 1, normal control, A; 2, II-8, A; 3, II-2, J + E; 4, III-4, A + J; 5, III-5, A + J; 6, I-2, E; 7, II-6, A + E; 8, III-3, propositus, A + J.

bins A and E. The combined incidence in the Thai sample of 42.2 per cent for the E and A + E genotypes, which is higher than those reported previously, has been confirmed in recent studies among North-eastern Thais by Wasi and NaNakorn<sup>18</sup>.

Subject III-3 was the original case with type A + J haemoglobins found in our study of Thai subjects; his 2 brothers also had the same combination. Their mother, II-8, possessed only normal A-type haemoglobin; their father, II-2, possessed genes for both J and E haemoglobins, and the J haemoglobin genes in the sons were apparently inherited from him. Subject II-3, the brother of subject II-2, also had types J and E haemoglobins; his wife, subject II-9, had normal haemoglobin A. Among the children of subjects II-3 and II-9, one daughter, III-6, had types A and J haemoglobins and 2 sons, II-7 and II-8, had types A and E.

All 5 of the living siblings in the second generation of the kindred had E haemoglobins in combination either with A or J haemoglobin. Their mother, I-2, was homozygous for the E haemoglobin gene as illustrated in Fig. 2. Their father is presumed to have had A and J haemoglobins.

The present family provides the first reported examples of haemoglobin J in Thais and also of an association within a kindred of haemoglobins E and J. Due to the high density of the gene for haemoglobin E in the Thai race as described here, it is obvious that any other abnormal haemoglobin occurring in Thais would occur rather frequently in the same kindred with haemoglobin E. Among those members of the family studied thus far, subjects II-2, II-3 and II-4 are the only examples of the E + J haemoglobin combination in one individual. In future work this family will be examined clinically and haematologically.

We thank Dr. H. Lehmann for his help in identifying one of our haemoglobin J samples, and Drs. Prawase Wasi and Oscar Thorup for their generous contributions of abnormal haemoglobin samples. We also thank the medical officers and technicians of the Royal Thai Army Medical Corps for their aid in procuring blood specimens from the Thai family. Likewise the support of Capt. R. A. Phillips, Commanding Officer of NAMRU-2, and Col. James L. Hansen, director of the U.S. Army Component of the SEATO Medical Research Laboratory, Bangkok,

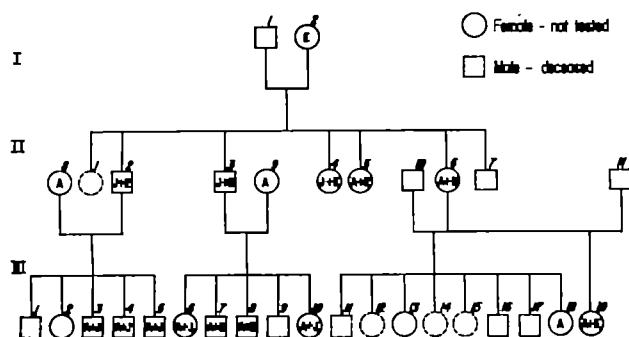


Fig. 1. Distributions of haemoglobin types E, A + E, A + J, and J + E among 3 generations of Thai kindred. Subject III-3 is the propositus.

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### Haemoglobin E in Vietnamese

SINCE its simultaneous discovery a decade ago in an American family of mixed Spanish, Guatemalan, and Indian ancestry<sup>1</sup> and in Thais<sup>2</sup>, haemoglobin E has been found in a variety of ethnic groups a majority of which are located in the Middle East and Asia. It is particularly common among the peoples of south-east Asia where it had been found in relatively high incidence in Burmese<sup>3</sup>, Thais<sup>4-7</sup> and Cambodians<sup>8</sup>, and in lower incidences in Vietnamese<sup>9</sup>, Malaysians<sup>10,11</sup>, Indonesians<sup>12</sup>, Chinese<sup>13,14</sup> and Filipinos<sup>15</sup>. The results in this communication confirm the occurrence of haemoglobin E among Vietnamese.

Subjects for this investigation were residents of Saigon and environs. At the time of the work, during February and March 1964, they were patients in the Cho Quan Hospital, Saigon, where they had been admitted with diarrhoea during the cholera epidemic and were under treatment for dehydration. Red cells were obtained from residual heparinized blood samples collected for other diagnostic purposes and were preserved with merthiolate and by refrigeration prior to their transfer by air to the Biochemistry Department Laboratory of this Unit for analysis.

On receipt in the laboratory the red cells were washed with saline, mixed with equal volumes of distilled water, and frozen until used for electrophoretic analysis. After thawing, the samples were centrifuged and the supernatant haemolysates examined by Smithies's vertical starch-gel electrophoresis procedure<sup>16</sup>. The gel buffer employed was the *tris*-EDTA-borate buffer, pH 9.0, at the concentrations recommended by Goldberg<sup>17</sup>.

Results of the study indicate that 17, or 3.53 per cent, of the 482 subjects had A + E haemoglobins. No other abnormal haemoglobins were detected. Although no quantitative determinations were made of the relative amounts of haemoglobins A and E in the samples, haemo-

globin E was always the minor component. By inspection the E component was estimated to comprise from one-fifth to one-third of the total; in all cases it exceeded the levels to be expected for A<sub>2</sub> haemoglobin, which has the same electrophoretic mobility as E in the buffer employed.

Three cases of haemoglobin E, 2.7 per cent, were reported by Albahary *et al.*<sup>9</sup> among their 113 Vietnamese subjects. Based on the results of both investigations the incidence of the gene for haemoglobin E in the Vietnamese appears to be lower than those in Thais, Cambodians, Burmese and Malaysians, similar to those of some of the Indonesians, and higher than those of Chinese and Filipinos.

Although our results with respect to haemoglobin E in Vietnamese agree with those of Albahary *et al.*<sup>9</sup>, we were unable to confirm their finding of a slow-moving component which they called haemoglobin 'Sud-Vietnam'. No slow component other than E or A<sub>2</sub> was found among our 482 subjects.

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### Xg<sup>a</sup> Investigations of the Family of a Child with a Ring X Chromosome

THE Xg blood groups reported under this title in the issue of November 21, 1964, presented some abnormal features<sup>1</sup>. We recently had the opportunity to test the family and found the father, the mother, the daughter with the ring X chromosome, the normal daughter and the normal son all to be straightforward Xg(a+). Two examples of anti-Xg<sup>a</sup> were used.

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# IMMUNOLOGY

## Immobilization Antigen in Heterozygous Clones of *Paramecium aurelia*

CLONES of *Paramecium aurelia*, heterozygous for two alleles at one of the loci specifying the immobilization antigens<sup>1</sup>, react to both parental type-specific antisera, but usually more strongly to one parental type than to the other. It is of considerable interest to determine whether these heterozygous antigens consist of one molecular species combining in varying degree the specific regions of the parental protein molecules or whether there is simply a mixture of the parental antigens. On the basis of refined serological tests, Finger and Heller<sup>2</sup> have suggested that a single hybrid molecular species is present and that the variation between clones (sometimes even derived from the same mating) is due to the formation of a hybrid combining in different ways the characters and presumably primary structure of the parental proteins.

Animals of stocks 60 and 90 of *P. aurelia* variety 1, expressing the D serotypes, were mated to produce clones of phenotype 60D/90D. About 10<sup>8</sup> of these cells were grown and the immobilization antigen extracted by methods which have been described elsewhere<sup>3</sup>. The antigen was freed from most of the contaminating protein by passing through a column of 'SE-Sephader' with a pH gradient between pH 4.2 and pH 5.6 in 0.05 M sodium acetate. This treatment produces no separation of any of the immobilization antigens which have been examined. 60 mg of antigen was then fractionated on a column of hydroxyapatite<sup>3,4</sup> at pH 6.8 at 2°–3°. Elution was with a series of sodium phosphate buffers 0.002 M, 0.010 M, 0.025 M and 0.050 M applied by a constant rate pump at 3–4 ml/h. Some 100–150 ml. of each buffer was used. Fig. 1 shows the optical density at 280 mμ. The fractions were also examined for their capacity to inhibit the immobilization reaction between both 60D animals and anti-60D serum and 90D animals and anti-90D serum (Table 1).

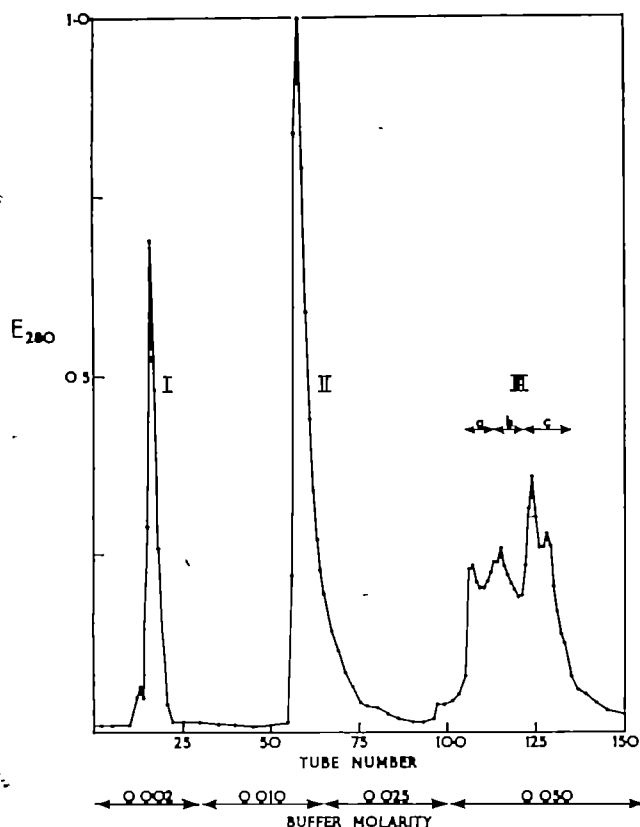


Fig. 1. Fractionation of 60/90D antigen on hydroxyapatite

Table 1 Inhibition of immobilization reaction		
Fraction	60D	90D
I	—	—
II	++++	—
IIIa	+++	++
IIIb	+++	++
c	+	+++

Peak I is completely inactive and is thus the remaining impurity. Peak II is eluted in the position characteristic of pure 60D antigen and gives no reaction for 90D. Pure 90D antigen would normally be eluted in the region IIIb–IIIc. Fractions IIIa and IIIc were each refractio-nated under the same conditions and were again eluted in the same region and showed the same activity pattern. No elution in any other region of the chromatogram was observed.

Thus the heterozygote antigen contains parental 60D material amounting to about 50 per cent of the total antigen. However, the remainder reacts with both anti-60D and anti-90D antisera, and consists of at least two molecular species. It was not possible to determine whether any pure 90D was present because the resolution in this region was not sufficiently good. When the original heterozygote antigen was examined serologically it appeared to contain 70–80 per cent 60D activity.

Other preparations of 60/90D antigen have given similar results.

The immobilization antigen is a protein of molecular weight 250,000 and contains two identical half-molecules held together by many disulphide bonds. These half-molecules in turn probably consist of several non-identical sub-units<sup>5</sup>. In the heterozygote the molecule might be constructed in two ways: (1) The half-molecules, made independently to give two species (60D)<sub>1</sub> and (90D)<sub>1</sub>, might then combine randomly to give three possible species—60D, 90D and (60D)<sub>1</sub>(90D)<sub>1</sub>. In the present experiment where 60D comprises 50 per cent of the total, 90D would be 10 per cent and the hybrid 40 per cent. (2) The half-molecules might be randomly assembled from the possible sub-units, and then form dimers. In the simplest case of only two sub-units, say (AB)<sub>1</sub>, the species formed would be (A<sup>60</sup>B<sup>60</sup>)<sub>2</sub>, (A<sup>60</sup>B<sup>90</sup>)<sub>2</sub>, (A<sup>90</sup>B<sup>90</sup>)<sub>2</sub>, and (A<sup>60</sup>B<sup>90</sup>)<sub>2</sub>, and the amounts in this instance 50, 10, 20 and 20 per cent. In both cases the total amount of 60D activity would be 70 per cent of the whole. Either of these simple hypotheses, particularly the second, can explain the results but, of course, it may be that the situation is more complex. Implicit in the second scheme is the suggestion that the sub-units are specified by separate genetic loci. Beale<sup>6</sup> was unable to demonstrate recombination between alleles at the D locus, but the resolving power of his methods was rather low and close linkage cannot be ruled out.

These results differ basically from those of Finger and Heller<sup>2</sup>, who suggested that only one species was formed in any clone and that the observed variation in the degree of parental type reaction was not a consequence of quantitative variation but of the exclusive formation of different hybrids in different clones. The present results suggest rather that the quantitative variation is basic and that hybrids arise in a purely random way. This quantitative variation will be examined more thoroughly. It can be considered as partial dominance at the molecular level or, in the broader sense, as an example of a very simple form of differentiation.

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## PATHOLOGY

## Effect of Blood-letting on Chronic Mountain Sickness

In 1928, Monge<sup>1,2</sup> described the occurrence in the Peruvian high-altitude areas of a disease characterized by an excessive polycythaemia (greater than usual erythropoietic response to the degree of existing hypoxia) and congestive symptoms, both relieved on descent to lower altitudes. This disease, which may develop after years of residence at high levels or in natives born and living in highlands, has been called chronic mountain sickness or Monge's disease<sup>3,4</sup>. The present report describes the effects of blood-letting on the oxygen saturation, CO<sub>2</sub> pressure and pH in the arterial blood of patients with chronic mountain sickness living in Cerro de Pasco, Perú, at an altitude of 4,300 metres above sea-level.

The studies were carried out in the Cerro de Pasco Laboratory (4,300 metres) of the Instituto de Investigaciones de Altura. Three cases of chronic mountain sickness were selected on the basis of congestive symptoms, an arterial oxygen saturation (HbO<sub>2</sub> percentage) of less than 81 per cent, which is the normal figure for a similar altitude reported by Hurtado and Aste<sup>5</sup>, and a haematocrit of more than 70 per cent. In addition, they all had an arterial pCO<sub>2</sub> above 32.5 mm Hg, which is the average figure for Cerro de Pasco found by Monge *et al.*<sup>1</sup>. This last characteristic corresponds to a condition of hypoventilation recently described by Hurtado<sup>6</sup> as existing in these cases. The subjects were high-altitude natives. They slept in the laboratory the night before the experiment in order to assure basal conditions. Puncture of a brachial artery was done using a standard anaerobic technique and the blood pH was read immediately in an Astrup 'M4' micro blood pH meter. The blood pCO<sub>2</sub> was determined using the micro-equilibration technique of Astrup<sup>7</sup>. The HbO<sub>2</sub> percentage was determined in the Van Slyke apparatus. The determination of the O<sub>2</sub> capacity was carried out by tonometer equilibration at pCO<sub>2</sub> 40 mm Hg and pO<sub>2</sub> 200 mm Hg. After the arterial puncture was finished an indwelling trocar was inserted in an arm vein and a variable amount of blood was withdrawn (see Table 1). The arterial blood measurements were repeated 24 h later.

Table 1 contains the data on HbO<sub>2</sub> percentage, pCO<sub>2</sub>, pH and haematocrit of each subject before and after bleeding. It can be seen that the values after bleeding do not show important differences in cases 2 and 3. Case 1 showed a drop in HbO<sub>2</sub> percentage from 66.2 to 60.8 with no significant change in pH or pCO<sub>2</sub>. This case is interesting because the patient had, in addition to congestive symptoms, a moderate degree of heart failure. He had a drop in haematocrit from 76 to 68 in spite of a moderate bleeding of 600 ml. The three patients did not show any improvement in their symptomatology after blood was removed.

The excessive polycythaemia of chronic mountain sickness has been attributed to the increased arterial blood unsaturation secondary to hypoventilation (Hurtado)<sup>6</sup>. The purpose of the present work was to test the hypothesis of a primary excessive polycythaemia and secondary hypoventilation due to embarrassment of the cerebral circulation and activity of the respiratory centre. Our results show that 24 h after venisection the changes in pCO<sub>2</sub> were not significant and in one case there was a drop in HbO<sub>2</sub>,

percentage. These preliminary results contradict the hypothesis of primary excessive polycythaemia and secondary hypoventilation. As bleeding is often followed by a sense of well-being in cases of chronic mountain sickness, future studies are needed, testing different post-bleeding periods and studying their effect on other physiological parameters such as the elevated pulmonary arterial pressure found by Rotta *et al.*<sup>10</sup> and Peñaloza *et al.*<sup>11</sup> in these cases.

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## Experimental Tumorigenesis in the Hamster Cheek Pouch

THE cheek pouch of the Syrian hamster (*Mesocricetus auratus*) has been used in many investigations involving circulation<sup>1-3</sup>, hetero- and homo-transplantability of normal tissues and neoplasms<sup>4-7</sup>, histochemistry<sup>8,9</sup> and chemical carcinogenesis<sup>5,6,10</sup>. Salley<sup>10</sup> showed that experimental squamous cell carcinomata could be produced in the hamster's cheek pouch by painting with 9,10-dimethyl-1,2-benzanthracene. Delarue *et al.*<sup>9</sup> reported that they obtained fibrosarcomata nine months after subcutaneous implantation of paraffin pellets containing DMBA in the cheek pouch. We have reported<sup>11</sup> that a single subcutaneous injection of 3,4,9,10-dibenzpyrene at the nape of the neck induced fibrosarcomata in the hamster. The primary purpose of this investigation was to determine whether the subcutaneous tissue of the hamster's cheek pouch was as susceptible to the action of the chemical carcinogen.

One hundred and twenty female hamsters, each weighing 75-100 g, were divided among six experimental groups; the animals were lightly anaesthetized and the right cheek pouches were exteriorized. Each pouch received a single subcutaneous injection of 3,4,9,10-dibenzpyrene which had been dissolved in triolein at doses of 0.25 mg, 0.50 mg, 1.0 mg, 1.5 mg, 2.0 mg or 2.5 mg/hamster in an injection volume of 0.2 ml. for five groups. At the maximum concentration it was administered at 0.4 ml. The number of usable hamsters was reduced to 81 due to host mortality in all injected groups. They were examined weekly until death and the tumour was removed at autopsy for microscopic examination. A diet of Wayne laboratory chow was provided, with water *ad libitum*.

The number, incidence and average latency period of all observed tumours and the survival of tumour-bearing hamsters are tabulated in Table 1. Administration of 3,4,9,10-DBP at the doses used resulted in the induction of subcutaneous fibrosarcomata at the site of injection by the seventh week. The cumulative incidence was as follows: 0.25 mg/hamster, 85 per cent; 0.50 mg/hamster, 87 per cent; 1.0 mg/hamster, 100 per cent; 1.5 mg/hamster, 100 per cent; 2.0 mg/hamster, 100 per cent; and

Table 1. ARTERIAL BLOOD VALUES IN CASES OF CHRONIC MOUNTAIN SICKNESS BEFORE AND AFTER VENISECTION

Patient	HbO <sub>2</sub> (%)	pH	pCO <sub>2</sub> (mm Hg)	Haematocrit (%)	Bleeding (ml.)
1 b	66.2	7.420	39	76.0	600
a	60.8	7.420	37	68.0	
2 b	73.6	7.503	41	76.0	750
a	74.5	7.419	42	74.0	
3 b	73.8	7.410	38	80.5	1,200
a	74.7	7.428	36	72.5	

b, before bleeding; a, 24 h after bleeding.

TABLE 1. TUMOR PRODUCTION AND SURVIVAL OF ETHAN SYRIAN HARBOR INFUSED WITH STRAIN DOGS OF 3,4,9,10-DIBENZOPYRANE IN CHALK POWD

Dose	Effective No. of animals		Weeks after induction																				
			4	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
0.25 mg 3,4,9,10-DBP/ 0.5 ml. trisectanone	13	No. tumours/No. survivors No. tumours/week Tumour-bearing survivors No. deaths/week	0/13	0/13	0/13	0/13	0/13	0/13	0/13	0/13	0/13	0/13	0/13	1/13	2/13	2/13	2/13	2/13	2/13	4/13	5/13	6/13	6/13
0.5 mg 3,4,9,10-DBP/ 0.2 ml. trisectanone	15	No. tumours/No. survivors No. tumours/week Tumour-bearing survivors No. deaths/week	0/15	0/15	0/15	0/15	0/15	1/15	1/15	1/15	1/15	1/15	2/15	2/15	4/15	6/15	7/15	9/15	10/15	10/15	10/15	10/15	10/15
1.0 mg 3,4,9,10-DBP/ 0.2 ml. trisectanone	13	No. tumours/No. survivors No. tumours/week Tumour-bearing survivors No. deaths/week	0/12	0/12	1/12	1/12	3/12	3/12	3/12	4/12	4/12	4/12	6/12	6/12	7/12	8/12	8/12	9/12	9/12	9/12	9/12	9/12	9/12
1.5 mg 3,4,9,10-DBP/ 0.2 ml. trisectanone	13	No. tumours/No. survivors No. tumours/week Tumour-bearing survivors No. deaths/week	0/13	2/13	2/13	2/13	3/13	3/13	3/13	4/13	4/13	5/13	6/13	8/13	10/13	12/13	12/13	12/13	7/13	5/13	5/13	5/13	5/13
2.0 mg 3,4,9,10-DBP/ 0.2 ml. trisectanone	8	No. tumours/No. survivors No. tumours/week Tumour-bearing survivors No. deaths/week	2/8	3/8	3/8	3/8	3/8	3/8	3/8	4/8	4/8	4/8	4/8	4/8	5/8	5/8	5/8	5/8	5/8	5/8	5/8	5/8	5/8
3.5 mg 3,4,9,10-DBP/ 0.4 ml. trisectanone	20	No. tumours/No. survivors No. tumours/week Tumour-bearing survivors No. deaths/week	0/20	3/20	3/20	6/20	14/20	14/20	14/20	15/20	15/20	16/20	16/20	18/20	18/20	18/20	19/20	19/20	0	0	8/10	8/10	8

[illegible]

Table 2. AVERAGE LATENCY PERIODS IN HAMSTERS RECEIVING SINGLE INJECTION OF 3,4,9,10-DIBENZOPYRENE SUBCUTANEOUSLY IN SUPRASCAPULAR AREA OR CHEEK POUCH

Agent	Dose (mg/hamster)	$\bar{X}$ Latency period (weeks) suprascapular	$\bar{X}$ Latency period (weeks) cheek pouch
3,4,9,10-DBP	2.0	9.8	14.4
	1.0	10.2	17.7
	0.5	13.0	19.5
	0.25	14.0	24.5

2.5 mg/hamster, 95 per cent. The average latency period and mean survival times were related to dosage, that is, decreasing doses of 3,4,9,10-DBP resulted in a gradual prolongation of the average latency period and mean survival time, although the tumour incidences were in close proximity for the six groups.

The average latency period of subcutaneous fibrosarcomata arising in the suprascapular area and in the cheek pouch were compared. The results shown in Table 2 demonstrated that at similar doses the average latency period was shorter when 3,4,9,10-DBP was injected in the suprascapular area.

Microscopically the 3,4,9,10-dibenzopyrene tumour (Figs. 1 and 2) was seen as a spindle cell sarcoma with areas of

pleomorphism. Multinucleated giant cells were seen in some areas and mitotic figures were present.

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### Structure and Role of Cytoplasmic Ribonucleic Acid in Malignancy

X-RAY diffraction investigations<sup>1-4</sup> have indicated that RNA of normal biological material exists as an oriented helical structure with the sugar-phosphate chains forming its circumference and the hydrogen-bonded bases forming its central core. Despite the irregularity in the sequence of its bases<sup>5</sup>, RNA derives a certain degree of regularity from repetition of the O<sub>5</sub>—O—P—O—O<sub>3</sub> internucleotide linkage and from the spatial configuration of the sugar-phosphate 'backbones'.

The purpose of this communication is to propose a different structure for cytoplasmic RNA of malignant material, and to present a theory concerning its biological role in the initiation and progress of cancer.

The basic feature of the proposed structure is an overall state of irregular molecular folding consequent to loss of the three-dimensional potentiality of the molecule at multiple sites determined by the sequence of the pyrimidine bases on the polynucleotide chains.

This is proposed on the ground of my earlier observations<sup>6-10</sup> on the difference in interaction of RNAs of normal and malignant material with the basic dye acridine orange *in vivo*. Evaluated by intravital fluorescence microscopy<sup>11</sup>, cytoplasmic RNA of normal living organs, whether resting or physiologically active, exhibited no affinity to the dye cations. (The intravital fluorescence microscope used was designed by me and constructed by J. D. Möller Optical Works, Wedel-Hamburg, Germany.) On the other hand, they were bound by cytoplasmic RNA of malignant tumours in relative orientation compatible with cationic dimerization. This has been indicated by the spectral range of emission of the dimers (5470 Å–5770 Å), by their reversible disaggregation with variations of temperature and solvents and their irreversible dispersion consequent to the action of ribonuclease *in vivo*<sup>10</sup>.

Fixation of the cations to the sugar-phosphate chains was ruled out since the phosphate residues are in effective combination with the proteins, and since precipitation of the phosphate groups that may exist in a free state have not interfered with the dimerization<sup>10</sup>.

This has suggested fixation of the cations to the nucleotide plates through a competition with the hydrogen ions for the possession of the weakly acidic groups of the bases, as has been demonstrated by the reversibility of the interaction in presence of hydrogen carriers and with increase in the hydrogen-ion concentration<sup>10</sup>.

Consequently, each dimer should be located at right angles to the axis of the  $\alpha$ -helix and would comprise two



Fig. 1. Subcutaneous fibrosarcoma in hamster cheek pouch ( $\times$  c. 180)



Fig. 2. Subcutaneous fibrosarcoma in hamster cheek pouch ( $\times$  c. 330)



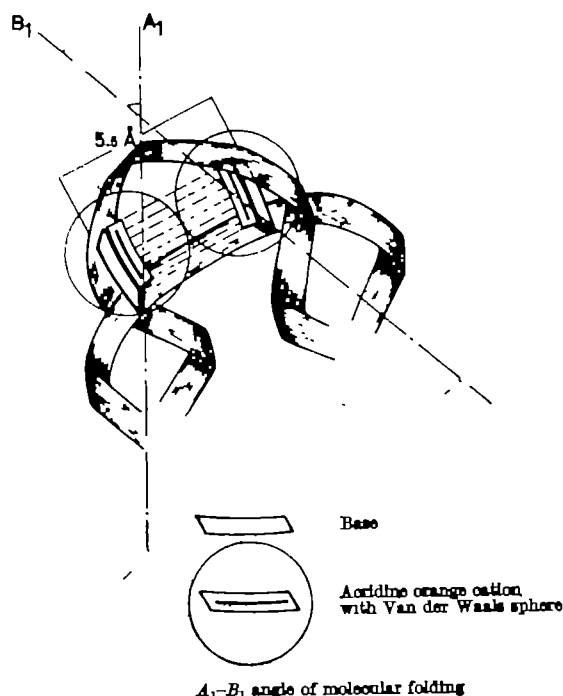


Fig. 1. Diagrammatic representation for the proposed disorientation of the nucleotide plates of RNA at the site of formation of acridine orange dimer

cations with an interplanar distance of 5.5 Å, which is the shortest distance for their approximation.

To account for formation of each dimer bound to cytoplasmic RNA of malignant material, I have conceived disorientation of two successive nucleotide plates in favour of an increase in the internucleotide distance demonstrated for RNA of normal material<sup>1-4</sup>. This has seemed to indicate deviation of the axis of the  $\alpha$ -helix at the binding site for each dimer (Fig. 1), with a repetition of the axial deviation that would be consistent with formation of multiple short-chain dimers.

I have found this indicative of an overall state of irregular molecular configuration such as may result from loss of the three-dimensional potentiality of the molecules at multiple sites. Recognizing that stabilization of the three-dimensional potentiality is basically a function of the primary covalent structure of the molecule, I have attributed the proposed disorientation to multifocal deformation of the  $C_5-O-P-O-C_5$  internucleotide linkage. The dispersive effect of ribonuclease on the dye complexes indicates that the enzyme has broken the continuity of the internucleotide linkage at points essentially related to the geometry of each dimer. Since ribonuclease is a highly specific phosphodiesterase which hydrolyses only secondary phosphate esters of pyrimidine nucleotide-3'-phosphate<sup>13</sup>, it is reasonable to conclude that the deformation is concerned with the  $C_5-O-P-O-C_5$  linkage, which joins a pyrimidine nucleotide with the following nucleotide in sequence. Since the deformation would most likely be a change of one or other of the bond angles, and since parallelism of the resonance systems of the cations is essential for their dimerization, I would consider that the linkage affected is pyrimidine-pyrimidine rather than pyrimidine-purine.

**Induction and biological role.** In spontaneous malignancy it is probable that the proposed structure would be a product of configurational isomerism of a normal RNA molecule, initiated by abnormal environmental fluctuations, which exaggerate the physiological minor rearrangements within the molecular structure yet permanently interfere with the reversibility to the basic configuration. A constitutional instability transmitted in the germ plasma at the molecular level could then

account for cancer susceptibility displayed by species, strains, tissues or organs.

In experimentally induced malignancy and that due to recognized aetiological agents, a multitude of mechanisms should be expected to operate. Whatever the nature of an agent or its mechanism of action, the ultimate biological effect should be interference with the three-dimensional potentiality of the RNA molecule in the manner specified.

Demonstration of the proposed structure in carcinogen-treated material as well as fully developed malignancy<sup>7,8,10</sup> has indicated its replication on the molecular level with transmission of a cancerous trait. With the extensive experimental bases for the acceptance of a nuclear hereditary system in the normal cell, this would signify a non-identical system of heredity in cancer determined by self-duplication of the proposed structure and acquisition of the folding deformity by the protein molecules. Replication of the proposed structure is conceivable in the light of the observations<sup>13,14</sup> on the self-propagating capacity of viral RNA and those on the possibility of replication of biological molecules with induced breaks in the sugar-phosphate backbone<sup>15</sup>.

The structure presumably underlies a functional disorganization at the level of the cytoplasmic organelles which would account for the faulty protein synthesis, the abnormal mitotic activity, and the disarrangement of the energy-yielding reaction which characterize malignancy.

I find the proposed structure and its suggested role compatible with earlier theories on the cytoplasmic origin of malignancy<sup>16-18</sup> and also in agreement with recent experimental observations suggestive of a qualitative change of RNA in certain tumours<sup>19</sup>.

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## Complement-fixing Antibody to Colloid and Epithellum In Monkey Thyroiditis

HASHIMOTO's disease exhibits features not demonstrable in the autoimmune thyroiditis of guinea-pigs<sup>1</sup>, rabbits, dogs<sup>2</sup> and rats<sup>3</sup>. Diffuse lymphoid infiltration of the thyroid gland and humoral antibodies against epithelial antigens are not found in the experimental lesion<sup>4,5</sup>.

To evaluate the histological and serological characteristics of experimental primate thyroiditis, rhesus monkeys were injected with saline extract of monkey thyroid gland according to the following schedule: four monkeys received intramuscularly 2 c.c. of an emulsion composed

of equal parts of saline extract of thyroid gland (30 mg of protein/ml.) and complete Freund's adjuvant at 7-10 day intervals, and were killed 10 days after the last injection. Two monkeys received three injections and two monkeys four injections. As controls, two monkeys were injected with 'APL' (brand of human chorionic gonadotropin supplied by Ayerst Laboratories) and Freund's adjuvant, and two monkeys with 'Pergonal' (PR2016, brand of human menopausal gonadotropin supplied by Cutter Laboratories) and Freund's adjuvant. Control monkeys received four injections.

Small focal infiltrations of mononuclear cells were observed in thyroid glands of monkeys receiving three injections of thyroid emulsion. Occasionally thyroid acini were destroyed. In monkeys receiving four injections, extensive thyroid infiltrations of large mononuclear, lymphocytic and plasma cells were associated with destruction of thyroid parenchyma (Figs. 1a and b). Control monkeys exhibited rare foci of mononuclear cell infiltration.

Sera were assayed for antibody to thyroid antigens by the indirect fluorescent antibody technique. Acetone-fixed cryostat sections of normal monkey thyroid were treated with test sera followed by fluoresceinated rabbit antihuman  $\gamma$ -globulin, shown to cross-react with monkey  $\gamma$ -globulin by immunoelectrophoresis. Complement-fixation reactions were carried out by applying fresh guinea-pig serum or fresh human serum to cryostat sections of thyroid previously treated with a test serum, followed by fluoresceinated rabbit anti-guinea-pig complement or fluoresceinated rabbit anti-B<sub>1</sub>C globulin, respectively. Both reactions gave similar results, although fluorescence observed with human complement was brighter. Incubation at 5° C eliminated complement fixation (Table 1).

Sera of monkeys with severe thyroiditis contained antibodies to colloid (Fig. 2a) and epithelial antigens (Fig. 2b). Antinuclear antibodies were not observed.

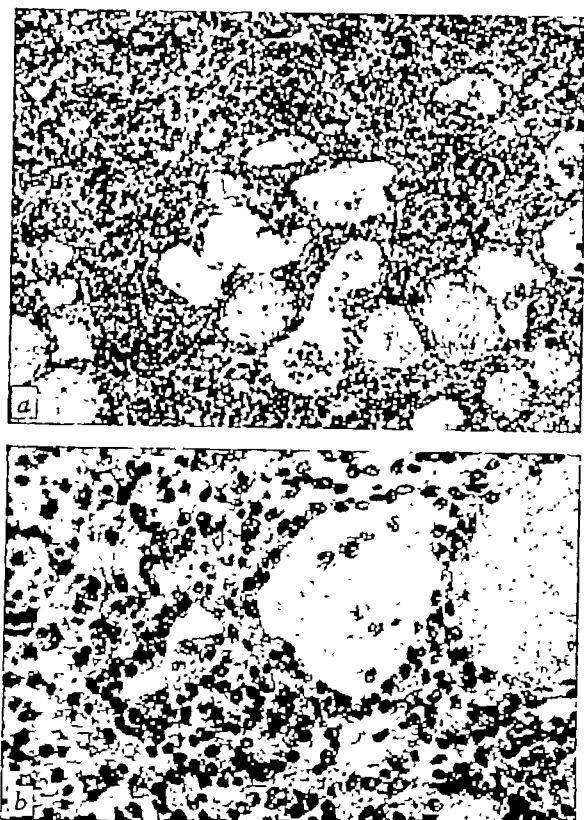


Fig. 1. Thyroid gland from a monkey receiving four injections of thyroid extract-adjuvant emulsion. a, Infiltration of cells and destruction of parenchyma ( $\times 675$ ), b, mononuclear cells surrounding a partially disrupted acinus ( $\times 6190$ ).

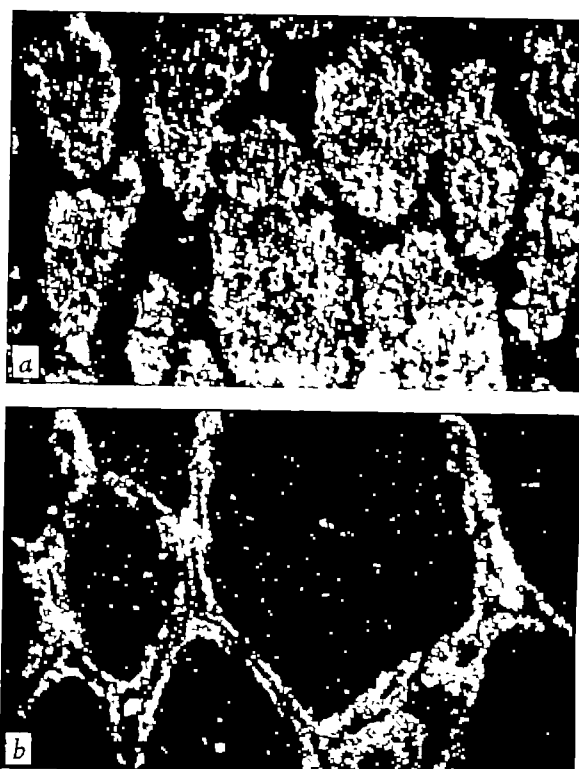


Fig. 2. *In vitro* complement fixation by antibody from serum of a monkey with thyroiditis. a, Colloid fluorescence ( $\times 6210$ ); b, epithelial fluorescence ( $\times 6210$ ).

Table 1. IMMUNOFLOUORESCENT DEMONSTRATION OF HUMORAL ANTIBODY TO MONKEY THYROID ANTIGENS

No. of injections	Material injected	No. of monkeys	Antibodies to colloid antigen	Antibodies to epithelium	Complement-fixation reactions Colloid antibody	Epithelium antibody
3	Thyroid adjuvant emulsion	2	Weakly positive*	Negative	Negative	Negative
4	Thyroid adjuvant emulsion	2	Strongly positive	Positive	Positive	Positive
4	Hormone adjuvant emulsion	4	Negative	Negative	Negative	Negative

\* Refers to fluorescent staining observed.

Antibodies to epithelium exhibited complement-fixing properties similar to those against colloid antigens.

In human thyroiditis complement-fixing antibody to epithelium has previously been demonstrated<sup>4-6</sup> and shown to exhibit cytotoxicity in tissue culture<sup>7</sup>. The presence of complement-fixing antibodies to colloid and epithelium in sera of monkeys with thyroiditis may indicate that both these antibodies have cytotoxic potential. Although serological similarities between primate and human thyroiditis are evident, the early histological lesions resembled those in guinea-pigs, rats, rabbits and dogs. An examination of more chronic lesions in primates may, however, reveal greater morphological similarities to the human disease.

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# RADIOBIOLOGY

## Evidence for Radio-Ruthenium in Milk

DURING routine counting for the determination of caesium-137 levels, an unexpected gamma-ray peak at about 0.51 MeV was observed in a number of milk samples from many different locations throughout Utah (Figs. 1 and 2). It was detected in milk produced on all dairy farms which we sampled between March 1962 and March 1963.

To determine whether the activity was actually in the milk or due to external contamination, a sample containing significant activity at 0.51 MeV was transferred into two clean one-gallon plastic bottles and re-counted. It was found that the activity at 0.51 MeV was still associated with the milk. The original bottles in which the sample was first counted showed only a small trace of the photo-peak when filled with distilled water and re-counted. No evidence of zirconium-95 was observed in the milk. Because all our samples of vegetation and soil showed appreciable zirconium-95 during this period, the absence of this nuclide suggested that the unknown emitter was in the milk and not a result of contamination with dirt during handling.

A study was conducted to determine the half-life of the unknown nuclide using this same milk sample. Counting procedures were identical to those used in routine milk

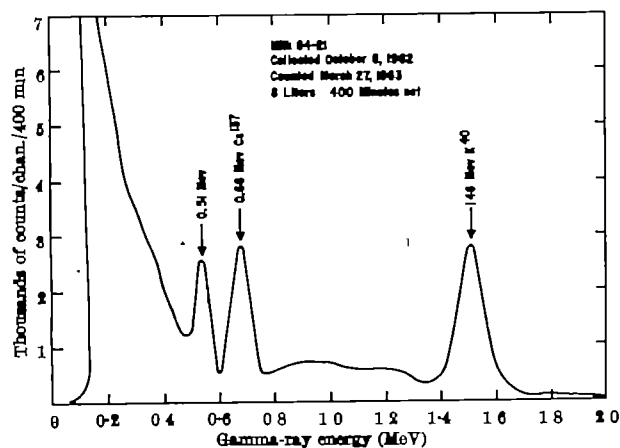


Fig. 1. Gamma-ray spectrum of the milk sample used in the half-life determination

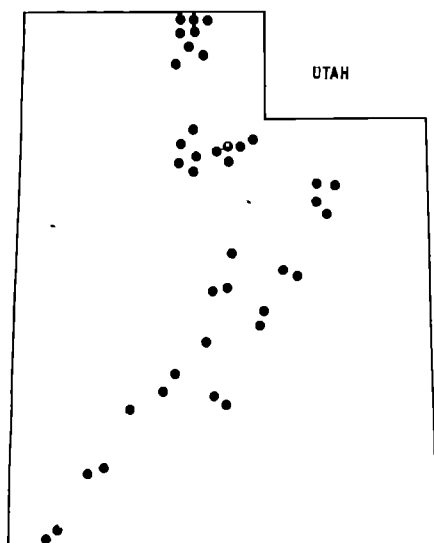


Fig. 2. Locations of dairy farms from which milk showing a detectable photopeak at 0.51 MeV was collected

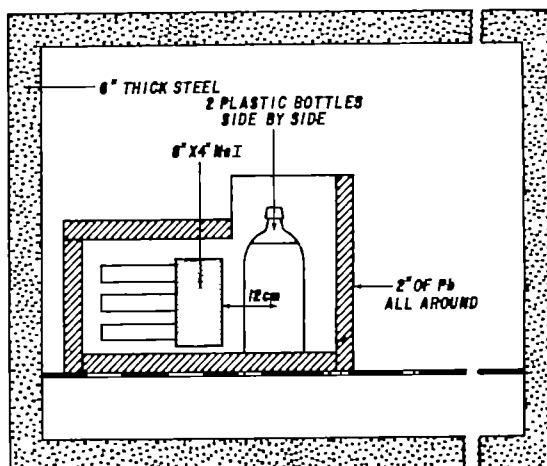


Fig. 3. Positions of sample and detector for milk counting. The 8 in.  $\times$  4 in. NaI (Tl) crystal is operated in conjunction with a 400-channel pulse height analyser

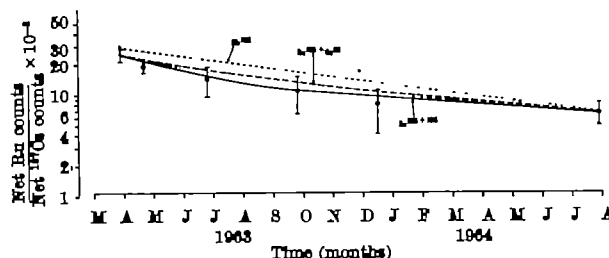


Fig. 4. Data points for the six determinations with their standard errors compared to the theoretical curves described in the text. ....,  $^{106}\text{Ru}$ ; ----,  $^{106}\text{Ru} + ^{106}\text{Ge}$ ; —,  $^{106}\text{Ru} + ^{106}\text{Tl}$

analysis (Fig. 3), but the counting time was lengthened to 400 min, and later to 800, 800 and 8,000 min for improved counting statistics in the 0.51-MeV band. The milk sample was counted 6 times during the period of March 1963–July 1964.

Calculations of relative activity at 0.51 MeV for the sample were based on a ratio of the net counts in this band (0.46–0.54 MeV) to the caesium-137 counts in the 0.66-MeV band (0.62–0.70 MeV). This ratio was selected because it would correct for any change in counting geometry between determinations and because the sample contained enough caesium-137 to be counted accurately. In all computations each energy band was corrected for the appropriate contributions from caesium-137 and potassium-40.

The ratios of the corrected net counting rate in the 0.51-MeV band to that in the 0.66 MeV (caesium-137) band were plotted on semi-log paper to show the radioactive decay of the nuclide with time. The slope of the resulting curve suggested that two emitters were present in the 0.51-MeV band (Fig. 4). These results are not incompatible with the assumption that this gamma activity was due to 865-day ruthenium-106 and 40-day ruthenium-103. A theoretical curve was constructed and plotted of combined ruthenium-103 + 106 counts expressed as a fraction of caesium-137 (Fig. 4). This was accomplished by correcting our last point back to the first determination using the physical half-period of ruthenium-106. The residual counts at the first point were assumed to be ruthenium-103 counts. Referring to commonly used lists of gamma-ray emitting nuclides, the number of possible nuclides fitting the criteria of  $\gamma$  energy and photon abundance was narrowed to seven<sup>1-4</sup>. This was done assuming that the data in the Table of Isotopes<sup>1</sup> are correct (Table 1).

Of the seven nuclides under suspicion, only three display half-lives comparable with the long-lived component of our data. Theoretical mixtures of the possible nuclides were

Table 1. NUCLIDES WITH HALF-LIVES OVER 20 DAYS WITH PHOTONS BETWEEN 0.47 AND 0.55 MeV

Nuclides	Half-life	Principal photon energy in MeV and abundance (% or relative †)	Reason for exclusion
<sup>143</sup> Ba	40 days	0.50 (90%), 0.51 (6%)	
<sup>146</sup> Br	53 days	0.48 (11%)	
<sup>146</sup> Br	64 days	0.51 (100%)	
<sup>146</sup> Br	83 days	0.52 (100%)	
<sup>146</sup> Br	206 days	0.12 (†50), 0.20 (†60), 0.48 (†80), 0.51* (†), 1.06 (†60)	
<sup>68</sup> Ge + <sup>68</sup> Ga	280 days	0.51 (170%), 1.07 (8%)	
<sup>104</sup> Ru + <sup>104</sup> Rh	365 days	0.51 (21%), 0.52 (10%), 1.04 (2%)	
<sup>114</sup> Sm	43 d	0.49 (†10), 0.54 (†74), 1.30 (†31)	No 0.04 or 1.30 MeV photon
<sup>114</sup> Sm	43 d	0.12 (†8), 0.25 (†2.8), 0.48 (†14)	No 0.12 MeV photon
<sup>114</sup> Sm	50 d	0.19 (†100), 0.55 (†19), 0.72 (†19)	No 0.19 or 0.72 MeV photon
<sup>140</sup> La	60 d	0.51* (0.6%), 0.78 (2.7%)	No 0.78 MeV photon
<sup>140</sup> La	60 d	0.16 (9%), 0.48 (0.6%), 0.53 (1.0%), 0.88 (0.3%), 0.93 (0.4%)	No 0.16 MeV photon
<sup>140</sup> La	71 d	0.51* (30%), 0.80 (100%)	No 0.80 MeV photon
<sup>140</sup> La	74 d	0.51 (†170), 0.47 (†64), 0.60 (†30)	No 0.51 MeV photon
<sup>140</sup> La	77 d	0.51 (40%), 0.84 (†100), 1.22 (†70), 1.76 (†17), 2.56 (†16)	No 0.84, 1.22, 1.76 or 2.56 MeV photon
<sup>140</sup> La	245 d	0.51* (3%), 1.12 (44%)	No 1.12 MeV photon
<sup>140</sup> La	240 d	0.06 (†100), 0.48 (†0.53)	No 0.06 MeV photon
<sup>140</sup> La	2.1 y	0.47 (†2), 0.57 (†22), 0.60 (†100), 0.80 (†110)	No 0.57, 0.60, or 0.80 MeV photon
<sup>140</sup> La	2.6 y	0.51* (178%), 1.28 (100%)	No 1.28 MeV photon
<sup>140</sup> La	10.4 y	0.52 (0.7%)	A noble gas should escape
<sup>140</sup> La	>30 y	0.08, 0.18, 0.23, 0.42, 0.54, 0.72, 0.82	No 0.08, 0.18, 0.23, 0.42, 0.72, 0.82 MeV photon
<sup>140</sup> La	7.4 × 10 <sup>2</sup> y	0.51* (168%), 1.83 (96%)	No 1.83 MeV photon
<sup>140</sup> La	8.0 × 10 <sup>2</sup> y	0.51 (†90), 0.52 (†100)	No 0.52 MeV photon

\* Indicates annihilation radiation from β<sup>+</sup> emission.

plotted with the curve being forced through our last point, and when possible through our first point. Then, depending on the decay of the mixture, it could be seen how compatible with the actual data line was the theoretical line. If correction for decay of rhodium-102 is made from our last determination to the date of the first determination, it changes too rapidly to lie within our standard errors. This leaves only germanium-68 and ruthenium-106 as suspects. Plotting mixtures of germanium-68 plus the short-lived component as a function of caesium-137 decay, it can readily be seen that they all lie well above the data line and the ruthenium-103 + 106 line. Of the germanium mixtures, that most compatible with our data would be germanium-68 + ruthenium-103. This is shown in Fig. 4. However, the germanium-68 + ruthenium-103 mixture still lies above the intermediate data points. The best fit to the data is a mixture of ruthenium-103 + ruthenium-106. It is logical to expect that if one isotope of ruthenium were present, the other would also be present.

Ruthenium-103 has a gamma ray at 0.498 MeV and a half-life of 40 days. Ruthenium-106 has a 365-day half-life, and its 30-sec daughter, rhodium-106, emits gamma-rays of 0.513 and 0.62 MeV. The 0.62 MeV peak is difficult to detect in the presence of large amounts of caesium-137, since it would be masked by caesium-137 gamma-rays at 0.66 MeV. If ruthenium-106 were present in this sample, some contribution would be made to the caesium-137 band from the 0.62 MeV gamma-ray of rhodium-106. The amount of this contribution can be estimated by calculating a ratio of net counts in the caesium-137 band to net counts in the 0.51 MeV band for a ruthenium-106 standard. By multiplying this ratio by the net counts for ruthenium-106 in the 0.51 MeV band of the sample, this number of counts for the six determinations can be obtained. The total net counts in the caesium-137 band attributable to the 0.62 MeV peak of ruthenium-106 ranged from 5 per cent on the first count to 2 per cent on our last count. Appropriate corrections were made. An estimated value of 7 pc. ruthenium-106/l. was calculated for the sample, for the day of collection, correcting from the last count using a 365-day half-life. The ruthenium-103 in the sample was also computed for the first count based on the calculated contribution from ruthenium-106. This value was then corrected back to the day of collection, using the 40-day half-life and gamma-ray abundances of ruthenium-103, and this was 34 pc. ruthenium-103/l. For comparison, this milk contained 54 pc. caesium-137/l.

Ruthenium is generally considered to be poorly absorbed by living systems<sup>1</sup>. However, all our hay samples, collected during 1962 and 1963, showed appreciable amounts of radio-ruthenium. Fodder heavily contaminated with relatively fresh fission products could provide a source of

ruthenium-103 and ruthenium-106 for dairy cattle, but the absence of ruthenium in milk produced in the latter part of 1963 suggests that perhaps a different chemical form of ruthenium resulting from local tests might be involved.

Further studies are needed to verify that dietary ruthenium can appear in milk and to determine the influence of the chemical form of the ruthenium. This could be accomplished by feeding various chemical forms of radio-ruthenium to cows. Analyses of the milk, excreta, blood and organs would determine the uptake, metabolism, excretion and secretion of ruthenium by dairy cattle and its significance to man.

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### Intracellular Oxygen and Radiation Sensitivity

It is well known that the sensitivity of cells to ionizing radiation is commonly increased two- to three-fold by the presence of oxygen in their immediate surroundings. A great variety of plant and animal cells show this effect, but it has apparently not been demonstrated in chlorophyll-containing cells. This report describes such a demonstration and also presents evidence that oxygen generated intracellularly in photosynthesis leads to the same sensitization as oxygen from extra-cellular sources.

Vegetative chains of the freshwater green alga *Oedogonium cordiacum* (female line), collected from soil-water cultures, were irradiated in a flattened water-filled glass vessel through which gas could be continuously bubbled at about 400 ml./min. Back-diffusion of air was prevented by a narrow gas exit connected to a flow-meter. The X-ray beam (300 kVp., half-value layer 20 mm Cu, dose rate ≈ 240 rads/min) entered from one side and a light beam ('Photoflood', 150 watt, directed through a glass and water heat-filter and giving about 270 ft.-candles at the surface

of the vessel) could be passed from the other. Alternatively, the vessel could be blacked out during X-irradiation.

The condition of light or darkness, as well as equilibration with gas, was started twenty minutes before and continued during irradiation. After exposure the cells were transferred to culture medium (inorganic salt solution plus soil extract) in the dark for about 60 h and then given light from below. This treatment stimulated the transformation of the cells, without mitosis, into motile zoospores. The spores were collected on gridded slides placed on the bottoms of the vessels, as described by Horsley and Fucikovsky<sup>1</sup>, and about 240 healthy and well-attached spores in each sample were located and their positions noted. After seven to nine days' growth in culture medium at  $\approx 100$  ft.-candles (warm daylight fluorescent tubes, 16 h illumination per day) the selected cells were scored for survival. The criterion of survival was the attainment of a chain of more than twelve cells during the growth period. This criterion was chosen in preference to eight or ten cells used in other work<sup>1,2</sup> because it gave more consistent results.

There has never been any indication that the X-ray or other treatments used had any effect on the number or visible morphology of the zoospores which could be collected after stimulation, and it is assumed that the samples scored were unselected except that visibly abnormal or poorly attached spores were excluded. In some experiments a variable proportion of the selected cells failed to start growing and died at an early time: this behaviour was also unrelated to treatment. Such non-viable cells were omitted from the samples.

The effects of X-irradiation under four conditions were compared and the results are shown in Fig. 1. Unirradiated controls treated with any of the experimental gases, in the light or in darkness, gave more than 90 per cent survival, and values have been normalized to the control placed at 100 per cent. The four conditions of exposure to X-rays were: (a) air in light or in darkness with or without CO<sub>2</sub> (curve A); (b) O<sub>2</sub>-free N<sub>2</sub> in darkness or in light (curve B); (c) O<sub>2</sub>-free N<sub>2</sub> + 2 per cent CO<sub>2</sub> in the light, giving points lying near curve A; (d) O<sub>2</sub>-free N<sub>2</sub> + 2 per cent CO<sub>2</sub> in darkness, giving points lying near curve B.

The results show an increase in radiation sensitivity due to oxygen with an oxygen enhancement ratio of approxi-

mately 2.5. It can also be concluded that the increased sensitivity of cells irradiated in the presence of CO<sub>2</sub> and light is due to oxygen generated in photosynthesis. Neither light alone nor CO<sub>2</sub> alone affected sensitivity: both had to be supplied, and when this was done the sensitivity was increased to the same extent as by the presence of air dissolved in the medium.

It may further be concluded that the oxygen generated within a cell exerts its effect on radiosensitivity within that cell. Any oxygen which diffused into the surrounding water would be rapidly diluted and swept away by the stream of anoxic gas which was bubbling continuously through the irradiation vessel. This is consistent with the view that when cells show increased radiation sensitivity, due to oxygen supplied from outside, it is the oxygen which enters the cell which is important in determining sensitivity.

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## BIOLOGY

### An Evaluation of Inbred Rabbit Populations by Skin Homotransplantation

ALTHOUGH the classical tissue transplantation investigations by Medawar were performed on rabbits, the lack of isogenic strains such as exist in mice and in guinea-pigs has limited the usefulness of the rabbit in experimental transplantation. Since inbred populations of rabbits are rare, we wish to report preliminary experiments carried out in such a colony.

Among the most inbred rabbit lines in the United States are those lines developed over the past thirty years by Dr. Max B. Lurie at the Henry Phipps Institute of the University of Pennsylvania. His most inbred race, the 'O' family, was started in 1938 from the Swift stock in Michigan and maintained by sibling and parent-offspring mating with occasional back-crossing necessitated by the family's low fertility<sup>1</sup>. The complexity of inheritance in this family makes difficult the precise calculation of the inbreeding coefficient.

This family is noted for its extremely low resistance to tuberculosis; during the course of the disease, the bacillus multiplies readily, and both circulating antibodies and delayed hypersensitivity to tuberculin develop slowly<sup>2</sup>. A small number of individuals of the eighteenth and nineteenth generations of this family were made available for testing their response to skin homografts. In addition, the 'AD' family, an inbred race developed in a closed colony and known to be intermediate in its resistance to tuberculosis, was tested in its tenth generation.

Skin homograft transplantation was carried out to determine the approximate degree of genetic identity present in the colony and to determine the ability of the rabbits to respond to the antigenic stimulus of skin grafts from individuals of the same family and of a foreign strain.

Under pentobarbital and ether anaesthesia, full thickness orthotopic skin grafts 12 mm in diameter were transplanted to a 5 cm  $\times$  6 cm open area overlying the panniculus carnosus of the dorsolateral thorax of the recipient. A dressing of petrolatum gauze, dry gauze, and plaster of Paris protected the graft area. In general, two homografts from a single donor and two autografts were transplanted on to the prepared bed. The open-fit method was used because it made possible more accurate estimation of prolonged graft survival times. Graft rejection was determined grossly by the onset of necrosis. This onset was characterized by changes in the appearance, texture,

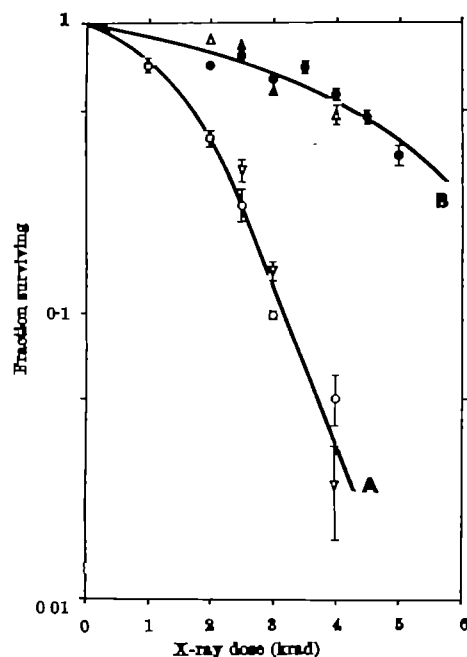


Fig. 1. Survival of spores irradiated under different conditions. ●, nitrogen, oxygen-free, in light or darkness; ○, air or air + CO<sub>2</sub>, in light or darkness; △, nitrogen + CO<sub>2</sub>, oxygen-free, in darkness; ▽, nitrogen + CO<sub>2</sub>, oxygen-free, in light

and size of the graft and by the necrotic appearance of the outgrowth of epithelial cells from the graft, as described by Medawar<sup>3</sup>.

Transplantation of skin grafts in the 'O' family was designed to emphasize any genetic disparity existing between the donor and the recipient: no grafts were exchanged between siblings except from O19-38 to its non-litter-mate O19-37; a large graft dosage was used: two grafts from each donor, totalling 2.3 cm<sup>2</sup>; and, in most cases, simultaneous grafting of skin from two donors increased the dosage of any shared histocompatibility antigens.

The graft survival time was distinctly longer for grafts from donors of the 'O' family than for simultaneous grafts from the control foreign strain ('Flemish Giant') rabbits, as shown in Table 1. Excluding grafts to recipients which died with intact grafts, the average survival time of grafts from 'O' family rabbits was 30 days, compared with 12 days for grafts from the 'Flemish' rabbits. The longest survival times obtained were 58 days and 60 days.

Table 1. GRAFT SURVIVAL TIME (IN DAYS) IN THE 'O' FAMILY

Recipients	Donors' graft survival time	
O18-20	O19-10: 20 d.; O19-26: 20 d.; OH998-1 ('Flemish'): 12 d.	
O19-10	O18-20: 60 d.; O19-26: 48 d.; OH998-1: 12 d.	
O19-26	O18-20: 27 d.; O19-10: 25 d.; OH998-1: 12 d.	
O19-38	O19-37: 58 d.; O19-12: 18 d. (approximate)	
O19-18	O19-27: 18 d. (approximate)	
O19-27	O19-18: 18 d. (approximate)	
O18-22	O17-21: over 41 d. (rabbit died, no cause apparent at autopsy)	
O17-21	O18-22: over 16 d. (rabbit died, no cause apparent at autopsy)	

The 'AD' family was also tested with skin homografts. To each of five siblings were applied single grafts from the remaining four siblings and a graft from a 'Flemish Giant' rabbit. The recipients were challenged with second-set grafts six weeks later. Rabbits AD10-6 and AD10-7 and rabbits AD10-14 and AD10-15 were litter-mates; this had no observable effect on the graft survival time. However, in a separate experiment, a skin graft from rabbit AD8-30 to a non-sibling AD9-16 survived 14 days, whereas a graft from AD9-15 to its litter-mate AD9-14 survived 42 days. The latter graft survival time was the longest observed in the 'AD' family and suggested a close genetic relationship between donor and recipient.

The graft survival times for multiple simultaneous grafts in the 'AD' family siblings is shown in Table 2. The average time of sibling graft survival was 18 days for the first set and 8 days for the second set. The 'Flemish' skin grafts transplanted simultaneously with multiple 'AD' grafts survived an average of 12 days.

Table 2. GRAFT SURVIVAL TIME (IN DAYS) IN THE 'AD' FAMILY SIBLINGS (First set/second set)

Recipients	Donors' graft survival time					OH242-8 ('Flemish')
AD10-4	AD10-4	AD10-6	AD10-7	AD10-14	AD10-15	
AD10-4	—	15/6	15/6	15/6	15/6	11
AD10-6	16/8	—	18/8	15/7	16/8	11
AD10-7	28/16	19/7	—	10/8	35/7	14
AD10-14	15/7	15/7	15/7	—	15/7	12
AD10-15	*TF/20	16/11	*TF/11	15/11	—	11

\* TF: technical failure ("Second set" graft survival time was not averaged in the results for the group.)

These preliminary experiments suggest that the 'O' family and the less inbred 'AD' family are both capable of responding to the antigenic stimulus of a foreign skin graft in a normal manner. The extremely low resistance and poor immunological response to tuberculosis found in members of the 'O' family did not appear to be reflected in their response to skin homografts. The graft rejection time for recipients of both the 'O' and the 'AD' families was 12 days for a graft from a foreign ('Flemish') strain. This is slightly longer, but probably not significantly different from the average of 10.3 days (range 8-11 days) for grafts transplanted according to this method from 'New Zealand White' to 'Flemish Giant' and from 'Flemish Giant' to 'New Zealand White' rabbits.

The graft survival times for skin grafts between individuals of the 'O' family and, to a lesser extent, between

siblings of the 'AD' family suggest that progress has been made in inbreeding toward homozygosity, as determined by transplantation antigens. This is especially true of the 'O' family where the graft survival time was prolonged up to five times the value of the control grafts. However, it is evident from these studies that the 'O' rabbits cannot be considered isogenic. This is further substantiated by the fact that this family is still segregating at at least one of the five known gene loci which determine the red cell antigens, though it appears to be no longer segregating at the *a* and *b* loci which determine  $\gamma$  globulin allotypes<sup>4</sup>. The less inbred 'AD' family is still segregating at the *a*  $\gamma$ -globulin allotype locus. Other experiments have indicated that neither homozygosity for the  $\gamma$ -globulin allotypes nor for the red cell antigens alone is a sufficient condition for permanent skin homograft survival<sup>5</sup>.

Although the 'O' family shows progress toward genetic fixation, at the present time the most promising inbred strain of rabbits for homotransplantation studies appears to be the Bar Harbor line developed by Sawin, Cohen and Chai and reported by Chai<sup>6</sup>.

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## Occurrence of Myogenic Hearts in Arthropods

A DIRECT demonstration of the myogenic or neurogenic origin of the heartbeat in a given animal requires morphological identification of the structures involved and proof of their pacemaker function by surgical or electrophysiological means. In small animals this is difficult to achieve, and in such cases pharmacological evidence is often used. This indirect line of argument originated with Prosser<sup>1</sup>, who showed that the hearts known at the time to be innervated myogenic were slowed by acetylcholine, that purely myogenic hearts were not affected and that neurogenic ones were accelerated. Similarly, Needham<sup>2</sup> suggested that neurogenic hearts are inhibited by ether while myogenic ones are resistant to it. Although both authors made it clear that such evidence is suggestive rather than conclusive, often in practice no other approach is feasible, and acetylcholine in particular is made to carry the full weight of the argument.

Scorpions are a case in point. Kanungo<sup>3</sup> reported that the heart of *Palamnaeus* was inhibited by acetylcholine (ACh) and insensitive to ether. This put scorpions among the small group of arthropods thought to have non-neurogenic hearts as adults. Their relatively large size permitted us to carry out a more detailed analysis of the pacemaker.

*Urodoacus* is a genus of Scorpionidae restricted to Australia. The mesosoma of our animals, and hence the tubular heart extending through it, reached a length of about 25 mm. The tergum with the heart attached was removed, inverted and the preparation perfused with

saline and ACh in varying concentrations. The inhibiting effect of ACh is dependent on concentration and, at least at lower concentrations, reversible (Fig. 1). This is the reaction attributed to an innervated myogenic heart. However, the ACh concentrations are even higher than in Kanungo's experiments, showing values characteristic of neurogenic hearts.

Ten intact animals were exposed to ether vapour and their heart rates determined. With one exception, their hearts were less resistant than the somatic musculature; even when the heart had completely stopped the legs twitched in response to puffs of air or when the table was made to vibrate. On the other hand, heart failure was a decline of amplitude only; the heart rate actually rose in all cases within a minute and before the animal became agitated—a response for which no allowance is made in Needham's scheme, but one which is in keeping with his interpretation of ether as an antagonist of ACh.

The innervation of the heart consists of the 'epicardiac nerve' of Pollee<sup>4</sup>. It runs throughout the length of the heart along the dorsal surface and is in close contact with it. Histological preparations revealed the presence of at least two types of cells: a large number of cells with small elliptical nuclei, and a smaller number (of the order of 60–100) of large cells with large round nuclei staining less strongly with both haematoxylin and borax carmine. The large cells were at first suspected of being neurones; but *in vivo* staining with methylene blue showed that it is the small cells that innervate the myocardium. Nerve fibres supply each semi-circular striated muscle bundle as well as the thin superficial layer of smooth muscle. The dorsal or cardiac nerve is therefore a cardiac ganglion.

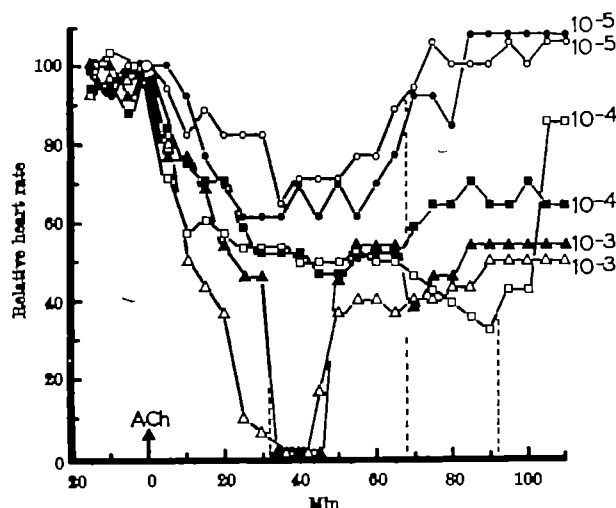


Fig. 1. Inhibition of the heart of *Urodaeus* by acetylcholine at three concentrations. Drug added at time 0 and removed at the time indicated by dashed lines

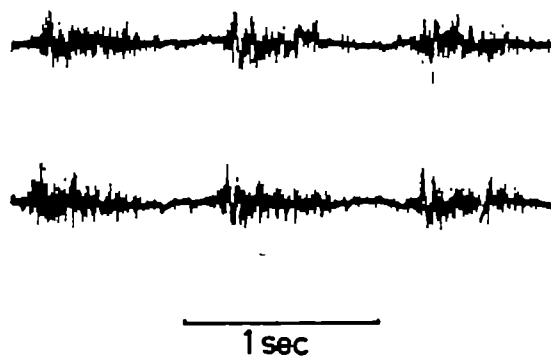


Fig. 2. Spontaneous volleys from the detached cardiac ganglion of *Urodaeus*, recorded with external platinum electrodes. a.c. recording

The ganglion is easily detached from the heart. If this is done over a limited region, contractions stop immediately in that region, while the adjacent parts continue to beat in phase. Complex volleys (Fig. 2) can be recorded with metal hook electrodes, each volley corresponding to a systole. The complexity and variability of the discharge pattern are what one would expect from the large number of neurones involved.

Thus the heart of *Urodaeus* is clearly neurogenic, in contradiction to the pharmacological evidence. Previous claims for myogenic hearts in arthropods, so far as they rely solely on acetylcholine and ether, will have to be reconsidered in the light of this finding.

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### Seasonal Changes in the Blood and Thyroid of the Grass Snake, *Natrix natrix*

CHEMICAL and physical changes in the blood during both natural hibernation and experimental hypothermia have been followed in a number of mammalian species<sup>1</sup>. Similar investigations do not appear to have been extended in any detail to the reptiles, although fish and amphibians have received some attention. The grass snake is less active during the winter, and this condition is often referred to as torpor. In preliminary investigations carried out in this laboratory during 1961, the blood from five specimens, which had entered normal winter torpor in a vivarium, was analysed in February. The ambient temperature was 7° C and some intermittent activity was observed. In July of the same year eleven specimens were available for analysis, the ambient temperature being 18° C. The results, together with comparable data on the hedgehog<sup>1</sup>, are presented in Table 1.

So far as a truly hibernating mammal is concerned quite well-defined changes are to be observed. First, there is some degree of haemoconcentration, as illustrated by the haematocrit and sodium concentration figures. Severe hypoglycaemia and leucopenia are, however, the most noticeable effects, together with a virtual doubling of the calcium:magnesium ratio, due principally to the rise in the magnesium concentration—calcium varying but little. The changes to be observed in the snake are similar in nature, but generally are of a smaller order of magnitude. Some haemoconcentration occurs, the calcium:magnesium ratio rises a little, again due chiefly to a rise in the magnesium value, but leucopenia is again severe. The glucose concentration in the blood of snakes is a highly variable quantity. Little seasonal difference is to be

	Snake		Hedgehog	
	Summer	Winter	Summer	Winter
Average body weight (g)	52.5	71.0	—	—
Haematocrit (%)	32.2	34.2	45	58
±	±1.6	±2.8		
Red blood cells (× 10 <sup>6</sup> )	0.23	1.08	8.9	9.2
±	±0.05	±0.12		
White blood cells (× 10 <sup>6</sup> )	5.4	1.56	6.0	1.6
±	±0.7	±0.13		
Glucose (mg%)	44.0	44.6	125	44
±	±8.0	±9.0		
Sodium (mM/L)	156	166	156	168
±	±3	±4.5		
Potassium (mM/L)	5.1	4.2	6.6	7.0
±	±0.36	±0.3		
Calcium (mM/L)	3.0	2.8	2.4	2.5
±	±0.16	±0.09		
Magnesium (mM/L)	1.8	2.1	1.3	2.5
±	±0.09	±0.13		
Ca:Mg ratio	1:0.6	1:0.76	1:0.52	1:1.0
±	±0.03	±0.04		



seen in the average values obtained, but the variation within the sample is enormous, being 20–60 mg per cent in winter and from 20–111 mg per cent in summer. In an endeavour to find a possible explanation for this wide variation, the seasonal changes in the histology of the thyroid gland were investigated. In mammals there is a marked change in the thyroid during the course of hibernation: the glandular epithelium of the follicles becomes flattened and there is an increase in stored colloid. Thyroids of grass snakes, killed by decapitation, were fixed in Bouin, stained in haematoxylin and eosin and cut from wax blocks at  $6\mu$ . The proportions of the principal components of the thyroid tissue were determined using the quantitative method of Uotila and Kannas<sup>2</sup>, whereby the amount of a given tissue touching parallel lines drawn on projected sections is expressed as a percentage of the total tissue in the visual field.

Data are available for a 12-months period which included two winters. Fig. 1 shows the seasonal changes in the amount of colloid and in the height of the glandular epithelium. Colloid storage is at a maximum in summer and autumn whereas in mammals it is maximal in winter. The height of epithelial cells varies markedly, being at a peak of  $15.2\mu$  in the first winter, which was mild, decreasing to a low level of  $4.5\mu$  by the following October and then increasing somewhat during the succeeding cold winter to a height of  $5.8\mu$ .

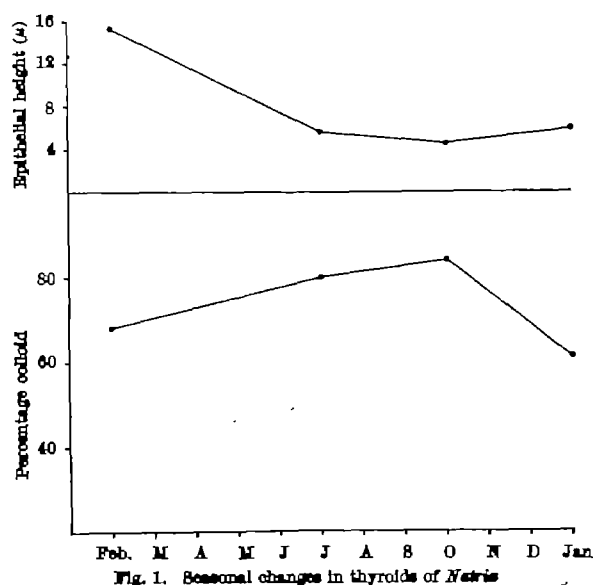


Fig. 1. Seasonal changes in thyroids of *Natrix*

In hibernating mammals the glandular epithelium is high in summer and late autumn and low in winter, the summer height being approximately double that of the winter height. It is interesting to observe that in the minnow the maintenance of animals at a low temperature does not result in an increase of cell height, nor does high temperature result in a decrease. The results obtained by Barrington and Matty<sup>3</sup> indicate that cell height in minnow increases with rise in temperature, the relationship being a direct one instead of an inverse one as is found in mammals. While there is generally good agreement that hibernation is accompanied by thyroid involution, small mammals in deep hypothermia often exhibit a very active thyroid although accompanied by minimal oxygen consumption.

In conclusion, two important factors associated with reptilian physiology must be pointed out. First, the enormous variation to be found between individuals, making comparative work extremely difficult, and, secondly, the evolutionary aspect. The typically mammalian homeostatic influence of the thyroid on metabolism is obviously yet to be developed, as witnessed by the

range of blood glucose values and the typically poikilothermic picture of the thyroid itself, although some control is already apparent. The seasonal variation in the white cell count is clearly demonstrated, as also is the increasingly narcotizing effect of the enhanced calcium: magnesium ratio.

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### Delayed Implantation in an Equatorial Fruit Bat

At Kampala, Uganda (latitude  $0^{\circ} 20' N.$ ), there is an enormous roost of the fruit bat, *Eidolon helvum* Kerr. Numbers vary seasonally, with a maximum of about 250,000 during October–November.

A sample of about 50 bats was collected from this roost once a month from October 1962 until November 1964. Breeding is seasonal, births taking place only in February and March. The weight of the testes varies seasonally, reaching a peak in April–June, when they weigh 3–4 times as much as at their lowest weight. Histological examination shows that spermatozoa are most abundant when the testes are at their peak weights. The males thus exhibit a sexual cycle suggesting that mating occurs in April–June. This is confirmed by the presence of spermatozoa in the genital tracts of all adult females in April–June. Implanted embryos do not occur until October. Examination of the uteri of the adult females by cross-section and by dissection showed that during July–September unimplanted blastocysts were present. Hence there appears to be a delay of about three months between fertilization and implantation.

There appears to be no record of delayed implantation in equatorial mammals. Delayed implantation, however, does occur in mammals in temperate regions, for example, in the badger, *Meles meles* L.<sup>1</sup> Storage of spermatozoa and delayed fertilization occur in some insectivorous bats in temperate regions<sup>2</sup>. At  $0^{\circ} 20' N.$  seasonal change in day-length is trivial. Rain at Kampala occurs all the year round, with peaks during October–November and April–May. The bats feed on a large variety of both cultivated and wild fruits which do not appear to vary in abundance seasonally. The ecological significance of delayed implantation and seasonal breeding in *Eidolon helvum* therefore remains obscure.

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### Growth of Excised Embryo Shoot Apices of Wheat *In vitro*

EMBRYO apices of the spring wheat cv. 'Koga II' were used in the investigation described here. The shoot apical meristem together with its three leaf primordia was excised under aseptic conditions, but in some experiments the coleoptile was also removed with the apex. The excised apices were inoculated on to the surface of 5 ml. of a semi-liquid basal medium containing the mineral

salts of Baldev<sup>1</sup>, 2 per cent sucrose and 0.3 per cent Difco 'Noble' agar in 3 in. x 1 in. soda-glass tubes and grown in the dark at 25° ± 1° C.

The aim in this investigation was to ascertain as precisely as possible the simplest metabolic requirements for sustained growth and organogenesis of the shoot apex separated from the more mature tissues lower down the axis. The growth response of excised apices is dependent on the number of leaves associated with the apex. Apices inoculated on to the foregoing medium, supplemented with 1 mg/l. gibberellic acid, showed a decrease in meristematic activity which is directly related to the number of leaves associated with the apex (Table 1). The possibility that the coleoptile may be supplying some special growth factor to the meristem cannot be ruled out; it is a well-known field observation that cereal seeds with damaged coleoptiles show decreased germinative energy.

Table 1. INCREASE IN LEAF NUMBER AND DRY WEIGHT OF EXPLANTS OF MEDIUM CONTAINING GIBBERELIC ACID (1 MG/L.) AFTER FOUR WEEKS' CULTURE

	Apex with coleoptile	Apex without coleoptile	Apex without coleoptile or leaf 1
Dry wt. (mg)	9.3	1.8	0.2
Increase leaf number	1.4	0.7	0

Provided the explant apex had three leaves, the inclusion of gibberellic acid in the medium produced a significant increase in apical activity, measured by the increase in leaf number, and this is accompanied by an increase in vigour of the explant. This supports the hypothesis of Ball that in angiosperm shoot apices the actively growing leaf primordia are involved in the supply of metabolites essential for the continued growth of the meristem.

The incorporation of indolyl-3-acetic acid in the concentration range of  $1 \times 10^{-8}$ – $8 \times 10^{-8}$  mg/l. had an increasing inhibitory effect on growth with increasing concentration. Below 0.05 mg/l. kinetin has no effect on growth but above this concentration it inhibits growth generally. When adenine was included in the medium, buds were frequently produced in a position on the opposite side of the shoot to the leaf axis but associated with the node of the leaf, generally just below the node (Fig. 1). Occasionally buds were produced both in the leaf axil and diametrically opposite. On odd occasions buds have been produced in this anomalous position on explants grown on media containing gibberellic acid.

Like most monocotyledons, with the exception of *Asparagus*<sup>2</sup>, the shoot apices of wheat are difficult to grow *in vitro*. However, the stimulation of apical activity by 1 mg/l. gibberellic acid and the decrease in the variability of the response when the acid is present suggest that this

hormone plays an important part in the regulation of growth in the apical meristem. Simpson<sup>3</sup> has shown that a gibberellin-like substance occurs in the shoots of wheat seedlings, particularly in the basal parts.

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## Bud Initiation in Excised Roots of *Linaria vulgaris*

VEGETATIVE reproduction in *Linaria vulgaris* is effected by the formation of root-buds at the junction of the parent and some of the lateral roots. Up to four buds may be formed in the superficial layers of the lateral root within the cortex of the parent root<sup>1</sup> (Fig. 1), though parent and lateral root tissues are often not separately recognizable in this region (Fig. 2). Bud initiation does not occur until some secondary growth has occurred in the parent root.

Roots were excised from seedlings under aseptic conditions and cultured in a modified White's medium<sup>2</sup> containing thiamine and pyridoxine at 0.1 mg/l.; nicotinic acid at 0.5 mg/l.; and 2 per cent sucrose. In the first 3-weeks' passage, 11 of 183 roots grown in the dark, and 45 of 55 roots grown under 600 ft.-candles white fluorescent light, initiated buds at lateral root bases as in the intact plant.

Clones were obtained from those roots which had grown well in the first passage by sub-culture of lateral root apices. Not all roots gave satisfactory clones; apical growth or lateral root initiation sometimes ceased or

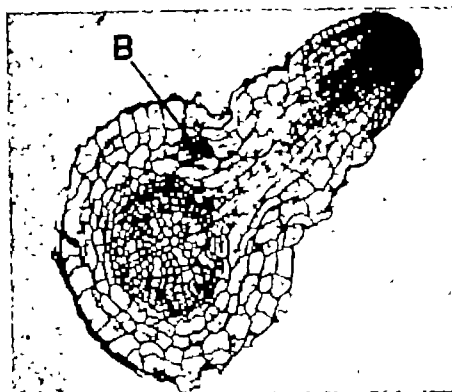


Fig. 1. Root-bud (B) of *Linaria vulgaris* initiated in the outer layers of the cortex of a lateral root, within the cortex of the parent root. (x c. 133)

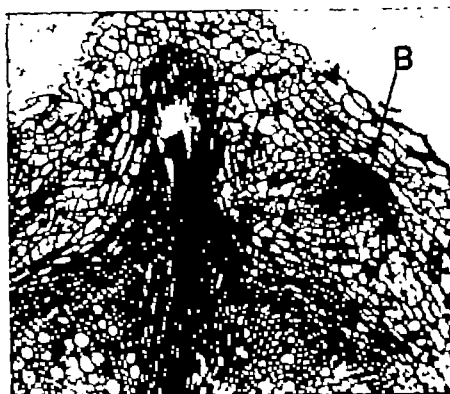


Fig. 2. Root-bud (B) of *Linaria vulgaris* at a lateral root-base where the demarcation of parent and lateral root-bases is not visible. (x c. 67) of



Fig. 1. Buds in anomalous position on explants grown in medium containing adenine, after 4 weeks' culture. Scale in cm

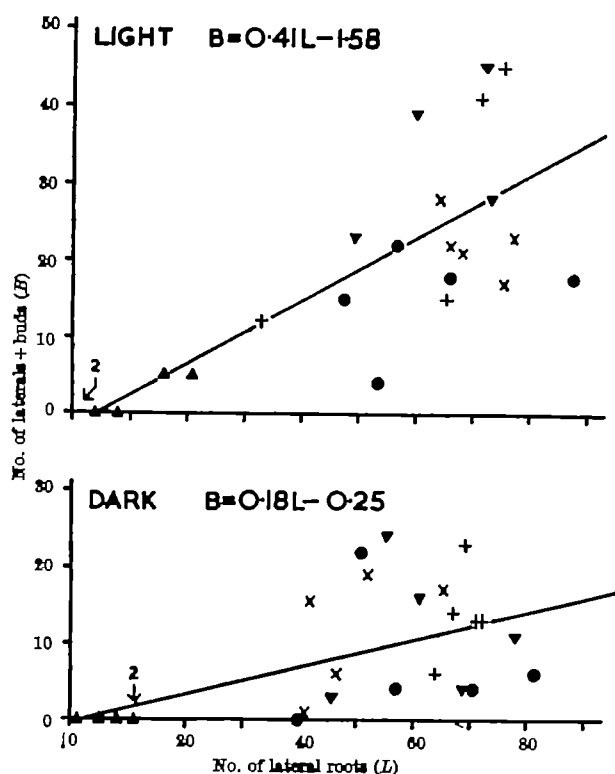


Fig. 3. The relationship between number of lateral roots ( $L$ ) and number of lateral roots with associated buds ( $B$ ) in excised roots of *Linaria vulgaris* grown in a range of kinetin treatments in light and dark. Multiple points are indicated.  
 $\times$ , No kinetin;  $\bullet$ , 0.025 mg/l. kinetin;  $\blacktriangledown$ , 0.05 mg/l. kinetin;  $+$ , 0.10 mg/l. kinetin;  $\blacktriangle$ , 0.25 mg/l. kinetin.

became poor. Bud formation did not normally occur after the third 3-weeks' passage in light or dark, though after this time it sometimes occurred if a passage was prolonged beyond 4 weeks. Similar losses of the capacity to initiate buds have been reported in studies of other excised roots<sup>3,4</sup> and kinetin has been found to re-induce bud formation<sup>4</sup> or, in *Convolvulus arvensis*, to enhance bud initiation<sup>5</sup>.

Kinetin treatment, at 0.025–0.75 mg/l. in light or dark, of *Linaria* clones which had ceased bud initiation did not result in bud formation in the normal 3-weeks' passage. In longer experimental passages bud initiation occurred in kinetin and control treatments, but interpretation in terms of direct effects on bud initiation presented some difficulty since kinetin at or above 0.25 mg/l. inhibited root growth. Two modes of appraisal were used, and the results are presented of one experiment in which a dark-grown clone was used in a 6-weeks' experimental passage in light and dark, at 5 roots per treatment.

The number of buds per lateral root-base was not significantly increased by kinetin treatment but was greater in the light (Table 1). A decrease at 0.25 mg/l. is probably related to the general inhibition of root growth and lateral root initiation. The regressions of the number of lateral roots with associated buds ( $B$ ) on the total number of lateral roots ( $L$ ) were calculated as  $B = 0.41L - 1.58$  and  $B = 0.18L - 0.25$  (Fig. 3) for light- and dark-grown roots respectively (using the values from individual roots and including all kinetin treatments). Both regressions were significant at the  $P = 0.01$  probability-level. It was concluded that kinetin treatment had no effect on the rate of bud initiation relative to the rate of lateral root initiation, but that light relatively increased the rate of bud initiation.

In other experiments it was found that indolyl-3-acetic acid (IAA), gibberellic acid, adenine and adenosine, and combinations involving kinetin, IAA and adenine had no stimulatory effects on bud initiation. Changes in carbohydrate level were also ineffective.

Table 1. EFFECT OF KINETIN ON THE NUMBER OF BUDS PER LATERAL ROOT BASE IN EXCISED ROOTS OF *Linaria vulgaris*

Kinetin mg/l.	0	0.025	0.05	0.10	0.25
Buds per lateral root-base					
light	1.24	1.21	1.19	1.16	1.06
dark	1.02	1.02	1.09	1.00	—

Bud initiation at the base of lateral roots of *Linaria vulgaris* is a function of the lateral roots; so far it has not been positively affected by any external treatment except white light. Yet the number of buds per root-base is variable, and many root-bases do not have buds. Also, in the excised roots, bud initiation is variable in the sense that it decreases with passage number. It seems likely that bud initiation is dependent on some unknown factor(s) present in roots at excision but not synthesized to any great extent in the roots. The factor(s) are not likely to be related to conditions determining the onset of secondary growth since some cambial activity is present in clones more than three years old. Since experimental treatment with the usual growth substances is ineffective on bud initiation it seems that there may be some unknown system which controls bud initiation; this system may be susceptible to the influence of light.

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### Genetic Recombination in a Blue-green Alga, *Cylindrospermum majus* Kuetz.

THE blue-green alga, *Cylindrospermum majus* Kuetz., forms a characteristic filament with single terminal heterocyst and sub-terminal spore at both ends of the filament. The alga was isolated on Allen and Arnon's nitrogen-free medium<sup>1</sup> in axenic cultures. At one stage of the growth cycle most of the filaments of the alga form characteristic spores, which are ellipsoidal with sculptured dark-brown exospore wall.

Five clones of the alga grown from spores were isolated on the above medium. Out of these clones, one is non-sporulating (Fig. 1a) in successive repeated cultures for the past three years. It thus appears to be a mutant which has lost a genetic marker  $S$  involved in sporulation. The non-sporulating mutant clone is therefore designated as  $S^-$ . Constant attempts to induce sporulation by a variety of methods including one of addition of culture filtrate from a sporulating clone and the parent strain has so far been unsuccessful. The remaining four clones are sporulating like the parent strain (Fig. 1,b). They are designated as  $S^+$ .

These clones, along with the parent strain, were screened for drug-resistance on media supplemented with a gradually increasing concentration of penicillin and streptomycin. A streptomycin-resistant strain (0.2  $\mu$ g/ml.)



Fig. 1. Photomicrographs of *Cylindrospermum majus* Kuetz.; a, non-sporulating clone; b, sporulating clone. (Phase contrast,  $\times 180$ )

of the non-sporulating clone was isolated by repeated subcultures on the antibiotic-supplemented medium. In a similar manner a penicillin-resistant strain (100 µg/ml.) of one of the sporulating clones was derived. Both these strains seem stable. They have been passed through a number of subcultures in the absence of their respective antibiotics without having lost their resistance.

Each of the resistant strains was tested for cross-resistance to the other antibiotic and showed no growth on antibiotic-supplemented media. They were also inoculated—separately or simultaneously—into media which contained both the antibiotics. No growth was found to have occurred in any case even after two months. However, when the two strains (designated as *S-Sm<sup>r</sup>P<sup>r</sup>* and *S-Sm<sup>r</sup>P<sup>r</sup>*) were grown together in the basal medium containing no antibiotic and transferred five times in the same medium, and then inoculated in the mixture of the two antibiotics, growth occurred in two out of 47 culture tubes. This points to a case of genetic recombination due to the chance inclusion and rare distribution of recombinants in the two culture tubes where growth had occurred in the presence of both the antibiotics. The double-resistant recombinant also formed spores at low frequency. It has been passed five times in antibiotic-free medium without loss of resistance and spore character. It is highly probable that the recombinant strain (designated as *S-Sm<sup>r</sup>P<sup>r</sup>*) is formed by the transfer of the genetic marker *Sm<sup>r</sup>* of the non-sporulating clone to the sporulating clone showing penicillin resistance. These results seem to indicate the occurrence of sexual or parasexual phenomena in the blue-green alga, *Cylindrospermum majus* Kuetz.

The only other previously reported case of an apparent genetic recombination in a blue-green alga is that of *Anacystis nidulans* by Kumar<sup>2</sup>, who found it impossible to grow clones of this alga.

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### Chloramphenicol Inhibition of the Growth of Green Algae

VÁZQUEZ<sup>1</sup> has recently stated that D-threo-chloramphenicol does not inhibit the growth of protozoa (with the exception of *Tetrahymena pyriformis*) and plants.

Tamiya *et al.*<sup>2</sup> have given an account of the effect of chloramphenicol on the green alga *Chlorella ellipsoidea*, and shown that at the relatively high concentration of 323 µg/ml. the rate of growth was decreased after a lag period. At ten times this concentration, growth was virtually inhibited, but at 32.3 µg/ml. no effect was observed. The reduction in the growth rate was accompanied by a decrease in the size of the cells. *Chlorella* therefore appears to be a more resistant organism to chloramphenicol than the bacteria, but still shows similar effects.

Kumar<sup>3</sup> has found that with the blue-green alga *Anacystis nidulans*, between 1–6 µg/ml. the growth rate is unaffected, but there is an increasing lag period with increase in concentration. At 8 and 10 µg/ml. no growth was observed after twelve days.

It may also be observed that the report concerning *Tetrahymena*<sup>4</sup> is somewhat ambiguous. It is stated that "growth was considerably delayed or completely arrested at 25–150 µg/ml.". This may be taken to mean that there was a lag period before growth was commenced, but it does not specifically state that the growth rate itself was affected.

I have recently carried out some experiments on the effect of D-threo-chloramphenicol on the growth and metabolism of *Scenedesmus quadricauda*, a green alga. Here,

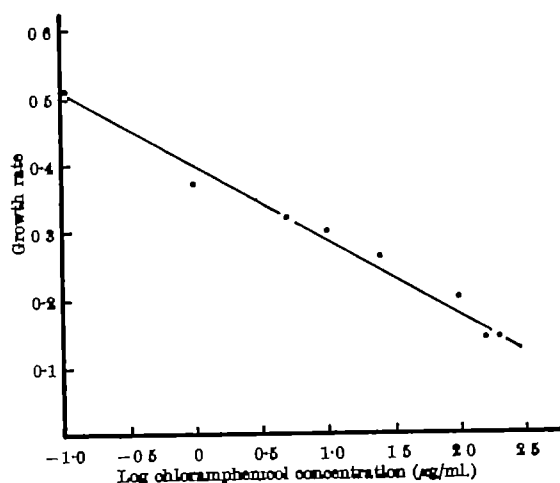


Fig. 1. Effect of D-threo-chloramphenicol concentration on the growth rate of *Scenedesmus quadricauda*. Control growth rate = 0.59. Correlation coefficient = 0.90; significant at  $P = 0.001$ . Growth rates are the coefficients  $b$  in the equation  $\log N = a + bt$ , where  $N$  = number of colony units (of four cells) per unit volume, and  $t$  is the time in days. The equation is calculated for the period of unrestricted growth only.

increasing the chloramphenicol concentration over the range 0.1–200 µg/ml. results in a progressive reduction in the growth rate (Fig. 1). This is accompanied by an increase in the lag period before unrestricted growth commences.

We have, therefore, two cases of the growth of green algae being affected by D-threo-chloramphenicol, though the levels which are effective differ considerably in the two organisms. *Scenedesmus* has more in common with the chloramphenicol-sensitive bacteria in this respect than with *Chlorella*.

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### MICROBIOLOGY

#### Isoenzymes of Lactate-dehydrogenase in Micro-organisms

It is generally accepted that lactate-dehydrogenase (LDH) exists in more than one molecular species. LDH isoenzymes have been separated by (among other methods) using their eventual heat stability at 58° C (ref. 1). It has been shown that the LDH fraction prominent in heart muscle is more stable to heat than other fractions from, for example, liver tissue<sup>2</sup>. There is good correlation between this heat-stable isoenzyme and the fastest moving, anodal LDH I (nomenclature of Wieme<sup>3</sup>) in electrophoresis.

In the present investigation, we examined suspensions of *Staphylococcus pyog.*, var. *aureus* (1 ml. containing approximately 10<sup>8</sup> bacterial cells, as measured by turbidity) by the method of King<sup>4</sup> for LDH activity. Aliquots of the suspension were simultaneously exposed to 58° ± 0.2° C for 60 min in a water-bath. Twenty-four samples were examined, all determinations being made in duplicate. After heating, the LDH activity of the samples decreased by 83 per cent ( $s_x \pm 15.9$ ) with very good reproducibility.

In a second experiment, we examined strains of *Staphylococcus pyog.*, var. *aureus*, resistant to a variety of antibiotics (penicillin, streptomycin, chloromycetin, tetracycline). After exposure to 58° C, practically no LDH

activity could be shown. We conclude that *Staphylococcus* strains contain at least two LDH isoenzymes, the greater part being of a heat-labile type, similar to human 'liver' LDH (or the isoenzyme derived from malignant cells), typical of metabolism with anaerobic glycolysis. Strains of *Staphylococcus*, resistant to various antibiotics, contain less or none of the heat-stable isoenzyme. The possibility of prevalent activity in the LDH 3 zone (embryonic type\*) cannot be ruled out by the method used, and requires further attention.

It can be assumed that the isoenzyme pattern, generated in bacterial cells, is a result of differential gene formation.

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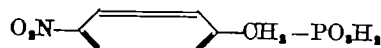
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## VIROLOGY

### Antiviral Activity of *p*-Nitrobenzylphosphonic Acid

*p*-NITROBENZYLPHOSPHONIC ACID (I), first prepared by



Litthauer<sup>1</sup> in 1889, has attracted little attention from biologists or biochemists. In a recent investigation<sup>2</sup> this compound was found to inhibit the activity of intestinal alkaline phosphatase. In continuation of our programme of work on phosphonic acids in various biological systems<sup>3-4</sup> we have investigated the effect of *p*-nitrobenzylphosphonic acid (*p*-NBP) on the propagation of encephalomyocarditis virus (EMC) *in vitro* and *in vivo* and found it to possess antiviral activity.

The source of EMC virus for our experiments was a 10 per cent suspension of infected mouse brain in broth which was stored at  $-15^\circ\text{C}$ . Mouse embryo tissue cultures used in this study were grown in a medium containing 10 per cent bovine serum, 0.5 per cent lactalbumin hydrolysate, Hanks's solution and standard amounts of penicillin and streptomycin. The infected cultures were maintained in Eagle's medium supplemented with 2 per cent of horse serum.

The titration of virus was performed according to the method of Tytell and Neuman<sup>5</sup>, in a modification adapted for polyoma and EMC viruses in mouse embryo tissue cultures<sup>6</sup>. The cultures, about  $4 \times 10^4$  cells/2 ml. in wide round-bottomed serological tubes, were infected with

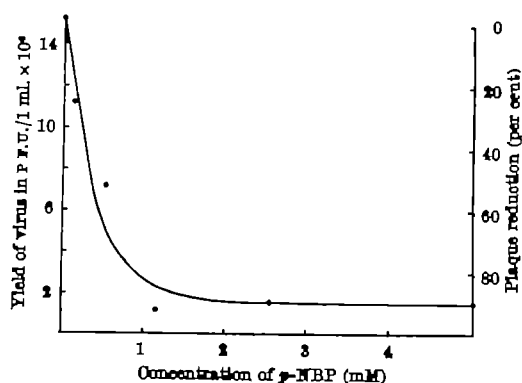


Fig. 1. Relation of *p*-NBP concentration to inhibition of EMC virus in mouse embryo cell cultures. All cultures (about  $6 \times 10^4$  cells/3 ml.) were infected with 0.8 ml. EMC virus ( $3 \times 10^6$  P.F.U./ml.). After 2 h adsorption the maintenance medium (3 ml. per culture) and indicated amounts of *p*-NBP were added. The yield of virus, with and without *p*-NBP, was determined 20 h after infection.

virus and shaken mechanically for 1-2 h at room temperature. The cultures were then overlaid with Eagle's medium containing 1 per cent methylcellulose (British Celanese, Ltd.) and 2 per cent horse serum. After incubation for 24 or 48 h at  $37^\circ\text{C}$ , 0.1 ml. of a 0.1 per cent solution of neutral red was added to the overlay and the plaques were counted after further incubation for 3 h at  $37^\circ\text{C}$ .

The inhibition of the production of virus by varying concentrations of *p*-NBP is shown in Fig. 1.

At a concentration of  $1 \times 10^{-4}$  M of *p*-NBP 90 per cent reduction of plaque number was obtained and further increase of concentration had no additional effect. As much as a 99 per cent reduction in the number of plaques was observed when the effect of *p*-NBP was evaluated after incubation for 48 h. It is conceivable that in this case several consecutive cycles of virus replication were inhibited.

The cytopathogenic effect of mouse embryo cultures infected with EMC virus and treated with *p*-NBP was delayed and partially diminished.

Some information on the mechanism of action of *p*-NBP can be gained from the experiment presented in Fig. 2.

It appears that *p*-NBP inhibits the production of virus only when added to the cultures not later than 3 h after infection with EMC virus. The yield of virus in cultures treated with *p*-NBP 4-5 h after infection was only slightly reduced. Preincubation of the cultures with *p*-NBP for 24 h before infection did not affect the degree of inhibition.

In view of the known fact that the formation of EMC virus particles requires 3-4 h<sup>7</sup>, it may be assumed that *p*-NBP is active throughout the whole of the latent period of infection.

*p*-NBP did not inactivate the virus after 24 h contact at  $4^\circ\text{C}$ . Also the adsorption of virus to the cells was not

Table 1. EFFECT OF *p*-NBP ON THE VIRAEIA AND PERMEATION OF VIRUS INTO BRAIN OF V MICE INFECTED WITH EMC VIRUS

Exp. No.	Inoculum (P.F.U./ml.)	Time after infection (h)	Title of virus in P.F.U./ml.			
			Treated with <i>p</i> -NBP*		Treated with saline	
			serum	brain	serum	brain
1	$3 \times 10^4$	48	$1.25 \times 10^4 \dagger$	0,0,0,0 ‡	$2.5 \times 10^4 \dagger$	$7.0 \times 10^4$ , $1.0 \times 10^4$ , $4.0 \times 10^4$ , 0,0,0,0 ‡
2	$3 \times 10^4$	48	$6.0 \times 10^4 \S$	$4.0 \times 10^4 \S$	$2.0 \times 10^4 \S$	$2.0 \times 10^4 \S$
3	$1 \times 10^4$	20	$2.0 \times 10^4 \dagger$ , $9.0 \times 10^3$ , $6.0 \times 10^3$ , $6.0 \times 10^3$ , $1.0 \times 10^4$	$<1.0 \times 10^4 \dagger$ , $<1.0 \times 10^4$ , $<1.0 \times 10^4$ , $<1.0 \times 10^4$ , $<1.0 \times 10^4$	$4.5 \times 10^4 \dagger$ , $2.0 \times 10^4$ , $1.7 \times 10^4$ , $7.5 \times 10^3$ , $6.0 \times 10^3$	$1.0 \times 10^4 \dagger$ , $1.5 \times 10^3$ , $<1.0 \times 10^4$ , $<1.0 \times 10^4$ , $<1.0 \times 10^4$

\* Virus injected intraperitoneally (0.5 ml./mouse). *p*-NBP administered in Exp. No. 1 and 2 intraperitoneally (0.5 ml./mouse) together with virus, and in Exp. No. 3, one hour after virus.

† Pool from 5 mice.

‡ Every sample of brain and serum tested separately.

§ Pool from 3 mice.

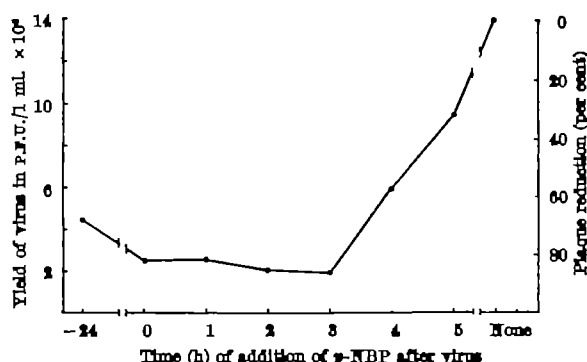


Fig. 2. Effect of time of addition of *p*-NBP on the yield of EMC virus in mouse embryo cell cultures. Tissue cultures (about  $4 \times 10^6$  cells/2 ml.) were infected with 0.2 ml. EMC virus ( $1 \times 10^6$  p.f.u./ml.) for 1 h at zero time. The unadsorbed virus was removed from the cultures by washing prior to the addition of the maintenance medium. At intervals *p*-NBP was added directly to the medium. The yield of virus was measured 20 h after infection.

affected by *p*-NBP. Even when EMC virus was added to the cells together with *p*-NBP and after 1 h the inhibitor was removed by repeated washing the production of virus was not inhibited.

*p*-NBP in concentration ten times higher than those inhibiting virus multiplication did not cause any visible damage of tissue cultures observed for 1 week. Another indication of the low toxicity of *p*-NBP was obtained from an examination of its effect on respiration and aerobic glycolysis of chopped mouse embryo tissues. It was found that *p*-NBP does not affect the consumption of oxygen or production of lactic acid of tissues suspended in Krebs-Ringer solution with 0.2 per cent of glucose for 3 h. However, a slight inhibition of oxygen uptake and increase of glycolysis were observed after 4–5 h. *p*-NBP in a concentration of  $10^{-4}$  M reduced by 40 per cent the activity of alkaline phosphatase in mouse embryo homogenate. The respiration was measured manometrically<sup>8</sup>. Lactic acid was determined by the method of Barker and Summerson<sup>9</sup> and the activity of alkaline phosphatase was estimated according to King<sup>10</sup>.

In the experiments carried out *in vivo*, albino Porton mice weighing 12–16 g were used. Groups of mice were injected intraperitoneally with 0.5 ml. virus and 0.5 ml. 0.1 M solution of *p*-NBP (sodium salt, pH 7.4). This dose of *p*-NBP was not toxic to mice. The control mice received 0.5 ml. virus and 0.5 ml. physiological salt solution.

Antiviral effect of *p*-NBP was estimated by comparison of cumulative mortality (Table 2) and of the amounts of virus in blood and brain (Table 1) in groups of mice treated with *p*-NBP and untreated.

Table 2. EFFECT OF *p*-NBP ON INFECTION OF MICE WITH EMC VIRUS

Exp. No.	Inoculum* (p.f.u./1 ml.)†	Time after infection (days)	Mortality	
			+ <i>p</i> -NBP‡ dead/inoculated	Control dead/inoculated
1	$3 \times 10^6$	4	0/5	1/5
		5	0/5	3/5
		6	0/5	4/5
		7	0/4	4/5
		10	0/4	4/5
2	$1 \times 10^6$	4	1/15	0/10
		5	3/15	2/10
		6	8/15	6/10
		8	11/15	9/10
		10	12/15	9/10

\* 0.5 ml. intraperitoneally.

† *p*-NBP administered together with virus only once intraperitoneally 0.5 ml. 0.1 M sodium salt, pH 7.4.

‡ Plaque-forming units.

It appears that a single injection of *p*-NBP has some protective action against death of mice caused by infection with a small dose of EMC virus given intraperitoneally. However, the protective effect was much smaller with larger doses of virus. Striking evidence of the effect of *p*-NBP on the course of infection was obtained when the

amounts of virus in blood and brain were measured. In the group of mice infected with a small dose of virus and treated with *p*-NBP the viraemia was reduced and the penetration of virus into the brain was blocked.

It is our hope that *p*-nitrobenzylphosphonic acid and related compounds will prove to be new selective antiviral chemicals.

We thank Dr. A. C. Allison and Prof. T. Baranowski for their advice, and Mrs. M. Albin for assistance.

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<sup>1</sup> Lethbridge, S., *Ber.*, **23**, 2144 (1890).

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## GENETICS

### Synthesis of Ribonucleic Acid by the X-Chromosomes of *Drosophila melanogaster* and the Problem of Dosage Compensation

INVESTIGATIONS of the expression of various sex-linked genes in *Drosophila* have shown that for most of them their phenotypic manifestation is identical in male and female. Stern<sup>1</sup>, Muller<sup>2</sup> and later Muller *et al.*<sup>3</sup> by use of mutations such as bobbed and apricot in various doses showed that the identity and equality of genic expression between the two sexes are not associated, *a priori*, with the development of sex, but rather involves a system of genes, plus and minus modifiers, which regardless of sex tends to repress the action of extra doses of sex-linked genes. Muller proposed the name 'dosage compensation' for this effect and concluded that for each sex-linked gene there is a set of 'compensator genes'.

Dosage compensation has been a subject of modern genetic research in *Drosophila*<sup>4</sup> and also in mammals<sup>5–7</sup>, but the cytochemical examination of the mechanism of dosage compensation has been initiated only recently by Rudkin *et al.*<sup>8</sup>.

It has long been known that the single X-chromosome in the male salivary gland is almost as wide as the autosomes of the same nucleus, as are the two paired X's in the female salivary gland. Dobzhansky clearly established this fact in the examination of salivary glands of *F*<sub>1</sub> hybrids from the cross of *Drosophila inularis* × *Drosophila tropicalis*<sup>10</sup>. Feulgen as well as ultra-violet absorption spectrophotometry shows that the single X in the male, in spite of its being as thick as the two X's in the female, contains an amount of DNA equal to a 'haploid' X in the female<sup>9,11</sup>.

It therefore seems pertinent to ask two questions at this stage: First, if what is true for individual genes on Muller's theory of dosage compensation is also true for

the whole sex chromosome, at what level of genic action does the mechanism of compensation work? To be more specific, with what stage of information transfer do the compensator genes interfere? The second question is, what underlies the cytological behaviour of the X-chromosome in the male (1X2A) salivary gland. Is it part of the mechanism of dosage compensation at the molecular level?

Experiments were designed to find out whether RNA synthesis by the X-chromosomes of male and female salivary glands operates at recognizably different levels, and if so, whether it is possible to evaluate the results in terms of a repressing effect in females or an enhancing effect in males.

For this purpose, short-pulse labelling with tritiated uridine was used. The dissected salivary glands from the third instar larvae of wild-type *Drosophila melanogaster* ('Oregon R') were incubated in tritiated uridine (2  $\mu$ .) in 10  $\mu$ l. of *Drosophila* Ringer for 5 min. The usual procedure of staining, squashing and covering with stripping film (Kodak 'AR 10') was followed. The slides were exposed for 14-17 days. After development, fixation and washing, the slides were again stained in buffered Giemsa after Schmid<sup>12</sup>, dried and mounted with 'Euparal'. Grains on cytoplasm-free chromosomes were counted with an oil immersion objective at a magnification of approximately 1,280 times. Timed larvae were used in all experiments so that only chromosomes with similar puffing patterns were compared.

The following observations were made: (1) Grains were counted on single male- and paired female-X-chromosomes (tip to 3B and whole X). The autosome 3L was used as standard. Grains on the segments 61 and tip-68B of 3L were counted and the ratios of the grains on part (tip-3B) and whole X to those on the two segments of 3L were used for comparison of the values in the two sexes. (2) Grains were counted over unpaired X-chromosomes of female, and unpaired 3L autosomes of male and female nuclei. These served as controls to check the effect of physical factors such as stretching, flattening and the geometric configuration of the chromosomes. Grain distribution along various unpaired segments of X's as well as autosomes was recorded. (3) A comparison has also been made of the grain distribution on an unpaired single X and paired double X's in the same nucleus. The 3N nuclei from the triploid stock  $y^{sc} w_1 sc = FM_4 (sc^1 Y); cr/cr/cr$  formed the material for this purpose. The triploid stock was prepared by Dr. Justin Frost, and was made available to us from Dr. C. Stern's laboratory.

Table 1 gives the means of the ratios of: (a) X(tip-3B)/61; (b) X(tip-3B)/tip-68B; (c) X(whole)/61; (d) X(whole)/tip-68B for males and females in two categories. In one, relatively high labelled chromosomes, based on the absolute number of 70 or more grains on the X-fragment (tip-3B), have been considered; in the other, the ratios from low labelled chromosomes (those with less than 70 grains on the same fragment) have been taken into account. It will be seen from the table that there is some difference between the ratios in high and those in the corresponding low labelled types but the differences in the values for (a), (b), (c) and (d) between males and females are not significant. Comparisons of the grain number on unpaired and that on paired chromosomes show that the sum of the corrected number of grains on unpaired chromosomes is considerably higher than that on the paired chromosomes. However, grain distribution on individual unpaired chromosomes or segments thereof is identical within statistical limitations (for example, for 4C-16B of X, one chromosome had 102 and the other had 105 grains; for segments 41-57 of 2R one had 220 grains, and the other 215; and for 62-68B of 3L one had 60 and the other 61). The additional observation on the grain distribution on the single unpaired and double paired chromosomes of the 3N nuclei shows that the single unpaired chromosome has a relatively higher number of grains than half the number of grains on paired double

chromosomes, the difference being of the order of 10-20 per cent. Two examples will illustrate this: case 1 (chromosome 3R): the unpaired single had 102 grains, the paired double had 183 on the same segment; case 2 (chromosome 2L): the unpaired single had 53 grains, the paired double had 88. It should be emphasized that even on correction of the ratios, in males, for this possible 10-20 per cent excess due to unpairing, the mean ratios for males presented in Table 1 are still far in excess of those expected for a single X in the 2X2A genotype.

These results show that at the level of chromosomal RNA synthesis the single X-chromosome in males works with an efficiency close to that of the two X's in females. This suggests that dosage compensation operates at the level of information transfer from DNA to RNA. From our results, however, we cannot draw any definite conclusion as to whether dosage compensation is a phenomenon involving repression or activation, that is, works in the female or in the male. Preliminary studies on the grain counts on similar lengths of X-chromosomes and autosomes, in both sexes, show that, ignoring the very big puffs, the grain numbers on similar lengths of X-chromosomes and autosomes are not significantly different (for example, X:3L=250:259 in the female and X:3L=154:158 in the male). Assuming that the overall capacity of RNA synthesis per unit length is similar in the X and autosome, this result points to an enhancing effect in the male rather than a repressing effect in the female. An exact evaluation of these alternatives has to await further investigation. The present communication also does not take into account those genes which do not show any compensation as known from genetic investigations<sup>4,8</sup>.

Table 1. MEANS OF THE RATIOS OF X(tip-3B) AND X(whole) TO 3L(61) AND 3L(tip-68B)

Type of nuclei and chromosome labelling	(a)		(b)		(c)		(d)	
	X/t/61	N	X/t/tip-68B	N	X(whole)/61	N	X(whole)/tip-68B	N
(1) Female (high)	1.67	47	0.89	23	8.78	37	1.94	18
(2) Male (high)	1.6	18	0.83	8	9.15	16	1.93	7
Variance (between groups 1 and 2)	(0.063)		(0.001)		(1.551)		(0.006)	
Total var.	(5.32)		(0.19)		(162.91)		(5.23)	
(3) Female (low)	1.62	23	0.82	19	9.20	20	1.85	15
(4) Male (low)	1.65	20	0.84	9	9.55	16	1.75	8
Variance (between groups 3 and 4)	(0.014)		(0.002)		(0.601)		(0.046)	
Total var.	(5.33)		(0.15)		(161.39)		(5.23)	
P (high, 1 versus 2)	> 0.4		> 0.7		> 0.3		> 0.8	
(low, 3 versus 4)	> 0.7		> 0.5		> 0.7		> 0.4	

M/R, mean ratio; N, total number of nuclei examined, X/t, X(tip-3B).

In answer to the question of cytochemical behaviour of the X's in the male and female, it is possible that a different protein composition causes both the enlargement of the X and its higher synthetic activity in the male salivary gland. It has been shown by Rudkin *et al.*<sup>9</sup> that the single X in the male contains 11 per cent more proteins (with end-absorption at 231 m $\mu$ ) than either of the two X's in the female. This aspect of dosage compensation in *Drosophila* requires further biochemical study.

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## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

**MAP CURATOR** (preferably geography graduate with an interest in maps and bibliographic work) in THE DEPARTMENT OF GEOGRAPHY—The Registrar, The University, Leeds, 2 (August 16).

**RESEARCH DEMONSTRATOR IN ANIMAL NUTRITION**—The Registrar, The University, Leeds, 2 (August 20).

**ADMINISTRATIVE ASSISTANT** (man or woman, preferably with some familiarity with the notations and terminology of higher mathematics) in THE DEPARTMENT OF MATHEMATICS—The Registrar, The University, Leeds, 2 (August 21).

**CHEMIST or BIOCHEMIST WITH THE NEW ZEALAND MEDICAL RESEARCH COUNCIL**, to join its research group at the University of Otago Medical School, Dunedin, working on the metabolism of fatty acids and the relation of diet to atheroma—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or The Administrative Officer, Medical Research Council, Box 913, Dunedin, New Zealand (Dunedin, August 22).

**ASSISTANT LECTURER IN HORTICULTURE**—The Principal, Yorkcote (W.R.) Institute of Agriculture, Askham Bryan, York (August 23).

**SENIOR DEMONSTRATOR** (Ph.D. or near completion of Ph.D.) in THE SCHOOL OF PHYSICS—The Registrar, University of Melbourne, Parkville, N.2, Victoria, Australia (August 23).

**BIOPHYSICIAN ENGINEER** (able to cope with a wide range of practical problems in varied fields of physics and electronics, and with a high degree of initiative and experimental ability) in THE DEPARTMENT OF METEOROLOGICAL ENGINEERING—The Registrar, The University, Manchester, 13, quoting Ref. 164/65/Na (August 25).

**RESEARCH AGRONOMIST; a SENIOR LECTURER/LECTURER IN AGRICULTURAL BOTANY; a RESEARCH BOTANIST/PLANT BREEDER; a PLANT INTRODUCTION OFFICER; and a PLANT PATHOLOGIST** in THE INSTITUTE OF AGRICULTURAL RESEARCH and the FACULTY OF AGRICULTURE, Ahmadu Bello University, Northern Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.O.1 (August 26).

**SENIOR LECTURER or LECTURER in GEOLOGY** (Petroleum Geologist) at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (August 27).

**SENIOR RESEARCH ASSOCIATE** (with a knowledge of geology or interested in applying chemical techniques to geological problems) in ORGANIC GEOCHEMISTRY—Prof. T. S. Westoll, F.R.S., University of Newcastle upon Tyne, Newcastle upon Tyne, 1 (August 27).

**ASSISTANT LECTURER** (with an honours degree in psychology or equivalent academic qualifications, and preferably experience in teaching and/or such fields as clinical psychology, educational guidance, vocational guidance, or remedial education) in CHILD PSYCHOLOGY in the School of Education, University of Melbourne—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 30).

**LECTURERS and ASSISTANT LECTURERS** (with a recognized honours degree in mechanical or electrical engineering and experience in research, and preferably practical experience and experience in teaching at degree level) in ENGINEERING at the University of Malaya—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Kuala Lumpur and London, August 30).

**LECTURERS or ASSISTANT LECTURERS** (with a recognized honours degree in mechanical or electrical engineering and experience in research) in ENGINEERING at the University of Malaya—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Kuala Lumpur and London, August 30).

**ASSISTANT LECTURER** in THE DEPARTMENT OF BIOCHEMISTRY—The Secretary, The University, Edinburgh (August 31).

**KEMPER** (with a good honours degree) of PREHISTORY—The Director, Rhodes-Livingstone Museum, P.O. Box 498, Livingstone, Zambia (August 31).

**ASSISTANT** (graduate in medicine or science) in THE DEPARTMENT OF BACTERIOLOGY, Queen's College, Dundee—The Secretary, University of St. Andrews, c/o Queen's College, Dundee (September 1).

**LECTURER** (molecular chemist/physicist) in THE DEPARTMENT OF PHYSICS—The Assistant Registrar (Science), The University of Birmingham, Birmingham, 16, quoting Ref. P/N (September 1).

**LECTURER** (science graduate) in BACTERIOLOGY, Queen's College, Dundee—The Secretary, University of St. Andrews, c/o Queen's College, Dundee (September 1).

**LECTURER** (with some basic training in clinical pathology such as is required for the Primary M.C. Path.) in BACTERIOLOGY—The Secretary, The University, Edinburgh (September 1).

**HORTICULTURIST** (with qualifications equivalent to a pass degree or the Royal Horticultural Society's final diploma, and an interest in applied entomology and in other topics relating to crop pests) in THE DEPARTMENT OF AGRICULTURAL AND FOREST ZOOLOGY, to manage the plots and experiments at the Pen-y-fnidd Field Station, Bangor—Mr. J. Hobart, Department of Agricultural and Forest Zoology, University College of North Wales, Bangor, North Wales (September 3).

**SENIOR LECTURER** (with a Ph.D. in physics, or equivalent in research publications, and some university teaching experience) in PHYSICS at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (September 3).

**SENIOR LECTURERS (2); and LECTURERS/ASSISTANT LECTURERS** (with at least a good honours degree with suitable teaching and research experience) in THE DEPARTMENT OF MATHEMATICS, University of Malaya—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Kuala Lumpur and London, September 6).

**ASSISTANT LECTURER** in THE DEPARTMENT OF MEDICAL PHYSICS—The Secretary, Royal Free Hospital School of Medicine (University of London), Hunter Street, London, W.O.1 (September 7).

**POST-DOCTORAL RESEARCH ASSISTANT** in THE DEPARTMENT OF CHEMISTRY for investigations concerning the synthesis and study of organometallic compounds containing fluorocarbon groups, in collaboration with Dr. R. D. Chambers—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (September 10).

**SENIOR LECTURER/LECTURER in NUMERICAL MATHEMATICS; and a PROGRAMMER in THE COMPUTING CENTRE**, University of New England, Armidale, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 10).

**RESEARCH SCIENTIST or SENIOR RESEARCH SCIENTIST** (biochemist, with a Ph.D. degree or postgraduate experience of equivalent standard and duration supported by satisfactory evidence of research ability, and preferably previous

experience in the field of fungal biochemistry) in THE BIOCHEMISTRY SECTION of the Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization, Canberra, A.C.T., Australia, to initiate studies on the biochemistry of spore germination with particular reference to *Peronospora tabacina* (Blue Mould) in tobacco—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.O.2, quoting Appointment No. 180/723 (September 11).

**LECTURER in VETERINARY PHYSIOLOGY**—The Secretary, Trinity College (University of Dublin), Dublin, Republic of Ireland (September 18).

**LECTURER/SENIOR LECTURER in AERONAUTICAL ENGINEERING** at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 15).

**LECTURER in CYBERNETICS** in THE DEPARTMENT OF APPLIED PHYSICAL SCIENCES—The Registrar, Room 22 (O.R.B.), The University, Reading (September 18).

**LECTURERS (2) in GEOGRAPHY** at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 24).

**LECTURER in THE DEPARTMENT OF PSYCHOLOGY**, University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 25).

**LECTURER/SENIOR LECTURER** (preferably specialist in the fields of chemical or process metallurgy) in METALLURGY at the University of Newcastle, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 30).

**RESEARCH ASSISTANT** (with a degree in zoology or in forestry, and preferably previous experience in research involving field studies) to work with the Lecturer in Forest Zoology on investigations into the ecology and biological control of foreign insects—The Secretary, The University, Aberdeen (September 30).

**CHAIR of GEOGRAPHY** at the University of Otago, Dunedin, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 15).

**CHAIR of GENETICS**—The Registrar, University College of Swansea, Singleton Park, Swansea (October 30).

**SCIENTIFIC OFFICER** (preferably specialising in biochemistry, animal genetics or invertebrate zoology)—The Director, Tropical Fish Culture Research Institute, Batu Berendam, Malacca, Malaysia (October 31).

**AGRICULTURAL CHEMIST** (national of the United Kingdom or the Republic of Ireland, with an honours degree in chemistry and a reasonable amount of experience) in MALAWI, to organize and control a fairly large chemical laboratory working on various problems connected with the development of agriculture—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RO 213/134/66.

**CHEMIST** (with a pass degree or equivalent qualification, and preferably previous experience in handling radioisotopes) for interesting work on the preparation of short-lived radioisotopes from the cyclotron—The Director, Medical Research Council Cyclotron Unit, Hammermith Hospital, Du Cane Road, London, W.12.

**BIOPHYSICIAN SPECIALIST** (with experience of transistor techniques) in THE DEPARTMENT OF VETERINARY PHYSIOLOGY, to take charge of the Electronic Workshop—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh.

**GRADUATE RESEARCH ASSISTANT** (preferably with experience in the field of tissue culture) to work in an organ culture laboratory—The Secretary, St. Mary's Hospital Medical School, Paddington, London, W.2.

**LECTURER or ASSISTANT LECTURER or (exceptionally) SENIOR LECTURER in MATHEMATICS**—The Assistant Registrar (Establishment), University of Sussex, Stanmer House, Stanmer, Brighton, Sussex.

**LECTURER or ASSISTANT LECTURER** (preferably with research interests in forensic chemistry, toxicology or enzymology) in CHEMISTRY—The Secretary, The Royal Veterinary College (University of London), Royal College Street, London, N.W.1.

**RESEARCH ASSISTANT in THE DEPARTMENT OF CHEMISTRY AND BIOLOGY**, to assist in research on the nutritional aspects of smoking—The Secretary, Welsh College of Advanced Technology, Cathays Park, Cardiff.

**RESEARCH ASSISTANT** (with a diploma in technology, a degree or their equivalent) in THE PHYSICS DEPARTMENT, to work on magnetic and related properties of materials over a wide range of temperatures—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 33/AF.

**RESEARCH ASSISTANT** (with a good honours degree in animal physiology or zoology or an equivalent qualification, and preferably an interest in biochemistry) in THE PHYSIOLOGY DEPARTMENT, for an investigation of blood levels of oxytocin in relation to reproduction and lactation—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, Berkshire, quoting Ref. 64/N/16.

**RESEARCH ASSISTANT** (with a good honours degree in psychology) in THE DEPARTMENT OF PSYCHOLOGY for an investigation of associative aspects of human verbal learning—The Deputy Secretary, The University, Southampton.

**RESEARCH ASSISTANT** (with qualifications in physics or chemistry) in THE DEPARTMENT OF CHEMISTRY, to work on dielectrics with Dr. Mansel Davies—The Registrar, University College of Wales, Aberystwyth.

**RESEARCH LECTURER in CLINICAL PSYCHOPHARMACOLOGY**—The Secretary, Trinity College (University of Dublin), Dublin, Republic of Ireland.

**SENIOR II TECHNICIAN** (preferably with experience in bacteriology and haematology)—The Secretary, Birmingham and Midland Eye Hospital, Church Street, Birmingham, 3.

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## ROAD, RAIL AND WATER TRANSPORT

THE debate in the House of Lords on urban planning and development, opened by Lord Llewelyn-Davies on May 19, was notable for the authority of the speeches, particularly those of Lord Llewelyn-Davies and Lord Holford; Lord Mitchison replied on behalf of the Government. Lord Llewelyn-Davies spoke as chairman of a committee advising the Minister of Housing and Local Government on research in urban planning and also as a member of a similar committee advising the Minister for Land and Natural Resources. Among other things, he referred to the work which Lord Holford, as technical adviser in the Ministry, had done to build up theoretical structures on which the whole practice of British planning now rests.

He began by pointing out that the subject was urgent principally because of the great increase in population, the almost equally large increase in the use of the motor-car, and the fact that such a high proportion of houses and other buildings were now due for renewal. It was essential that we should attempt to guess at what life should be like in the future and design for it. Even better, we should attempt to imagine or to envisage a pattern of urban development and city construction sufficiently flexible to absorb and to ride with the changes in the pattern of human life and living which would occur within the life of the buildings which we propose to erect.

Lord Llewelyn-Davies urged that decisions as to planning were not matters for technologists, planners or architects: they were matters for the elected representatives of the people who should, however, take the decisions on the information and advice which could be supplied, although they were ultimately political issues. The concepts of planning were concerned with the limitation of the size of cities by establishing green belts and deliberately restricting development; with providing for increased population, so far as possible in self-contained, fairly small new towns some distance away from existing centres; and with the control of development within the cities in accordance with a plan for the use of land. He urged that an important criterion of effective planning should be that wise choices were made possible for the life of the institutions, such as universities and hospitals, which served the great cities. He did not believe that we needed to suffer the desolation of important and valuable areas of countryside to meet the demand of increasing population; there was enough space, but organization was needed to ensure that the buildings were put in the right and not the wrong place. He believed that a solution of the problem of urban planning and development was only possible by a massive direction of our universities towards research into the social, economic and architectural problems of urban affairs. He referred especially to Lord Silkin's advocacy of the establishment of an architectural research council for the built environments—this has since been supported by the Royal Institute of British Architects and other bodies, and by Lord Holford.

Lord Llewelyn-Davies spoke, of course, some weeks before the report of the Heyworth Committee was published (*Nature*, 207, 559; 1965) and before the Government had announced its acceptance of the recommendation to establish a Social Science Research Council, with which the proposal for a research council for the built environment is closely related. Lord Holford himself, in a maiden speech, began by expressing regret that we

separated development and preservation and did not consider preservation of historic and other buildings or urban open spaces as an integral part of development and not an opposing thing. He pointed out, moreover, that we seemed no longer to anticipate change and move to meet it, but merely to struggle to keep up with changes that had already overtaken us. We could only prepare for change by setting the stage for industrial building of various kinds; however, such planning could make all the difference to the quality of the environment and to its sensible distribution, and he added that the quality of our urban environment depended very largely on our attitude towards preservation. A preservation policy was important because the more imaginative and more modern architects in Britain, and especially landscape architects, were just as keen on keeping the creative work of the past under economic conditions as were those concerned solely with preservation. Problems of new accommodation could not be solved merely by rehabilitation and preservation of old buildings; however, we could minimize our loss by using these buildings in imaginative ways.

Preservation was also needed on the regional scale, and the need was for three things: first, for some financial help for local authorities, particularly in regions which were severely damaged during the Second World War, and where the rates were inadequate to carry all the social burdens; secondly, for an intelligent sub-regional plan; thirdly, for trained advice (which was very scarce)—advice which was independent and objective, independent not only of the developers but also of the local authority who might be a little too zealous in making clearance areas throughout the controlled district. He supported Lord Llewelyn-Davies in urging that the Ministry should have disinterested technical assessors to give some stature and substance to the decisions which it had to make in such large numbers. He urged that we should begin to think on all these problems: the giving of advice on development plans; the making of schemes within development plans; the combination of preservation and development on a basis which, of necessity, went beyond the boundaries of individual local authorities.

Lord Esher, who followed, strongly supported Lord Llewelyn-Davies on the overriding need for research and, like Lord Holford, referred to the report from the Royal Institute of British Architects. This report had pointed out that 58 of the chief officers in charge of planning in 109 county boroughs had no planning qualifications, while 449 out of 531 in the second-tier authorities were without such qualifications. Of staff working on planning in local authorities fewer than half had any planning qualifications and only 4 per cent were architects. So far as this arose from a shortage of qualified people, the professional institutions were doing all that they could to remedy the situation, but this took time. There also seemed to be a shortage of demand, and planning consultants were not being fully used; our available resources were not either fully or effectively deployed. He suggested that the Minister should remove delegated planning powers from authorities who did not possess qualified planning staff.

The Earl of Gosford, who followed, referred particularly to the transport aspect, quoting Lord Hastings's emphasis that the Buchanan Report provided a framework within which a programme of urban redevelopment could proceed. We could not expect to do everything at once and

our resources were already fully committed for the next few years, but the Earl of Gosford thought that the recent statements of the present Minister of Transport suggested that less and less was to be expended on the problem of roads, both outside the cities and, particularly, on roads inside the cities. He thought the urban programme was the key to urban renewal, in the context both of quality and progress. If our towns were not renewed in a context of planning which accepted the motor-car, there was a real danger that their prosperity would diminish. Lord Arwyn also strongly supported the suggestion for massive research at universities, and particularly for effective liaison between the universities, the professions and the building industry. Lord Hastings, however, was doubtful whether we should, as Lord Llewelyn-Davies had suggested, try to base our cities on institutions than on houses and people in houses. He thought we must try to work out as soon as possible a technique for redeveloping town centres, and that we must recognize that human considerations and economic planning were equally important. Lord Mitchison, replying for the Government, said that with scientific developments and other changes, town and country planning should be as flexible as one could possibly make it. It was not, however, merely a matter of science or population, but also of the kind of life people might wish to live in twenty or thirty years time, when their values also might have changed greatly. He agreed with what the Minister of Transport had said on April 14, that the only satisfactory basis on which local authorities could develop their plans with the necessary degree of financial realism would be on the assumption that the future scale of investment in urban road construction was unlikely to be very different from that programmed and already announced for the period up to 1970. While agreeing with earlier speakers as to the importance of land planning, he thought that we could only obtain the kind of planning we desired with the help of an authority specifically set up to see that land was put to the right use when development took place. As regards organization, he said that the Minister would look at the machinery of planning through a planning advisory group and that the report of this group would shortly be issued. A Directorate of Urban Planning had been set up under the Minister's technical adviser on urban planning. This body would consist partly of administrative and partly of professional people and was intended to lead a new drive into town planning and design, and to advise on what could be done on such questions as using the very limited amount of skilled manpower now available. He welcomed the report from the Royal Institute of British Architects on manpower, and said that the Government had already asked the University Grants Committee to consider the best ways and means of stimulating a greater output of trained planners both in the short term and in the long term. Local government finance, and central finance, were also being thoroughly investigated, including questions of rates and of shifting some of the burden from the rates to central finance.

The development not only of roads but also of waterways and of the major railway trunk routes should be considered with a hard-headed approach; and if we could, within five years, build up a national fleet of 450 pairs of narrow boats carrying a million tons a year we would remove 100,000 lorry loads from the roads. Quite apart from the amenity aspect, this possibility seems to be worth serious examination. The study of canal transport

now being prepared for the Inland Waterways Association should be carefully studied by Ministers, as well as by British Waterways. Lord Hinton, who is investigating the co-ordination of national transport, has before him now a three-stage programme for a modern waterway system for Britain, which was submitted by the Association early in August. Even at the present moment the £170 million could well be a most profitable investment.

The recent Beeching Report issued in February (British Railways Board. Pp. 100. 15s.) provides a critical examination of the trunk railway routes with the idea of selecting routes for future intensive use, but not selecting lines for closure. The report was made to enable the selection of trunk routes to be subjected to constructive criticism, to permit good planning of the next phase of development expenditure, and to enable any future proposals for closure of trunk lines or decrease in the utilization of lines to be seen in a broader context, while permitting the proper development of commercial policies, and giving customers a clearer view of the future. It thus complements the report on *The Reshaping of British Railways*, published in March 1963 (*Nature*, 198, 1233; 1963), and develops further the constructive proposals of that report which have largely been ignored in public discussions. Essentially these discussions have been centred on the proposed abandonment of the parts of Britain's railway system deemed 'unsound'. Like the earlier report, it is concerned with the economic aspect, stressing that the level of annual expenditure on through-route maintenance, renewal and improvement is likely to continue at the present level of about £85 million per annum for many years if the whole through-route system is maintained. Substantially lower expenditure on suitably selected routes is estimated as likely to provide all the capacity required to meet foreseeable future demand. It is thus open to the criticism that it is concerned almost solely with the economic facts, and also that the position of the railways with respect to other forms of travel is not considered. Like the debate in the House of Lords already noted, the Report refers to reports already issued, such as *The South-East Study, 1961-1981*, dealing with the growth and distribution of population and industry in the South-East region, issued in 1964, and it refers also to the Hall Report on *The Transport Needs of Great Britain in the Next Twenty Years* published in 1963. Considering the changes in the economy which are likely to affect transport over the next twenty years, the Report expects an increase in population from 54.2 millions to 62.5 millions: its proposals are based on the assumption of rates of growth of 3 and of 4 per cent. Two important assumptions are made on the distribution of population and industry. The rate of growth of the former is assumed to be comparatively even throughout Britain with no major redistribution and, although some change is foreseen in the commodity pattern of production, it is assumed that the geographical spread of general industrial activity would remain the same and that growth would also be uniform. These assumptions may not pass unchallenged. However, the further assumptions as to the pattern of transport demand between the main centres in 1984 are likely to be accepted generally; for example, that the total internal consumption of coal will remain fairly steady over the next 24 years (if anything, it is more likely to decline than increase), and that steel production will grow at a lower rate than the average for all industries, rising to some 40 million tons by 1984. It is estimated that some 19 million tons per annum of the existing freight traffic

could be transferred from roads to liner-train services as they build up, but for passenger traffic the assumptions and forecasts are much more questionable. It is taken for granted that public services on trunk lines will decline, and consideration of passenger traffic is limited to inter-city passenger movements. Nevertheless, the assumption that, in the main, passenger traffic will increasingly go by road in private cars ignores entirely the desirability of doing everything possible to take even private car traffic off the roads, at least in certain areas, and ignores other considerations entirely, such as were powerfully argued by Mr. C. D. Foster in *The Transport Problem*, in 1963, and more recently by Mr. R. Calvert in *The Future of Britain's Railways*\*.

The conclusions of the second Beeching Report are that the cost of providing the railway track and signalling per unit of traffic passing over it should be reduced to a level which enables services to be provided on a competitive basis. If such changes were made, the railways would be able to increase their freight carrying and the overall demand for rail transport on the trunk routes would appreciably increase over the next twenty years. The present network of through-routes is under-utilized, but if advantage is taken of technological progress in signalling and movement the disparity between capacity and use will increase. The through-movement could be channelled through a reduced network of lines amounting to some 3,000 route miles instead of the 7,500 miles provided to-day, and this would reduce route costs to about half their present level. If the railways concentrate on cheap bulk movement their prospects as trunk carriers can be revived. The real choice, Dr. Beeching argues, is between an excessive and increasingly uneconomic system with a corresponding tendency to fall into disrepute and decay, or the selective development and intensive utilization of a more limited trunk-route system.

It is claimed that the study provides a basis for positive planning and that it should be possible to concentrate railway investment on the selected trunk routes in the confidence that here the essential dense flows of traffic will be maintained. Even if that claim is conceded, the second Report is too pessimistic and even defeatist. It ignores, except for goods traffic, the overriding need to take off the roads, even from the point of view of safety, as much traffic as is reasonably possible. It fails to recognize that much passenger traffic is on the roads simply because of the failure of the railways to offer attractive transport for passengers in the mid-twentieth century. The Report does not consider rail and road and even waterway traffic as complementary; it fails completely, except perhaps for the liner trains, to consider the railways in terms of what they could be. An obsession with long-distance and bulk traffic leads in both Reports to the possibilities of the Diesel rail bus and new operating methods for local and intermediate traffic being disregarded.

For all these reasons, Dr. Beeching's reports should be read in the light of such a book as that by Mr. Calvert. Mr. Calvert's imaginative, but constructive, look at the inherent possibilities of modernization provides the essential corrective and relates railways to the wider and more general problem of planning development in which roads and waterways as well as railways have their place. He looks, also, at the implications of the Channel Tunnel and at the rationalization of operating methods as well as at modernization. Much of his book is technical, but the

broad issues are plain enough and no reader is left in doubt as to the very real asset which the railways still represent. Wisely and imaginatively used, they could make a vital contribution not merely to easing congestion and danger and waste on the roads, but also to safeguarding the erosion of the countryside and to restoring a standard of living in which man can regain some of the control over his environment that he has at present lost. There is evidence in the House of Lords debate that the possibilities and the opportunities are already being recognized.

## AN INTRODUCTION TO NUCLEAR STRUCTURE PHYSICS

### Nuclear Physics

An Introduction. By W. E. Burcham. Pp. xiv+739. (London: Longmans, Green and Co. Ltd., 1963.) 70s.

PROF. BURCHAM'S *Nuclear Physics: an Introduction* is a good book, carefully and thoughtfully written, that does, admirably, the job it sets out to do. It is a teaching book, a text-book; it does not pretend to take the reader to the growing points of nuclear physics or to infect him with the excitement of research. I wish it had, both because I think that every teaching book should be open-ended and give major glimpses of the next stage of the game and what it is all about, and also because I know that Prof. Burcham could have done this very well; but he has done a considerable service: he has provided us with the first really good undergraduate text in nuclear physics available in English. This is a strong statement, but it is true. Until this book came along there was no single undergraduate text that could be recommended with confidence as being not positively misleading. I believe that Prof. Burcham's text is still a little unbalanced: in my view it contains too much about detectors, accelerators and classical radioactivity and not enough about nuclear models, symmetry properties and reactions. But this is a personal point of view, and the present book is very much better in its balance than any of its predecessors. There is one very important matter, however, that must be mentioned here, though not as a criticism. It is that Prof. Burcham's text is about nuclear structure physics. The great excitements of the sub-atomic world are now sub-nuclear rather than nuclear, in the field of what we used to call elementary particles but which we now recognize as entities much more essentially complex than the nucleus itself. This world of high-energy physics is not treated in Prof. Burcham's text, but it must be an important part of any teaching course in nuclear physics that, on the nuclear structure side, attains the level of this book. Nuclear physics has its two sides, nuclear structure and high energy, and a properly-constructed course must contain both. High-energy physics is at an earlier stage of its development than nuclear structure physics and does not yet lend itself to the consolidated type of treatment of Prof. Burcham's text, but many basic features are nevertheless well established and must be taught at the undergraduate level because of the enormous intellectual importance of the subject. This, then, is a warning rather than a criticism.

Prof. Burcham's treatment of his subject is at the undergraduate level but probably contains more detail than will be accommodated in most 'finals' syllabuses. It is not a suitable graduate text because it does not look outwards to the unsolved problems and is too elementary in its approach, but it could still be useful as a bridge between the undergraduate course and research; many of its references are to research and graduate-type works. The book's use of references is excellent and enables a picture to be built up of how the subject developed; it is part of Prof. Burcham's approach which rather

\* *The Future of Britain's Railways*. By Roger Calvert. Pp. 176+16 plates. (London: George Allen and Unwin, Ltd., 1965.) 35s. net.

successfully combines the historical and the logical methods.

A considerable portion of the book is devoted to matters that are not properly nuclear physics: 48 pages to the spectroscopy of atoms and molecules, 69 pages to properties of ionising radiations, 53 pages to instruments, 43 pages to the measurement of radiation, and 59 pages to accelerators. All this is important if you want to know how it comes about that we know what we do about the nucleus, but of no interest if we simply want to understand the nucleus. As Prof. Burcham emphasizes, his is an "experimentally-oriented" book. My own view is that it is more important to teach undergraduates what we know about the nucleus itself than about the devices, apparatus and techniques by which that knowledge is obtained, but again this is a matter of opinion. At least Prof. Burcham distinguishes clearly between the wood and the trees and does not attempt to give the impression that the passage of electrons through thick absorbers has something to do with the physics of the nucleus.

Although Prof. Burcham's approach is experimental it is far from purely descriptive. He is quite free in his use of formulae and analysis and, although he works out nothing in detail, he gives the bare bones of the steps by which the theoretical results are achieved. He also attaches, wherever he can, a physical interpretation to the formal quantum mechanical result—for example, in explaining that the ortho-helium levels lie lower than the corresponding para-helium levels because the spatially anti-symmetric ortho-helium states inhibit the close approach of the two electrons that corresponds to large Coulomb energies whereas the spatially-symmetric para-helium states allow such close approach.

There are lacunae—for example, Prof. Burcham misses a good opportunity of showing how simple and general some aspects of nuclear behaviour are by omitting all mention of the photo-nuclear giant resonance, perhaps the only piece of nuclear dynamics obviously common to all nuclei and which is simply a child on a swing. But in bringing together the various aspects of nuclear behaviour, putting into them as much physical content as possible and making them assimilable at the undergraduate level (forgetting his occasional oracular remark such as "This is a result of quantum field theory"), Prof. Burcham has done an excellent and commendable job; we thank him for it.

D. H. WILKINSON

## IN DEFENCE OF METAPHYSICS

### *Is Metaphysics Possible?*

By Pratima Bowes. Pp. 237. (London: Victor Gollancz, Ltd., 1965.) 42s. net.

### *The Foundations of Metaphysics In Science*

By Errol E. Harris. (Muirhead Library of Philosophy.) Pp. 512. (London: George Allen and Unwin Ltd., 1965.) 68s.

THESE two books are very different in character as well as in scope: at the same time, however, they display certain aspects of knowledge which make them more valuable together than separately. Both contain traces of apologetics, which indeed are almost inescapable in the present state of the philosophical climate. The reader will try to discover what the bridge is which spans the gap between any metaphysical system (if such exists) and science. Clearly, this is entropy, a concept which becomes more and more significant as the authors' themes develop. Why this should be so will appear in the sequel; for the moment we see biophysics and its partners literally 'daring' entropy to increase as a matter of course. Epistemologically, perhaps this is the greatest break-through of synoptic thinking, even if there are many limitations and question marks. Nevertheless, in general, the second law of thermodynamics is still in command.

Mrs. Pratima Bowes's *Is Metaphysics Possible?* is distinguished by a becoming humility, accompanied by a conviction from the start that the answer to her own question is 'yes'. Prof. Acton, in a most readable introduction, puts his finger on a major point by remarking that logical positivism has flourished by the negative policy of denying meaning to transcendental metaphysics because empirical verification is lacking. Mrs. Bowes goes further, and pins down this antipathy to a dislike of mystery. Such matters—so the argument runs—are not genuine, and their discussion is pointless. One may well agree that all this is a little too easy: for example, the wildly improbable states (as judged statistically) that come not only into existence, but also into continued existence, in the biological sciences, are thoroughly mysterious. There is, as yet, no down-to-earth reason for such occurrences. One day there may be, but meanwhile some discipline akin to metaphysics (which never presumes to unearth anything) may so keep active minds intent on broad issues—without neglecting details when relevant—as actually to hasten discovery.

Metaphysics emerges as individual rather than collective, an art rather than a science; in this it resembles aesthetics, and tends to stand on the touch-line in preference to becoming involved in the rough and tumble of ordinary life. In a word, all this is not far removed from Polanyi's "personal knowledge", and the passion which is the prerequisite of all true advance. There must be conviction that there is a problem to be solved, coupled with the will to solve it. (Incidentally, the joy attending success is almost wholly aesthetic, in a realization of the beautiful.) And this cannot be done from inside. Mrs. Bowes might have mentioned Godel's theorem as her perfect example: 'inside' arithmetic we are plagued with incompleteness, whereas 'outside' we can at least do our sums. Which is as much as to say that, as such, metaphysics is unlikely to make progress, and the common transliteration as 'beyond physics' is seriously misleading.

Mrs. Bowes refers, however, to the work of David Bohm, who concludes that Nature is qualitatively infinite. He propounds the existence of various levels in physics (both classical and quantum), and suggests a further very deep level at which determinate factors are effective, resulting in the accepted laws of probability and the quantum higher up. Whether this is so or not remains to be seen, but one thing is certain—and in tune with all metaphysical thought—and that is that no event in Nature is isolated, so that if in fact something unknown is going on down below, then known phenomena, and the laws which we believe they obey, are influenced accordingly.

The contrast comes out strongly between metaphysics and common sense. The point is well taken in that it is unwise to identify science with common sense, for much goes to show that the most fantastic prognostications of theoretical physics have turned out to be correct at the expense of ordinary explanation. Nevertheless, applied science functions, but this is largely because it is like doing a large-scale exercise or example, based on theoretical knowledge, from which success follows consequentially from a grasp of essentials.

*The Foundations of Metaphysics in Science* is written from a different standpoint. Prof. E. E. Harris is not primarily concerned with the existence of metaphysics as with the way in which he finds it embedded in much of science itself. In fact, what empirical content the subject possesses resembles the natural philosophy of Scotland, which indeed covers much observation and experiment. It is clear that a comprehensive view, synoptic in character, is necessary—as Plato maintained—if whatever total knowledge is accessible to us is not to be partly lost through specialist fragmentation. Besides, it is salutary to examine scientists' own credentials, which they are unlikely to do for themselves beyond the immediate needs of their own investigations. This is the general



task, divided into four main parts—(1) "The Physical World"; (2) "The Realm of Life"; (3) "Mentality"; (4) "Outline of a Metaphysic". These headings are enough to show the scope which the book is intended to embrace. It is very readable, even though there is a fair proportion of paste and scissors, with a slight tendency to write round the references. In dealing with the question of intelligence, a warning is given of the effect of drawing a sharp line between logic and psychology, which is so marked a feature of recent discussion. This is the result of a determination to avoid psychologism, the pet aversion of the phenomenologists and the Husserl school. The principles of organization are eliminated, and with them purposeful thought. Broadly, the *Gestalt* concept bears up well: it has seen many modifications since the days of Wertheimer and Koffka, but its integrative features are still valuable, much more indeed than mere 'and-summations'. Mozart (quoted by the author) would survey a musical composition "all at once", the whole being instantly established.

The unique position of entropy, to which reference has already been made, appears as the connecting link not only between metaphysics and science but also between our conception of the living and the non-living, which is roughly that of its dominance in open and closed systems respectively.

The wonder is that the living organism is so constructed as to be a means whereby entropy can not only be held constant but also in some cases even decreased. This is what the communications engineer strives to do by making use of negative entropy,  $K \log 1/D$ , wherein  $D$  is a measure of noise. Apart from the mathematical formalism, we begin to see in organization a signpost to a better understanding of meaning and perception. Naturally, once this connexion between entropy and living activity is realized, there is scarcely any limit beyond which it is not applicable.

Prof. Harris makes the point that modern symbolic logic, with its great capacity for preventing mistakes, adds no contribution to the pressing need for a science which treats of what organization is supposed to do, namely to set up the forms and principles of thinking. Ethically, as the author says, it is only at the heights of human effort that, due to such vast complexity, aberrations can occur at all. It is still true that man tends to kill the object he loves.

The thesis which these two books are written to defend—essentially a study of contrasts—is best summed up metaphorically in the words which "My Lady Philosophy" used to console Boetius in his prison cell at Pavia in A.D. 524. She said to him, "I will show thee the way which shall carry thee home", which is good and useful science. But she added, "I will give thy mind wings to be raised aloft", which is metaphysics. F. I. G. RAWLINS

## TEACHING RELATIVITY

### Introduction to General Relativity

By R. Adler, M. Bazin and M. Schiffer. (International Series in Pure and Applied Physics.) Pp. xv + 451. (New York, Toronto, London and Sydney: McGraw-Hill Book Company, 1965.) 100s.; 12.50 dollars.

GENERAL relativity has become a popular and widely understood subject in the past 20 years. As a result there have been a large number of books on the subject, some of them presenting collections of original work, and others presenting a view of the whole subject from one particular point of view. The present book differs from all recent books (except perhaps that of Synge) in being a genuine text-book. It is based on a course of lectures, given at Stanford University, by Schiffer. The aim of the authors was both to direct the attention of mathematicians to a fruitful field of research and simultaneously to provide the physicists with a simple introduction to the powerful mathematical methods used in general relativity.

Great care is taken to explain the motivation for everything that is being done, and the whole theory is set out with great clarity. After an introduction dealing with non-Euclidean geometry the tensor calculus is set up. The notation is the classical one with suffixes, no attempt being made to bring in the exterior differential calculus. The relationship of tensor calculus to physics is introduced by means of Maxwell's equations in a flat space and also through a discussion of gravitational red shifts in terms of a description of gravitation by means of a curved space. This brings the reader up to the position of having "general relativity without field equations". The usual arguments are now brought forward for the Einstein field equations as generalizations of Laplace's equation. Here, however, the authors do not neglect to use the argument from the equation of geodesic variation which exhibits the connexion with Laplace's equation in its clearest form.

Having now formulated the theory, discussion on the spherically symmetric static solution and the linearized field equations follow. After the derivation of the Schwarzschild solution there is a very careful description indeed of the present position of the theory with regard to the three so-called 'crucial tests'. A particularly interesting chapter follows, on the mathematical structure of the field equations in relation to the initial value problem. The characteristics and the bi-characteristics of the equations are exhibited with great clarity. The authors elect to omit the general (Fourès) proof of the existence and uniqueness, but they do give the particular result of Lichnerowicz (that a static empty universe which is flat at infinity is flat everywhere). It will give an idea of the completeness of the treatment to mention that this chapter concludes with a proof of the maximum principle for the generalized Laplace equation (that is, Earnshaw's theorem in curved space).

There are also chapters on conservation laws, cosmology and electromagnetism (including Weyl's theory and the already-unified theory of Rainich and Wheeler). The book is beautifully printed and has an excellent index.

C. W. KILMISTER

## THE SUN

### A Star Called the Sun

By Dr. George Gamow. Pp. xiii + 208. (London: Macmillan and Co., Ltd., 1965.) 35s. net.

DR. GAMOW has written an entirely new book on the subject of his earlier work "The Birth and Death of the Sun", and has incorporated an account of the advances in solar research which have occurred in the interim.

The book begins with a survey of man's attempts through the ages to answer such questions as how far the Sun is from the Earth, how large it is and how hot. Then follow chapters on the solar spectrum and what it tells us about the composition of the Sun, the turbulent solar surface, its atmosphere and its corona, and its hot interior. We are reminded of Dante on his journey to the inferior regions: "I would have cast me into molten glass to cool me, when I enter'd, so intense rag'd the conflagrant mass". After analysing the sources of stellar energy at different stages of the life of a star, the author compares the Sun with other stars and explores the nature of such old ones as red giants, white dwarfs and supernovae, the stars that "die glamorously". The last section of the book, on the origin of stellar families, shows that at least one billion stars in the Milky Way may possess planetary systems like that of our Sun (an American billion is a thousand million). Dr. Gamow is an expert in this branch of astrophysics and, as is expected, the sections that deal with the evolution and decay of stars are accurate, written with conviction, and fascinating.

Some of his statements concerning fundamental physics are not always so happy. For example, the 'decay period' [sic] of radium is given as 1,840 years, and two pages



further on the 'half-life' appears as 1,590 years. Dr. Gamow seems to have forgotten that the original product of the mines of Joachimsthal (given as Joschischal) was silver—and hence the name of his country's best-known money unit—and not uranium. The style is popular and sometimes racy. Occasionally it will offend the English reader who has some feeling for his Mother tongue, but on the whole, in spite of his "oodles and oodles", the author has avoided the ambiguities and half-statements which are a feature of so many attempts to vulgarize science.

The book is well produced, with good plates, diagrams and end-plates. W. L. SUMNER

## VIRUSES INFECTING PLANTS

### Plant Virology

Edited by Dr. M. K. Corbett and Dr. H. D. Sialer. Pp. xvii + 527. (Gainesville: University of Florida Press, 1964.) 12.50 dollars.

THIS valuable summary of modern information regarding viruses infecting plants and the diseases caused thereby was compiled from a series of lectures by specialists, delivered during the 1963 Southern Regional Graduate Summer Session in Plant Virology at the University of Maryland. After a historical introduction by one of the editors the various symptoms by which virus infection of plants is expressed are summarized by F. O. Holmes and the means by which viruses can be transmitted from plant to plant are described by R. W. Fulton, with comments on inhibiting factors and on the mechanism of infection of an individual plant cell. The technique of identification of a plant virus is discussed by A. F. Ross, followed naturally by a chapter on virus strains, mutations, acquired immunity on the part of the host and interference by one virus with multiplication in the host by an unrelated virus (W. C. Price). Next B. D. Harrison summarizes recent information regarding the transmission of plant viruses in soil. Two groups of nematode-transmitted viruses are recognized, one with polyhedral particles, the other with tubular particles. Both the viruses and the nematodes have wide host ranges, ensuring survival on weed hosts while virus-immune crops are being grown on the contaminated soil. Transmission of lettuce big-vein virus and tobacco necrosis virus appears to be assisted by zoospores of the chytrid genus *Olpidium*. Susceptible plants may become infected when grown in soil to which tobacco mosaic virus has been added, but it is still not known whether any vector is involved in this instance.

Two chapters follow which deal with the mechanism of virus transmission by insect vectors. R. H. E. Bradley concentrates on the precise method of stylet transmission on the part of aphids, a process apparently beset by so many difficulties and inhibiting factors that it seems astonishing that any aphid ever succeeds in transmitting any virus. K. Maramorosch summarizes the less-extensive work on so-called 'circulative' and 'propagative' viruses, which are not carried immediately on the stylets but are accumulated in the insect's tissues, either multiplying there or not, and afterwards re-introduced into a host plant by the insect. The group includes a few aphid-borne viruses, notably that of potato leaf-roll, and also viruses transmitted by treehoppers, leafhoppers, whiteflies, mealy bugs, thrips and mites. Some of these viruses appear to be in some degree pathogenic to their vectors, others may possibly be in some way beneficial to an insect which ingests them.

The following seven chapters are perhaps those of most general interest since they deal with the nature of viruses themselves. Methods for assaying plant viruses are described by D. A. Roberts, with emphasis on the local lesion technique, virus purification techniques by R. L. Steere and serology by E. M. Ball. The principles of electron

microscopy and their application to virus research are summarized by C. E. Hall, followed by a detailed account by D. L. D. Caspar of modern inferences regarding the structure and function of regular virus particles. These consist essentially of a ribonucleic acid chain (or deoxy-ribonucleic acid in some animal viruses) enclosed in a regularly constructed coat of protein units, called rather unhappily the 'capsid', the shape and structure of which are characteristic of the virus. The shape of virus capsids is comprehensible if it is accepted that the most probable minimum energy designs for surface crystals composed of a large number of units are tubes with helical or cylindrical symmetry and closed shells with icosahedral symmetry. Each of these types of pattern is now known to be adopted by and characteristic of different plant viruses and, even though the morphologies of helical and icosahedral virus particles appear very different, it seems that the principles applied in their construction are the same. Unfortunately the morphology of a virus particle is not necessarily an indication of its relationship with other viruses. Since there are only 230 types of crystal lattices it is not surprising that chemically unrelated compounds can form crystals with identical symmetry and the structure of biologically unrelated viruses, for example, poliovirus and turnip yellow mosaic virus, may be based on very similar designs. The helical structure of the tobacco mosaic virus rod is described in great detail, with interesting indications of small differences in the arrangement of protein units in different strains of the virus. Next C. A. Knight elucidates the structural biochemistry of plant viruses, both as regards the construction of viral proteins and the fine structure of the ribonucleic acid chain. Finally, W. N. T. Takahashi discusses the biochemistry of virus infection and such information as exists regarding the synthesis of viral proteins and virus particles within the host cell.

Three final chapters summarize methods for control of plant virus diseases (L. Broadbent), frankly facing the fact that quarantine may delay but cannot prevent the spread of viruses around the world to a maximal distribution determined by the geographical distribution of their hosts and vectors. F. C. Bawden speculates on the origins and nature of viruses and the application to virus studies of the principles of molecular taxonomy is discussed by F. Lanni.

An appendix contains two papers on non-plant viruses: virus diseases of arthropods by K. M. Smith and an introduction to the tumour viruses by R. A. Manaker.

Each chapter is followed by a comprehensive bibliography. R. W. G. DENNIS

## GOLD

### Gold

Recovery, Properties, and Applications. Edited by Edmund M. Wise. Pp. x + 367. (Princeton, New Jersey, Toronto, New York and London: D. Van Nostrand Company, Inc., 1964.) 93s.

THE metal gold has had a special place in civilized communities even from the most ancient times. It has been known to man for 6,000 years, and rivals copper as the first metal ever to be used. Early applications became possible because the gold occurred naturally in the metallic state in the beds of streams and alluvial sands in an easily recognizable form. Large pieces could be hand-sorted from neighbouring rocks, and fine particles trapped by washing gold-bearing sand over fabrics or animal fur. Sheepskin was popular for the latter purpose, and it seems probable that the 'Legend of the Golden Fleece' arose in this way. The gold was used in the earliest times exclusively for ornament, and was sufficiently ductile to be hammered into the required shapes without the need for sophisticated metallurgical treatment. Its attractive appearance, relative rarity, and chemical inertness led naturally to it being a prized possession, and to its use as

a medium of exchange for goods, and as a coinage. The function of gold as a monetary standard has continued up to the present time, and it has become the yardstick by which national currencies are measured.

The monetary uses of gold, and their influence on human behaviour (for example, as a brief glance at almost any television Western will show), have tended to obscure the applications of the metal in other fields. Broadly speaking, these applications stem from the ability of gold to resist many corroding environments, and from the ease with which it may be worked. Such properties lead directly to its use in electrical contacts, electron tubes, conductors, resistors, and thermocouples, where great precision and reliability are vital. A large part of the world production of gold, in fact, now goes into non-monetary uses. Dr. E. M. Wise, the editor of *Gold*, has studied and worked on the properties of the metal for many years, and he and his contributors have set out here to collect together for the attention of engineers, chemists, metallurgists, physicists, dentists, and those in the medical profession, the properties and applications which are of relevant interest.

The book contains twenty-eight chapters and includes descriptions of the sources of gold, the extraction, mechanical, physical, chemical, and metallurgical behaviour, the applications in electrical engineering, dentistry, jewellery and medicine, and special uses such as an additional element to glass, in the form of very thin films, and as a solder. Methods of chemical analysis, an important subject in view of the necessity of keeping account of the metal, are also included. The book is provided with ample lists of references, and I am not aware of such a comprehensive treatment in any other volume. It is expensive, but as it is a reference work rather than a book for the general reader this is probably not a disadvantage. It will certainly find its way to the library shelves of all organizations dealing with the metal, since it is useful to remember:

"Foul cankerous rust the hidden treasure frets,  
But gold that's put to use more gold begets".

J. A. CATERBALL

## THE BIOCHEMIST'S 'VADE-MECUM'

### Methods In Enzymology

Vol. 6. Edited by Sidney P. Colowick and Nathan O. Kaplan. Pp. xxiii+1054. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1963.) 200s.

THIS volume completes the supplement to the original treatise "Methods in Enzymology" (Nature, 177, 810 (1956); 178, 509 (1956) and 181, 1431 (1958)) and, like the earlier volumes, has been edited by S. P. Colowick and N. O. Kaplan. The editors have brought together an impressive list of 147 authors who have between them contributed 127 articles. These contributions are grouped together under three major headings; the first two deal with the preparation and assay of enzymes and substrates, and the third with special techniques used in enzymology. The enzymes included in this volume are those concerned with nucleic acid, phosphate, coenzyme and vitamin metabolism and the respiratory enzymes. Other enzymes have been dealt with in the previous volume. The preparation and assay of substrates were not included in the first volume of the supplement and so there is complete coverage of the recent progress in this field. The final section is entitled "Special Techniques" and, besides descriptions of more general methods of protein analysis, includes articles on the esoteric techniques involved in neutron-activation chromatography of phosphorus compounds and in the application of nuclear magnetic resonance and electron spin resonance to enzymology.

With such a large number of authors contributing to this volume there is some variation in the style and standard of the individual articles, although in general

the latter is high. There is usually an introductory paragraph to each contribution describing the enzyme or substrate and giving the principles involved in the assay. The experimental procedures are given in detail with adequate reference to the original literature so that, if problems arise in following methods given in the book, the individual worker can readily turn to the original paper. The grouping of methods under the general titles 'enzymes' and 'substrates', although in general it is satisfactory, does lead to minor inconveniences. For example, polynucleotide phosphorylase, RNA polymerase and cell-free protein synthesis directed by messenger RNA, all occur in the first 25 pages, yet we have to turn to page 713 for the synthesis of synthetic polyribonucleotides. Similarly, the isolation of DNA is on page 726, the enzymatic preparation of polydeoxyribonucleotides on page 718 and the DNA polymerase on page 34.

Throughout this volume the editors have allowed the use of trivial names for the enzymes, and it is unfortunate that, two years after the publication of the report of the Commission on Enzymes, a book of this importance can be written in which the systematic names and numerical code numbers for the enzymes are not used. Apart from this criticism this volume maintains the standard set by the editors in the previous volumes and together with them provides an excellent laboratory manual for all biochemists who require detailed working instructions for the preparation and assay of enzymes. D. KERRIDGE

## A SURVEY OF MANAGEMENT ATTITUDES

### Thrusters and Sleepers

A Study of Attitudes in Industrial Management. (A PEP Report.) Pp. 295. (London: Political and Economic Planning; and George Allen and Unwin, Ltd., 1965.) 35s.

WHILE much is said and written about the influence of management on productivity, little systematic investigation has been carried out to determine how far managers in fact achieve a high and sustained growth rate for their firms. There should be general approval, therefore, for this report of a survey carried out by P.E.P. into management attitudes and practices and their effects on a company's prosperity. Firms selected for study included those whose management attitudes and practices were likely to be conducive to growth and these were compared with firms whose managers were thought not to possess such attitudes.

The choice of firms was decisive. Expert opinion was consulted about those firms which were expected to be prospering in ten years time and, in contrast, the survey included firms of similar size and operating conditions but whose prospects appeared to be unpromising. Altogether, eight firms were visited in each of six varying types of industry which included old and new, science-based and non-science-based, contracting and expanding, capital and consumer goods, craft and mass production industries, localized and non-localized industries and American-influenced and wholly British companies. The industries finally selected were wool textiles, machine tools, ship building, electronics, domestic appliances and earth-moving equipment. Each company was visited for two days, executives were interviewed, much published material about the companies was read, and accounts were analysed.

The research teams investigated a number of factors which are in some way associated with improved productivity. Among them are the systematic development of junior and middle managers and the introduction of modern management techniques. The latter includes mechanization, work study, operational research and accounting techniques like budgetary and cost control. Frequently these are introduced into companies by management consultants and, consequently, the use made

of consultants was taken as a further means of determining management attitudes. The attention paid to research and development, marketing and exporting was also examined. In all, a total of 59 factors was built up. Firms whose managers reacted positively to them were described as 'thrusters' and those reacting negatively as 'sleepers'; there were many intermediate grades.

In itself, such a survey would have been inconclusive. 'Sleepers' could fairly claim that, in practice, the aggressive and busybody 'thrusters' might achieve no greater commercial success than the firms whose pace was based on that of traditional slow growth. Despite all the difficulties of measurement, therefore, the investigating teams analysed the companies' financial results to determine the compound average increase in capital employed and the average return of earnings (pre-tax profits) to capital employed over the previous 5 and 10 years. Comparisons showed that, in nearly all cases, 'thrust' in management is accompanied by commercial success and that 'sleepy' management leads to little company financial growth or reward.

Despite all its inherent difficulties and limitations, this is another example of the kind of survey which P.E.P. does superlatively well. Prof. J. R. Parkinson, Anthony Gater, David Insull, Harold Lind, Peter Seglow and all who have contributed to it deserve well of those who are concerned with the productivity of British industry. The survey has been well devised, and conducted and the findings should be used on all possible occasions as a means of prodding 'sleepers' into wakefulness.

The limitations of the book, however, become clearly apparent in the second half where the authors try to show how the findings could be applied to promote productivity. They describe Government measures which should be used to promote management education as well as incentives to encourage the acceptance of management techniques and the release of actual and potential managers to attend management courses. They recommend the setting up of "Industrial Boards" which could stimulate the development of modern techniques in recalcitrant firms in the same way that the "Industrial Training Boards are already giving assistance in the field of industrial training". In point of fact, no Industrial Training Board has yet made any impact on industry and the few inaugurated have not appointed their main officials. This, and other remarks, suggest that the survey has been carried out by men whose real knowledge of industry is slender and much of what they have accepted would have emerged differently from men possessing more detailed awareness of what really happens in industry. A great deal, for example, of what they call management development is open to question; their 'work study' is most often little more than the fixing of wage rates. The real opposition to marketing comes less from production managers and more from the sales managers who have no time for it. These are the kind of issues which make the book ring less than true and, unfortunately, detract from the value of what could have been one of the most penetrating analyses of British management conducted so far. T. H. HAWKINS

## INTRODUCTIONS TO GEOLOGY

### Geology

By W. C. Putnam. Pp. xii + 480 (illustrated). (New York and London: Oxford University Press, 1964.) 55s.

### An Illustrated Elementary Classification of Minerals, Rocks and Fossils

By H. C. Curwen. Pp. xi + 183. (Oxford, London and New York: Pergamon Press, 1965.) 42s.

SHORTLY before his death in March 1963, Prof. W. C. Putnam had all but completed the text of a course in geology for elementary students, which his colleagues in the University of California, Los Angeles, have

now seen through the press. The work is a fitting memorial to a distinguished scholar. Viewed as an introductory textbook for university classes it is unfortunately somewhat restricted in its coverage; for example, there is a full chapter on petroleum geology but no mention at all of ore deposits. Viewed as a text for collateral study, it merits high commendation. The style of the author is eminently readable if sometimes a little discursive, and it is with an infectious enthusiasm that he guides the reader through the study of earthquakes, volcanoes, deserts, glaciers, and so on, sometimes with digressions into literary and historical by-ways which text-book writers rarely tread. The book is graced by more than 200 illustrations, most of them half-page or full-page photographs of superlative quality which have not appeared elsewhere. The publishers are to be congratulated on the production, at a reasonable price, of a work which will do much to stimulate the interest of young people in earth science and which can be advocated unreservedly as a very suitable addition to every senior school library.

The Pergamon Press claims, for *An Illustrated Elementary Classification of Minerals, Rocks and Fossils*, that "the book will prove invaluable for use in universities, technical colleges, and schools of mines". It consists in large part of a collection of poorly reproduced and badly captioned geological photographs, principally culled (though this is not stated) from the albums of the Geological Survey of Great Britain and supplemented by tabular presentations of the properties of some rocks and minerals, cribbed from elementary text-books with the introduction of many mistakes, misapprehensions, and mis-spellings. Few pages are free from errors, some of which (for example, the claim that phosphate rock is metamorphosed guano) are repeated several times; and the standard of technical editing is poor.

C. F. DAVIDSON

## DATING EARLY MAN

### Frameworks for Dating Fossil Man

By Dr. Kenneth P. Oakley. Pp. x + 355. (London: Weidenfeld and Nicolson, 1964.) 45s. net.

A FOSSIL human skull has been discovered: how is it possible to tell its geological age? The various methods that can be used, their possibilities, limitations, difficulties, and results, are discussed in Dr. Oakley's most up-to-date book on this fascinating topic: *Frameworks for Dating Fossil Man*.

The classical method is by relative dating, where we can rely on geology, stratigraphy, palaeontology, palynology, geomorphology and archaeology. But an entirely new chapter has been opened by absolute dating—called by Oakley 'chronometric dating'—where radioactivity can be used, as in the carbon-14 and the potassium argon methods. While the first can only be used to date samples younger than about 50,000 years, the latter method covers a much wider range and can be used for the whole of the stratigraphical column. Here, at last, we have a yardstick for measuring geological time absolutely.

The book is subdivided into two equal parts, one on stratigraphical dating and one on archaeological dating. The reader is introduced in the first part to the problems of the chronology of Quaternary deposits in general, and separate chapters deal with the glacial chronology, the interglacial correlation and palynology, the correlation by fauna, the palaeotemperatures recorded in deep-sea cores, the changing sea-levels and the formation of river terraces. After this come more general chapters, in which Dr. Oakley treats certain important geographical regions: the terraces of the Somme in France, the glacial stages in Latium, Italy, the Pleistocene subdivision in Holland, and the post-glacial Baltic stages. Africa is discussed in

detail: pluvials and interpluvials, ancient lake-levels, fossils, soils, palaeoclimatology and climatic changes. In the second part mesolithic and palaeolithic cultures are considered, first in Europe and the Near East, then Africa, and finally Asia.

Palaeontologists will be pleased to find that for faunistic reasons the Günz glaciation as well as the whole of the Red Crag of East Anglia are now included in the Lower Pleistocene—the Villafranchian. Two curves demonstrating the palaeotemperatures (Figs. 5 and 12) have been taken from Emiliana, whose dates, as Dr. Oakley remarks, are not generally accepted. I myself believe that the estimated age for the Günz glaciation of 260,000–300,000 years is too small (the date given by Eriksen and Wollin in *The Deep and the Past* (1964) is 1,400,000). Leakey's Challeen skull from Olduvai, according to an improved dating by Curtis and Evernden, is now considered to be the same age as the *Pithecanthropus erectus* skulls from Java (500,000 years).

An important part of the book is the appendix. In the "Fossil Hominid Dating Tables" all known fossil hominids are listed according to group, country, site, date of discovery, name, stratigraphical and cultural and, if known, also the absolute date. Heidelberg Man is classified among the *Pithecanthropinae*; however, I am of the opinion that the name *Pithecanthropus* should be preserved, as Dubois gave a definition of the genus when describing the first specimen in 1894. The list of the *Australopithecines* to me seems open to discussion. *Meganthropus* from Java certainly does not belong here; I also think it premature to place '*Homo habilis*' from Olduvai here (this one name covers at least two different forms). It is regrettable that Dr. Leakey has already announced in the Press his new discoveries—even trying to give a new definition of the genus *Homo*—before making available a scientific description: it is, as yet, impossible to confirm or dispute his conclusions. From the list is missing the large upper molar from Olduvai, Bed II, attributed by Leakey (1958) to '*Homo sp.*', but which definitively has australopithecine affinities; it came from the same level as did Chellean handaxes and demonstrates the coexistence of Hominines and *Australopithecines*.

The foregoing should serve to demonstrate the broad base of Dr. Oakley's study, which indeed covers all modern aspects of dating our Pleistocene ancestors: tertiary deposits and possible human ancestors have been excluded. This readable book contains many tables and well-selected illustrations as well as maps and charts which make it easy for the reader to find his way through a great variety of subjects. But what makes the book especially valuable are the many illuminating notes and the extensive lists of modern references. The book presents an excellent review of the present state of affairs not only for the specialist but also for the interested layman.

G. H. R. VON KÖNIGSWALD

## LOOKING INTO THE FUTURE

### The Conquest of the Material World

By John Nef. Pp. xii+408. (Chicago and London: The University of Chicago Press, 1964.) 8-95 dollars; 67s.

DR. J. NEF has devoted many years to the study of social history of industrialization from the earliest times, and he has thrown much fresh light on the factors that have been involved by his great learning and penetrating analysis. He is the founder and chairman of the Committee on Social Thought in the University of Chicago, and is the driving force behind the recently formed Centre for Human Understanding at Washington. The subject of its last international meeting was "The Building of Bridges of Understanding" between nations at different stages of development. Dr. Nef seems to have been

surprised that the questions uppermost in the minds of the emergent nations in Africa, Asia and Latin America were concerned with the industrialization of their countries—how fast should it proceed and how far should they retain their old traditions even if this involved slower achievement of the material wealth that they are all seeking.

In *The Conquest of the Material World*, Dr. Nef has collected a number of key essays relevant to these problems, several of them having been re-written. In them, he traces the development of our industrialized civilization in Western Europe, which Nature has endowed with such varied and abundant mineral resources. "The conquest of this underground wealth by the Western peoples has been inseparable from the unprecedented power obtained by men in recent centuries over the physical world." The early chapters deal with mining and metallurgy in medieval society, with industrial Europe at the time of the Reformation, with the growth of large-scale industry in Britain between 1640 and 1840, and the contrast between industrial growth in Britain and in France. Dr. Nef then comes to the heart of the matter in his studies of historical interrelationships, in which he relates novel developments in industry to other kinds of endeavour, religious, monetary, scientific and governmental. His aim is to bring these diverse strands of history into a new unity that has hitherto been missed. This ends with an account of the influence of the political and industrial development in Britain and France on the drafting of the American Constitution. It omits, however, any detailed consideration of the quickening of the rate of change of the whole world economy due to the impetus given by the two great World Wars to the effort devoted to scientific research and development and to the desire for national independence.

In his epilogue, Dr. Nef looks into the future and states very clearly his own faith. He rejects the cyclic point of view of historians like Gobineau and Spengler, who regard history as the inevitable result of predictable forces.

He ranges himself with de Tocqueville against Gobineau in his belief "that the conquest of the material world has come out of human efforts never made before and out of a new hope in human nature which the future will either destroy or sustain". "What is my position?" he asks; "essentially this: the rise, particularly during the 17th and 18th Centuries in Europe, of a society transcending national and even European boundaries, putting the tender virtues in the foreground as ideals to be sought after, a society bent for a time on settling by limited warfare the issues that divide parts of it, a society open to all the societies of the world, cannot be accounted for without the attempts made to realize in the world something of the love emanating from Christ. . . . Men and women everywhere are now confronted with dangers and blessed with opportunities greater than any that existed in the times of Gobineau. The way in which they meet these dangers and opportunities depends not only on the scientific and technological knowledge they possess but upon the beliefs they hold. . . . It seems probable that the future of the human race rests with conduct based on higher ethical and aesthetic standards than have ever prevailed in either private or public relations. If this is so, how can we reject de Tocqueville's testimony concerning Christianity? . . . However dangerous dogmatism based on religious faith may be, it is less dangerous than dogmatism based on the extension of science to matters upon which science cannot legitimately pronounce. . . . Having conquered the material world, men and women have now the mission of making it a more decent place for human beings to live in. In order to do this, they will have to conquer themselves."

This is a challenging book by an idealist, that comes at an opportune moment for this troubled and divided world.

HAROLD HARTLEY

### Nuclear Power Systems

An Introductory Text. By C. D. Gregg King. Pp. xiv+480. (New York: The Macmillan Company; London: Collier-Macmillan Limited, 1964.) 100s.

WHAT real purpose is served by adding yet another to the already considerable number of introductory texts in nuclear engineering is difficult to see. There is little new to say and no really different way of saying it, but still the books come.

*Nuclear Power Systems* adds nothing at all to the existing texts. It covers the, by now, conventional ground of nuclear and reactor physics, reactor control, shielding, materials, reactor types, thermodynamics, steam turbines, fluid mechanics, heat transfer and nuclear power plant design. It is even more conventional than most, in that much of the material in the latter half of the book is standard revision of the topics and is scarcely related to nuclear reactors at all.

There is a certain lack of exactness in some of the sections, which must inevitably lead to wrong impressions being given to someone using the book as an introduction to the subject. For example, in the first mention of the multiplication factor, the critical condition is given that it shall be equal to unity, without any qualification that this either relates to its effective value or to an infinite system. There is a statement that "all neutrons in the moderator entering the fuel lump having the resonance energies are absorbed in the outer portions of the fuel lump". Then, as another example, what is now almost a standard schoolboy howler is repeated. This is the mistaken belief that the reason that Xe-135 builds up on shut-down of a reactor is because the I-135 half-life is less than the Xe-135 half-life. Despite the fact that the book is well produced it cannot really be recommended, bearing in mind the number of excellent texts there are already covering very similar ground. J. F. HILL

### Die Rohstoffe des Pflanzenreichs

Von Julius von Wiesner. Fünfte Auflage herausgegeben von Constantin von Regel. Lieferung 3: Organic Acids. By G. C. Whiting. Pp. 194. (Weinheim: Verlag von J. Cramer, 1964.) n.p.

THIS paper-backed volume reviews the properties, distribution and, in some cases, the industrial importance of the organic acids occurring in plants. Aliphatic and alicyclic acids are accorded the most space (some 54 and 20 pages, respectively) while aromatic, heterocyclic and amino-acids take up the remainder (6, 8 and 10 pages, respectively). The literature list includes 1,157 references, but few publications subsequent to 1961 are given. Plant names and the organic acids are separately and fairly fully indexed.

The book should be of considerable value as an information source, but the coverage is rather uneven and is clearly not comprehensive; unfortunately, no indication is given of the intended scope of this monograph. The approach is classical in its factual emphasis and there is little mention of the overall biochemical significance of the acids and their distribution. G. EGLINTON

The Problem of the Minimum of a Quadratic Functional By S. G. Mikhlin. Translated by A. Feinstein. (Holden-Day Series in Mathematical Physics.) Pp. ix+155. (San Francisco, London and Amsterdam: Holden-Day Inc., 1965.) 8.95 dollars.

THE process of solving a linear partial differential equation with given boundary conditions by minimising a suitable integral has a history which goes back at least as far as Riemann's method for solving Dirichlet's problem for Laplace's equation; Euler's variational condition leads back from the integral to the differential equation. More generally, there is a connexion between the linear differential equation and the minimal condition for a quadratic functional; and quadratic functionals at

once suggest the use of Hilbert space and the cognate theory of operators. Moreover, it is reasonable to hope that this concept may help in dealing with a delicate existence problem: Does the minimising function possess derivatives of an order sufficiently high to ensure that the terms in the differential equation have a meaning and that the equation is satisfied?

A number of Prof. Mikhlin's books have already been translated into English, and this is a welcome addition. In the first chapter he sets the variational scene; the second chapter contains some preliminary work, chiefly about operators. The meat of the book is in Chapters 3 and 4, where the method is first applied to equations of elliptic type and then shown in action in solving the basic partial differential equations of elastic equilibrium under prescribed conditions on the boundary displacements or stresses.

The reader needs to know something about the theory of operators in Hilbert space, and in addition might well be helped by making a parallel study of Sobolev's monograph on the applications of functional analysis in mathematical physics, recently translated for the American Mathematical Society. The clarifying and systematizing power of functional analysis makes it one of the most efficient tools of the present-day mathematician, in both pure and applied mathematics. T. A. A. BROADBENT

### Annual Review of Microbiology

Vol. 18. Edited by Charles E. Clifton, in association with Sidney Raffel and Mortimer P. Starr. Pp. vii+394. (Palo Alto, Calif.: Annual Reviews, Inc., 1964.) 8.50 dollars.

THIS volume continues the annual series of reviews which by now is so well known among research workers and university teachers as to require no introduction. The reviews, as is usually the case with volumes in this series, are written in an extremely condensed manner, which means that, although most of the relevant literature is adequately discussed, the reviews make rather daunting reading.

The precedent of the earlier issues is continued by covering a wide range of microbiological topics of interest to workers in both the pure and applied aspects of the subject. In all, fourteen topics are reviewed, three mainly of virological interest, two genetic, five biochemical, one immunological and two taxonomic.

It is virtually impossible for one person to give a critical review of a collection of articles of this kind, quite apart from the question of whether it is desirable to review a review. Enquiry among specialists in the various topics covered by the volume suggests that each one is valuable and covers the ground well. The articles on the "Genetic Aspects of Metabolic Control" by W. K. Maas and E. McFall and on "Biochemical Mechanisms of Drug Resistance" by H. S. Moyed are of particular interest to me. The review on metabolic control is valuable in that it attempts to define the terms 'feed-back inhibition', 'repression' and 'induction', and then to present the experiments which throw light on the biochemical nature of these processes. In this field, the work of the group at the Institut Pasteur on the synthesis of  $\beta$ -galactosidase in *E. coli* has, in the past, often been discussed in reviews of this kind at the expense of work on other inducible and repressible enzyme systems in bacteria. This review goes a long way to correct this imbalance.

In the review on the "Biochemical Mechanisms of Drug Resistance", the author gives a full account of the methods whereby micro-organisms may become resistant to growth inhibitors. The review fails to consider, however, the frequency with which these various types of resistant strain arise. This is unfortunate, since information is highly important when considering the properties of successful antibacterial agents.

M. H. RICHMOND

# REMOVAL OF RADIONUCLIDES FROM THE BODY

By D. HYLTON SMITH and T. H. BATES

Health and Safety Department, Atomic Energy Authority, Chapelcross Works,  
Annan, Dumfriesshire

**DIRECT** evidence that incorporated radioactivity induces pathological change in humans includes, for example, the case-histories of the radium dial painters of the 'thirties, together with knowledge accumulated from the clinical use of isotopes. Indirect evidence has accumulated from investigations on animals, though much of this work is unrealistic in terms of human exposure. Few cases of human contamination in the nuclear industry have been recorded. However, as Catsch so rightly remarks, "even if the number of internal contaminations or poisonings by radioactive contamination could be minimized by means of effective protective measures and precautions, it would not exempt the scientist and physician from the necessity to test and develop appropriate therapeutic measures".

In contrast to external radiation where exposure occurs only for the period within the radiation field, internally deposited radionuclides provide a radiation dose continuously until they physically decay or unless they are removed from the body by a metabolic process. The relative danger depends on the effective half-life, the type of decay and the concentration of the radionuclide in a radiation-sensitive tissue. It is beyond the scope of this review to discuss detailed metabolism of the radionuclides, but it is important to stress that the critical organ can differ according to several physical and biological factors as well as the mode of entry into the body. Entry can be by inhalation, ingestion or wound contamination and, in practical terms, when considering radionuclides such as strontium-90 and plutonium-239 there are certain critical organs to take into account—lung, lymph nodes, bone and liver. Thus, inhaled plutonium dioxide is firmly fixed in lung and thoracic lymph nodes, injected or inhaled monomeric plutonium nitrate aerosol accumulates in bone while polymeric plutonium concentrates in liver. Strontium rapidly enters the skeleton whatever the mode of entry, being incorporated into bone crystals.

When an insoluble particle is inhaled, its site of deposition in the lung depends on its size. Particles less than  $1\mu$  in diameter will enter the alveoli. Here, they may be ingested by phagocytes which are able to migrate into the lymphatic circulation or they may become embedded in fibrous tissue. Larger particles tend to deposit in the bronchioles. The 'non-respiratory' bronchioles are lined with ciliated columnar epithelium and particles in this region are removed from the lung by ciliary action. The hazard then becomes one of ingestion if the particle is swallowed.

Bone metabolism is vaguely understood. Bone tissue is predominantly made up of hydroxyapatite crystals and collagen. Three types of cell are identifiable, each associated with a specific function. Osteoblasts are located on the growing surface of bone and synthesize collagen; osteocytes are osteoblasts that have become surrounded by their own calcified, secretory products; osteoclasts are giant cells associated with resorption of bone. The fundamental unit in bone is the osteon, and the degree of calcification of these units alters with age. The mechanism controlling bone growth is obscure, but it appears to be influenced by several humoral stimuli. One of three different metabolic states can exist at any time within bone. On active surfaces, the matrix is laid down as a border of uncalcified osteoid covered by active osteoblasts.

If the surface is quiescent, non-active osteoblasts lie against a calcified matrix. Finally, if the surface is resorbing it is covered with osteoclasts. The alkaline earth radionuclides, which follow the movement of stable calcium ion closely, concentrate on active surfaces, whereas plutonium-239 tends to concentrate on resting surfaces, possibly in combination with mucoproteins.

When a soluble radionuclide enters the body, transport and metabolic processes lead to its deposition in various sites including the critical organ. A knowledge of how this occurs is of fundamental importance in studies aimed at the therapeutic removal of the radionuclide. Such investigations have been carried out at Chapelcross using strontium-85 and plutonium-239 as an example of a transuranic element.

Mature female Sprague-Dawley rats were killed at intervals up to 48 h following the intraperitoneal injection of carrier-free strontium-85 as chloride or  $^{239}\text{Pu}$  as nitrate. Blood and tissues were analysed for activity in order to follow the kinetics of the distribution of the radionuclides and clearance rates from body compartments. Some of these results are summarized in Figs. 1 and 2. The specific activities of strontium-85 and plutonium-239 in blood reached maxima at 0.25 h and 1 h respectively. After approximately 2 h the linearity of the clearance curves plotted on a log-log scale indicates compliance with a power law. Very rapid transfer of

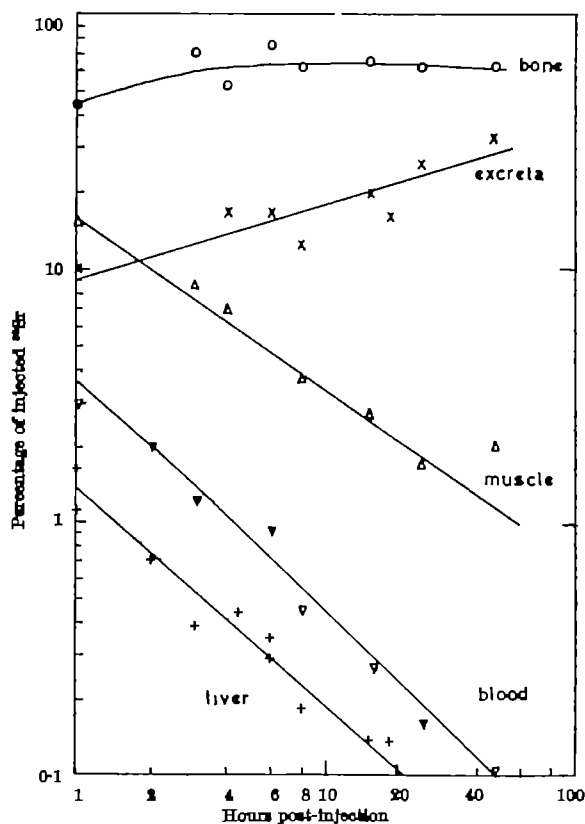


Fig. 1. Tissue fractionation of  $^{85}\text{Sr}$  in the rat

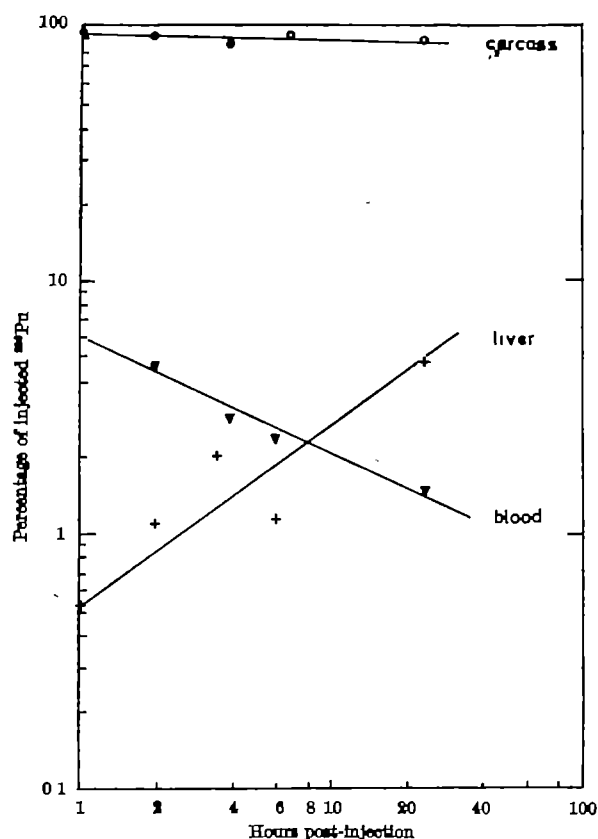


Fig. 2. Tissue fractionation of  $^{239}\text{Pu}$  in the rat

both isotopes is indicated but the longer duration of plutonium-239 in blood is of significance from the point of view of its therapeutic removal. Strontium is present predominantly in the plasma fraction of blood, both as free ion and protein bound; but the latter complex is easily dissociated since plasma samples subjected to dialysis were completely decontaminated. Hence the nuclide is readily available for clearance. Plutonium, on the other hand, is more strongly bound to protein in plasma since only 15 per cent was ultrafilterable. It appears to exist as a  $\beta$ -globulin or  $\beta$ -lipoprotein complex.

The clearance rates of the two isotopes from liver also illustrate a distinct difference. Whereas the clearance rate of strontium paralleled that from blood, the liver burden of plutonium increased over the period examined. Using the monomeric form, the liver contained 4.7 per cent at 48 h, although up to 70 per cent can be concentrated if the polymeric form is injected. The uptake figures into bone for both radionuclides show a rapid transfer from extracellular fluid, so that effective therapy should ideally be initiated during this period.

#### Use of Chelating Agents to mobilize Radionuclides

The principles of chelation chemistry have been applied to the problem of removing radionuclides from critical organs in recent years. Success depends on the formation of a blood-soluble metal chelate which is capable of crossing cellular membranes and of excretion in the unionized form by the kidneys.

The following remarks are intended only as an elementary explanation of chelation chemistry for those not acquainted with the principles. In a solvated metal ion  $M(\text{H}_2\text{O})_x^+$ , the formation of co-ordination compounds is dependent on the co-ordination number ( $x$ ), that is, the number of molecules of water bound to the ion. If this number is greater than unity and the metal ion forms one or more heterocyclic rings, the complex is called a metal chelate. Its formation depends not only on the co-

ordination number but also on the number of donor atoms of the ligand ( $L$ ). Because of its basic nature, being an electron donor, the ligand is associated with protons over a wide range of pH and thus the formation of a chelate is influenced by competition between metal ion and protons for the ligand.

Catech<sup>1</sup> has derived a formula for expressing the biological effectiveness of chelating agents. In the simplest circumstances, he postulates that the alkali metals and the trace elements in a biological fluid do not form chelates which can effectively compete with calcium ion. Thus, in measuring the ability of a chelating agent to combine with a radioactive metal,  $M$ , calcium ion is the only significant competitor. Catech, in a first approximation, assumes a simple  $LM:1L$  chelate and a  $1Ca:1L$  chelate to exist at pH 7.4, but the effectiveness of the ligand for the metal ion  $M$  is dependent on whether  $M$  is carrier-free or isotopically diluted. How this effectiveness varies with concentration of metal and ligand is shown in Fig. 3. The ordinates show the ratio of chelated to non-chelated metal ion  $(ML)/(M)$ , the abscissae show concentration (in moles/l.) of either  $M$  or  $L$ . If the metal ion concentration is low, the effectiveness of chelate formation increases linearly with ligand concentration (curve a). Conversely, this effectiveness decreases if the ligand concentration is constant (say,  $10^{-4}$  moles/l.) and the metal ion concentration increases (curve b).

Marked deviations from this theoretical concept are found in the living organism. The therapeutic effectiveness of a chelating agent demands a fairly constant and high extra- and intra-cellular concentration of the ligand. Unfortunately such levels are commonly associated with toxic effects. The maintenance of a high concentration is influenced by the rate of metabolism of the chelate, the rate of excretion and the stability of the chelate under extreme conditions of pH (for example, in the renal tubules). The ideal chelate should be metabolically inert and this is so for the polyaminopolycarboxylic acids. However, they are not stable at extremes of hydrogen ion concentration and may dissociate in both renal tubules and bone cells. No ligand has yet been synthesized which

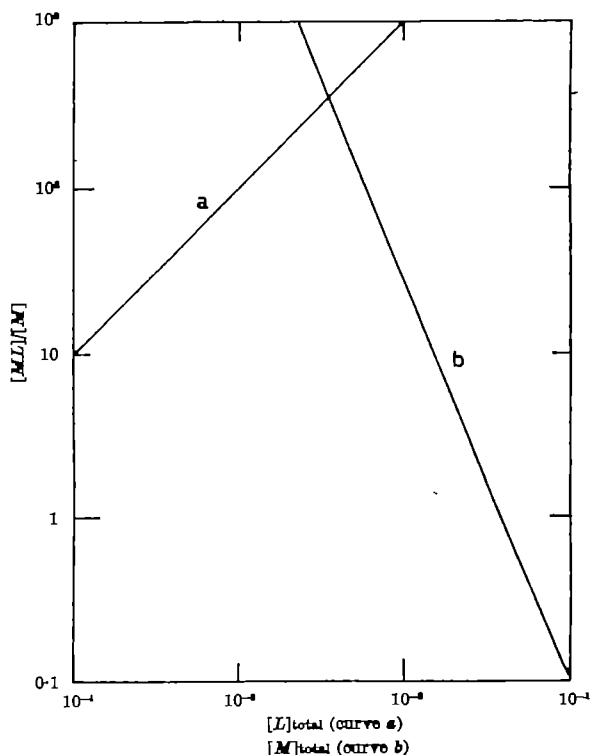


Fig. 3. Dependence of chelation on the total concentration of the chelator and the metal  $a$ ,  $[M]_{\text{total}} = 10^{-10}$ ;  $b$ ,  $[L]_{\text{total}} = 10^{-4}$



will react specifically under biological conditions with a particular metal ion. In a complex biological environment such as plasma, many metal chelates can be formed at differing velocities of formation and stabilities. Thus the presence of competitive physiological chelators must influence the effectiveness of the artificial chelating agent, particularly if the radionuclide to be removed is carrier-free.

All the chelating agents claimed to be useful in metal mobilization contain oxygen, sulphur or nitrogen as electron donor atoms; a group at Aldermaston has studied the chemical characteristics of many of these chelators. The polyaminopolycarboxylic acids, in particular, ethylenediamine tetraacetic acid (EDTA) and diethylenetriamine pentaacetic acid (DTPA), are considered on chemical grounds to be applicable to biological media. These compounds are water-soluble, metabolically inert, but are relatively toxic when used at the highest permissible dose consistent with maximal removal of the radionuclide. Apart from toxicity, one serious limitation of the known chelating agents is that their effectiveness is profoundly reduced if treatment is delayed. Even the use of a pharmacologically active substance in combination with a chelating agent (for example, parathyroid hormone plus EDTA to remove plutonium-239 (ref. 2), diamox plus DTPA to remove cerium-144 (ref. 3)) does not modify this efficiency. DTPA may be cited as an exception to this in certain circumstances. Although disappointing results have been obtained using aerosol-DTPA to reduce lung burdens of plutonium-239, this radionuclide can be mobilized from bone after delayed intravenous or intraperitoneal DTPA. Results from a typical experiment in our laboratory are shown in Fig. 4 where increased excretion was produced after a delay of forty-three days. DTPA accumulates in intracellular fluids so that success in delayed treatment may depend on this fact. Esterified DTPA, which can penetrate cellular membranes more easily than DTPA, is possibly more efficient because of this property<sup>4,5</sup>.

Recently, a chelating agent, desferrioxamine methane sulphonate (FAB), has been claimed to be more effective than DTPA in removing plutonium-239 from bone<sup>6</sup>. This is at present being confirmed.

#### Attempts to Modify the Excretion of Radioactive Strontium after Modification of Certain Physiological Parameters

From the investigations, by many workers, on the removal of radionuclides deposited in the body, there has emerged the fact that the synthetic chelating agents so far

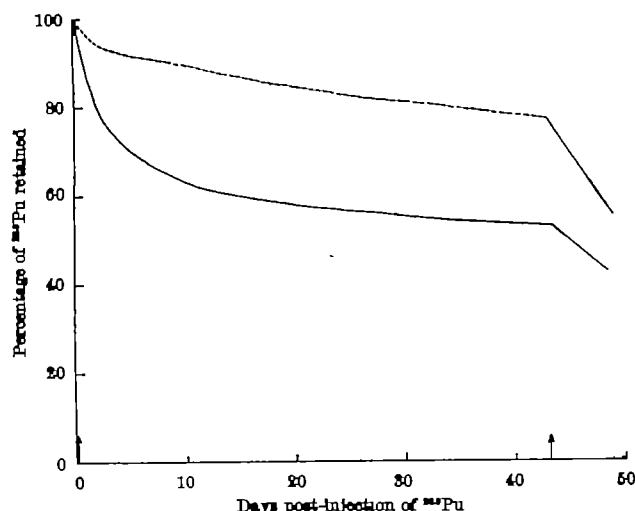


Fig. 4. Effect of DTPA on the retention of <sup>239</sup>Pu injected in the rat initially and after 43 days. Injected dose, 15 mld/kg. ---, Controls, —, treated.

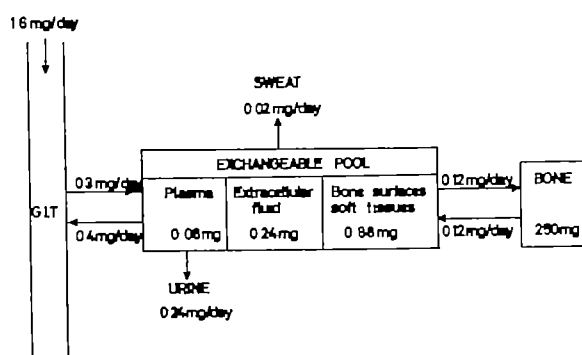


Fig. 5. Compartmental model for strontium metabolism in human adults

available are limited in application. In some cases, notably that of strontium deposited in bone, there are theoretical limitations to the efficiency of chelating agents because of the similar chemical properties of calcium and strontium. It is believed that the alteration of certain physiological mechanisms offers the most promising approach and a summary of our work is included. All experiments were designed to modify calcium deposition and resorption processes in the rat.

The rapidity with which radioactive strontium is transferred to bone is vividly shown in Fig. 1, 40 per cent being associated with skeletal tissue 30 min after injection.

Nordin *et al.*<sup>7</sup> envisage a two-compartment model in the metabolism of calcium. The first compartment is referred to as the exchangeable pool and includes the extracellular fluid calcium, the exchangeable intracellular calcium and a small fraction of skeletal calcium; it reaches equilibrium within 24 h. The second compartment comprises the skeletally fixed 'non-exchangeable pool'. Stable calcium leaves the exchangeable compartment by three routes—excretion, new bone formation and slow physical diffusion or exchange. Excretion is irreversible and is assumed to proceed at constant rate under normal physiological conditions. Bone formation and the physical processes, on the contrary, are potentially reversible and are variable parameters capable of determining the net transfer of calcium from the first compartment to the second. As a result of new bone formation and physical exchange, the bulk of the isotope is incorporated into bone mineral and, once this has occurred, the removal of calcium and, of course, associated radioactive isotopes is very slow. In the mature human, new bone formation is believed to occur at the rate of 0.01–0.02 per cent per day with the laying down of crystals in osteons at about 1 μ per day. Continual accretion and resorption are occurring, but the rate of this process declined with increasing age. The metabolic turnover of bone in a young adult is estimated at 10 per cent per annum. The metabolism of stable strontium, for comparison to calcium, is shown in Fig. 5. This is a compartmental model as designed by Dolphin and Eve<sup>8</sup>.

Success in removing bone-seeking radionuclides from the body is obviously dependent on initiation of therapy as soon as possible in order to prevent the transfer of loosely bound radionuclide to the firmly incorporated form. Thus the time-factor has an important bearing on the possible use of simple prophylactic measures before human exposure to short-term high occupational hazard and on the clinical value of any necessarily delayed therapeutic procedure.

Trusta<sup>9</sup> postulates that the formation of new bone begins with the maturation of the cartilage cell and the alteration of the matrix surrounding it to contend with the development of a vascular system which supplies the materials required for calcification. It is from the blood vessel walls that osteoblasts are laid down and lamellar bone formed. The factor inducing vascularization may be chemotactic in nature. Sobel<sup>10</sup> has shown that bone

collagen fibrils *in vitro* possess the property of nucleating hydroxyapatite crystals. Inhibition of this mechanism, which is responsible for calcification, would therefore prevent new bone formation. Fleisch and Neuman<sup>11</sup> have identified an inhibitor of this process in plasma which is inactivated by bone alkaline phosphatase. They suggest that normal calcification in bone is dependent on the local destruction of the inhibitor which they have tentatively characterized as a pyro- or poly-phosphate.

Fluoride and fluoracetate have also been shown to inhibit calcification *in vitro*, the former by blocking enolase activity at a concentration as low as  $10^{-4}$  M, the latter compound inhibiting enzymes in the tricarboxylic acid cycle, causing the accumulation of citrate in tissues<sup>12,13</sup>.

All these inhibitors were examined by us. Fluoride, fluorinated derivatives of metabolic precursors, pyro- and poly-phosphates were administered to rats either before, simultaneously with or after the intraperitoneal injection of strontium-85. The rate of excretion of the radionuclide was then determined by a whole-body counting technique. Fluoracetate, the phosphates and fluoride at a concentration in drinking-water below 75 p.p.m. did not accelerate the rate of excretion of strontium-85 compared with a control group of animals, but pre-medication for fourteen days with fluoridated water at a concentration of 150 p.p.m. significantly increased the excretion rate (Fig. 8). At this concentration no pathological changes were observed.

The process of bone resorption and remodelling is dominated by the osteoclast. This cell is controlled by parathyroid hormone which acts primarily by maintaining a steady state of supersaturation in relation to calcium and phosphate between bone and plasma, possibly by the localized production of citrate ion in areas of resorption<sup>14</sup>. Oestrogens and succinate ion may also create citrate accumulation<sup>15,16</sup>. In this respect, evidence is forthcoming which shows that citrate can dissolve hydroxyapatite crystals effectively<sup>17</sup>. Vitamin A may also be involved, directly or indirectly, in bone resorption<sup>18</sup>, either by modifying the action of parathyroid hormone or by increasing cellular permeability to allow migration of larger molecules.

Citrate is located in significant amounts on the surface of bone crystals, thus creating the conditions necessary for the localized metabolic acidosis necessary for solution of bone crystal. Citrate ion has unique biochemical properties. It has three carboxyl groups all of which are ionized at the pH of body fluids. It forms unionized but soluble complexes with the alkaline-earth metals. Therefore it behaves as a physiological chelating agent for mobilizing calcium and strontium ions.

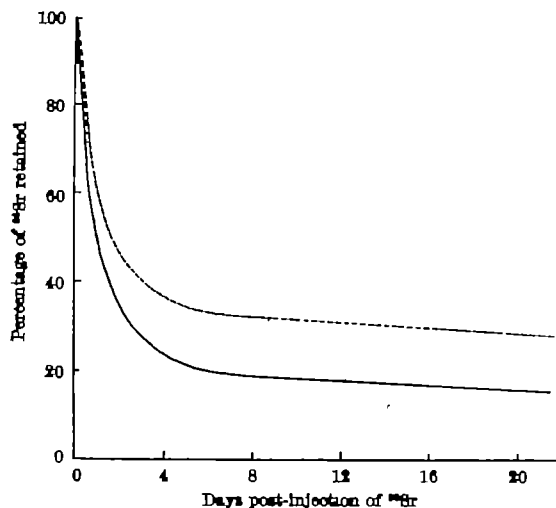


Fig. 6. Effect of sodium fluoride in drink on  $^{85}\text{Sr}$  retention in the rat. ---, 1, 10 or 75 p.p.m.; —, 150 p.p.m.

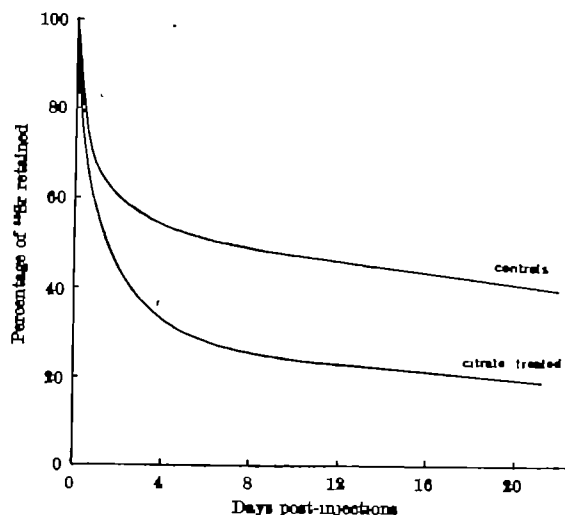


Fig. 7. Effect of sodium citrate (8.5 mM/kg body-wt.) on  $^{85}\text{Sr}$  retention in the rat

Injection of trisodium citrate into animals given strontium-85 showed that provided it was injected intraperitoneally and within 30 min of the radionuclide, then a significantly increased excretion rate of the nuclide was possible (Fig. 7). We believe this effect to be due to chelation with that strontium contained in the exchangeable pool. Citrate is rapidly metabolized in liver and kidney and thus remains effective for a limited period after injection. Introduction of citrate prior to the radionuclide is valueless if the time-interval between injections allows for metabolism of the citrate. High intracellular concentrations of citrate can be maintained by prolonged intraperitoneal or intramuscular injection, but neither of these techniques proved to be superior to a single intraperitoneal injection.

The administration of citrate, parathyroid hormone, vitamin A, the induction of a generalized metabolic acidosis with ammonium chloride or a carbon dioxide respiratory acidosis had no effect whatsoever on the excretion of that strontium-85 firmly incorporated into bone. The results obtained with parathyroid hormone are surprising and further investigations with this hormone are in progress.

Attempts to stimulate increased excretion of extracellular fluid before the injected strontium-85 became firmly incorporated were made using a mercurial diuretic and hyaluronidase. The former substance acts by blocking renal tubular reabsorption and hyaluronidase increases cellular permeability, thus increasing the volume of the extracellular compartment to produce a diuresis. Neither of these compounds altered the rate of excretion of strontium-85, alone or combined with the various stimulators or inhibitors of calcification.

In the proceedings of a scientific meeting in 1962 held in Vienna, Loutit<sup>19</sup> emphasized the role of physiological mechanisms in removing radioactive strontium: "even when strontium is injected intravenously into the normal adult, only about 10 or 15 per cent is retained after a year, so that all these small factors (referring to renal and intestinal excretion) of the normal physiological function provide 70, 80 to 90 per cent removal of strontium. If each of the factors could be increased slightly, the gain by summation would be considerable".

This fact is illustrated in Table 1, which shows the excretion of radioactive strontium from rat. An attempt has been made to modify one of the excretion routes by selective blocking of renal tubular reabsorption which would reduce the body burden even further, provided that re-equilibration occurs rapidly between extracellular fluid and exchangeable calcium, and assuming that some radioactive strontium is present in the exchangeable pool.

Salicylate ion appears to induce this mechanism of tubular block as a delayed phenomenon. If salicylate is administered immediately after injection of radioactive strontium, the eventual excretion of this radionuclide is reduced compared with controls. However, if given after 24 h, it induces enhanced excretion. Factors other than renal may therefore be involved, but alteration of kidney function is suggested as the primary event. The influence of salicylate is shown in Table 2. Although isotopic dilution of radioactive strontium has been found to be ineffective if delayed<sup>20,21</sup>, some synergistic effect can be observed if it is injected with citrate or salicylate. A summary of the various treatments used is given in Table 3.

Table 1. DISTRIBUTION OF <sup>88</sup>Sr IN EXCRETA OF RAT (MEANS OF 20)

Period (days)	Per cent injected dose Urine	Per cent injected dose Faeces
0-1	18.8±6.8	12.0±1.9
1-2	4.5±0.5	8.0±2.0
2-3	2.4±0.5	2.5±0.7
3-4	1.0±0.1	1.1±0.1
4-7	1.5±0.4	1.5±0.3
7-9	1.1±0.5	1.1±0.7
9-11	0.7±0.3	0.4±0.1
11-14	0.6±0.1	0.7±0.1
14-16	0.7±0.1	0.4±0.1
16-18	0.5±0.1	0.5±0.1
18-21	0.4±0.1	0.5±0.1

Table 2. RATIOS OF <sup>88</sup>Sr ACTIVITY IN EXCRETA RELATIVE TO CONTROLS

Period	Na salicylate (200 mg/kg) daily		Sodium citrate 3.5 mM/kg initially and daily salicylate	
	Urine	Faeces	Urine	Faeces
0-1	0.89	0.69	2.24	0.49
1-2	3.25	1.08	4.17	0.78
2-3	3.75	1.03	4.80	0.81
3-4	3.51	0.88	4.09	0.74
4-7	2.20	1.13	2.20	0.72
7-8	2.01	0.96	1.88	1.10
8-9	1.57	1.10	2.50	1.00
9-10	1.66	0.99	1.68	0.96
10-15	1.37	1.20	1.60	1.18
	Best period		Best period	
15-17	0.74	0.68	1.10	0.37
17-18	1.38	0.96	0.66	0.72
18-21	1.60	0.80	1.80	0.21
	Weekend		Weekend	
21-22	1.12	0.61	1.00	0.91

Table 3. CLASSIFICATION OF TREATMENTS TRIED TO LIMIT UPTAKE AND REDUCE THE BURDEN OF RADIOACTIVE STRONTIUM

Inhibition of calcification	Stimulation of decalcification	Increase of membrane permeability	Selective renal tubular reabsorption
Sodium hexameta-phosphate	NH <sub>4</sub> Cl acidosis	Hyaluronidase	Acetazolamide
Sodium dihydrogen pyrophosphate	Respiratory acidosis	Anoxia	Mercuryl Sodium bicarbonate
Sodium tripolyphosphate	Parathyroid hormone	Vitamin A	Sodium salicylate
Sodium decapolyphosphate	Vitamin A		
Sodium fluoride	Oestrogens	DOCA	Isotope dilution
Magnesium fluoride	Sodium citrate		Stable strontium
Sodium fluoracetate	Sodium succinate		Stable calcium
	Sodium tricar-ballylate		

In conclusion, the most fruitful approach at present is that of combined therapy—early citrate treatment in the presence of carrier strontium, followed by late treatment with sodium salicylate. Efforts are now being directed to finding the optimum conditions of treatment and our most successful experiment is shown in Fig. 8. A significant reduction in body burden is possible after twenty-one days.

### Attempts to modify the Removal of Plutonium-239 by Modification of Certain Physiological Parameters

Several centres are studying the problem of plutonium-239 removal from the body. Progress in this field is reviewed in the Hanford Symposia of 1962 and 1964 (refs. 22 and 23). Success in mobilizing plutonium-239 depends on the critical organ in which it is deposited, and the development of a series of chelating agents has resulted in the evolution of a form of therapy for its removal from skeleton and soft tissues. However, the limitations of chelating agents have been mentioned previously in this review. No published record of attempts to alter

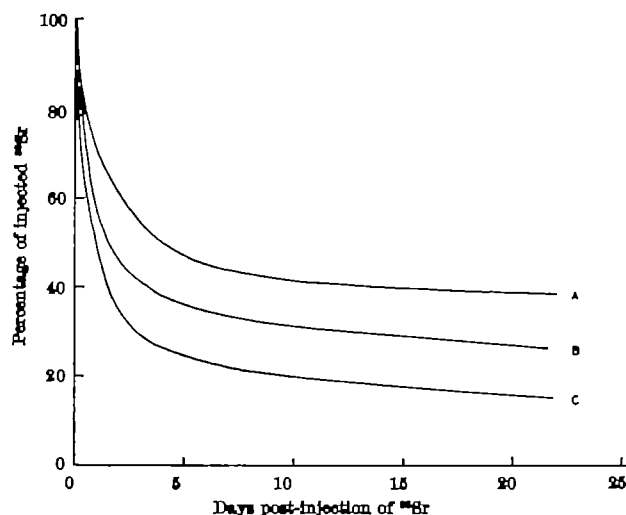


Fig. 8. Effect of combined treatments on retention of <sup>88</sup>Sr in the rat. A, Controls; B, citrate-succinate then salicylate; C, as B but with carrier strontium

the factors which control the metabolism of plutonium-239 is known and work in progress at Chapelcross is directed towards the application of such a concept as a form of therapy. A more serious problem of plutonium-239 contamination is that of the concentration of inhaled, insoluble <sup>239</sup>PuO<sub>2</sub> in the lungs and lymph nodes (Fig. 9). Little success can be claimed for the chelating agents<sup>24</sup>. Labelle and Brieger<sup>25</sup> were able to increase the clearance rate of radioactive particles from lung by inducing phagocytic activity, but Bair and Hennacy<sup>26</sup> using carbon aerosols were unable to confirm this finding.

Pulmonary vasoconstrictors, expectorants and mucolytic agents have been used without success, but the bronchodilator protocatechayl alcohol and the surface-acting polymer 'Phuronic F68' may have a marginal effect<sup>24</sup>. Some recent work by Heppleston<sup>27</sup>, with whom we are collaborating, suggests that phagocytic action may be induced within fibrous areas of the lung by means of physiologically occurring molecules. This work is in the initial stage of development.

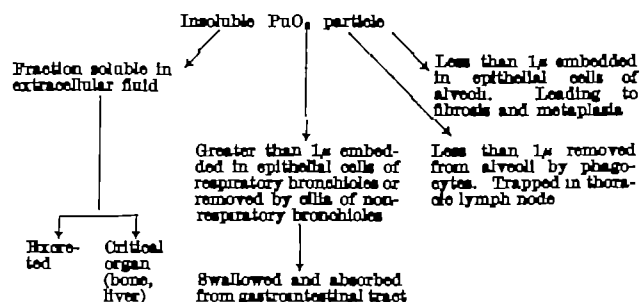


Fig. 9. Fate of inhaled plutonium dioxide

### Conclusion

This review is intended to place the problem of radionuclide contamination into perspective. Any treatment initiated by a clinician must be planned with the welfare of the patient at heart, so that risk and trauma are reduced to a minimum. The synthetically produced chelating agents have a place in therapy; but it is suggested that such treatment will not assume the same importance as it has in the mobilization of the stable metals from the body.

Successful elimination of any radionuclide should be possible, but it is essential to understand the physiological processes involved in radionuclide transfer and deposition. Only then can therapy be applied which is based on the delicate control of metabolic function so that selective

inhibition or accentuation of a particular pathway is achieved.

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## OPERATION OF A 'LAW' OF PARSIMONY IN SHAPING ANIMAL LIFE-CYCLES

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"Divvie a search," said the Irishman.

"Sure God can search for me if he wants me. My own belief is that he's not all that he sets up to be. He's not properly made and finished yet. There's somethin in us that drivin at him, and somethin out of us that drivin at him: that's certain; and the only other thing that's certain is that the somethin makes plenty of mistakes in thyrin to get there. We've got to find out its way for it as best we can, you and I . . ."

George Bernard Shaw: *The Black Girl in Search of God*

"In metaphysics my creed is short and simple. I think that the external world may be an illusion, but if it exists, it consists of events, short, small and haphazard. Order, unity and continuity are human inventions just as truly as are catalogues and encyclopaedias."

Bertrand Russell: *The Scientific Outlook*

IT is possible to class animal life-cycles either as relatively 'compact' or 'diffuse', giving these terms their normal respective meanings: 'closely packed together or condensed' and 'spread out, or not concentrated'.

As examples of the more 'compact' life-cycles we can turn to those animals the life-habits of which are generally considered to be prime examples of natural selection's tendency to favour parents which are thrifty and prudent regarding the welfare of their offspring. Here the mammals are obvious models. Bringing forth their young alive and often well-equipped for free life almost from birth, mammalian parents appear to safeguard their young against the rigours of the environment. Some care in rearing the young is also common to them, so by one means or another mammals are generally assured of a good start in life. Moreover, the young usually occupy much the same territory as the adults, often living side-by-side with them from birth. But if the mammals are such obvious examples of life-cycle 'compactness', so, too, are the many protozoans which reproduce by fission to give rise to two nearly identical individuals occupying essentially the same space that their 'parents' occupied. What could be more 'compact' than this? This also illustrates the non-phyletic nature of 'compactness' and 'diffuseness'.

I must make it clear that by 'diffuse' life-cycles I do not want to restrict my meaning only to animals with stages in their life-cycles which are spatially wide apart, but also wish to include those the life-cycles of which demand a more or less complex régime of conditions for the successive stages if they are to go to completion, and which may also involve considerable ontogenetic variation

between the stages. Thus, while birds that have breeding and rearing grounds far separated from the regions where the non-breeding adults pass their lives are obvious examples of the spatial separation of different parts of life-cycles, insects, molluscs, coelenterates and parasites can provide examples of the kind of 'diffuse' life-cycle in which varying conditions and much ontogenetic difference between various stages are seen. Some cicadas may have doubly 'diffuse' life-cycles in that the adults are markedly separated both in ontogeny and time from the egg stage, and indeed, many animals provide examples of these several aspects of 'diffuseness' within their own single life cycle.

Clearly, only the more extreme forms of relatively 'compact' or 'diffuse' life-cycle may be located readily within this system. All others will fit somewhere into the spectrum of possibilities between the two extreme conditions.

In contemplating animals with 'diffuse' life-cycles many people appear to feel either that there must be something (albeit often very subtly expressed) in their peculiar life-cycle characteristics of peculiarly high survival value to the species. Alternatively, they may think of such forms as biological curiosities—surviving forms the aberrancies of which reflect a strangely tortuous evolutionary history. Such views may be quite valid so far as they go, but what I wish to propose here is that animals with 'diffuse' life-cycles are evidences of the working of a 'law' of parsimony in evolution.

In philosophy the law of parsimony states that no more causes or forces should be assumed than are needed to account for the facts of a situation or set of events—which is scarcely a new concept. Aristotle enunciated it for biology and it lies at the basis of 'Occam's razor' and, therefore, of the scientific method itself. The law's operation in evolution depends on two principles basic to biological thought: the tendency of animal species continually to produce variation, and the action of natural selection.

Now the actual details of the multitude of life-cycles in existence, whether species are free-living or parasitic, whether growth occurs continuously or in a series of steps that involve moults or metamorphoses, are of secondary importance in the present context. Nor does it really matter whether reproduction is sexual, parthenogenetic, or the result of simple fission. What does matter is that, for a species to maintain itself in the face of both internal

and external factors which cause mortality, it must produce enough new recruits to replace the dead, and to do this enough older members of the species must remain alive to produce eggs or young. Of course, in certain species or at certain times the adults may all be dead long before the young they produce will themselves be able to reproduce.

Let us now consider the life-cycle of the Atlantic eel, typical of the more 'diffuse' type. The young eels migrate, or are passively transported by ocean currents, to the land masses around the Atlantic, when they may move up rivers and become mature. During this trip, profound physiological changes accompany the changing external conditions that the migrating or transported individuals must encounter. Adult eels, which cannot reproduce in fresh water, have to return to one main region in the Atlantic to breed, again involving a trip of remarkable length and duration (though it is only fair to point out that Tucker<sup>1</sup> recently proposed that only the North American adults of the Atlantic eel actually complete this journey). How can we make any sense of a life-cycle involving a journey of this sort?

Suppose we assume the ancestral eel stock to have been wholly marine. According to Bertin<sup>2</sup> this would probably be a reasonable assumption, as he proposed that eels (marine in origin) have become dispersed throughout the world's oceans, the adults gradually having acquired tolerance to changes in climate and salinity enabling them to invade many fresh waters, while the eggs and very young free-living stages are still confined to what are essentially their ancestral salinity and climatic types, which are themselves now found only in certain sections of the oceans. Indeed, Bertin actually wrote that: "Because their need of warmth at the time of spawning brings them back every year into the warm waters, it has been necessary for their larvae to adapt themselves to longer and longer journeys." This statement is probably correct, but it fails to satisfy analytically. Why has it been necessary for the larvae to adapt themselves to progressively greater journeys? After all, many fish of fairly recent marine origin have successfully invaded fresh waters with all the stages of their life-cycle.

I think this illustrates what every biologist knows full well: that the forces of natural selection act on animals at every stage of their life-cycle but that these forces are often quite different at the different stages, and of course the different stages represent different kinds of raw material on which natural selection can act. For example, an aquatic insect could succumb to the influence of some environmental factor during its nymphal aquatic existence or while it is a winged aerial adult or as an egg. Yet the environmental forces that can impinge on these three stages may differ profoundly from one another. Here I suggest that almost any degree of complexity, oddness—in short 'diffuseness'—may be present in a life-cycle which does not interfere with its completion at an ecologically satisfactory level. This is the parsimony principle at work.

Here, then, I am really suggesting that a way of thinking about life-cycles that seems prevalent to me should be inverted. For example, in considering the intricacies of a parasite's life-cycle or the spatially spread out life-cycles of many birds, many seem to believe them to be principally delicate and subtle adaptations to habitat or environment; perhaps, too, as indicative of trends towards certain kinds of biological perfection. But I would say merely that they should be regarded as causal outcomes of evolutionary development, given the particular characters of the evolving stock from which they came at the moment of its exposure to effectively selective forces. This does not mean that such intricacies are not cause for wonder or that they are not true adaptations in the usual sense. But I would like to escape from the notion (teleological in overtones) that Nature is always driving at something, which I think colours so much thought on these subjects

even in an age when science is supposed to have emancipated itself from teleology.

The essential task in species survival—completion of the life-cycle—can be done simply and 'compactly' or complexly and 'diffusely'. From the animal's point of view, that it is done at all is what matters. The details may be essentially irrelevant, in themselves (however interesting to us, in themselves), to this central and, perhaps, only purpose of survival in evolution.

Perhaps we can also apply this general method of thinking in a way which will prove useful in considering certain other features of animal life. In a recent discussion with colleagues at which some aspects of the life-cycle of a parasite were raised the question was posed of the utility to the parasite of a complexly multi-layered cyst. It was certainly valid to enquire, as was done, as to the various functions implied by the presence of this complex layer. But I wondered why the enquiry seemed limited by the assumption that several aspects of the parasite's integrity within the cyst were being safeguarded by the layer. I thought that there was no *a priori* reason for thinking that a multi-layered structure must function in more than one capacity. It may do; but this is a matter which only further investigation can decide. The parasite in question, when in the cyst, is very small, with a very low total number of cells. It seems to me that it might easily be more energetically efficient for it to secrete what impresses us as a complex covering, using more than one group of its small number of cells to do the job, than to opt for a simple covering which, if it were to be of the requisite thickness or strength, would be beyond the capacity of any one of its groups of secretory cells. The analogy between this problem and the question of life-cycles is just this: apparently complex life-cycles, which we may suspect express in some subtle fashion peculiar advantages in the lives of their possessors, may merely be doing a basically simple job in a roundabout manner. But doing it: that is the point.

Actually, it appeared likely that a main function of the cyst wall is to safeguard loss of water. It was, however, objected that evidence suggested that the cyst material was not an effective water-proofing agent, and that an additional layer of wax or fat would be needed for that. But again, this argument could be invalid. So long as the cyst wall is sufficiently effective in preventing loss of water for the encysted animal to survive in the actual conditions of its encystment and carry on to the next stage in its life-cycle, then the cyst wall has done its task, whether it be made of lipidal material, chitin, plastic foam or feather dusters!

Now what the eventual outcome of the investigation I have referred to here will be is irrelevant for my purposes. It will, in the end, be decided in the objective empirical manner of all biological investigations. But it is important that we formulate a correct approach to such investigations, and I have tried to show that there are two ways of looking at this problem. The first of these rather makes assumptions as to detailed and intricate functioning, arguing from *our* conception of the complex, the second allows for a simpler explanation, at least as a possibility. An investigator armed with both approaches would, I believe, be readier to design a programme of analytical research, that could consider several possibilities, and would be less likely to reach a possible impasse in his research because of looking for functions that may not in fact be implied, except in his own mind.

To return to life-cycles, it may be, of course, that a mammal, with elaborate associations of juvenile and maternal parts of the life-cycle, will manifest some general advantage over another animal which occupies much the same territory but the life-cycle of which is much more 'diffuse'. But there is surely no fixed rule about this. Many splendidly successful bird species breed in one hemisphere, but spend at least half of their lives in the other. The forces in evolution producing such life-

cycles have been the subject of endless debate, and may have to do with changing climates, movements of land masses and the like. But the interesting thing here is that whenever we postulate that this kind of stretching out of a life-cycle is to be explained in some such terms—within an evolutionary frame—we can cite other examples where animal groups have simply become separated from parent stocks, or represent surviving forms of a stock many of whose earlier representatives have become extinct, and yet do not have diffuse life-cycles.

Perhaps then we can put the matter like this: natural selection will be free to act independently on the various parts of the life-cycle of an animal; in some animals this will mean that entirely different selective forces will act on different parts of the life-cycle; yet so long as the selective forces are not so severe as to break any part of the life-cycle the species will be able to maintain itself. The particular (or peculiar) feature of a life-cycle that we examine will be the evolutionary result of a particular group of biological responses of a particular life-history phase to certain particular effects of the environment.

L. C. Cole<sup>3</sup> has attempted to show that life-histories of organisms always have meaning and significance in terms of the survival of the species. He dealt particularly with intrinsic rates of increase, with iteroparity and semelparity, to show the profound effects that these properties can have in the maintenance and growth of populations, and he sketched the evolutionary strengths and weaknesses which might be inferred about a species through their consideration. Nothing I have written in any degree disparages what Cole put forward, and I regard as great

the significance of his essay to ecology and evolution. But Cole was dealing with parameters of populations which govern numbers, and through numbers evolutionary success. In another sense Cole clearly showed that these parameters are also adaptations of the species to its environment and way of life, ecological adaptations rather than the morphological and physiological ones we are, even to-day, more used to, but adaptations nevertheless.

In the last analysis the continuance of both populations and species turns on the effectiveness with which life-cycles are completed, despite changing conditions. The extinction of both populations and species is a matter of broken life-cycles rather than death of individuals; that is what makes habitat destruction so sore a point with modern conservationists, and also what frequently places so difficult a burden on them in delimiting and defining habitat.

The ability of a life-cycle to maintain the existence of a population or species or an evolving stock of animals will depend on the genetics of the animal, the particular features of the life-cycle (including adaptations of greater or lesser perfection) at the moment of selection, and these determine what fraction of the population survives and, therefore, the character of the future population or those derived from it. But, as there are many ways to skin a cat, there are many ways to complete a life-cycle, and no animal group has a mortgage on the way to do this successfully.

<sup>1</sup> Tucker, D. W., *Nature*, 133, 495 (1959).

<sup>2</sup> Berlin, L., *Kels* (Oliver-Hume, London, 1956).

<sup>3</sup> Cole, L. C., *Quart. Rev. Biol.*, 29, 103 (1954).

## OBITUARIES

### Dr. Ricardo Zariquiey Alvarez

DR. RICARDO ZARIQUIEY ALVAREZ, who died at his home in Barcelona on January 27, 1965, was not only a well-known paediatrician but also an authority on certain Spanish Coleoptera, notably the Bathyscininae, and on the decapod Crustacea of the Iberian Peninsula.

Ricardo Zariquiey Alvarez was born in Barcelona on January 3, 1897, and was educated at the Jesuits school. After graduating with distinction in medicine at the University of Barcelona in 1918, he studied in Paris and Lausanne in order to specialize in paediatrics. Like his father, Dr. Ricardo Zariquiey Oenarro, whose medical practice he eventually took over, he was keenly interested in natural history. Together with his father, he made extensive collections of Coleoptera, especially cavernicolous ones, and in a series of papers published during 1917–27, he described many new or interesting species from a wide area of northern Spain. Other arthropod specialists have paid tribute to the two Zariquieys by naming three genera or sub-genera and twenty-eight species or sub-species after them. Their collection of Coleoptera has been bequeathed to the Instituto Español de Entomología, Madrid.

About 1934 his father became interested in the decapod crustaceans of which he amassed an extensive collection and on which he published nine papers before he died in 1943. Dr. Zariquiey Alvarez then devoted his leisure time almost exclusively to the Decapoda, although in 1944 he published a paper on the distribution of the dipteran genus, *Phlebotomus*, in Spain. When the civil war ended he built a holiday bungalow near Cadaqués on the Costa Brava, and during the next twenty years he made a thorough investigation of the decapod fauna of that coast from Port de la Selva to the Bay of Rosas. Visits to the fish markets of Rosas and Barcelona yielded some decapod species new to science or new to the Spanish fauna. Local fishermen brought him any unusual finds,

and various marine and fishery stations in Spain sent him material for identification.

Dr. Zariquiey was always rather diffident about his hobby, regarding himself as an amateur carcinologist, and frequently consulted specialists in the Paris, Leiden and British museums and elsewhere. He also invited a few specialists to his home in Cadaqués and it was there that the small "Groupe d'Études carcinologiques" was formed in 1955. The main object of the group, which met again in Barcelona in 1955 and in Naples in 1959, was the compilation of a critical check-list of the Mediterranean decapod Crustacea. Zariquiey's suggestion, based on an unsurpassed knowledge of the living animals, that some of the so-called variable species of the museum taxonomist were in reality mixtures of two or more species, met with initial scepticism. But later on specialists had to acknowledge the correctness of this view. His results were published in a series of twenty-five papers during 1945–64; his book on the decapod Crustacea of the Mediterranean coast of Spain (1946) will soon be superseded by an up-to-date revision of the Decapoda of the Iberian Peninsula to be published posthumously. The whole of the Zariquiey collection of decapods has been bequeathed to the comparatively new Instituto de Investigaciones Pesqueras, Barcelona.

In addition to his presidency of the Institución Catalana de Storia Natural, Zariquiey was closely associated with numerous scientific societies in Spain; he was a life-member of the Entomological Society of France and a member of the board of advisory editors of *Crustaceana* from its inception. A rather shy and retiring man, he was exceedingly kind and took a genuine interest in the work and personal affairs of the simple fisher folk from whom he obtained specimens. One had only to see him with his grandchildren to realize how good a children's doctor he was. Those of us who were privileged to visit him in his happy and most hospitable home will always

cherish the memory of those days at Cadaqués; his enthusiasm for crustaceans was most stimulating. Our deepest sympathy goes to his widow, Señora Mercedes Colom de Zariquiey, to whom he was devoted, and to his son, his four daughters and their families.

ISABELLA GORDON

### Mr. I. Izsak

On April 21, 1965, Imre Izsak, chief of satellite research and analysis of the Smithsonian Astrophysical Observatory and lecturer at Harvard University, Cambridge, Massachusetts, died of a heart attack at the age of thirty-six. He was in Paris attending a COSPAR symposium on trajectories of artificial celestial bodies as determined from observations.

In the brief years of his scientific career, he had established himself as a pre-eminent authority on geodesy and had made significant contributions to the study of celestial mechanics. His death is both a deep personal tragedy and an irreparable loss to the scientific community.

Born in the small town of Zalaegerszeg, some two hundred miles from Budapest, Izsak attended the University of Budapest, where he worked in astronomy under the late Karoly Lascovszky (who also later joined the Smithsonian Astrophysical Observatory) and specialized in the investigation of variable stars and galactic clusters. Fleeing Hungary during the 1956 revolution, he began work on solar physics at the Zurich Observatory in November of that year. Two years later he emigrated to the United States and, after a brief period at the Observatory of the University of Cincinnati, joined the staff of the Smithsonian Astrophysical Observatory. On February 24, 1964, he became a citizen of the United States of America. He lived in Cambridge with his wife Emily and an infant son, Andrew.

Izsak pioneered in the development of both theoretical and practical aspects of the application of satellite data to geodesy. He directed the writing of a complex tesseral harmonics programme, which served as a powerful tool in his investigation of geodesy. From it he made determinations of the gravitational potential of the Earth. These results are the best representation so far of the Earth's potential.

Izsak also applied himself to the problem of refining the determinations of the positions of the Observatory's twelve Baker-Nunn camera stations for tracking satellites. In a dynamic approach, he used the Observatory's differential orbit improvement programme to obtain residuals in satellite position, and then analysed these residuals in his own tesseral harmonics programme to determine changes in station co-ordinates and the tesseral harmonics of the geopotential. These latter quantities are strongly correlated, and one cannot solve for one without solving for the other. Shortly before his death, Izsak directed the production of a new computer programme to combine the data from Dr. George Veis's geometric approach with that of his own dynamic approach. This has the effect of reducing the correlation of station positions and the tesseral harmonics. These investigations have shown that the geometric and dynamic methods agree well and have produced the most accurate global measurements of points on the Earth: the locations of the stations are all now known to within a few tens of metres, and those with the best determinations to within 10–20 metres. At the beginning of the programme, the corresponding precision was about 100 metres.

As a by-product of these undertakings, Izsak developed techniques for making numerical and analytical calculations using electronic computers. These programmes, which involve problems in celestial mechanics and have significant implications for interplanetary navigation, carry out algebraic operations that would take a life-time to do by hand.

The geodetic work in which Izsak played such a fundamental part will culminate in the publication of the Smithsonian Standard Earth, a concept he and others initiated. That model, which is expected to be the most accurate representation of these features yet available, will incorporate the results of his investigations of the geopotential and of the station co-ordinates.

Izsak wrote a number of scientific papers, including analyses of satellite orbits, a determination of the ellipticity of the Earth's equator, a second-order solution of Vinti's dynamical problem, and various notes on the mechanization of the tedious algebra of celestial mechanics.

Izsak was a warm, charming, witty, gentle person. He gained not only the deep respect but also the spontaneous affection of his colleagues. His association with them was one of mutual inspiration.

### Dr. R. F. Farquharson, M.B.E.

DR. R. F. FARQUHARSON died suddenly on June 1 at the age of sixty-eight while attending a meeting of the Medical Research Council in Ottawa.

With his death, Canada has lost the pre-eminent figure of its medical profession. To his work as teacher, research worker, consultant and statesman he brought a fine mind and a great heart. That he cared for people was obvious and everyone felt that his wise judgment was always available to them, as indeed it was. He gave the impression that he was prepared to be generous with his unusual talents, and this was coupled with an equally unusual ability to put them to work.

Early in his career it was apparent that he was a man who could accept responsibility, and over the years it was given to him to a degree that will not be seen again. For almost twenty years his advice was called for in connexion with almost every new medical enterprise. Both professional and lay organizations sought his counsel at their inception and some were fortunate enough to be able to continue to count him as one of their officers. Equally deftly he helped other and older groups who were in trouble and who, as they searched for new courses, needed his wisdom and the backing of his moral authority. It was the good fortune of the Royal College of Physicians and Surgeons of Canada to have him as its president in the crucial post-war years 1945–47, when the entire structure of the College was altered so that it could accept its responsibilities to the country for medicine at the specialist and consultant level. He presided at the transformation in 1960 of the Division of Medical Research, National Research Council, into the virtually autonomous Medical Research Council. During the Second World War he was consultant in medicine to the Director of Medical Services of the Royal Canadian Air Force. Immediately after the War he worked in association with the Director-General of Treatment Services, Department of Veterans' Affairs, and had much to do with the high standard of patient care which was evolved in association with the medical schools. From 1949 until 1962 he was a member of the Defence Research Board. During the discharge of these and innumerable other duties he was held in high esteem as a teacher. He was a brilliant consulting physician who remained to the end quietly and privately surprised that others did not have the skill at the bedside which was his.

It was natural that many honours should come his way. These he carried as lightly and with as little sense of self-importance as he carried the many secrets which were shared with him. In 1946 he was made a member of the Order of the British Empire, and in 1949 came the first of eight honorary degrees. He was elected a Fellow of the Royal Society of Canada in 1960. The clinical investigation unit in his own hospital has carried his name



for some years. A new science building in York University (Canada) will shortly bear it.

Dr. Farquharson is survived by his wife and two daughters.

G. M. BROWN

#### Prof. H. B. Gilliland

PROF. H. B. GILLILAND died on June 23 in Pietermaritzburg, at the age of fifty-four. From 1935 until 1954 he was a member of the staff in the Department of Botany, University of the Witwatersrand. During the Second World War he served in North Africa, working his way from private to major. From 1955 until

1965 he was professor of botany at the University of Singapore.

During 1964 Prof. Gilliland served as acting vice-chancellor of the University of Singapore. He was created professor emeritus in June 1965. Early in 1965 he took up the post of senior lecturer in botany in the University of Natal, Pietermaritzburg.

Prof. Gilliland was well known for his investigations in taxonomy and ecology, having published important contributions to these subjects both in Africa and in Singapore. He leaves a widow, a son and a daughter. His passing is greatly regretted by a wide circle of colleagues and old students.

J. F. V. PHILLIPS

## NEWS and VIEWS

### Chairman of the Social Science Research Council

IN a written reply to a question in the House of Commons on August 5, Mr. Anthony Crosland, the Secretary of State for Education and Science, announced that the Government had accepted in principle the recommendation of the Heyworth Committee to establish a Social Science Research Council. Mr. Crosland said that an Order in Council was being prepared, specifying the objects of the new body and declaring it to be a Research Council for the purposes of the Science and Technology Act 1965; the draft would be laid before Parliament, in accordance with Section 1 (4) of the Act, after the new Council had been established by Royal Charter. Mr. Crosland also stated that Dr. M. Young had been appointed chairman of the new Research Council. [See also *Nature*, 207, 559, 576; 1965.]

#### Dr. M. Young

DR. M. YOUNG has been a Fellow of Churchill College, Cambridge, since 1961, and director of the Institute of Community Studies since 1953. He was born in 1915 and was educated at Dartington Hall School and at the University of London. Later he was called to the Bar at Gray's Inn. Dr. Young was director of Political and Economic Planning from 1941 until 1945, and secretary of the Research Department of the Labour Party from 1945 until 1951. In 1956 Dr. Young became chairman of the Consumers' Association, and in 1959 he assumed the chairmanship of the Advisory Centre for Education. He has been a member of the Central Advisory Council for Education since 1963. Dr. Young is the author of *Family and Kinship in East London* (with Peter Willmott) (1957), *The Rise of the Meritocracy* (1959), and *Family and Class in a London Suburb* (with Peter Willmott) (1960).

### Natural Environment Research Council

MR. D. J. MACLEAN has been seconded from the Road Research Laboratory to the Natural Environment Research Council as a deputy chief scientific officer. As the senior officer (scientific) under the secretary of the council, Mr. Maclean will be responsible mainly for matters of scientific policy and for scientific programmes and for the Council's research grants and training awards. Until his secondment, Mr. Maclean was deputy director of the Materials and Construction Division of the Road Research Laboratory, Ministry of Transport, at Harmondsworth. He is best known for his work on soil mechanics and was largely responsible for the very successful book on soil mechanics for road engineers which was published by the Road Research Laboratory in 1951.

### Applied Physical Sciences in the University of Reading: Prof. P. B. Fellgett

DR. P. B. FELLGETT, head of the Astronomical Instrumentation Division of the Royal Observatory, Edinburgh,

has been appointed to a professorship in applied physical sciences in the University of Reading. Dr. Fellgett's first experience of research was towards the end of the Second World War, when he was a member of a group dealing with infra-red problems, under the leadership of Dr. (now Sir Gordon) G. B. M. Sutherland, in the Department of Colloid Science, Cambridge. Experience gained there stood him in good stead when in 1947 he became a research student at the Cambridge Observatories, where he obtained a Ph.D. for work on infra-red magnitudes of stars and the theory of ultimate sensitivity of radiation detectors. His thesis included a proposal for, and theoretical discussion of, an interference spectrometer of original design. He spent 1951-52 at the Lick Observatory and then returned to Cambridge, to follow up the ideas developed in his thesis by constructing a multi-channel interference spectrometer for use on stellar spectra in the near infra-red. In addition, with Linfoot, he applied information theory to the evaluation of optical images. In 1955 he joined the Cambridge Observatories staff as a senior observer. About this time he became convinced of the importance of using modern machine methods for the measurement of astronomical photographs taken with Schmidt telescopes, where traditional methods are much too slow to extract all the recorded information. He investigated the problems associated with designing a suitable machine, processing its output, and storing in a readily accessible form the large quantities of numerical data that would be produced. In 1959 he became a principal scientific officer at the Royal Observatory, Edinburgh, and there has taken a leading part in the development of such a machine (*Galaxy*), now under construction by Ferranti. He has also built up a strong electronics group at Edinburgh, has been responsible for elaborate control equipment for the new twin 18-in. telescopes, and has converted most of the Royal Observatory's measuring equipment, so as to give digital outputs suitable for immediate processing by an electronic computer. Dr. Fellgett has always been an enthusiastic advocate of the use of the most advanced optical and electronic techniques in observational astronomy, and an outspoken critic of those reluctant to depart from traditional methods and conventional instrumental design.

### Engineering Production at the Bradford Institute of Technology: Prof. R. C. Brewer

MR. R. C. BREWER, senior lecturer in the Production Engineering and Management Studies Section of the Department of Mechanical Engineering, Imperial College of Science and Technology, has been appointed to the newly established chair of engineering production at the Bradford Institute of Technology (proposed University of Bradford). Mr. Brewer was, for some nine years, lecturer, and later senior lecturer, at the Royal College of Advanced Technology, Salford. His research there was mainly concerned with the fundamentals of the machining

processes and the development of refractory oxides as tool materials. He is a British member of the International Institution for Production Research (C.I.R.P.) and is a United Kingdom member of the C.I.R.P./O.E.C.D., Co-operative Research Programme in Machining, for which he is chairman of the Statistics Sub-Group and secretary of the Surface Finish Sub-Group. In addition to research papers and *Reports of the Machine Tool Laboratory, Imperial College*, Mr. Brewer has published the following books: *The Numerical Control of Machine Tools* (1958), and *Manufacturing Properties of Materials* (1963). At Imperial College Mr. Brewer has been responsible for teaching and research of manufacturing processes. The chair in engineering production at Bradford has been established with the aim of applying mechanical engineering to production, as distinct from industrial or management aspects of production which are studied at the Institute's Management Centre. Mr. Brewer's responsibilities will include developing teaching and research in this field.

#### Polymer Chemistry In McGill University, Montreal:

Prof. L. E. St. Pierre

DR. LEON E. ST. PIERRE, manager of the Polymer and Interface Studies Section, General Electric Research Laboratory, Schenectady, New York, has been appointed the first professor of polymer chemistry in McGill University, Montreal, which is situated in the Otto Maas Chemistry Building, a new laboratory on the campus at the corner of Sherbrooke and University Streets. The new laboratory will pursue fundamental research on the chemistry of polymers and expand opportunities for graduate students to train themselves in this specialized field. Industries based on polymers, notably in textiles, plastics and elastomers, will thus have an increased pool of already specialized talent from which to recruit staff, in addition to the benefit of additional knowledge of the chemical processes and mechanisms of polymerization. Dr. St. Pierre is a native of Edmonton and was educated at the University of Alberta, where he graduated *magna cum laude*, and the University of Notre Dame, Notre Dame, Indiana, where he earned his Ph.D. degree in 1954. Since that time he has conducted research on various aspects of polymer chemistry at the General Electric Research Laboratory, and he has published numerous scientific articles in professional journals.

The new laboratory occupies almost all of the fourth floor of the two blocks forming the east wing of the Otto Maas Chemistry Building, and in addition to normal service and equipment, it will have its own constant-temperature and instrument rooms. Although organized as a separate, discrete unit, it will be situated close to those specialized laboratories of particular use in polymer chemistry: radiochemistry, mass spectrometers, ultra-centrifuge and electrophoresis laboratories. Nuclear magnetic resonance, ultra-violet and infra-red equipment are also situated nearby. The new chemistry building, although not yet formally opened, was occupied by the end of June, and all parts, including the polymer laboratory, are expected to be working when classes commence in September. Twenty industries and associations, with an interest in polymer chemistry, have contributed to the capital costs and some operating expenses of the new Laboratory. In addition to its new research facilities, the Department of Chemistry, under Dr. C. B. Purves, chairman, has existing research laboratories of inorganic, physical and organic chemistry, radiochemistry and pulp and paper chemistry, the last-mentioned being also an integral part of the Pulp and Paper Research Institute of Canada.

#### Organic Chemistry In the University of Reading:

Prof. D. Bryce-Smith

DR. D. BRYCE-SMITH has been appointed to the newly established chair of organic chemistry in the University

of Reading. He was at Bancroft's School, Essex and afterwards studied at the South-west Essex Technical College, the Sir John Cass Technical Institute and the West Ham Municipal College. In 1945 he became a research assistant with Powell Duffryn Research, Ltd. and from 1946 until 1948 was a research chemist for Dufaycolour, Ltd. For the following three years he carried out research for a Ph.D. at Bedford College, University of London. From 1951 until 1955 he held an Imperial Chemical Industries postdoctoral research fellowship at King's College, London, and in the latter year was appointed assistant lecturer at the same College. He took up his post as lecturer in the University of Reading in 1956, being promoted reader in 1963. He was awarded a D.Sc. of the University of London in 1960. Dr. Bryce-Smith's main research interests are in the development of new synthetic methods in organic chemistry and in the study of reaction mechanisms; his principal fields of work have been the photochemistry of organic compounds and organometallic compounds. Other topics of his work falling outside these two main fields include catalytic reactions of acetylenes on noble metals, the chemistry of tropylium salts, syntheses of carboxylic acid anhydrides and *p*-quinones, and the reactions of silver benzoate-halogen complexes with aromatic compounds.

#### Physical and Inorganic Chemistry In the Chelsea College of Science and Technology: Prof. H. J. V. Tyrrell

DR. H. J. V. TYRRELL has been appointed to the chair of physical and inorganic chemistry at the Chelsea College of Science and Technology. Dr. Tyrrell was educated at Newport (Monmouthshire) High School, and at Jesus College, Oxford, which he entered in 1938 as a Welsh Foundation Scholar in chemistry. After graduating in 1942 with honours in chemistry, he held several industrial appointments before going to the University of Sheffield as assistant lecturer in chemistry in 1947. His appointment coincided with the beginning of the post-war expansion of the Department, and he has had long experience of the problems associated with such developments. During his period in industry, Dr. Tyrrell had been concerned mainly with the electrochemical production of metal powders, and with corrosion problems, and it was experience in the latter field which led him to begin an investigation of electrolytic thermocouples at Sheffield. This led, in turn, to a general interest in non-isothermal systems, and in the thermodynamics of irreversible processes, reflected in his monograph on "Diffusion and Heat Flow in Liquids", published in 1961. He has also been interested in the thermodynamics of complex equilibria in solution and in spectroscopic topics. In 1963 he was awarded the degree of D.Sc. by the University of Oxford, and has recently become reader in physical chemistry in the University of Sheffield. For many years he has been closely associated with the work of the Chemical Society, first as local representative for Sheffield and then as a member of the Publications Committee. Dr. Tyrrell's experience in industry and in the academic field will be valuable in his new appointment to a College which is strongly orientated towards technology and which will undergo many changes during the next few years.

#### The Overseas Development Institute

THE annual report for the year April 1964-March 1965 of the Overseas Development Institute records the firm establishment of the Institute as a centre dealing with most aspects of aid and development (Pp. 24. London: The Overseas Development Institute, Ltd., 1965). A large two-day conference on the economic and social development work of the United Nations was sponsored by the Institute. It is recorded that the library has collected about 2,500 books and pamphlets and receives more than 100 periodicals regularly. The basis of all the work of

the Institute remains its study programme, and the year was the last full year in which financial help will be received from the Nuffield Foundation for studies of development problems. Five studies have been published dealing with British aid, one with German aid, another with Japanese aid, and a third on the Jeanneney Report dealing with French aid, as well as others dealing with India at 'mid-passage', aid to Africa, aid to the West Indies and with assistance rendered 'Not by Governments Alone'. Other studies during the year have included a special examination of private investment and one of British aid to agriculture. Projected investigations include one of recipients, another of donors, an annual survey of Britain's aid problem and others dealing with trade and with the private sectors. Financed by a special grant from the Nuffield Foundation, the first three of the Institute's Nuffield Fellows went to Uganda, Kenya and Tanganyika in the summer of 1963, and three more Fellows were sent out in 1964. A further and increased grant from the Nuffield Foundation has made it possible to appoint six Fellows this year for 1965-67 and six next year for 1966-68. The Governments which will employ the Fellows are asking for twenty new Fellows this year, and applications have been received from about fifty candidates in the United Kingdom.

#### Analysis and Planning of Management

In an article reprinted from *Personnel Management*, June 1965, Mr. M. Hull argues that the analysis and planning of manpower have been neglected both at the level of the firm and over the economy as a whole. After describing the technical potential of the computer in this field, he points out that standardized codings of labour are required for educational and occupational purposes. He suggests that a statistical manpower grid, derived from the records of firms with mechanized payrolls, ideally coding on similar lines, could provide valuable information on the utilization of manpower, including correlations between industrial usefulness and academic qualifications. Such a system, he considers, is both technically and economically possible and would enable the supply of labour at all levels to be related better to needs.

#### Abbé Lazzaro Spallanzani

It is just 200 years since the Abbé Lazzaro Spallanzani (1729-99) anticipated Pasteur's work and that of John Tyndall when he banished the idea of spontaneous regeneration of living matter, a contribution soon forgotten since it was beyond the comprehension of the scientists of his day. Philosophers such as Diderot had directed attention to this question, discussing in his *Interpretation of Nature* whether living matter could combine with living matter, whether "the energy of a living molecule could vary by itself or according to the dead or living matter with which it united". The two priests Needham and Spallanzani took up the theme—after Needham had studied "very small organisms" with Buffon. Needham visualized a productive or vegetative force charged with the formation of organic matter. Spallanzani examined Needham's method of enclosing putrescible matter in vessels warmed to produce animalcules (as he thought), and the controversy between the two opened. Spallanzani realized that Needham had not used a sufficiently high temperature to destroy bacteria or "seeds" of living matter as he called them. By avoiding porous cork or stoppers on vessels, and using sealed vessels maintained for an hour in boiling water, Spallanzani found on opening them that "not the slightest trace of animalcules remained, though I had examined with a microscope the infusions from 19 different vessels". Despite his accurate observations and the controlled conditions which he used to verify his results, Spallanzani was little appreciated, and the full significance of his work

had to await the work of Pasteur and of Nicolas Appert. Even the vigorous attack on Needham by Voltaire, who was ever ready to cross a nib to deride "the Irish Jesuit" as he called him, did not help recognition of Spallanzani's work. Voltaire jested at a race of so-called eels which had their origin in gravy from boiled mutton; at philosophers who claimed there is no germ, that all is regenerated by a vital force of nature. He derided "clever scientists taken in by a Jesuit", although Needham had been "so triumphantly refuted by M. Spallanzani".

#### Museum of Comparative Zoology, Harvard University

THE annual report of the Museum of Comparative Zoology at Harvard University directs attention to the considerable expansion which has taken place during 1963-64 both in teaching and research activities (Pp. 40. Cambridge, Mass.: Museum of Comparative Zoology, Harvard University, 1965). As in previous years, many members of staff were invited to give lectures at congresses, conferences and seminars. Field research included expeditions to East Africa, Anatolia, Turkey, and an Indian Ocean investigation aboard the R/V *Anton Brum*. Research centred around evolutionary biology and such problems as: How did the enormous diversity of life on this world evolve?; What are the mechanisms, the causal determinants and the environmental conditions? Recognizing that it is as true to-day as it was 200 years ago that sound taxonomy is the indispensable basis of all research in zoology and botany, at least part of the time of the staff is devoted to basic taxonomic researches. In this connexion Prof. F. M. Carpenter has nearly completed his insect volume for the *Treatise on Invertebrate Paleontology*, and Dr. B. W. Evans has completed his revision of the genus *Rhabdopyris*, probably the most generalized group of the large sub-family *Epyrinae*.

#### The University Museum, Oxford

ON May 1, 1964, the Delegacy for the University Museum, established in 1853, ceased to exist, its place being taken by the Delegacy for the Science Area. Fuller responsibility for the Museum proper is now vested in the Committee for the Scientific Collections. In view of serious overcrowding and the need to provide for the proper display and expansion of the collections, the suggestion in the Holford Report that new accommodation for the Pitt-Rivers Museum should be provided, in association with the Institute of Social Anthropology and the Research Laboratory for Archaeology, was welcomed. For the immediate future, a modern, artificially-lit exhibition gallery, of approximately 600 square feet, has been built immediately inside the Pitt-Rivers Museum court at the foot of the main entrance stairs from the University Museum (Seventy-sixth Report of the Delegates of the University Museum and First Report of the Delegates of the Science Area. (For the year ending July 31, 1964.) Supplement No. 4 to the *University Gazette*, March 1965. Pp. 133. Oxford: The University. 10s.).

#### Catalogue of Bronze Implements

THE card catalogue of British bronze implements, started by the British Association in 1913, has since 1955 been on loan to the Department of European Archaeology at Oxford, where it has been brought up to date under the supervision of Prof. C. F. C. Hawkes. Early in October this year it will be returned to the British Museum and installed in the new Students' Room of the Sub-department of Prehistory and Roman Britain.

#### Diamonds In Metalworking

THE use of diamond tooling in machining very hard materials has been known for many years. The problem of sintered tungsten carbide in this connexion was faced during the 1920's and only diamond was found hard enough to solve it satisfactorily. From this inception

came the idea of research into design of suitable diamond tools, embodying stones of different grades and sizes, to machine many other hard materials from ceramics to concrete. But to-day diamond has many industrial uses where reliance solely on the hardness of the workpiece is the chief criterion of its efficiency in metalworking. For example, it is frequently used in machining soft materials such as copper or brass, where a particularly high degree of mirror finish is required; it is specified on long automated production runs to overcome time lost in replacing worn tools, thus saving cost of idle plant; in deep grinding, the use of suitable diamond tools can achieve a very good finish after large quantities of stock in a single pass have been removed. Diamonds now used in metalworking fall roughly into five groups, in descending order of stone or particle size, according to a recent publication *Diamonds in Metalworking* (Pp. 20. London: Industrial Diamond Information Bureau, June 1965). These groups comprise: (1) large stones, which are almost of gem quality except for imperfect colouring, used for wire-drawing dies; (2) slightly smaller stones, again near gem quality, incorporated into single-point tools for turning or dressing; (3) very small stones applied in clusters to dressing tools, this design to meet requirements of less skilled and experienced operators; (4) the well-known diamond grit, graded by sieves into a wide range of particle sizes, available in both natural and synthetic form, and conventionally bonded to surfaces of grinding wheels or profiling rollers; (5) diamond powder of fineness outside normal sieve range, which is mixed with light oils to form the basis of extremely efficient polishing paste. This well-illustrated brochure conveys adequately the information it seeks to impart on the wider use of industrial diamond. In fact, wherever speed must be coupled with precision engineering, the impression is that diamond tooling will probably provide the most frequent answer to the problem.

#### Summer School in Crystallography in Pyongyang

A SUMMER school in crystallography was held in Pyongyang, North Korea, during July 5-16. The arrangements were made under the auspices of the Association of Democratic Scientists of Korea. Some twenty persons took the course, most of whom were students of physics in their fourth year at the Kim Il Sung University of Pyongyang. The text of the lectures had been submitted in advance by Dr. W. A. Wooster, and the lectures were delivered in Korean by Assistant-Prof. Jung Kuen. A suitable room for the course was provided in the centre of the city in the Library of the Academy of Sciences. The course consisted of twenty lectures in X-ray crystallography, each lecture being followed by a 2-h period of practical exercises by the students. The emphasis in the course was on these practical exercises in the interpretation of all kinds of single-crystal data, and the lectures were written mainly as an introduction to the exercises. The students were remarkably diligent and seemed interested in their work. Their previous studies had not included much single-crystal work. Very few members of the course knew English, but with the help of interpreters this difficulty was largely overcome. Dr. Nora Wooster assisted with the practical demonstrations.

#### Geology of the Wealden District

THE eighteen handbooks in the *British Regional Geology* series, designed to provide a simple but authoritative account of the geology of various parts of England, Wales and Scotland, have proved to be among the most popular publications of the Geological Survey and Museum since they were first published in 1935, and they have all passed through two or more editions. Thirty years, however, is a long time for a work to remain in the same format, and the fourth edition of the handbook to the Wealden district has had a face lift (Department of

Scientific and Industrial Research. Geological Survey and Museum. *British Regional Geology—The Wealden District*. Fourth edition (based on previous editions by the late F. H. Edmunds). By R. W. Gallois. Pp. xii+101+13 plates. London: H.M.S.O., 1965. 6s. net). The guide covers the geology of Kent, Sussex and parts of Surrey and Hampshire. It successfully relates the geology of the region in a readable manner which can be understood by students, naturalists, and the enquiring layman; and with the aid of excellent photographs of the countryside and of typical fossils it should do much to evoke an intelligent tourist's interest in the local scenery. Unfortunately it does not contain an electron-microscope photograph of the marvellously minute coccoliths which make up the Chalk cliffs portrayed on the front cover, representations of which peculiarly English organisms have found their way into foreign text-books but not yet into British ones. At 6s. the handbook is most reasonably priced, and it is to be hoped that it will shortly be followed by similar attractively produced volumes covering the rest of Britain.

#### E-route International Highway Network

PLANNED as long ago as 1950 at a meeting of various Ministers of Transport in Geneva, a network of twenty-six main international routes, each with the designation 'E' for Europe, plus a number, was mapped out, including branch and link roads connecting all important cities to the system. Already 4,600 miles of this system have been completed, and by 1970 it is anticipated that about 10,000 miles will have been achieved, involving the six Common Market countries together with Austria, Britain and Switzerland, and others to follow. This enormous project involves not only many spectacular bridges already open or under construction, but also the two longest automobile tunnels in the world, the Great St. Bernard Tunnel at the Swiss-Italian border on route E2 between Lausanne and Turin (completed in 1964) and the 7.2-mile Mont Blanc tunnel linking France and Italy, recently opened. A striking article by Jack Long entitled simply "E" and published in *The Lamp* by the Standard Oil Company, New Jersey (New York, N.Y. 10020; 47, No. 1; April 1965), gives some interesting details of this project and includes an excellent map depicting many of the proposed E routes. Part of the scheme involves the Channel Tunnel (if and when constructed) with the E5 linking London, Budapest, Belgrade, Istanbul and Ankara, and E2 the direct route from London to Milan and Bologna and beyond, while E1, presumably involving a cross-Channel ferry-service via Southampton, is routed through Paris to Florence, Rome, Naples and Palermo. Other prominent routes are E4, linking ultimately Lisbon, Madrid, Barcelona, Basle, Frankfurt, Hanover, Hamburg, Copenhagen, Stockholm and Helsinki; E8 from Amsterdam via Berlin, Warsaw to Moscow; E12, from Paris via Frankfurt, Prague and Warsaw to Leningrad; and E3 from Lisbon to San Sebastian, Paris, Brussels, Hanover, Hamburg and so to Stockholm. It is concluded that the forces drawing Europeans together are stronger than those separating them, and one unifying force is certainly this 'E' road system, not only as it may ultimately be realized, but also as the links in the chainwork are slowly but progressively forged.

#### System-built Houses

IN recent years the development of new techniques and machines has made available better and more economical supplies of such everyday commodities as food, clothes, furniture and many other necessities of modern life; in comparison, the building industry in Britain has in many respects remained strangely conservative. It still tends to cling to traditional materials and methods, and speed of construction is seldom achieved on the same scale as, for example, in Scandinavian countries and the United

States, where what is known as 'system building' has been successfully adopted for many years past. It is abundantly clear, however, that with the present and seemingly endless shortage of houses to meet an increasing population and higher standards of living, the next few years will witness revolutionary changes in the building industry brought about by the adoption of factory-made units, at least for certain types of housing. In a recent issue of *Ciba Technical Notes*, entitled "Ciba Glues in System-Built Houses" (Pp. 8. Duxford, Cambridge: Ciba (A.R.L.), Ltd., April 1965), a brief illustrated account of the 'system' in operation is presented. Among the advantages claimed for this form of prefabrication, apart from the speed of construction, are: a smaller labour force is needed on site; building sites, being notoriously unpleasant, especially in bad weather, are not conducive to good work, whereas inside a factory components are carefully fashioned regardless of weather, and supervision of working processes can be more strictly exercised; such units can more readily incorporate modern materials to give the building good weathering and thermal qualities; compared with traditional walls made of bricks and mortar, many units are light in weight, economies thereby being made in cost of foundations; plumbing, heating and ventilation can be standardized using well-conceived and tested designs; finally, overall costs are reduced because production can be planned in detail, no time is lost owing to bad weather, and seasonal employment of building workers is largely avoided. The Housing Development and Construction Co., Ltd., Stratford upon Avon, has developed such a system, suitable for a wide range of buildings: houses, schools, hospitals, old people's houses, etc., of which brief details are given, including use of Ciba wood glues, 'Aerodux' and 'Aerolite', which are fundamental to the strength and durability of certain units. It is, however, stated that "... factory-made houses are generally suited to estates, and it is often uneconomical to erect single houses on sites far removed from the factory."

### Rural England

THE Sheffield and Peak District Branch of the Council for the Preservation of Rural England maintains constant vigil on behalf of all who may be affected. In the annual report for 1965 many examples are given of how the vigour of the Branch and its officers has resisted encroachment on surrounding amenities (Pp. 28. Sheffield: Council for the Preservation of Rural England, Sheffield and Peak District Branch, 1965). The report also describes the recent gift to the National Trust, by two local donors, of some 8 acres under and including a part of Baslow Edge at Curbar Gap which provides magnificent views over the Derwent Valley. Also included is a reference to the work of the Council for the Conservation of Sheffield Antiquities in restoring the Abbeydale Works. This is the last remaining place where steel-making and forging processes of the 1750's can be studied in their original forms. Their scenic setting is attractive. An appeal has already brought in more than £30,000, and the completed project should add much interest to a city which has lost many historic features.

### World Health Organization Publications

Among the *Technical Report Series* issued recently by the World Health Organization, No. 300 is of special interest in that it discusses *The Effects of Labour on the Fetus and Newborn* (Pp. 32. Geneva: World Health Organization; London: H.M.S.O., 1965. 2 Sw. France; 3s. 6d.). Pre-natal mortality has, in many countries, remained almost stationary for the past ten years at the level of about 30 per 1,000 births—a considerable reduction since the beginning of the present century. Experience, however, suggests that it could be reduced still further. Apart from the children who die, many survive with such permanent handicaps as spasticity or mental deficiency

(attributable to the effects of labour). This report examines the complex factors involved in childbirth, the ways in which the process of labour may be upset to the detriment of the infant, defects in intra-uterine and postpartum environment which affect the child's capacity to survive and its future health. Perinatal infection is examined in relation to labour and caution is expressed about the use of drugs and other forms of therapy before and during labour. Recommendations for research are made and the report pleads for the provision of qualified and adequately trained personnel to care for the mother and child.

Among other publications recently published are a useful 1947-1964 *Catalogue of World Health Organization Publications* (Pp. 114), and the third report of the World Health Organization Expert Committee on Bilharziasis (*Technical Report Series*, No. 299. Pp. 56. 3 Sw. France; 5s.). The latter describes, among other features, the planning in detail of a bilharziasis control programme. Another valuable publication entitled *Snail Control in the Prevention of Bilharziasis*, 30s., discusses the chemical composition and relative merits of the newer molluscicides now in use or on trial together with methods of applying them and the risks of some of them to the operators and to agriculture. Types of cercarial larvae that may be encountered are described, so that they can be differentiated from the cercariae which affect man. Studies of man-made and natural snail habitats are recorded together with the methods and apparatus used for collecting snails. This publication should interest all biologists and should be useful to veterinarians, who have to control diseases of farm stock, such as fascioliasis.

### Vascular Plants of South Georgia

THE only previous major work on the botany of South Georgia, a comparatively small and remote but nevertheless interesting island of the South Atlantic, was written more than a half-century ago by Carl Skottsberg (1912). It is therefore particularly gratifying to note how much work has been done by S. W. Greene (*Vascular Flora of South Georgia*. Pp. 58+24 maps and 6 plates. London: British Antarctic Survey, 1964. 32s. 6d.) not only in bringing the systematic botany of the vascular plants of the island up to date, but also in dealing with topics of historical, geological and ecological importance. In this publication the author has set out to provide students of the area, as well as intending scientific visitors, with a concise and compact account of its botany. He gives most useful, if rather brief, accounts of the topography and geology, and also the various ecological communities relevant to the vascular plants. A very extensive and interesting section is devoted to the history of botanical exploration of the island from its earliest visit by Cook in 1775 up to the time immediately prior to this publication. In this latter section, not only is each individual visit to the island recorded, but also the extent of the collections made and their present location—information most valuable to students of the flora of Antarctica. In addition to these individual entries, tables are provided showing the location in the major world herbaria of all the plants known from the island. The systematic section of the work is very concise with clear and positive descriptions of the plants, although it is a great pity that, of the 24 taxa regarded by the author as native to the island, only seven have been illustrated in any form. There can be no doubt that Dr. Greene's volume will be regarded as one of the most valuable on the flora of the island, and it is hoped that at some future date he will supplement this with a similar systematic treatment of the lower plants, particularly the Bryophyta, in which South Georgia is very rich.

### A New Species of Catfish from the Zambesi

IN 1955 a strange clariid was taken from the Zambesi River, about 15 miles above the Victoria Falls, by Mr.

T. E. Davidson, then warden of the Wankie National Park. The specimen was put aside while attempts were made to discover others, but although several collections have since been made in the Zambezi and its tributaries, none has been found. With the aid of photographs, this unique specimen has been discussed in considerable detail by R. A. Jubb, Freshwater Fish Section, Albany Museum, Grahamstown, with Dr. P. H. Greenwood, whose papers have contributed much to knowledge of the Clariidae and the comparative anatomy of their suprabranchial organs (*Ann. Mag. Nat. Hist.*, 7, No. 79; 1964). As a result of this discussion, it has been concluded that this Zambezi clariid is a new species for which provision should be made in the genus *Clariallabes*, Boulenger, 1900. The genotype *Clariallabes melas* has the dorsal and anal fins united with the caudal, a feature which is also evident in the type *Cl. petricola*, Greenwood, 1956. This feature, however, is not consistent, as Greenwood now has specimens of *Cl. petricola* which have a distinct gap between the caudal, and the dorsal and anal fins.

### Lady Tata Memorial Fund Awards

THE Trustees of the Lady Tata Memorial Fund for research in leukaemia and allied disorders have made the following awards for the academic year 1965-66: *Fellowships*, Dr. V. Buonassisi (Rome); Dr. M. Matsuyama (Aichi Cancer Center, Nagoya, Japan); *Scholarships*, Dr. G. Corneo (Laboratory of Molecular Biopathology, Milan); Dr. D. Quaglino (Institute of Medical Pathology, Modena); Dr. G. Tridante (Division of Experimental Cancer, Bari); *Expenses Grants*, Dr. A. Agostoni (Medical Clinic, Milan); Dr. J. de Maeyer (Department of Virology, Louvain); Dr. F. Squartini (Institute of Pathological Anatomy and Biology, Perugia).

### University and College News: Birmingham

THE following appointments have been made: *Senior Lectureships*, H. F. Downton (mathematical statistics). *Lectureships*, Dr. N. T. Bailey (mining and minerals engineering); J. M. Joffe (psychology); A. Trainor (materials science); Dr. A. E. Williams (microbiology); Dr. P. M. H. Raab (physiology). *Research Fellowships*, Dr. J. Baugh and Dr. D. G. Ryan (physics); T. Pratt (electronic and electrical engineering).

### Newcastle upon Tyne

THE following lecturers have been appointed: J. D. Simmett (experimental pathology in the Department of Pathology); Dr. D. T. Edmonds (hydraulics and hydrology in the Department of Civil Engineering).

### Salford

Dr. J. G. HATHCOTE has been appointed reader in biochemistry at the Salford Royal College of Advanced Technology.

### Swansea

THE following lecturers have been appointed: T. H. Walton (pure mathematics); Dr. J. W. Barnes (geology).

### Announcements

LORD MURRAY OF NEWHAVEN, chancellor of the University of Southampton, has been appointed to the Board of Trustees of the Wellcome Trust.

A SYMPOSIUM on "Elastohydrodynamic Lubrication", arranged by the Institution of Mechanical Engineers, will be held in Leeds during September 20-22. Further information can be obtained from H. Umpleby, Institution of Mechanical Engineers, 1 Birdcage Walk, London, S.W.1.

THE thirteenth symposium on "Vertebrate Palaeontology and Comparative Anatomy" will be held at Royal Holloway College, Englefield Green, during September 20-22. Further information can be obtained from P. M. Butler, Department of Zoology, Royal Holloway College, Englefield Green, Surrey.

AN international conference on "Elementary Particles", organized by the Rutherford High Energy Laboratory, will be held in Oxford during September 19-25. Further information can be obtained from Mr. R. C. Pepperell, Scientific Conference Secretariat, Rutherford High Energy Laboratory, Chilton, Didcot, Berkshire.

THE third international symposium on "Natural Mammalian Hibernation" will be held in the University of Toronto during September 13-16. This will mark the opening of the Ramsay Wright Zoological Laboratories. Further information can be obtained from Dr. A. R. Dawe, Office of Naval Research Branch Office, 219 South Dearborn Street, Chicago, Illinois.

AN international conference on "Thermionic Electrical Power Generation", organized by the Institution of Electrical Engineers under the auspices of the European Nuclear Energy Agency of the Organization for Economic Co-operation and Development, will be held at the Institution of Electrical Engineers during September 20-24. Further information can be obtained from the Secretary, Institution of Electrical Engineers, Savoy Place, London, W.O.2.

## THE NIGHT SKY IN SEPTEMBER

All times are in Universal Time

MOON		CONJUNCTIONS WITH THE MOON	
New Moon	25d 03h	Venus	28d 07h, 8° S.
Full Moon	11d 00h	Mars	29d 01h, 2° S.
		Jupiter	18d 20h, 2° S.
		Saturn	10d 12h, 8° N.

### PLANETS

Times of Rising (R) and Setting (S) during the month

Name	R/S	Beginning	Middle	End	Mag.	D <sub>p</sub> (10 <sup>6</sup> miles)	Zodiacal position
Mercury	R	8h 36m	4h 50m	Unfavourable	—	116	—
Venus	S	19h 55m	19h 15m	18h 40m	-8.5	106	—
Mars	S	20h 35m	19h 40m	19h 10m	+1.8	168	Libra
Jupiter	R	22h 00m	21h 50m	21h 00m	-1.9	476	Taurus→Gemini
Saturn	S	—	4h 35m	3h 25m	+0.8	807	Aquarius

D<sub>p</sub> is the distance of planet from the Earth on the 15th of the month

### OCCULTATIONS OF STARS BRIGHTER THAN MAGNITUDE +6 AT GREENWICH

Star	R/D	Time	Mag.
51 Ari	R	15d 04h 03.8m	+5.7
• Tau	R	17d 00h 52.7m	+4.8
227 B. Tau	R	17d 04h 18.8m	+6.9
57 Gem	R	20d 03h 16.6m	+5.1

(D, disappearance; R, reappearance)

OTHER PHENOMENA: 7d 15h, Venus 2° N. of Spica. 17d 21h, Mars 5° S. of Neptune. 23d 06h, Autumnal Equinox

## PROF. J. L. W. THUDICHUM (1829–1901)

JOHN LEWIS WILLIAM THUDICHUM is regarded to-day as the father of neurochemistry. During 1865–82 he carried out a series of brilliant pioneer investigations on the analysis and characterization of brain tissue. This began the process of defining the nervous system in chemical terms—a scientific endeavour which has continued and is still gaining momentum to-day. Although Thudichum carried out virtually all his scientific work in London he was born in Büdingen, a small 'medieval' town in the old Grand Duchy of Hesse. It was here that after a recent colloquium in Mosbach, Baden, representatives of the Gesellschaft für Physiologische Chemie gathered to unveil a plaque on his birth-place (Fig. 1).

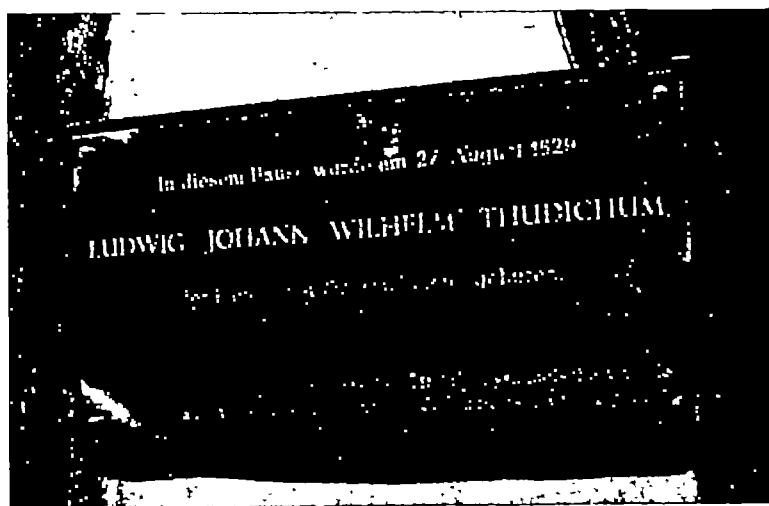


Fig. 1

Thudichum's family, distinguished in scholarly attainments, originated in the town of Marbach, the birthplace of the poet Schiller, and in this atmosphere he derived a great love for the classics. This latter expressed itself not only in his naming of the substances which he discovered but also in his non-scientific writing. Thudichum studied medicine at Heidelberg and later at Gießen where he was encouraged by his teacher, the great chemist Justus von Liebig, to develop a deep interest in the chemistry of natural substances.

Soon after graduation, Thudichum came to London where he married and settled for the rest of his life. Until his death he actively pursued the clinical practice of medicine, being a skilled otologist and rhinologist. He devised many surgical instruments and published a treatise on nasal polyps (which ran to several editions) and one on gall stones.

However, his interest in the application of chemistry to medicine steadily grew, he lectured in pathological chemistry at the old Grosvenor Place School of Medicine and then in 1865 he was appointed as lecturer in pathological chemistry in St. Thomas's Hospital, London. He also devoted himself to his chemical researches; urochrome, the principal colouring matter of the urine, was isolated in 1864, and he also made many important contributions to our knowledge of the carotenoids or 'luteins' as he called them.

These investigations, however, were probably not of such sustained importance as his work on the chemistry of the brain. These were carried out in his private laboratory and were supported by the Privy Council, the medical officer of which, Sir John Simon, quickly appreciated the value and brilliance of Thudichum's work. Sir John believed that eventually all diseases of the brain would be explained in chemical terminology and to do this it was first necessary to understand the chemistry of the normal brain. This in those days was remarkably far-sighted and is a line of reasoning not unfamiliar to many present-day neurochemists filling out applications for grants to support their work.

Thudichum continued his work on the brain during 1865–82, carrying out systematic analyses which resulted in the isolation of many new and important compounds, for example, sphingomyelin, cephalin, phrenasin, kersasin, sphingosine and cerebronic acid. Although in these days of chromatography we have become quite familiar with the rapid and easy separation of lipids, in the context of the times this was indeed a remarkable achievement. His work was published in a series of reports to the

Privy Council and in 1884 his classic work on the *Chemical Constitution of the Brain* appeared in English, followed by a revised edition in German.

On his retirement from active chemical work, Thudichum wrote two most unusual works: the *Spirit of Cookery* appeared in 1895 and a *Treatise on Wines* in 1896. This illustrates the great diversity and originality characteristic of the man. His ever active mind and spirit appeared tireless to his contemporaries; he invented, wrote poetry, painted, sang with a fine voice and, like so many scientists, had a deep love of music. This combined brilliance often proved too much for lesser mortals and evoked unjust criticisms of his work. Nevertheless, his contribution has stood the test of time and his pioneering work still continues to act as a lead and inspiration to present-day neurochemists.

H. DEBUCH

R. M. C. DAWSON

## SCIENCE IN PARLIAMENT

### Metric System

IN a written answer in the House of Commons on May 24, Mr. D. Jay, the President of the Board of Trade, stated that the Government was impressed with the case which had been put to it by the representatives of industry for the wider use in British industry of the metric system of weights and measures. Countries using that system now took more than half Britain's

exports and the total proportion of world trade conducted in terms of the metric unit would probably increase. Against that background the Government considered it desirable that British industries on a broadening front should adopt metric units, sector by sector, until that system became in time the primary system of weights and measures for Britain as a whole. The Government had therefore asked the British Standards Institution—and the Institution had agreed—to pay special attention



to the provision of metric standards, wherever possible internationally recognized, and to press on with this as speedily as possible. The Government would take this new commitment into account in determining the amount of future grants-in-aid to the Institution. It was also considering how best to encourage the educational work to familiarize future school generations and students in technological establishments with working in terms of metric units. The Government would also encourage a change to the metric system as and when this became practical for particular industries, by seeking to arrange that tenders for procurement by the Government and other Public Authorities should be in terms of metric specifications. The Government hoped that within ten years the greater part of Britain's industries would have effected the change and to this end it proposed to establish a small standing joint committee of representatives of Government departments and industry to facilitate the removal of obstacles and keep under constant review the progress which was being achieved.

In the House of Commons, on June 15, the Minister of Technology stated that his Department would give every possible assistance to the British Standards Institution through its research stations and advisory staff in preparing the new series of British Metric Standards foreshadowed by the President of the Board of Trade. The Ministry of Technology, through its research stations and the grant-aided research associations, was represented on more than a thousand committees of the British Standards Institution. The measures under discussion included production of a comprehensive manual of international metric units used in industry. In collaboration with the Central Office of Information, assistance had been given to the Publishers' Association to revise engineering text-books for the domestic and export markets.

#### Machine Tool Industry

In a statement in the House of Commons on June 14, Mr. F. Cousins, the Minister of Technology, said that his Department was engaged in a full study of the machine tool industry. He welcomed the undertakings given by manufacturers' representatives to press for action on increased capacity, on building up research and development and qualified staff, on increased specialization and elimination of wasteful duplication, and on development of British machines to replace some types that are imported. Similar important undertakings had been given about the improvement of deliveries, and the manufacturers had also agreed to initiate a review of the factoring of improved machine tools by British manufacturers. Mr. Cousins said that the Government would also play its part and, to assist the industry to develop and produce more of the most advanced and efficient types of machine tool, research and development contracts would be considerably increased. The National Research Development Corporation was already examining a number of projects and the new Act would enable it to make a greater contribution on terms which should find wider acceptance in industry. The Government was prepared to order pre-production models for approved new types, and the National Engineering Laboratory at East Kilbride would be built up to give further support in the research field: its expanded activities should yield ideas for development projects. The Laboratory would mount a major effort, supported by appropriate machine tool control engineering and user firms, on applying numerical controls to manufacturing process and using computers to assist design. His Department was negotiating for a suitable British-made powerful computer for this purpose.

Mr. Cousins emphasized that the machine tool industry was too fragmented and must be concentrated into stronger units which could better meet the needs of industry. The Machine Tool Trades Association had agreed that the industry itself must take the

lead in concentration and rationalization. One of his industrial advisers would direct particular attention to methods of promoting concentration and placing development contracts and pre-production orders to this end. His Department had also studied the serious difficulties created by the cyclical pattern of ordering machine tools, and a working party was studying the question of Government participation in financing building to stop its flat periods. The Government had also accepted the recommendation of the Machine Tool Economic Development Committee that machine tool holdings of Government establishments should be considered with the view of replacing older types with more modern machines when economically advantageous. It also accepted the recommendation that Government contracts should encourage advanced methods by stipulating the use of particular manufacturing techniques. Mr. Cousins referred to the important part that universities had to play in advancing machine tool technology: the Government had directed the attention of the University Grants Committee to the recommendation of the Economic Development Committee in this connexion. Mr. Cousins stated that he was setting up at his headquarters an expert machine tool unit the functions of which would include acting as a focal point for co-ordinating research and development in Government establishments with that in research associations, the National Research Development Council, universities and industry. This new unit would be developed to provide a technical advisory service to Government and public users of machine tools and, if desired, for private purchasers.

#### Greenland Fishing of Atlantic Salmon

In reply to a question from Lord Balfour of Inchrye in the House of Lords on July 5, regarding the Greenland fishing of Atlantic salmon, Lord Hughes, the Joint Parliamentary Under-Secretary of State for Scotland, said that the increase in exports of Atlantic salmon from Greenland from 2 metric tons in 1957 to 1,400 metric tons in 1964, mentioned by Lord Balfour, was quite correct. To that figure, however, had to be added the amount retained for consumption in Greenland itself, probably some hundreds of tons, but the total left out *grilse*, which were not reflected in the Greenland figures. As there was only one salmon river in West Greenland, it seemed that salmon caught off the coast must have bred elsewhere: and salmon tagged mostly as smolts in Britain and in other countries on either side of the Atlantic had been caught in this fishery.

Lord Hughes stated that of 64 salmon caught in Greenland up to May this year, just more than half had been tagged in the United Kingdom, but the information was still very scarce in some respects and insufficient for a final and reliable judgment. Partial information was to some extent reassuring. All the salmon caught in Greenland that had been tagged in Scottish waters came from half a dozen batches of smolts, totalling 38,000, and had been tagged between 1961 and 1963. Up to May 1965, 45 had been recaptured, but nearly all on their return to Scottish waters and only 13 off Greenland. It was not known, however, whether tagged salmon were easily recognized as such in the Greenland fishery and almost 90 per cent of the tagged Scottish smolts came from one river.

Lord Hughes referred to the difficulty and the importance of persuading Greenland fishermen that control was in their own interests and not just in the interests of people in other countries: international action was not within the control of Britain or even of Britain and Denmark. So far as the Danes were concerned, it had been confirmed that they were prepared to be helpful. For the joint tagging programme in Greenland waters, planned in co-operation with Danish scientists, and designed to show where the salmon in those waters went

if they were not caught there, Britain was receiving considerable help from the Danish authorities, who were contributing substantially to the cost of the equipment required and providing many of the facilities needed. They had also been co-operative in supplying the information about the Greenland fishery. For the same reason, at the recent meeting in Canada of the International Commission for the North-west Atlantic Fisheries, Britain joined with other nations in urging that all countries concerned should step up their investigation on salmon fisheries. After full discussion the Commission had drawn up a schedule of further information required for a proper assessment of the effect of the Greenland fishery on stocks

of the Atlantic salmon and commended it to all countries concerned. There had not yet been time to find out the full extent to which the Danish authorities could co-operate, and if remedial measures proved to be necessary they could only be taken by international action. There was a similar commission for the North-east Atlantic which relied for scientific advice on the International Council for the Exploration of the Sea, which was concerned, among other things, with salmon stocks native to European countries. Lord Hughes emphasized that Britain would keep in touch with developments, but he did not think that Britain could do more on the international side than she was doing at present.

## THE INSTITUTION OF GAS ENGINEERS

THE 102nd annual general meeting of the Institution of Gas Engineers was held at Solihull, near Birmingham, Warwickshire, during May 24-28, when several communications on diverse subjects of interest to members were read and discussed.

Among the papers presented\* was one by Dr. N. J. Sander and Dr. W. E. Humphrey (Exploration Department, American International Oil Company, New York) entitled "Why Look for Oil and Gas in the North Sea?" (now issued as Publication No. 677), one of the most informative, both geologically and technically, descriptions of the off-shore drilling areas in this basin yet to appear; the reasons for the venture are clearly stated, chances of success cautiously assessed, and inclusion of many maps and diagrams of simplified geological sections enables the reader to grasp without difficulty the fundamentals of North Sea geology and to assess for himself the ultimate possibilities of oil and gas recovery here.

J. W. Kerr (president, Canadian Gas Association) contributed a paper (678) on "Natural Gas in Canada", which described the phenomenal growth rate of the industry during the past seven years; while it is admitted that this rate cannot be repeated or maintained, the future growth prospects of the industry are viewed with justifiable

optimism. A paper (679) on "The Application of Work Study and Associated Techniques to Plant Maintenance" was presented by H. R. Hart (Scottish Gas Board), and confirms that, on the basis of results so far achieved, work study within the field of plant maintenance and having due regard to forward developments has more than proved its worth.

O. E. Mills discussed "Some Special Features of the Recent Developments in the East Midlands Gas Board" (680), which emphasized the enormous importance to the gas industry of the grid-main system in Great Britain, with special reference to the Killingholme Grid extension. A communication (682) by F. Bell, R. O. Emmony and P. E. Gallaher (West Midlands Gas Board), entitled "Keeping up the Pressure", concerns much that is administrative in the industry, especially since the advent of gas grids, the implication being "... pressure on staff, contractors, and suppliers of material and equipment". P. J. Savage (North Thames Gas Board) discussed (683) "The Production of Gas from Hydrocarbons, using the O.N.I.A. Continuous Autocaloric Process". "Part and Parcel" (684) is the title of a paper by W. V. Olsson and J. K. Mitchell (West Midlands Gas Board), which dealt with appliance spare-part service.

Finally, a paper on "Progress in Management Techniques" (685), by R. J. Maher (Australian Gas Light Company, Sydney), describes his company's "... effort to develop the conditions under which each sub-system and its objectives are compatible with and adaptive to the total Company objectives". The tremendous potentialities of the electronic computer for efficiently handling large and involved logical systems in management in the industry are particularly stressed.

\* The Institution of Gas Engineers. Publication No. 677: *Why Look for Oil and Gas in the North Sea?* By Dr. N. J. Sander and Dr. William E. Humphrey. Pp. 17. No. 678: *Natural Gas in Canada*. By James W. Kerr. Pp. 11. No. 679: *The Application of Work Study and Associated Techniques to Plant Maintenance*. By H. R. Hart. Pp. 16. No. 680: *Some Special Features of the Recent Developments in the East Midlands Gas Board*. By O. E. Mills. Pp. 21. No. 682: *Keeping up the Pressure*. By Fred Bell, R. O. Emmony and P. E. Gallaher. Pp. 17. No. 683: *The Production of Gas from Hydrocarbons, Using the O.N.I.A. Continuous Autocaloric Process*. By P. J. Savage. Pp. 17. No. 684: *Part and Parcel (An Appliance Spare-Part Service)*. By W. V. Olsson and J. K. Mitchell. Pp. 16. No. 685: *Progress in Management Techniques*. By R. J. Maher. Pp. 18. (London: The Institution of Gas Engineers (1965).

## INDUCTION OF ADRENAL DAMAGE AND CANCER WITH METABOLITES OF 7,12-DIMETHYLBENZ(a)ANTHRACENE

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A CARCINOGENIC hydrocarbon, 7,12-dimethylbenz(a)anthracene (DMBA), differs from other members of this group of carcinogens in a number of ways that include:

(1) Being non-planar in the crystalline state, whereas most carcinogenic hydrocarbons are planar<sup>1</sup>. But the deviation from planarity of DMBA is not sufficiently great to prevent formation of donor-acceptor complexes with nitroaromatics.

(2) The ability to produce cancer in mice and rats in shorter periods of time than do other aromatic hydrocarbons.

(3) The ability to combine with DNA *in vivo* to a greater extent than do other hydrocarbons<sup>2</sup>.

(4) The property of "invariably, selectively and totally destroying two zones of adrenal cortex of the adult rat and the induction of adrenal apoplexy"<sup>3</sup>. Rats can be protected against this effect and from the lethal action of

large doses of DMBA by previous treatment with certain aromatic hydrocarbons and amines<sup>4</sup>. The protective compounds are those which cause increases in the activities of the microsomal enzymes that metabolize foreign compounds including many carcinogens.

(5) Whereas phenanthrene, benz(a)anthracene, dibenz(a,h)anthracene and other unsubstituted aromatic hydrocarbons are metabolized by oxidation of the double bonds to extents varying with the activity of these bonds in the molecules, DMBA is metabolized mainly by oxidation of the methyl groups to the hydroxymethyl derivatives, 7-hydroxymethyl-12-methylbenz(a)anthracene (7-OHM-12-MBA) and 12-hydroxymethyl-7-methylbenz(a)anthracene (12-OHM-7-MBA)<sup>5</sup>. The amounts of the hydroxymethyl derivatives accumulating in rat-liver microsomes are reduced if the animals from which the microsomes are prepared are first treated with substances that induce increases in microsomal enzymes and that reduce the adrenal damage in rats caused by DMBA.

Because DMBA appeared to be unique in its action on the adrenal glands and in its metabolism and because these characteristics were changed by compounds that induce microsomal enzymes, it appeared probable that one or other of the metabolites of DMBA might be responsible for the specific effect on the adrenal.

Groups of female rats of the Sprague-Dawley strain at about 50 days of age were given single doses of DMBA, 7-OHM-12-MBA or 12-OHM-7-MBA, dissolved in sesame oil, by gastric instillation. The rats were killed three days later and the adrenals weighed and examined. The results (Table 1) show that DMBA, and one of its metabolites, 7-OHM-12-MBA, cause adrenal damage as indicated by histological change (apoplexy), increase in weight of the gland and increase in the haemoglobin content of the gland. 7-OHM-12-MBA was more active than the hydrocarbon, as 5 mg of 7-OHM-12-MBA caused about the same amount of damage as 30 mg of DMBA. The isomeric 12-OHM-7-MBA was inactive even in 60-mg doses. The potency of DMBA to induce adrenocorticalysis is thus enhanced by hydroxylation at position 7, whereas it is eliminated by introduction of OH group on the methyl group at position 12.

7-Hydroxymethylbenz(a)anthracene (7-OHMB) did not destroy adrenals, indicating that the methyl group in the 12-position is essential for this biological activity. DMBA increases the activity of the enzyme menadione reductase in the livers of rats<sup>6</sup>; 7-OHM-12-MBA (Table 2) also increases this enzyme, but not so effectively as DMBA.

The carcinogenic activities of the metabolites of DMBA have been tested by gastric instillation into female

Table 1. EFFECT ON ADRENAL OF DIMETHYL- AND HYDROXYMETHYL-DERIVATIVES OF BENZ(a)ANTHRAcene

Benz(a)anthracene derivative	No. of rats	Dose (mg)	Adrenal		
			Weight (mg)	Apoplexy	Haemoglobin* (g)
None: controls	10	—	22 ± 2	0/20	63 ± 15
7,12-Dimethyl	10	30	43 ± 8	20/20	499 ± 101
7-Hydroxymethyl	1	60	21	0/2	45
7-Hydroxymethyl-12-methyl	3	2	24 ± 1	0/6	53
Same	3	5	23 ± 6	6/6	655
12-Hydroxymethyl-7-methyl	6	30	21 ± 3	0/12	76

Sprague-Dawley female rats, age 47 days, weight 147–150 g, were given by gastric instillation a single dose of the aromatic dissolved in sesame oil. The adrenals were collected at age 50 days.

\* Standard deviation.

\* When 1 adrenal of a rat contains less than 125 µg of oxyhaemoglobin it is considered to be free from haemorrhage. Values greater than this are diagnostic of adrenal apoplexy.

TABLE 2. INDUCTION OF MENADIONE REDUCTASE IN LIVER

Benz(a)anthracene derivative	Dose (mg)	No. rats	Menadione reductase (units/g)	(%)
Control: no compound	—	15	20 ± 2	100
7-Hydroxymethyl	30	10	22.7	113
7-Hydroxymethyl-12-methyl	10	3	28.4	142
7-Hydroxymethyl-12-methyl	30	3	34.4	172
7,12-Dimethyl	10	3	50.7	253

Rats were given by gastric instillation a single dose of aromatic dissolved in sesame oil at age 47 days; a sample of liver was obtained under ether anaesthesia 24 h later. One unit of menadione reductase is defined as the enzyme activity which reduces 1 µmole of DPNH/1 min under stated conditions.

Table 3. INDUCTION OF CANCER BY FEEDING DMBA AND DERIVATIVES TO RATS

Benz(a)anthracene derivative	Dose (mg)	No. of rats	Cancer yield	Appearance of palpable mammary cancer (days) Range	Mean
7-Hydroxymethyl-12-methyl	10	10	7/10	49–119	87
12-Hydroxymethyl-7-methyl	20	5	0	—	—
7,12-Dimethyl	10	19	15/19	28–76	43

Female Sprague-Dawley rats were fed a single meal of the aromatic dissolved in 1 ml. of sesame oil at age 50 days.

Table 4. INDUCTION OF SARCOMA IN RATS

Benz(a)anthracene derivatives	No. of rats	No. of rats with sarcoma 8 months after injection	Time of detection in days
7,12-Dimethyl	8	8/8	97–137 (110 ± 14)
7-Hydroxymethyl-12-methyl	8	2/8	153
12-Hydroxymethyl-7-methyl	8	0	—

Compounds (2.5 mg in 0.5 ml.) sesame oil were injected into the thigh muscle of 46-day-old Sprague-Dawley rats which were observed for 8 months.

Table 5. INDUCTION OF TUMOURS IN C57 MICE

Benz(a)anthracene derivatives	No. of mice	Sex	No. of mice with tumours	Time of appearance of tumours in days
7-Hydroxymethyl-12-methyl	20	Male	16	119
7-Hydroxymethyl-12-methyl	20	Female	14	127
12-Hydroxymethyl-7-methyl	10	Male	9	119
12-Hydroxymethyl-7-methyl	10	Female	4	119

Mice were injected with 1 mg of the compound in 0.5 ml. arachis oil weekly for 10 weeks.

Sprague-Dawley rats and by subcutaneous injection into rats and mice. DMBA and some other carcinogens induce mammary cancer in a few weeks in female Sprague-Dawley rats<sup>6</sup>. 7-OHM-12-MBA had about the same (but not greater) activity in inducing these tumours as DMBA, but the mean induction time was twice as long (Table 3). The other metabolite, 12-OHM-7-MBA, did not induce these tumours, although it was given in larger doses. This indicates that the oxidation of DMBA to 12-OHM-7-MBA is a detoxicating process. The mammary tumour-inducing activity of DMBA might be exerted after conversion into 7-OHM-12-MBA, but this is not certain.

The results of single injection experiments in rats (Table 4) in eliciting sarcoma show that DMBA is more active than are the metabolites. Tests in mice (Table 5), in which ten weekly injections were given, show that both the metabolites were carcinogenic under these conditions. DMBA injected as single 1-mg doses into mice caused a number of deaths, showing that the hydrocarbon is more toxic than the related hydroxymethyl compounds. It is of interest that 12-OHM-7-MBA appears to be an active carcinogen in mice, but did not elicit tumours in rats.

Both DMBA and 7-OHM-12-MBA are strong electron donors and gave red colours with a chloroform solution of 1,3,5-trinitrobenzene, whereas 12-OHM-7-MBA is a weak donor. It is not clear at the moment whether or not these properties are related to the biological activities of the compounds.

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# PHYTOHAEMAGGLUTININ-INDUCED CYTOTOXIC ACTION OF UNSENSITIZED IMMUNOLOGICALLY COMPETENT CELLS ON ALLOGENEIC AND XENOGENEIC TISSUE CULTURE CELLS

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IN a previous article we reported that lymphoid cells from spleen, lymph nodes, thoracic duct lymph or blood of non-immune humans or rats were cytotoxic for tissue culture cells of human or rat origin, when incubated together with such cells in the presence of phytohaemagglutinin (PHA)<sup>1</sup>. The medium from PHA-treated lymphocytes was not cytotoxic and separation of lymphocytes from tissue culture cells by means of a 'Millipore' membrane also prevented such an effect. Similar results have recently been reported by others<sup>2,3</sup>.

The target cell killing observed in these experiments may have been the expression of an immune response of the lymphocytes, induced by their close contact with the target cells and possibly potentiated by PHA. However, it was also feasible that cell damage was brought about in an entirely non-specific way. In all experiments performed at that time, lymphoid cells and target cells differed in regard to their histocompatibility antigens. It was anticipated that no effects would appear in syngeneic lymphocyte/target cells combinations, if the target cell killing indeed had an immunological basis<sup>1</sup>. Furthermore, if this was the case, it could be assumed that the cytotoxic activity of the lymphocytes should be related to their immunological competence. The experiments described in this article aimed at an elucidation of these important points.

Primary cultures and first sub-cultures were set up from kidneys of new-born rats or from embryonic rat or mouse fibroblasts. The rat cells were from rats of the highly inbred *R*-strain, reared by brother-sister mating for more than 40 generations and with no transplantation incompatibilities between different individuals. The mouse fibroblasts originated from inbred *A/Sn* mice (*H-2<sup>k</sup>/H-2<sup>k</sup>*). The culture conditions, isotope labelling (2  $\mu$ C-<sup>14</sup>C-thymidine (spec. act. ~30 mc./mmole) in 15 ml. of culture medium) and further handling of the material have been described<sup>1</sup>. In some experiments, kidney cells from outbred, new-born Sprague-Dawley rats were also used. These cells had been grown in suspension culture for 6 months. For isotope labelling,  $3 \times 10^7$  cells were grown for 2-3 days in 40 ml. of medium containing 2  $\mu$ C-<sup>14</sup>C-thymidine. After three washings, the cells could then be used for incubation with lymphocytes without further processing.

Lymphoid cells from rat (female) or mouse spleen, lymph nodes or thymus (*R*-strain or Sprague-Dawley rats, *A/Sn*, *UBA* (*H-2<sup>k</sup>/H-2<sup>k</sup>*) or outbred mice) were prepared by suspending the tissue in balanced salt solution, cutting it into small pieces and finally dispersing the cells by repeated pipetting. Clumps and debris were removed by sedimentation. Lymphocytes from human or rat peripheral blood were isolated by the gelatine method of Coulson and Chalmers<sup>4</sup>. <sup>14</sup>C-isotope-labelled and washed target cells were incubated in roller tubes under sterile conditions with an excess of lymphoid cells as previously described<sup>1</sup>. The procedure is outlined in Fig. 1. Samples containing more than 10 per cent dead cells at the beginning of incubation were discarded. Parker's Medium 199 contain-

ing 5 per cent foetal calf serum was used throughout. <sup>14</sup>C-thymidine (final concentration 10  $\mu$ g/ml.) was added in order to prevent isotope re-utilization. The lymphoid cells were added 2 h later immediately followed by PHA. The final concentration of PHA (Difco, phytohaemagglutinin M) was 0.3 mg/tube. The final volume of the incubation mixtures was 1.5 ml. The further handling of the samples followed the description given earlier<sup>1</sup>. As found earlier, the bulk of DNA-bound radioactivity from dead or damaged cells was first released after trypsin treatment of sediment (Fig. 1) and this release could then be used as a measure of cell damage<sup>5,1</sup>. A critical evaluation of this method will be found elsewhere<sup>6</sup>.

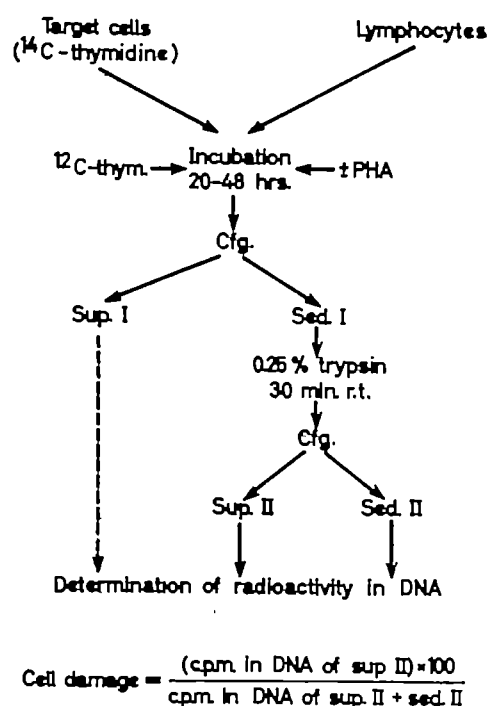


Fig. 1. Procedure for determining the cytotoxic action of lymphocytes on tissue culture cells

Table 1 shows the typical results of series of experiments in which *R*-strain fibroblasts were incubated with spleen cells from syngeneic *R*-strain rats or from mice of an outbred laboratory colony. Fig. 2 shows a summary of this and two similar sets of experiments, in which the target cells were either from inbred rats or inbred mice. The dashed line represents isotope release, and the shaded area its 95 per cent confidence limits, in those controls which contained lymphocytes but not PHA. The results of these and similar experiments may be summarized as follows: (1) Damage of the target cells in the presence of syngeneic lymphoid cells was the same, regardless of whether or not PHA was added to the tubes. It was

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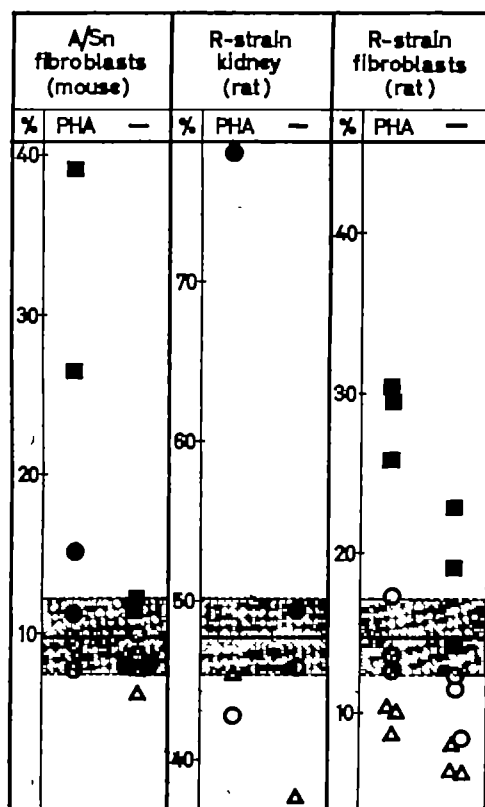


Fig. 2. Cytotoxic action of lymphoid cells of different genetic origin on various tissue culture cells. The three columns represent three sets of experiments performed with the target cells (primary cultures) indicated on top of each column. The ordinates give the percentage of isotope released from the target cells (Fig. 1). Each symbol represents a separate lymphocyte/target cell incubation. Squares, xenogeneic lymphoid cells (first column: spleen cells from Sprague-Dawley rats; third column: lymph node cells from outbred mice); black circles, allogeneic lymphoid cells (first column: spleen cells from OBA-mice; second column: lymph node cells from Sprague-Dawley rats); white circles, lymphoid cells (first and second column: lymph node cells; third column: spleen cells) syngeneic with the target cells indicated in the figure. Triangles, no lymphoid cells added.

sometimes, but not always, moderately elevated over that found in the absence of lymphocytes, probably due to cell crowding or other non-specific causes. (2) *Allogeneic* lymphocytes had the same effect as syngeneic lymphocytes when PHA was absent. However, in the presence of PHA, the target cell damage was significantly elevated. (3) *Xenogeneic* lymphocytes always gave a pronounced target cell damage in the presence of PHA. A slight, but significant, effect as compared with the controls sometimes also appeared without PHA. Finally, it should be stressed that the PHA-induced lymphocyte/target cell aggregation was always the same when observed in the microscope, regardless of the genetic relationships between the cell types.

Table 1 Target cells: Rat, R-strain fibroblasts (prim.). Lymphocyte/target cell ratio: 100:1. Incubation: 48 h			
Lymphoid cells	PHA	C.p.m. in DNA (Sup. II + Sed. II)	% Isotope release
Mice spleen	-	1,228 ± 92	18.9 ± 4.4
Mice spleen	+	1,121 ± 24	28.5 ± 2.4
Rat spleen (R-strain)	-	1,178 ± 220	10.7 ± 2.1
Rat spleen (R-strain)	+	1,217 ± 40	14.2 ± 2.9
-	-	1,223 ± 77	6.9 ± 1.1
-	+	1,220 ± 28	9.7 ± 0.9

The numbers represent mean values and S.D., each based on three different experiments.

These results strongly suggested that the cell-killing effect of the lymphocytes had indeed an immunological basis. Similar results have recently been reported by E. Møller<sup>2</sup>, using normal lymph node cells and tumour cells from mice in different H-2 combinations. This author

also made the very interesting observation that lymph node cells of semi-isologous F<sub>1</sub> hybrid origin killed tumour cells of parental strain origin. Since the F<sub>1</sub> lymphoid cells were considered to be 'genetically incompetent' of reacting immunologically against parental strain H-2 antigens, the cell-killing phenomenon was interpreted as reflecting a mutual contact inhibition of cells differing in surface structure.

In order to examine the mechanism of this reaction, we have performed several different types of experiments. In a first series, isotope-labelled rat tissue culture cells were mixed with excessive numbers of unlabelled tissue culture cells of syngeneic, allogeneic or xenogeneic origin. These mixtures were then incubated for 48 h in the presence or absence of PHA. When present, PHA was seen to cause the formation of mixed aggregates. In some cases, isotope release was high (35-40 per cent), regardless of the cell combination used, but increase of isotope release due to PHA was never seen. Similarly, no cell damage was obtained when a very large excess of mouse red blood cells and PHA were added to labelled rat tissue cells.

In a second series of experiments, it was attempted to establish close lymphocyte/target cell contacts without using PHA. Rosenau has previously shown that addition of polylysine and allogeneic lymphocytes to mouse fibroblasts in monolayers resulted in firm aggregations but no cell damage<sup>7</sup>. Similar experiments performed by us also yielded negative results (Table 2), in spite of strong lymphocyte/target cell aggregations. The relatively pronounced cell damage in the lymphocyte-free polylysine controls indicated that this substance was toxic by itself at the concentrations used. Rosenau and Moon have also shown that incorporation of hydrocortisone in the medium abolished the cytotoxic effects of immune lymphocytes without preventing their aggregation to the target cells<sup>8</sup>. The same was seen in our experiments with normal lymphocytes and PHA. Similarly, addition to the target cells of lymphocytes, killed either by heating (56°C, 30 min) or by freezing and thawing (4 times), also abolished their cytotoxic effects. A typical experiment is shown in Table 3.

Table 2					
Target cells: rat kidney (cell line). Lymphocytes: human (periph. blood).					
Lymphocyte/target cell ratio: 60:1. Incubation: 48 h					
	Exp. 1		Exp. 2		
Incubation	C.p.m. in DNA (Sup. II + Sed. II)	% Isotope release	C.p.m. in DNA (Sup. II + Sed. II)	% Isotope release	
Lymphocytes -	403 ± 20	17.5 ± 3.4	1,210 ± 198	11.7 ± 2.1	
Lymphocytes + PHA	452 ± 35	27.9 ± 3.4	1,116 ± 80	28.3 ± 7.8	
Lymphocytes + polylysine	390 ± 132	19.2 ± 1.6	1,025 ± 25	14.8 ± 1.0	
— + PHA	499 ± 35	5.7 ± 0.4	1,058 ± 49	19.9 ± 1.7	
— + polylysine	459 ± 34	23.0 ± 0.1	1,112 ± 120	25.1 ± 1.0	
— —	458 ± 12	17.7 ± 8.0	1,065 ± 11	21.5 ± 2.6	

Exp. 1: 1 µg polylysine/ml. Exp. 2: 5 µg polylysine/ml. The numbers represent mean values and S.D., each based on three separate experiments.

Table 3 Target cells: rat kidney (cell line). Lymphocytes: human (periph. blood). Lymphocyte/target cell ratio: 40:1. Incubation: 48 h			
Lymphocytes	PHA	C.p.m. in DNA (Sup. II + Sed. II)	% Isotope release
+	-	1,001 ± 100	8.7 ± 1.7
+	+	886 ± 84	25.9 ± 5.7
+	(killed)	887 ± 104	6.2 ± 1.3
+	(killed)	961 ± 153	4.0 ± 1.0
-	-	1,125 ± 103	8.8 ± 1.0
-	+	1,006 ± 89	7.9 ± 1.0

The figures represent mean values and S.D., each based on three separate experiments.

When taken together, these results suggested (but did not prove) that living lymphoid cells had to be added to the incubation mixtures if cytotoxic effects were to occur. In order to investigate the possible relationship of this cell-killing activity of lymphoid cells to their immunological competence, the action of peripheral lymphocytes was compared with that of lymphocytes from the thymus.

Such cells were isolated from young mice (randomly bred), and material from the same individual animals was used in each experiment. When added to tissue culture cells in monolayers in the presence of PHA, thymus lymphocytes were seen aggregated around the former to the same extent as peripheral lymphocytes (Fig. 3). However, as seen from the typical results of Fig. 4, thymocytes gave no or only weak cytotoxic effects in cases where peripheral lymphocytes were active. This difference was not due to a lesser viability of the thymocytes. When samples of these cells were kept separately for 48 h under otherwise similar conditions, the survival rates of both peripheral lymphoid cells and thymocytes was approximately 40 per cent in all cases.

The lesser reactivity of the thymocytes found in our experiments corresponds to that seen by others in different immunological situations (for references and discussion see ref. 9). It may be concluded that the cell-damaging activity of lymphocytes as measured here is related to their 'immunological competence'. Therefore, we believe that the target cell damage reflects a response of competent lymphocytes, started by their close contact with antigen which they recognize as 'foreign', and perhaps potentiated by PHA<sup>10</sup>. The action of lymphoid cells of  $F_1$  hybrid origin on parental strain tumour cells can probably also be explained on this basis. The notion of a 'genetic incompatibility', such that the  $F_1$  lymphoid cells do not react against parental cells<sup>6</sup>, is based on the fact that no such reactions occur in normal transplantation or graft-versus-host situations. It may be recalled that slight structural changes of a variety of antigens may sometimes give rise to a loss of natural tolerance and ensuing autoimmune responses<sup>11</sup>. In analogy, and as example only, small differences in cell surface structure of parental and  $F_1$  cells due to different arrangement or concentration of histocompatibility antigens may be sufficient to release a response of competent lymphocytes when the cells are tightly aggregated in this highly artificial manner.

The statement that the present reactions reflect an immune response of competent lymphoid cells requires qualification. While there is little doubt that the response is initiated by an immunologically specific recognition step, no evidence exists as yet for a corresponding specificity of the target cell destructing reactions. In other words, the response does not necessarily have to involve antibody formation. Although available evidence

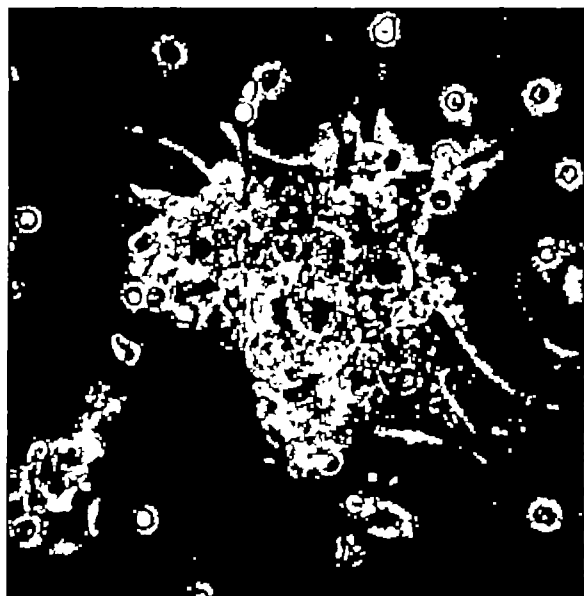


Fig. 3. Rat fibroblasts on cover slip, incubated with mice thymocytes for 12 h in the presence of PHA. Micrograph (phase contrast) of living cells ( $\times 400$ ).

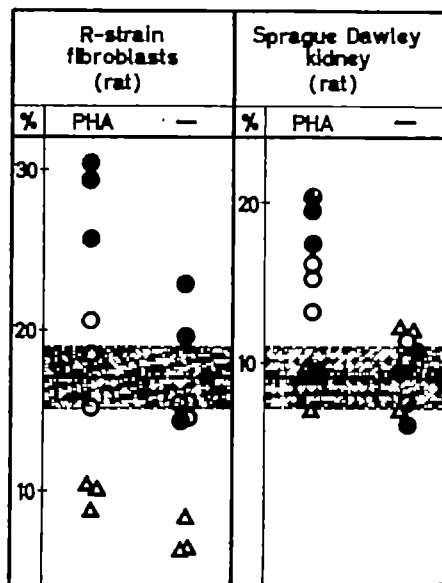


Fig. 4. Cytotoxic action of thymocytes and peripheral lymphoid cells on various tissue culture cells. The target cells were R-strain rat fibroblasts in the experiments of the first column and rat kidney cells (Sprague-Dawley, established cell line) in the experiments of the second column. Black circles, peripheral lymphoid cells from lymph nodes (first column) and spleen (second column) of randomly bred mice. White circles thymocytes from the same animals used as donors of peripheral lymphocytes. For further explanations see legend to Fig. 2.

speaks against the release of pharmacologically active agents from the stimulated lymphocytes to the incubation medium, a local action of such agents in the contact areas between the cells cannot be excluded<sup>1</sup>. A third alternative would be that the aggregated lymphocytes are metabolically stimulated by foreign antigen and therefore damage the target cells by depriving them of nutrients or oxygen. However, this explanation seems difficult to reconcile with the negative results seen in syngeneic lymphocyte/target cell aggregations in which PHA should have affected the metabolism of the lymphocytes in a similar way to antigen.

In our experiments, the lymphocyte response was most probably induced by their contact with foreign histocompatibility antigens. Obviously, as seen in syngeneic systems, the presence of organ-specific substances on the target cells was not sufficient to release a significant response. This raises the interesting problem as to the mechanism of target cell destruction, observed in similar experiments with lymphoid cells from 'autoimmune' animals<sup>12,6</sup> or human beings<sup>13</sup>. In those experiments, lymphocyte/target cell contacts were most probably established by antibody or antibody-like substances on the lymphocytes. However, in all cases described so far, lymphocytes and target cells were derived from genetically different individuals. Therefore, the target cell-damaging activities of the lymphocytes may also have been released by their close contact with foreign histocompatibility antigens. Experiments with lymphocytes from autoimmune animals, added to target cells of syngeneic origin, should throw light on this point. Such experiments are in progress.

Many other questions remain. In particular, one would like to know the relationship of the phenomenon described here to the lymphocyte transfer reaction<sup>14</sup>, or to the *in vitro* stimulation of lymphocytes, occurring on exposure to foreign cells<sup>15</sup> or antigen<sup>16</sup>. The type of the lymphoid cells which participate in the reaction is unknown at present and one also wonders whether the reactors are immunologically committed or uncommitted cells. The true function of PHA in the present system will also have to be investigated further. Nevertheless, experiments of the kind described in this article would seem to offer some means for investigating the mechanism by which lymphoid cells recognize 'foreignness', and the relation-

ship of the recognition step to the response evoked by it in the competent lymphocytes.

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## INHIBITION BY METHOTREXATE OF TRIIODOTHYRONINE STIMULATION OF OXYGEN CONSUMPTION IN MAN

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THYROXINE has been shown to stimulate the rate of incorporation of amino-acids into protein<sup>1-3</sup>. It has recently been suggested that the well-recognized effects of thyroid hormones on oxygen consumption may be related to their effects on protein biosynthesis<sup>4-6</sup>. Thyroxine administration to rats results in increased rates of amino-acid incorporation into protein only in organs (liver, kidney and heart) in which the hormone also stimulates oxygen consumption, but has no effect on amino-acid incorporation into protein in organs (brain, testis and spleen) in which oxygen consumption is unaffected<sup>4</sup>. Drugs such as puromycin and actinomycin D, which result in an inhibition of protein biosynthesis, also block the effects of thyroid hormones on oxygen consumption<sup>5,6</sup>. These experiments provide further evidence of a relationship between the effects of thyroid hormones on protein biosynthesis and on oxygen consumption.

The present investigation was undertaken to determine whether methotrexate (amethopterin) treatment of human subjects counteracts the effects of thyroid hormones in a manner similar to that shown for puromycin in experimental animals. Investigations were carried out on two male patients, aged sixty-two and fifty-four years, with advanced metastatic carcinoma of the lung. Two patients were hospitalized in a metabolic ward, and received constant caloric and protein intake during the investigation. Each patient was euthyroid at the beginning of the investigation, as demonstrated by clinical examination and by normal values of serum protein-bound iodine concentration, serum cholesterol concentration, and basal metabolic rate. The measurements of basal metabolic rate were made by standard methods, with standard corrections applied for changes in body temperature and weight during the course of the investigation.

After baseline evaluation of thyroid function of the first patient had been completed (protein-bound iodine = 6.4 µg/100 ml. serum; serum cholesterol concentration = 175 mg/100 ml. serum; basal metabolic rate = +6 per cent), triiodothyronine was given orally in doses of 75 µg four times a day for a period of nine days. During this interval there appeared a loss of weight of 8.5 kg, a decrease in serum cholesterol concentration from 175 to 115 mg per cent, and increased sweating, nervousness and tachycardia. Basal metabolic rate (Fig. 1) increased progressively from baseline levels of +6 and +10 to a level of +65 per cent after treatment with triiodothy-

ronine. From the tenth to the sixteen day, methotrexate was administered intravenously at a dosage of 0.2 mg/kg body-weight a day. Daily determinations of haemoglobin concentration, haematocrit, white cell count and differential count were performed to evaluate drug toxicity. Triiodothyronine treatment was continued until the eighteenth day. Basal metabolic rate declined from a level of +65 before methotrexate treatment to levels of +19 and +26 per cent following the completion of seven days of methotrexate therapy, despite the continuance of triiodothyronine.

To evaluate further the effects of methotrexate in the patient under investigation, the antibody response to two different antigens was measured. Four h after the first dose of methotrexate had been given on day 10, the patient received 0.4 ml. of V<sub>8</sub> antigen (*E. coli*) by subcutaneous injection. Serial samples of serum were obtained before injection of antigen on day 10, and on days 14, 17, 20 and 24. During the two-week period following injection of antigen, no antibody titre could be detected, in contrast to mean titres (log<sub>10</sub> serum dilution) found in patients previously studied<sup>7</sup>. On day 24, the patient received 1.0 ml. of whole, killed tularemia vaccine by subcutaneous injection. Serum samples obtained during the ensuing two weeks had normal antibody titres.

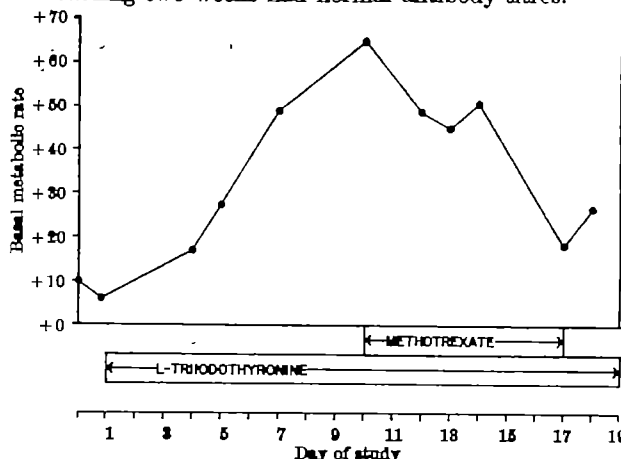


Fig. 1. Measurements of basal metabolic rate in the first patient studied, during administration of triiodothyronine, and of triiodothyronine in combination with methotrexate. Results are expressed as a percentage



In the second patient, baseline evaluation of thyroid function revealed that the serum protein-bound iodine concentration was  $6.9 \mu\text{g}/100 \text{ ml. serum}$ , cholesterol concentration was  $220 \text{ mg}/100 \text{ ml. serum}$  and basal metabolic rate was  $+9 \text{ per cent}$ . Constant caloric and protein intake were maintained in a manner similar to the first study. The measurements of basal metabolic rate are shown in Fig. 2. Methotrexate in the same dosage ( $0.2 \text{ mg/kg}$ ) was given by daily intravenous injection for a period of five days. Basal metabolic rate measurements before methotrexate did not differ significantly from values observed in the week after treatment. After an additional period of ten days had elapsed, which enabled the patient to recover from mild drug toxicity, triiodothyronine treatment was begun. At the end of nine days of treatment (day 24), loss of weight of  $4.6 \text{ kg}$  was noted, in association with tachycardia, nervousness, sweating, irritability and depression of the serum cholesterol concentration from  $220$  to  $135 \text{ mg}/100 \text{ ml. serum}$ . Basal metabolic rate levels had increased from control values of  $+5$  and  $+8$  to a level of  $+55 \text{ per cent}$ . Methotrexate was again administered for five days while triiodothyronine was continued. Following the completion of methotrexate administration, the basal metabolic rate had declined to  $+38 \text{ per cent}$ , and two days after discontinuing triiodothyronine treatment, the basal metabolic rate had declined still further to a level of  $+13 \text{ per cent}$ .

The work recorded here indicates that in the two patients investigated, the effect of triiodothyronine on elevating the basal metabolic rate was antagonized by treatment with methotrexate. In addition, during the period of methotrexate treatment, and for seven days following cessation of treatment, there was complete prevention of antibody synthesis after injection of Vi antigen. This effect may represent impairment of protein synthesis, although other factors are also involved in response to an antigenic stimulus. That prevention of antibody synthesis was only temporary was shown by our subsequent finding of a normal antibody response to tularaemia vaccine in the same patient.

By blocking the enzymatic reduction of folic and dihydrofolic acids<sup>1-3</sup>, methotrexate interferes with one-carbon transfer in many important biochemical reactions. Of particular importance is this effect on the synthesis of pyrimidines and purines. Following diminished formation of nucleotides, reduced synthesis of proteins would be expected to occur.

In the work recorded here, methotrexate treatment partially antagonized the stimulatory effects of triiodo-

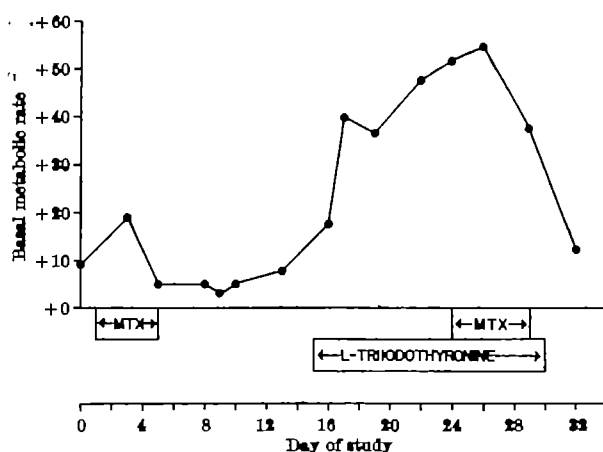


Fig. 2. Measurements of basal metabolic rate in the second patient studied, during administration of methotrexate and triiodothyronine separately, and of both drugs together. Results are expressed as a percentage.

thyronine on oxygen consumption. No effect on oxygen consumption was demonstrable in the absence of excess thyroid hormone. Since the actions of methotrexate on metabolism are numerous, it is not possible to attribute these results with certainty to a particular effect of the drug. However, the similarity of the present results to those previously obtained with agents which more specifically inhibit protein synthesis (actinomycin, puromycin) suggest that the pertinent effect of methotrexate may also have been on protein synthesis.

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## NEW MODEL FOR THE TROPICOLLAGEN MACROMOLECULE AND ITS MODE OF AGGREGATION

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THE molecular architecture of collagen has been intensively studied by a variety of physical and chemical techniques. According to current views collagen fibres are composed of fibrils which are themselves built up from a basic structural unit termed the tropocollagen macromolecule. Each tropocollagen unit is believed to have a three-stranded coiled-coil structure in which the most probable arrangement of the polypeptide chains would appear to be the collagen II model proposed by Rich and Crick<sup>1</sup>. The tropocollagen unit has been described as a rigid rod-like structure for which Boedtker and Doty<sup>2</sup>, using physico-chemical methods, originally found a diameter of  $13.6 \text{ \AA}$ , an average length of  $3000 \text{ \AA}$

and an average molecular weight of  $345,000$ . Recently, Rice, Casassa, Kerwin and Maser<sup>3</sup> have suggested a length of  $2800 \text{ \AA}$  and a molecular weight not greater than  $310,000$ .

One of the earliest observations made on biological material with the electron microscope was that native collagen fibres exhibited a conspicuous transverse banding with a repeat distance of approximately  $640 \text{ \AA}$  (Schmitt, Hall and Jakus<sup>4</sup>; Wolpers<sup>5</sup>). There have since been many reports on the structure of collagen using shadowing, thin sections and positive staining. These have been reviewed in detail by Harrington and Von Hippel<sup>6</sup>, Gross<sup>7</sup>, Fittin Jackson<sup>8</sup> and Veis<sup>9</sup>. Much research has been

devoted to clarifying the relationship of the basic tropocollagen unit to the native collagen fibre.

An important aspect of the problem is to explain how the observed periodicity of 640 Å of native collagen results from the combination of tropocollagen units of length 2800 Å.

It is well known that native-type collagen fibres may be reconstituted from acetic acid solutions of collagen by adjustment of pH and ionic strength. It is also possible to reconstitute collagen in a number of forms differing characteristically from the native fibre. A particularly interesting form is the segment long spacing (SLS) crystallites (Gross, Highberger and Schmitt<sup>10</sup>) produced by the addition of adenosine triphosphate (ATP) to an acetic acid solution of collagen. The SLS crystallites appear to consist of tropocollagen units aggregated side by side with the ends of the molecules in register. A large number of bands, arranged at right angles to the long axes of the tropocollagen units, may be seen with the electron microscope using either positive- or negative-staining techniques. The fact that these bands are distributed asymmetrically is strong evidence that the structure of the tropocollagen macromolecule is polarized.

Another type of collagen fibril, produced by dialysing an acetic acid solution of collagen (tropocollagen) containing acid glycoprotein or chondroitin sulphate (Highberger, Gross and Schmitt<sup>11</sup>; Schmitt, Gross and Highberger<sup>12</sup>) has been termed the fibrous long spacing (FLS) form. The repeat distance of this form has been found to vary with the method of preparation and the different varieties produced have been further designated FLS—I, II, etc., depending on the spacing observed. In the case of the FLS form of collagen a maximum repeat distance of about 2500 Å has been found. It has been suggested (Schmitt and Hodge<sup>13</sup>) that in the FLS form of fibre the tropocollagen units are aggregated end to end. The direction of polarization of the macromolecules was considered to be alternated in view of the symmetrical pattern obtained in the electron microscope after positive staining with phosphotungstic acid.

Application of the negative staining technique (Brenner and Horne<sup>14</sup>) to high-resolution studies of collagen with the electron microscope showed that it was possible to visualize, directly, tropocollagen macromolecules occurring in native collagen fibrils (Tromans *et al.*<sup>15</sup>; Olsen<sup>16,17</sup>). In their negatively stained preparations of collagen fibrils the most striking feature seen was the arrangement of slender, somewhat crooked filaments running roughly parallel to each other along the long axis of the fibril. These filaments, 15–20 Å across, were interpreted as being the tropocollagen macromolecules.

In a detailed study of negatively stained collagen specimens, Tromans<sup>15</sup> suggested that the light and dark bands resulted from differences in the density of the protein and in the penetration of the negative stain between the macromolecules. It was not found possible to demonstrate the precise positions of the ends of the macromolecules within the fibrils, nor was there any evidence in favour of a 'quarter staggered' arrangement as suggested by the model of Hodge and Schmitt<sup>18</sup>. The recent descriptions of native and FLS-type collagen by Olsen<sup>16,17</sup> and Kuhn and Zimmer<sup>19</sup> have indicated that the macromolecules are aggregated by end overlap. This view is not in agreement with the model proposed by Schmitt and Hodge<sup>18</sup>, in which it is suggested that end-to-end aggregation takes place by a coiling around each other of polypeptide chains projecting from each end of the tropocollagen macromolecule (Hodge and Petruska<sup>21</sup>).

This report is based on an electron microscope investigation on native, SLS and FLS forms of collagen, and on these same forms modified by treatment with various cross-linking agents. Our experiments have also included observations on the mode of aggregation of tropocollagen to the above forms. The effect of collagenase on native fibres was also investigated, in experiments which will be

reported in greater detail elsewhere. Acetic acid solutions of rat-tail tendon and guinea-pig skin collagen were used as sources of tropocollagen. From these investigations, a model has been developed which explains, in a relatively simple manner, the production of the majority of collagen forms. Their characteristic spacings can be considered to be determined by a basically random process of aggregation of tropocollagen units.

It was considered important to elucidate the mechanism whereby the light and dark bands, seen in negatively stained preparations of collagen, are produced. Treatment with a cross-linking agent, such as glutaraldehyde, resulted in an increase in the size of the light bands (A bands, Fig. 1, *b* and *c*; a well-defined light band also appeared in the dark or B bands. It was obvious from marked changes in the chemical and physical properties of the treated fibres (for example, no longer being soluble when autoclaved with water) that a number of strong cross-links had been introduced. Since the introduction of intermolecular cross-links by mild chemical means resulted in an increase in the size of the light or electron-transparent bands we may infer that the light bands, seen in native unaltered collagen, also result from regions of bonding between adjacent macromolecules. In native collagen this intermolecular bonding probably consists of polar and hydrogen bonds arising from polar amino-acid side-chains arranged in a structurally complementary manner. It thus appears that the tropocollagen units, forming the fibril, are not laterally bonded uniformly throughout their length. Such a concept is in conformity with the finding that the primary structure of collagen consists of inhomogeneous amino-acid sequences (Grassman *et al.*<sup>22</sup>).

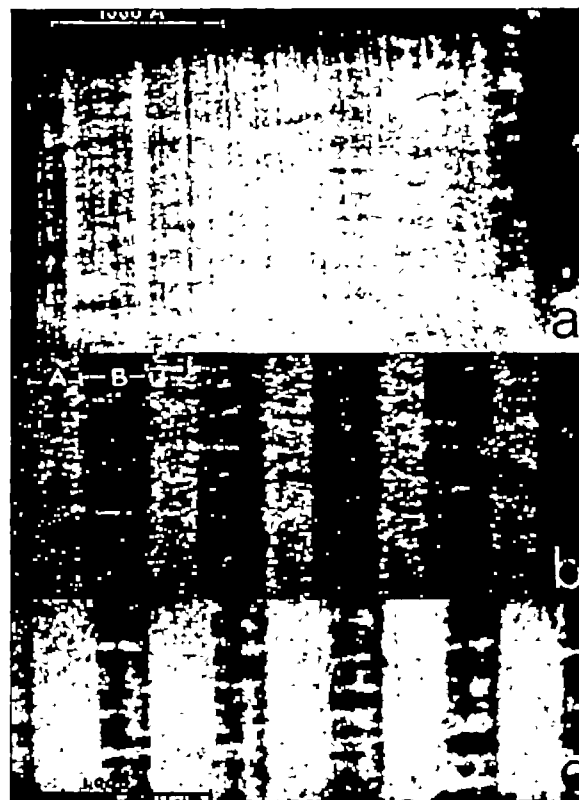


Fig. 1. *a*, Electron micrograph of negatively stained SLS crystallite. The length of the crystallite spans five A bands of the native fibril, shown in *b* at the same magnification. *b*, Negatively stained collagen fibril showing regions of light A bands and dark B bands. The rather flexible tropocollagen macromolecules are seen arranged roughly parallel to the fibril long axis. *c*, Negatively stained collagen fibril following treatment with glutaraldehyde. The increase in both size and density of the A bands is demonstrated. A well-defined light band within the B zone is also apparent.

In order to deduce a simple rational explanation of the relationship of the length of the tropocollagen macromolecule (c. 2800 Å) to the normal repeat distance ( $D$ ) of 640 Å, use may be made of the observation that the length of an SLS crystallite spans 5  $A$  bands of a native fibril when electron micrographs at the same magnification are compared (see Fig. 1, *a* and *b*). On average, the length of an  $A$  band (light) is about  $0.4D$ ; hence the length of the tropocollagen unit equals the total distance spanned by 5  $A$  bands plus the intervening  $B$  bands ( $=4.4D$ ). Careful examination of the electron micrographs gives no evidence of end-to-end junctions, all the macromolecules appearing to be aggregated together by lateral association with a good deal of cross-over in both  $A$  and  $B$  bands. The precise positions of the ends of tropocollagen macromolecules within the fibrils were difficult to determine. However, on careful inspection of the electron micrographs instances were noted of tropocollagen filaments appearing to end at the edge of an  $A$  band after passing through the band.

We suggest, on the basis of this evidence, that the tropocollagen macromolecule consists of alternating bonding and non-bonding regions, the bonding regions containing polar amino-acids which are arranged in a structurally specific manner. When two such bonding regions approach one another closely in proper alignment a strong lateral attraction results from the formation of many intermolecular polar and hydrogen bonds. All the 5 bonding regions may not be exactly equivalent and there is some evidence that the terminal bonding zones differ from the intermediate ones. Examination of the SLS form, by positive or negative staining, indicates that the tropocollagen macromolecule is polarized, and this is supported by the fact that in native fibres the band structure is asymmetrical. Tromans *et al.*<sup>12</sup> have suggested that the light bands be regarded as regions of crystallographic disorder. In view of the evidence from the cross-linking experiments we feel that this interpretation may be abandoned in favour of the more definitive explanation that the light bands correspond to regions of bonding between adjacent macromolecules.

The proposed model for tropocollagen (Fig. 2, 1) consists of a somewhat flexible filament of length  $c$ .

2800 Å, divided into 9 zones consisting of 5 bonding zones of length about  $0.4D$  (265 Å) separated by 4 non-bonding zones of length about  $0.8D$  (375 Å). It should be appreciated that the measurements quoted in this report represent approximate values drawn from our own experiments and other results cited in the literature.

It need not necessarily be assumed that the non-bonding regions are completely devoid of polar amino-acids. Indeed it appears from the cross-linking experiments that there is some lysine in these parts of the macromolecule. Nevertheless, it seems that in the native fibre only a few intermolecular bonds are formed between the non-bonding zones.

When tropocollagen units, conforming to the above model, are aggregated together so that there is an initial random choice as to which bonding region on one molecule cross-links in a structurally complementary manner with a bonding region on a different molecule, a fibre with 640 Å periodicity results (Fig. 2). A model of a collagen fibril constructed in this way shows a close resemblance to the structure of native collagen revealed by negative staining. Moreover, aggregation of tropocollagen macromolecules in an apparently random manner to form collagen fibrils has been observed by us in the electron microscope. There is no need to postulate a two-stage process in which tropocollagen macromolecules are first polymerized by end-to-end linkage through the interaction of terminal peptide chains to form protofibrils, which are then displaced relatively to one another by 0.25 of the molecular length (Schmitt and Hodge<sup>13</sup>; Hodge and Petruska<sup>14</sup>). Nor is it necessary to suggest an initial polymerization of tropocollagen units by overlapping which is limited to the ends of the molecules, to be followed by 'quarter staggering' of the protofibrils (Olsen<sup>15</sup>).

It also follows from the model for native collagen that all the tropocollagen units in one fibril will face in the same direction. The direction of polarization of a fibril, as determined from the band pattern, will depend on the direction of polarization of the first tropocollagen macromolecule. If, therefore, the tropocollagen macromolecules are not initially all pointing in the same direction, the resulting collagen fibrils will also be polarized in different directions. This is of interest in view of the recent finding

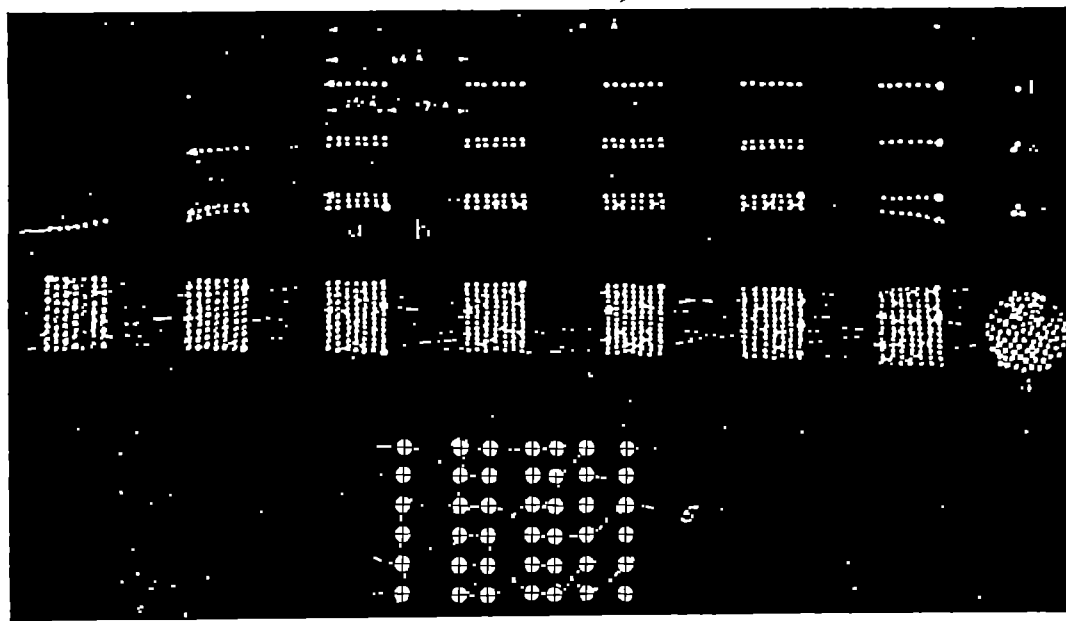


Fig. 2. 1. The diagram illustrates the proposed model for tropocollagen, consisting of a rather flexible filament divided into 9 zones. Five of these are bonding zones and four are considered as non-bonding zones. 2, 3. Association of tropocollagen by random aggregation through bonding zones is illustrated. The overlap between two adjacent macromolecules can cover one to five bonding zones. The direction of the macromolecules, indicated by arrows, determines the polarization of the collagen fibril. 4. The establishment of a repeat pattern of 640 Å results in the formation of light (*a*) bands as bonding zones and dark (*b*) bands as non-bonding zones. 5. For diagrammatic purposes, the linking with an (*a*) band is shown to illustrate the possible flexibility of the macromolecules.

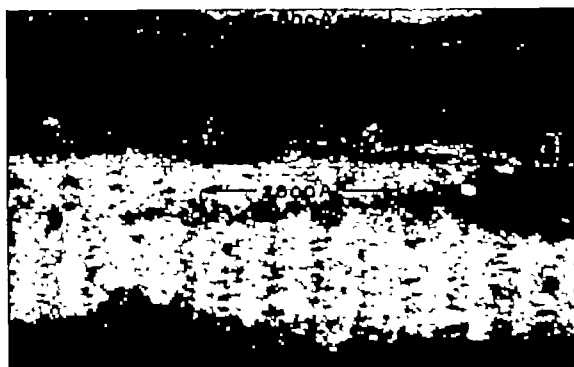


Fig. 3. a, FLS type 1, showing repeat period of about 2500 Å. b, FLS Type 1, following treatment with glutaraldehyde. The re-establishment of the 640-Å period is clearly visible. Both preparations were negatively stained.

of Braun-Falco and Rupeo<sup>24</sup> that in human dermis the collagen fibrils have an 'antiparallel arrangement'.

More direct evidence that the tropocollagen macromolecule consists of nine segments comes from experiments on the FLS form. In Fig 3a is shown an electron micrograph of FLS Type 1, made by dialysing an acetic acid solution of collagen containing serum glycoprotein; the repeat distance is about 2500 Å while the distance measured between the outer edges of the light bands corresponds to the length of an SLS crystallite (c. 2800 Å) or tropocollagen unit. This indicates that the FLS-1 structure is formed by overlap of the terminal bonding zones of tropocollagen as suggested by Olsen. However, the reason for the formation of this type of fibre may be that adsorption of negatively charged glycoprotein on to the tropocollagen units prevents the establishment of intermolecular bonds by the intermediate bonding zones, thus producing the wide spacing (Fig. 4; 1, 2). Moreover, when such an FLS-1 preparation was treated with glutaraldehyde the 640 Å repeat period was re-established (Fig. 3b). Presumably the glutaraldehyde was able to bridge between amino-groups in the blocked bonding zones to re-establish the native period. These experiments appear to provide clear evidence of the existence of 5 similar zones in the tropocollagen molecule separated by regions having different properties. The three intermediate bonding zones in tropocollagen can thus be suppressed

with glycoprotein but revealed by subsequent treatment with glutaraldehyde (Fig. 4; 2, 3).

In the case of the SLS crystallite, negative staining has revealed a marked asymmetry of the band structure. On the other hand, although Olsen<sup>17</sup> has attempted to deduce, by photographic methods, a relationship between SLS and the native fibril, a number of points remain obscure. The band pattern observed in the SLS form may in part be due to the presence of negatively charged ATP molecules interacting with positively charged groups in tropocollagen, outside as well as within the bonding regions. If this interpretation is correct, the SLS pattern could be regarded, in part at least, as an artefact with a band structure (by negative staining) not directly related to the native fibre. Furthermore, in the case of positively stained SLS form, the staining with phosphotungstic acid or uranyl acetate undoubtedly reveals the position of basic and acidic groups in the macromolecule. Positive staining, however, does not seem to indicate whether or not these groups are involved in the formation of bonds between adjacent macromolecules.

The fundamental difference between the model proposed here and other interpretations lies in the division of the tropocollagen filament into five more or less equal bonding zones separated by four regions apparently capable of forming few intermolecular links. It should be emphasized that the bonding zones mentioned in this report have no connexion with the subunits of Petruska and Hodge<sup>25</sup>. Collagen fibres have also been observed with a repeat period of only 210–220 Å (Gross<sup>7</sup>). By assuming that, under the conditions of pH and ionic strength necessary to precipitate this form, only a portion of each bonding zone is able to interact with another, it is possible to derive this repeat period from the new model (Fig. 5). It may be noted that the short period of 210–220 Å is less than the length of a bonding zone (A band) and also one-third of the native period as would be expected if this model is correct.

This investigation has been restricted to the longitudinal and transverse structure of collagen fibres in a two-dimensional sense. Work is at present in progress to include three-dimensional (cross-sectional) aspects of the problem and a three-dimensional model is being developed. Notwithstanding that this is only a two-dimensional model, since aggregation takes place in an essentially random fashion the criteria of Smith<sup>26</sup> appear to be satisfied.



Fig. 4. 1 and 2, The establishment of a repeat period of 2500 Å by tropocollagen macromolecules aggregated in the presence of glycoprotein. Linking between intermediate bonding zones is suppressed by glycoprotein, resulting in the observed long spacing. 2 and 3, FLS 1, showing re-establishment of 640-Å period by treatment with glutaraldehyde

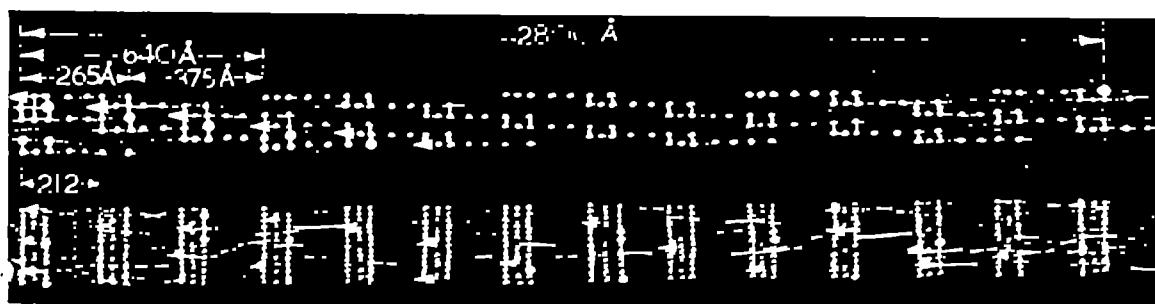


Fig. 5. The establishment of the observed short (210-220 Å) period is illustrated in the diagram. This can be considered to occur under conditions where each macromolecule is capable of interacting only with neighbours over a portion of the bonding zone.

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## FORMULATION AND USE OF EQUATIONS OF STATE FOR ELASTICO-VISCOUS LIQUIDS

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RHEOLOGY, which deals with the mechanics of continua, is a science which has come into prominence during the past fifteen years. Various disciplines, ranging from biology to chemical engineering, have been involved in its development. In the present communication I shall be concerned with the impact of applied mathematics on the science and I shall endeavour to trace the development of theoretical rheology. I shall direct particular attention to the study of incompressible elastico-viscous liquids, these being materials that are predominantly fluid but have some of the properties usually associated with solids. We have set ourselves no easy task, as a cursory glance of the literature will indicate, and newcomers to the field are faced with unnecessary hardships. These hardships are due to a number of factors, including the following:

(a) There is a confusing and ambiguous notation and terminology with an over-indulgence in definitions. This is perhaps best illustrated by considering a specific example. In a paper published in 1950, Oldroyd<sup>1</sup> defined certain kinematic tensors as 'rate-of-strain tensors'. Essentially the same tensors were re-derived by Rivlin and Ericksen<sup>2</sup> in 1955, and they have since become known as 'Rivlin-Ericksen tensors'. Noll<sup>3</sup> later introduced different 'rate-of-strain tensors' and Coleman and Noll<sup>4</sup> argued that Rivlin and Ericksen were the first to realize the importance of the 'Oldroyd rate-of-strain tensors'.

(b) There is a reluctance on the part of workers in the field to relate their work to what has gone before. The

result of this is that one gets the impression that theoretical rheology has had more than one beginning and one is left in doubt as to the merit of papers published before the various 'revivals'.

(c) There is often an unnecessary emphasis on mathematical rigour, and important physical principles are sometimes hidden by an abstract notation and analysis. In the present article it is hoped to show that although the development of the study of elastico-viscous liquids has been unsystematic and unnecessarily complicated, it is nevertheless possible to trace it.

The first major contribution that demands our attention is an article by Oldroyd<sup>1</sup> in 1950. In this paper, Oldroyd laid down certain principles that rheological equations of state have to satisfy. The two major principles may be stated as follows:

(i) The equations must be consistent with the requirement that the behaviour of a material element depends only on its previous rheological history and not in any way on the state of neighbouring elements.

(ii) The equations must be consistent with the requirement that the behaviour of a material element does not depend on the translatory or rotatory motion of the material as a whole in space. Although this principle was not given a name by Oldroyd, it has since become known by at least two names—'material objectivity' and 'material indifference'. The importance of this principle has been rightly emphasized by a number of later workers, who prefer to regard it as being concerned with indifference to an observer (identified with a moving co-ordinate

frame) rather than indifference to the absolute motion of the material in space (as in the formulation of Oldroyd), but the two approaches are precisely equivalent.

Oldroyd was able to satisfy the two principles mentioned here by introducing a convected co-ordinate system  $\xi^j$  drawn in the material and deforming continuously with it. This co-ordinate system has the property that a material element which is at  $\xi^j$  at time  $t$  will be at the same position (referred to the convected co-ordinate system) at all other times. There is an unlimited number of co-ordinate systems of the type envisaged here, but the equations  $\xi^j = \xi^j(\xi)$  relating two sets of convected co-ordinates  $\xi^j$  and  $\xi^j$  do not involve the time explicitly. This means that convected components of tensors associated with the same point of the material can be manipulated in the same way as the fixed components of tensors at a fixed point in space. Since principle (1) involves the consideration of a given material element over a period of time, the convected co-ordinate system is an ideal frame of reference to work with.

The convected co-ordinate system has the further advantage that it is unaffected by any superposed rigid-body motion (the co-ordinate surfaces being embedded in the material). This means that provided one works in a convected co-ordinate system and uses variables and operations which do not introduce any dependence on absolute motion in space, the resulting equations of state automatically satisfy the principle of material indifference (and principle (i), from that which was said earlier).

A suitable kinematic variable follows naturally from a description of the material properties in terms of a convected co-ordinate system. Since all quantities which refer to absolute motion in space are irrelevant, one is interested in the relative distance and the relative motion of the parts of an arbitrary element at  $\xi^j$ . The element of length  $d\xi$  between  $\xi^j$  and  $\xi^j + d\xi^j$  is given by:

$$[d\xi(t)]^2 = \gamma_{ji}(\xi, t) d\xi^j d\xi^i, -\infty < t < \infty \quad (1)$$

so that the metric tensor  $\gamma_{ji}$  of the  $\xi^j$  co-ordinate system is an obvious choice (but not the only one) as the kinematic variable. ( $t$  is taken to be the present time and  $t'$  an earlier time.) If  $D/Dt$  denotes a time derivative keeping convected co-ordinates constant, such quantities as  $D\gamma_{ji}(\xi, t)/Dt$ ,  $D^2\gamma_{ji}(\xi, t)/Dt^2$ , . . .  $D^n\gamma_{ji}(\xi, t)/Dt^n$  are also to be regarded as important kinematic quantities, these being measures of the relative motion of the parts of the arbitrary element at  $\xi^j$ .

Oldroyd took the covariant stress tensor  $\pi_{ji}(\xi, t)$  as the dynamic variable, and proceeded to develop his general theory on the basis of the variables defined here. The one disadvantage in working in terms of convected co-ordinates is that the equations of state should preferably be referred to axes fixed in space, since in any flow problem these equations have to be solved simultaneously with the familiar equations of motion and continuity. However, Oldroyd derived well-defined rules for transforming to a fixed system of co-ordinates. For example,  $\pi_{ji}(\xi, t)$ ,  $\gamma_{ji}(\xi, t)$  transform immediately into  $p_{ab}(x, t)$  and  $g_{ab}(x, t)$ , where  $p_{ab}$  is the stress tensor referred to a fixed system of co-ordinates  $x^a$  and  $g_{ab}$  is the metric tensor of this co-ordinate system; quantities such as  $(1/2)D^2\gamma_{ji}(\xi, t)/Dt^2$ , evaluated at  $t' = t$ , transform into rate-of-strain tensors  $e_{ab}^{(n)}(x, t)$ ,  $e_{ab}^{(1)}$  being the familiar (first) rate-of-strain tensor of classical hydrodynamics; quantities associated with the time  $t'$  transform into more complicated expressions involving the displacement functions  $x^a$ . For example,  $\gamma_{ji}(\xi, t')$  transforms into:

$$\frac{\partial x^a}{\partial \xi^j} \frac{\partial x^b}{\partial \xi^i} g_{ab}(x')$$

( $x^a$  is the position at time  $t'$  of the element that is instantaneously at the point  $\xi^a$  at time  $t$ .)

We have spent some time discussing Oldroyd's approach to the subject of formulation, in order to show that later contributions can in fact be regarded as applications of Oldroyd's general theory. These later contributions involve the writing down of what might seem to be appropriate equations of state relating suitable variables and the satisfying of the two principles mentioned here (the second being concerned with indifference to an 'observer') by means of well-defined mathematical rules. We might call this the 'mathematical' approach as against Oldroyd's more 'physical' approach. However, it must be emphasized that the two approaches are precisely equivalent.

The next contribution that requires consideration is a paper written by Rivlin and Ericksen<sup>2</sup> in 1955. These authors used the 'mathematical' approach to reduce a speculative equation of state into a form that satisfies the two principles mentioned here. Their initial equations were formulated on the basis that the stress is a function of the velocity gradients, acceleration gradients . . .  $(n-1)$ th acceleration gradients. Their derived equations can be effectively written in the form:

$$p_a = -pg_a + p'_a \quad (2)$$

$$p'_a = f[e_{ab}^{(1)}, e_{ab}^{(2)}, \dots, e_{ab}^{(n)}] \quad (3)$$

where  $p$  is an arbitrary isotropic pressure. From what was said earlier, it is clear that these equations could be immediately written down as a potentially useful set of equations of state from Oldroyd's general analysis. The actual merit of the work of Rivlin and Ericksen would therefore appear to be in the prominence the work has given to the useful equations of state given by (2) and (3), rather than in the formulation procedure used by these authors.

Later work by Rivlin<sup>3</sup>, and Rivlin and Spencer<sup>4,5</sup>, on the theory of matrix (tensor) polynomials can be regarded as a useful extension of the work of Rivlin and Ericksen.

In 1958, Noll<sup>6</sup> demonstrated that modern mathematics (particularly 'Functional Analysis') can be used with success in the formulation of equations of state. His initial work was concerned with systematizing the 'mathematical' approach and with the introduction of a very general class of fluids known as 'simple' fluids. In the notation of Oldroyd, these are defined by:

$$\pi'_{ji}(\xi, t) = \mathcal{F} [\gamma_{ji}(\xi, t')] \quad (4)$$

in convected co-ordinates, and:

$$p'_{ab}(x, t) = \mathcal{F} [G_{ab}(x, t')] \quad (5)$$

in fixed co-ordinates, where  $\mathcal{F}$  is a 'functional' and  $G_{ab}$  is given by:

$$G_{ab} = \frac{\partial x^m}{\partial x^a} \frac{\partial x^r}{\partial x^b} g_{mr}(x') \quad (6)$$

It is again apparent that although Noll's approach is different from that of Oldroyd, the same physical principles are involved in both, and either can be used to introduce the 'simple' fluids. In some ways, the Oldroyd approach has certain advantages in this respect.

Coleman and Noll<sup>7,8-11</sup> have given detailed consideration to the 'simple' fluids and have been remarkably successful in extending Noll's earlier work. For example, they have shown how the complicated equations of state given by (2) and (5) degenerate into manageable forms when the fluid has a fading memory and the flow is slow. They have also shown that it is possible to solve a limited number of flow problems in the general case.

The work of Coleman and Noll must be regarded as a very significant contribution to the subject. However, some of the importance of their work is to some extent

masked by an over-indulgence in definitions, axioms and theorems and an over-emphasis on mathematical rigour.

Many of the rheological flow problems of practical importance cannot be handled using the general equations of Rivlin and Ericksen and Coleman and Noll. It has therefore been necessary (see, for example, refs. 12 and 13) to construct simple equations of state that give an approximate description of observed behaviour and yet are simple enough to be useful in general flow problems. These equations must, of course, satisfy the general formulation principles. In this way, theoreticians have been able to supply experimentalists with a qualitative picture of rheological behaviour (see, for example, refs. 14-20). In favourable circumstances they have been able to predict important flow characteristics which have since been observed in the behaviour of real fluids. This line of research is therefore to be thought of as a practically useful extension of the formulation work discussed earlier.

Although there has been no systematic development in the study of isotropic elastico-viscous liquids, it is nevertheless true to say that the subject has reached a satisfactory stage of development. Notwithstanding the criticisms mentioned earlier, the problem of formulation

is now resolved; and the main avenues of present-day research involve the application of thermodynamic principles and wave propagation theory to rheology and the solution of flow problems of practical importance, the latter being more a problem in differential equation theory than anything else.

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## APPARENTLY SPONTANEOUS DECARBOXYLATION OF INDOLYL-3-ACETIC ACID

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**D**URING experiments on the metabolism of indolyl-3-acetic acid (IAA) in diseased plants, the breakdown of IAA was found to occur rapidly in the absence of plant tissues under some conditions in solutions of phosphate buffer at pH values between 4.5 and 5.3. This article describes some of the phenomena observed and shows how to avoid the problem. Breakdown was measured by the release of  $^{14}\text{CO}_2$  from carboxyl- $^{14}\text{C}$  labelled IAA using a similar technique to that described by Daly and Deverall<sup>1</sup>, in whose experiments spontaneous decarboxylation was negligible.

Radioactive IAA was prepared by Dr. G. W. Kirby, Department of Chemistry, Imperial College, from indole-acetonitrile- $1\text{-}^{14}\text{C}$  supplied by the Radiochemical Centre, Amersham, Bucks. Ascending chromatography of the IAA on Whatman No. 1 paper in either isopropanol/ammonia/water (85:10:15) or sodium chloride/acetic acid (100 of 8 per cent wt./vol. in water: 1) revealed one major radioactive peak, which gave a pink-purple colour after dipping in Ehrlich's reagent (1 of 10 per cent *p*-dimethylaminobenzaldehyde w/v in conc. HCl:4 of acetone) and had the same  $R_F$  as unlabelled IAA from L. Light and Co., Ltd. The ultra-violet absorption spectra of ethanolic solutions of radioactive and unlabelled IAA were identical. For experimental use, the IAA peak was eluted by ethanol from chromatograms developed in isopropanol, ammonia and water, and the eluate plus a few drops of ammonia were evaporated to dryness. The residue was dissolved in ethanol and double glass distilled water to give an ethanol concentration of 0.4 per cent. The solution was kept at  $-20^\circ\text{C}$ , in the dark, and exposed only for thawing and pipetting.

To 'Pyrex' brand beakers (3½ in. tall × 2 in. diam.) without spouts, 4.5 ml. of 0.1 M 'AnalaR'  $\text{KH}_2\text{PO}_4$  or appropriate mixtures with 'AnalaR'  $\text{Na}_2\text{HPO}_4$  were added. Flat nickel-plated mild steel planchets, 1 in. diam., containing disks of lens paper and 0.1 ml. 10 per cent KOH to capture  $^{14}\text{CO}_2$ , were suspended from rubber bungs. The planchets lay 1 in. above the base of the beakers. At the

start of the reaction, 0.5 ml. IAA solution was added to the buffer, and the vessels were transferred to a platform shaker moving at 100 strokes/min under a light-proof cloth at  $20^\circ\text{--}22^\circ\text{C}$ . Planchets were replaced when necessary, and dried at  $70^\circ\text{C}$  for 1 h before counting while hot in an Ekco sample oven beneath an end-window Geiger-Müller tube coupled to an Ekco scaler. Initial radioactivity of IAA in the reaction mixtures was obtained from 0.1 ml. aliquots pipetted on to 0.1 ml. 10 per cent KOH and lens paper in planchets. Thus all samples were counted at standard thickness and for 300 sec, and the decarboxylation expressed as a percentage of the counts from the IAA- $^{14}\text{C}$  appearing as  $^{14}\text{CO}_2$ .

The problem of the decarboxylation became apparent during a series of experiments using new beakers and tomato stem tissues. The beakers were routinely washed in hot mains water and glass-distilled water, but had received one overnight soak in a chromate cleaning solution followed by copious water washings and soaks. Table 1 shows the effect of pH on decarboxylation. Each replicate beaker contained 10 µg/ml. IAA, supplying 0.5 µg.

Excessive liberation of  $^{14}\text{CO}_2$  in the first hour was a pronounced feature of the reaction. All parts of the reaction system were checked to find the cause. First the same beakers were used after further water washing, and the effects of ethanol and unlabelled IAA, which was used to make up the concentration of IAA, were tested at pH 4.5. Results are given in Table 2.

Clearly the addenda were not the cause of the trouble. The competitive effect of unlabelled IAA and the surprising inhibitory effect of ethanol were recorded.

The glassware and the water used to make up the reaction mixtures were then tested for their effects on the stability of IAA.

Table 3 summarizes the results of experiments on the effects of treatments applied to the surface of the beakers used to obtain the results in Table 1 and which had been exposed to chromate once in their history. Glass-distilled water was used in these tests.



Table 1. EFFECT OF pH ON CUMULATIVE DECARBOXYLATION OF IAA

Time (h)	4.5	pH 4.7	5.3	6.0
1 h	18.7%*	17.3%	2.7%	0.6%
3 h	19.4%	18.1%	4.3%	0.9%

\* Initial radioactivity of IAA per beaker = 17,000 c.p.m.

Table 2. EFFECT OF ETHANOL AND UNLABELLED IAA ON CUMULATIVE DECARBOXYLATION OF IAA

Time (h)	Labelled IAA*	Labelled IAA* in 0.04% ethanol	Labelled IAA* in 0.04% ethanol plus unlabelled IAA†
1 h	6.4%	4.9%	5.6%
3 h	16.1%	10.9%	6.6%

\* 1.66 µg/ml. supplying 18,000 c.p.m. per beaker

† 10 µg/ml. giving final IAA conc. of 11.66 µg/ml.

Table 3. EFFECTS OF GLASSWARE TREATMENTS ON THE STABILITY OF IAA

Series*	Glassware treatments	Decarboxylation % - 1st h
I	H <sub>2</sub> O	13.4
	dichromate/H <sub>2</sub> SO <sub>4</sub>	21.4
	1N HCl	18.5
	1N HNO <sub>3</sub>	17.8
	1N NaOH	16.0
II	dichromate/H <sub>2</sub> SO <sub>4</sub> --- NaOH	1.9
III	hot 10% methanolic NaOH	30.0
IV	celloidin membrane	1.5

Reaction mixtures - 1.6 µg/ml. IAA at pH 4.5

\* I - 3 h soak in sol., repeated rinses in water and 1 h soak in water.

II - 18 h soak in sol., repeated rinses in water, 40 min soak in 0.1 N NaOH and further water rinses.

III - 10 min in sol. and repeated rinses in water.

IV - Rinse in sol. celloidin in ether and few drops ethanol, dried and rinsed in water.

Therefore further treatment with chromate accentuated the subsequent instability of IAA. Several treatments with acids and alkali were worse than mere water soaking, and the disastrous effect of methanolic NaOH is evident. However, removal of chromate by dilute NaOH using a recommended technique<sup>1</sup> or separation of IAA from the glass by a celloidin membrane greatly increased the stability of IAA.

Comparison of different types of water to make reaction mixtures showed that IAA was equally stable in single and double glass-distilled water and in water from a metal still. However, ion-free water from a laboratory ion-exchange demineralizer stimulated decarboxylation to 18.9 per cent in the first h when tested as part of series II in Table 3.

Finally, tests were performed on new 'Pyrex' brand beakers. Table 4 shows the stability of IAA in lots of glassware, which differed only in length of storage from manufacture to use, and which were prepared by washing in hot 'Stergene', hot mains water and double glass distilled water before drying at 90° C. Batches of the newest beakers from the warehouse of James A. Jobling and Co., Ltd., Sunderland, and sent by Mr. F. C. Sedgwick were also subjected to different desirable pretreatments which he suggested.

Table 4. EFFECT OF NEW BEAKERS AND THEIR PRE-TREATMENT ON THE STABILITY OF IAA

Source of beakers	Pre-treatment	Decarboxylation % - 1st h
College stores - old stock	'Stergene', H <sub>2</sub> O and 90° C	1.5 - 2.5
College stores - new stock	" " "	1.5 - 2.5
Factory warehouse	" " "	0.9
Factory warehouse	and repeated use and soaking in H <sub>2</sub> O	0.8
Factory warehouse	autoclaved (inverted) for 1 h at 121° C*	0.9
Factory warehouse	warm 5% acetic acid*	1.5
Factory warehouse	and "overnight" soaking in H <sub>2</sub> O	0.6

Reaction mixtures - 1.0 µg/ml. IAA at pH 4.5 (double glass-distilled water).

\* Then rinsed in water, acetone, drained and dried at 90° C.

Percentage decarboxylations obtained in the newest glassware after water washing or soaking were comparable to those recorded by Daly and Deverall<sup>1</sup>, who worked at 15° C and used solutions supplemented with labelled IAA to give final concentrations of 10 µg/ml. The greater instability of IAA in beakers which had been stored for longer periods and during a London winter may be attributable to the deleterious effects of atmospheric pollutants on glass surfaces.

Therefore, when working with IAA at pH values of 4.5-5.3, it is necessary to check glassware, which may have been chemically contaminated in storage or in prior use, and to avoid cleaning solutions which may damage the glass surface or be difficult to remove. Glass-distilled water may be preferable to ion-free water.

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## THE BRAIN OF THE HETEROSTRACI (AGNATHA)

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BENEATH the dorsal headplate of the heterostracan group of palaeozoic agnathan fishes there are often impressions which appear to represent the dorsal surface of the brain; a number of neural features have been described, particularly of cyathaspid Heterostraci, from this evidence. The structures revealed usually include an identifiable pineal organ, anterior and posterior semi-circular canals, and some dorsal areas of the brain, as shown in Fig. 1, which is based on illustrations of *Foraspis polaris* and *Homalaspisidella* (*Homalaspis*) *nitida* by Kiaer and Heintz<sup>1</sup>.

The cyathaspids were very early and so presumably very simply organized vertebrates. A modern brain with which this one might be compared would then be that of the lamprey (compare Goodrich<sup>2</sup>), or that of the primitive shark *Heptanchias* (compare Johnston<sup>3</sup>) which is seven-gilled, as were probably the cyathaspids<sup>4</sup>. But the brain of the larval or pride stage of the lamprey, shown in Fig. 2, is perhaps more suitable: as Watson has pointed

out<sup>5</sup>, the Heterostraci probably passed through a larval stage in which they would resemble the pride in many respects.

Several attempts to identify the regions of the heterostracan brain have been made, in particular those of Stensiö; his account of 1958 may perhaps be regarded as the definitive one<sup>6</sup>. The most recent is that of Denison<sup>7</sup>, who has made brief but useful comments on the brain in the course of a general description of cyathaspids. Interpretations differ, and it is possible that they have been made from too small a factual basis, but we consider that the visible regions can be identified safely if the comparative evidence is assessed.

Some non-skeletal tissue probably formed at least part of the impressions, for the roof of the chondrocranium in lower vertebrates is separated from the brain by wide tissue-spaces and may itself have extensive fontanelles, so that the roof could only give impressions of brain-shape in quite localized areas. Now in cyclostomes, the sur-

viving Agnatha, the meningeal envelope around the brain is simple: there is a compact meninx primitiva adhering closely to the brain; outside the meninx is a thick perimeningeal zone containing mucoid cells<sup>7</sup>. The impressions may therefore have been made at the boundary between meninx and perimeningeal tissue, where there would be considerable chemical and physical differences between the two tissues, or partly at the outer surface of the perimeningeal tissue, adjacent to skeletal parts<sup>8</sup>. Certainly the distinction between nervous tissue and choroid plexus, which shows up in the hind-brain region especially, would be difficult to account for on a basis of impressions made by skeletal parts alone, and indeed there seems little reason to suppose there was extensive internal cartilage in Heterostraci.

The medulla oblongata extended as far forward as the level of the junction between the anterior and posterior semi-circular canals, where the VIIIth nerve enters the brain; it is unlikely to have extended further forward, as will be seen later. The medulla is rather elongate, and tapers slowly towards the spinal cord; this is probably related to the large number of gills innervated by the Xth nerve, six as in the lamprey, for these involve a longer extent of the visceral components within the central nervous system. The central dorsal area evidently represents a choroid plexus, which would extend in life above the dorsal limits of the medulla, but might either collapse or fail to be preserved in fossil material (compare Kiaer and Heintz, Plate 30, ref. 3, with Goodrich's figure). The area of the hind-brain plexus is narrower than in lampreys, suggesting that there was a smaller concentration of grey matter here than in the lampreys; if so, the cyathaspid medulla was less developed and retained a greater resemblance to the spinal cord.

The otic capsules would closely surround the system of semi-circular canals; the horizontal canal if it existed would not be viable, but it is generally believed to be absent, since it is not developed (from the anterior canal) in modern Agnatha. The capsules extend forward as far as the caudal part of the mid-brain as they do in the adult lamprey. In the pride stage the capsules are very delicate; a similar condition in the Heterostraci would account for the canals, and not the capsules, forming the surface which provides the impression in the otic region.

The cerebellum in the lamprey is little more than a small transverse commissure of the VIIIth and lateralis components. In cyathaspids it is difficult to discern this region. There may have been a small cerebellar commissural band or merely a narrow choroidal 'neck' joining the

larger choroidal areas of the mid- and hind-brains: the latter would be a more primitive condition.

The mid-brain appears to have been like that of the larval and adult lamprey, retaining a central choroidal plexus; this is a stage through which the mid-brain passes in the development of jawed fishes. The condition is strongly indicated not only in Stensio's figures but also, for example, in those of Kiaer and Heintz of *Poraspis polaris*. This region has been identified by Kiaer and Heintz as mid-brain, and possibly by Stensio in Fig. 205A; but elsewhere Stensio identifies this rounded region with its flat centre as a part of the hind-brain, for example, Fig. 180.

The conformation of the visible parts of the mid- and hind-brain is then similar to that of the lamprey except for the apparent absence of a short hind-brain region in front of the VIIIth nerve. Tentatively, this difference would be explained very satisfactorily if the dorsal pre-otic nerves, that is V<sup>1</sup>, V<sup>2+3</sup> and VII, entered the brain dorsal to the corresponding somatic motor nerves III (and IV) and VI, that is, in what is believed to be their primitive cranial position, so that at least the trigeminal roots came from the mid-brain wall and not that at the front of the hind-brain. However this may be, the identity of the mid-brain seems unmistakable.

Turning to the fore-brain, the exposure of the brain-roof, between the mid-brain and the pineal complex, is longer than in most vertebrates. The diencephalon of the lamprey is also long, the pineal and parapineal organs extending forward from their origins in the pair of habenular ganglia. But the diencephalon of *Hepttranchias*, as illustrated by Johnston, has a roof that is remarkably like that of cyathaspids, and it has the symmetrical form that the lamprey, like many fishes, lacks.

The telencephalon (end-brain) is not visible in front of the diencephalon. Stensio has suggested that the front of the brain was sharply down-turned and so the end-brain would not be visible in the impressions: he pointed out that young myxinoids have a cephalic flexure of this sort. But in hagfishes this flexure is embryonic and is lost by the time of hatching<sup>9</sup>. Denison remarks that it is logical to assume that the pineal organ "lay, as in lampreys, at the anterior end of the diencephalon, and that the telencephalon lay antero-ventrally to it. There is no evidence that the fore-brain was crowded as in embryonic myxinoids, as claimed by Stensio (1958, p. 374)". In Stensio's reconstruction-figures, however, the end-brain is shown, not down-turned but strongly fore-shortened, and pushed back to either side of the diencephalon, while the rest of

the brain is elongate. His figure therefore follows the brain of the lamprey, and not that of the hagfish, where the whole brain is extraordinarily compressed. The compression in the lamprey is brought about by its nasohypophyseal system: in the pro-ammo-coete stage, where this system has not moved back and come to press on the brain, the end-brain still extends anterior to the diencephalon, as measured along the axis of the notochord<sup>10</sup>.

So it seems that compression antero-posteriorly, of either type, that of lamprey or hagfish, is not to be expected in cyathaspids. Stensio's reconstructions (Figs. 200 and 209) would raise many problems, particularly the whereabouts of the diencephalon and the identity of the constricted area of brain in front of the otic capsules, which is not indicated in these figures, although it is shown in figures of actual specimens, for example, Fig. 206.

We believe that the end-brain lay directly anterior to the diencephalon. The end-brain of *Hepttranchias* may be used for comparison: it is one of the least specialized

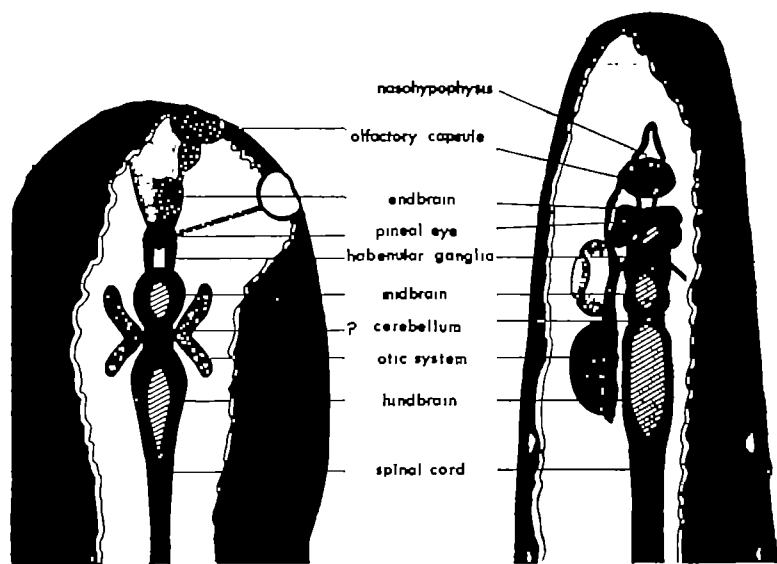


Fig. 1

Fig. 2

known among vertebrates, retaining a large telencephalon medium around the median ventricle. This end-brain has been drawn on the right in Fig. 1 (dotted white); at this scale the diencephala in the fossil and the living form are almost coincident in roof pattern. The olfactory system has also been drawn from *Hepiranchias*, although the length of the olfactory tract, which of course varies greatly, has not been copied; the position of the olfactory capsules in Heterostraci is not known precisely, but the capsules are sited by most workers in the position shown.

If the end-brain lay in this position it would not necessarily leave a clear impression; this would depend on whether the dorsal surface were nervous, as in *Hepiranchias*, or choroidal, and on the nature of the overlying tissues. There would apparently be room for the end-brain below the dorsal shield in this position; this can be seen from Denison's Fig. 105. In cyathaspid material, a trough, in the position where the end-brain of *Hepiranchias* would lie, has frequently been observed, for example, in Stensio's Fig. 205A. This may well be the impression of the telencephalon: the area of this trough is indicated by shading in Fig. 1.

So there seems good reason to suppose that cyathaspids, which are among the earliest vertebrates known, possessed, not a specialized, but on the contrary a very primitive form of brain. The brain was as primitive as that of the

pride but lacked the epithalamic asymmetry and compressed form of end-brain found in the latter. The brain of cyathaspids, and presumably also of other Heterostraci, may have been more primitive than that of any surviving vertebrate in its hind-brain organization: the anterior region of the hind-brain does not yet appear to have been built up dorsally by the caudalward migration of the pre-otic dorsal roots towards the post-otic dorsal roots. This migration must certainly have occurred during early vertebrate phylogeny; the condition in cyathaspid fossils may indicate where it occurred. We have previously pointed out that the somites of the pre-otic region were also organized in a more primitive manner than in any living vertebrate<sup>11</sup>.

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## TAIL TO TAIL AGGLOMERATION OF BULL SPERMATOZOA BY PHYTOAGGLUTININS PRESENT IN SOY-BEANS

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IT has been known for a long time that extracts of certain plant seeds (lectins) agglutinate erythrocytes of human as well as of animal origin. Many workers have shown that certain leguminous lectins are specific for certain blood group factors in human beings and chickens<sup>1-4</sup> and have demonstrated their usefulness in practical blood typing<sup>5</sup>. It has been previously demonstrated that mammalian spermatozoa exhibit antigenic characteristics in the identification of blood-group antigens in man and the bull or in the agglutination test of spermatozoa by antisera<sup>6-12</sup>. Sperm agglutination is a very general phenomenon, but any investigations of the action of natural heteroagglutinins on mammalian spermatozoa would be of interest in relation to spontaneous infertility in cattle and selective fertilization of animals if one were found that was specific for spermatozoa (see Tyler's discussion of the relation between immunological phenomena and fertility control<sup>14,15</sup>).

Experiments were designed to investigate the action of plant (soy-beans) extracts on fresh bull spermatozoa and were planned along the same lines as agglutination tests in blood grouping. Saline extracts of 21 strains of soy-beans, including 'Glycine max(L.)' and 'Glycine ussuriensis', were used. The procedure for extracting the lectin from soy-beans was a modification of that described by Boyd *et al.*<sup>16</sup>, and Leister and Kirk<sup>17</sup>. A 5-g sample in 2 volumes of saline was refrigerated for two nights and then thoroughly ground. The pulp was filtered through gauze, and centrifuged at 4° C for 15 min to remove debris. After the supernatant had been heated in a 100° C water bath for 30 min, the mixture was centrifuged and the final supernatant used in the test for the lectin. Sperm suspensions for the agglutination test were obtained by suspending artificially collected bull spermatozoa in 100 volumes of sterile saline. These were examined microscopically as well as macroscopically

30 min after mixing with equal volumes of lectins at room temperature (23°-25° C) using an agglutination slide for serology.

1,103 tests were carried out on the agglutinating effects of 21 soy-bean lectins on spermatozoa from 92 cattle. In 362 cases the spermatozoa showed a specific tail type of agglutination (Fig. 1). Spontaneous head-to-head agglutination of spermatozoa is a very general phenomenon and can be shown *in vitro* by saline dilution of mouse, horse, or fowl spermatozoa (it occurs less readily with bull spermatozoa). The present tail-to-tail type of agglutination is different from the head-to-head type of agglutination, and the tails of bull spermatozoa, which are highly motile, agglomerate in clumps showing a rim of heads on the outside in the suspension with soy-bean lectins. This tail-to-tail agglutination of bull spermatozoa

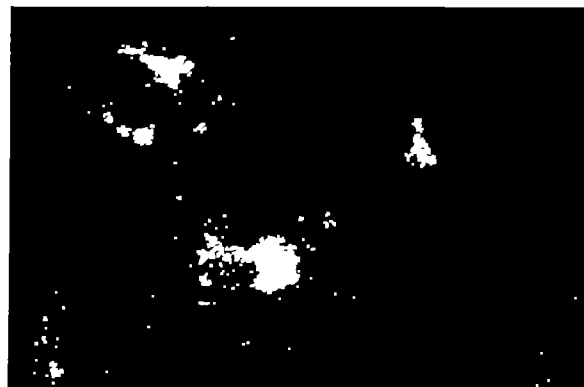


Fig. 1. X-type reaction of bull spermatozoa, tail-to-tail agglutination of sperm by phytoagglutinins of soy-bean. Phase contrast ( $\times 200$ )

resembles a 'three-dimensional cart wheel' and is similar to hetero-sperm agglutination in *Katharina tunicata*<sup>18</sup>, agglutination of bull spermatozoa in an antiserum<sup>19</sup>, tail agglutination of *Megathura orenulata*<sup>19</sup>, and agglutination of animal sperm with the normal sera<sup>20</sup>. This type of bull spermatozoa agglomeration, which occurs when a sperm suspension is mixed with soy-bean lectins, is a very simple  $Z_I$ -type agglomeration, does not occur spontaneously and is distinguishable from other complicated types of agglomeration or agglutination ( $Z_{II}$ ,  $Z_{III}$ , etc.)<sup>21</sup> observed in the test using other plant lectins, for example, from (*Phaseolus angularis*, *Ricinus communis*, etc.). It has been proposed that these tail-to-tail agglomerations should be called  $Z$ -type agglomeration, from the German *Zusammenball*, thus directing attention to their specificity without confusing them with usual agglutination<sup>18,19-20</sup>.

It was of interest to find that  $Z_I$ -agglomeration of spermatozoa did not occur in every sample of bull semen, but that it was individual-specific, and even breed-specific (Table 1). Some lectins specifically agglomerated sperms of Holstein-Friesian.  $Z_I$ -positive reactions occurred in 38 per cent of the Holstein-Friesian samples, 17 per cent Black Japanese (work cattle) and 11 per cent Brown Japanese (work cattle). In Table 1 the more interesting data are also shown as a percentage of the bulls tested whose spermatozoa reacted, but for which the results are not statistically significant (due to the small number of animals used).

Table 1.  $Z_I$ -TYPE AGGLOMERATION OF BULL SPERMATOZOA OBSERVED USING SOY-BEAN LECTINS

Breeds of cattle	% Tested bulls whose spermatozoa react			% Tests that show positive reactions		
	No. of bulls tested	Positive bulls	% positive	No. of total tests	$Z_I$ -agg.	% of $Z_I$ -positive
Holstein-Friesian (H)	68	48	70.6	808	312	38.6
Black Japanese (B)	12	6	50.0	106	18	17.1
Brown Japanese (R)	9	4	44.4	90	10	11.1
Jersey (J)	2	0		12	0	
Brown Swiss (BS)	2	0		5	0	
Total	92	58		1,020	340	

Statistical tests:					
	H	B	R		
No. positive bulls	48	6	4	No. positive reactions	18 10 312
No. negative bulls	20	6	5	No. negative reactions	87 90 496
Total bulls	68	12	9	Total tests	106 90 808
$\chi^2 = \begin{Bmatrix} 1.97 & 0.06 \\ 2.48 & \\ 25-10 & 90-75 \\ 25-10 & \end{Bmatrix}$					
$P: \begin{Bmatrix} 25-10 & 90-75 \\ 25-10 & \end{Bmatrix}$					

The lectins of soy-beans were found to be breed-specific in  $Z_I$ -positive reaction (Tables 2 and 3). It was interesting to find that soy-bean lectins showed almost entirely  $Z_I$ -type agglomeration (340  $Z_I$ -positive and only 12  $Z_{III}$  in 1,020 tests). The detailed results of  $Z_{III}$  reactions will be presented later. It has been suggested that the reaction of human blood with peanut lectin could be used in population studies<sup>22</sup>, so the present reaction with the soy-bean lectins could be useful for grouping bull sperm—especially if one were established that was specific for spermatozoa.

Further investigations are being made of the mechanism of sperm agglomeration, and of the chemical properties, including a molecular analysis, of the lectins. It may be noted that spermatozoa are observed only in motile conditions and disappear 2 h after the beginning of the test. Our present observations suggest that the agglutinins of the seed extracts may be of a protein nature and heat-stable. It would also seem possible that the lectins can be divided into a number of groups.

We thank the Ibaraki Agricultural Experiment Station and the National Institute of Animal Industry for the gifts of seed used in these investigations and the National Breeding Station of Livestock-Fukushima, Local Live-

Table 2.  $Z_I$ -REACTION OF BULL SPERMATOZOA AND BREEDS OF SOY-BEANS: SUMMARIZED DATA

Bull No.	No. of soy-beans extract																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	21	22	23	25
H 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
64	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
66	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
68	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
J 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BS 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+,  $Z_I$  aggregation; -, no aggregation; 2,  $Z_{II}$  aggregation; 3,  $Z_{III}$  aggregation.

Table 3. VARIETY OF SOY-BEAN USED FOR AGGREGATION TEST WITH BULL SPERMATOZOA

No.	Variety of glycine max (L.) Merrill, soy-bean seed extracts	% $Z_I$ -type agglomeration	No. of tests
1	Kinsume Ibaraki No. 1	22.2	36
2	Kongo Daiyu	27.8	36
3	Manchu	14.3	14
4	Tachisunari	41.8	67
5	Shinmeifuro	30.6	36
6	Okumefuro	14.3	14
7	Norin No. 1	30.6	36
8	Norin No. 8	39.0	82
9	Alex	39.0	77
10	Hatoguroshi	18.6	59
11	Norin No. 2	40.4	77
12	Tokachi-ohyo	25.0	36
13	Akabaya (Yamanashi)	40.9	22
14	Comet	35.9	78
15	Harosoy	38.9	36
16	Bonminori	37.1	70
17	Nemashirazu	38.8	80
18	Lee	30.2	84
19	Fujinsume	23.8	80

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## CIRCADIAN PERIODICITY IN SOME PHYSICO-CHEMICAL PARAMETERS OF CIRCULATING BLOOD

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THE view that an organism maintains physicochemical parameters at specific levels has impeded studies of their oscillatory nature and obscured the aims of some laboratory investigations. Endogenous rhythms of approximately 24 h duration have been termed 'circadian' by Halberg<sup>1</sup>. Circadian rhythms continue under adverse environmental conditions such as reversal of the light regimen or sleeping habits. A routine work schedule should synchronize the endogenous circadian rhythm with the 24-h scale. These circadian rhythms characterize a number of basic cellular processes as well as the endocrine and neural controls of functional co-ordination in time<sup>1,2</sup>. Halberg<sup>1</sup> has documented the role of a circadian adrenal cycle in the maintenance of external timing.

In man it has been shown that temperature<sup>3</sup>, blood volume and haematocrit<sup>4,5</sup>, total serum protein<sup>6,7</sup>, circulating blood cells and certain electrolytes and steroids<sup>8</sup> exhibit circadian rhythms. Circadian changes in the erythrocyte sedimentation rate remain debatable<sup>9</sup>.

In the present study a number of related physicochemical parameters have been measured at standard time intervals in order to determine the extent to which they exhibited a circadian rhythm. An appreciation of the oscillatory nature of the viscosity of blood and levels of certain components of the plasma may lead to a re-evaluation of the significance of 'normal values' and clarify some aspects in the periodicity of host-parasite relationships as exemplified by the cycle of microfilariae in the peripheral blood.

Three male volunteers participated in the study for a period of 72 h. (R.E. 59, 155 lb.; R.S. 55, 136 lb.; W.H. 27, 180 lb.) and one female (B.E. 26, 121 lb.) for two 24-h cycles. The volunteers were in bed from midnight until after the 0800 sample had been taken and were asleep at the time of withdrawal of the 0400 sample. The oral temperatures of the subjects in °F were measured just before each venepuncture. Blood samples (20 ml.) were collected in heparinized syringes at 4-h intervals. The times indicated under results represent midpoints covering a period of about 20 min. Subjects were fasting for the 0800 samples, meals being taken at 08.30, 1200, tea 1500, and 1800. Varying degrees of lipaemia were present in the plasma samples.

Screen filtration pressures were determined as described by Swank<sup>10</sup> and Swank, Roth and Jansen<sup>11</sup> immediately after sampling. Erythrocyte sedimentation rates in mm/h were obtained using Wintrobe tubes and these were then spun at 2,000g for 40 min to determine the haematocrit.

Coefficients of viscosity for each sample of blood were determined at shear rates of 2.30, 5.80, 11.5, 23.0, 46.0 and 115 sec<sup>-1</sup> in a Brookfield micro cone-plate viscometer maintained at 25.0 ± 0.1°. The viscometer was equipped with a torsion spring giving full-scale deflexion at 336 dynes cm and a standard spindle (cone-plate angle 1.57°, diameter 4.8 cm).

Calcium, potassium and sodium ions were estimated by means of a Beckman 'DU' spectrophotometer with a flame attachment and photomultiplier<sup>12</sup>, the procedures and calculations being performed as previously described<sup>13</sup>. Total plasma proteins were determined by the biuret method of Weichselbaum<sup>14</sup> and also, in the case of reasonably non-lipemic samples, with a Bausch and Lomb serum protein meter<sup>15</sup>. Protein-bound sialic acid was determined in the

trichloroacetic acid-insoluble fraction of plasma, using Svennerholm's resorcinol reagent<sup>16</sup>. Protein-bound carbohydrate was estimated using the phenol-sulphuric acid colorimetric method<sup>17</sup> adapted to the trichloroacetic acid-insoluble fraction of plasma with a mixed standard of 1:1 galactose mannose. Plasma-bound hexosamines were assayed by the procedure of Winzler<sup>18</sup>.

The withdrawal of 20 ml. blood every 4 h limited the extent of sampling in single individuals. It was assumed that the pooling of simultaneous observations on four individuals (mean of the population sample mean) over a period of about 72 h is equivalent to a theoretically continuous period of two weeks on a single individual (overall sample mean). The closer the mean of the population sample mean corresponds to the overall sample mean the more probably is this procedure valid. After withdrawal of the regular 0800 specimen on the last day of the experiment, two of the subjects engaged in special physical exercise by running and climbing stairs so as to observe the effect of strenuous muscular activity on the values of the physico-chemical parameters.

The circadian rhythm of temperature and of total plasma protein in relative values is shown in Fig. 1. The overall sample mean value for oral temperature was 97.63° F and the mean of the population sample mean 97.64° F; accordingly 97.6° F corresponds to 100 per cent. The range varied between 96.6° F and 98.6° F. Statistical analysis gave an *F* ratio of 2.079 with *P* = 0.021. Total plasma protein levels followed a similar course, with overall and population sample means of 8.42 g per 100 ml. The *F* ratio was 5.232, *P* < 0.001. Protein-bound carbohydrate and hexosamine levels follow the total protein rhythm. The overall sample mean value for hexosamine was 80.08 mg, the population sample mean 79.87 mg and the range from 65.1 mg to 98.2 mg per 100 ml., *F* ratio 5.170, *P* < 0.001. The overall sample mean value for protein-bound carbohydrate was 119.5 mg per 100 ml., and the mean of the population sample mean was 119.7 mg per 100 ml. The range varied between 102 and 135 mg, *F* ratio 3.919, *P* < 0.001.

Plots of the means of screen filtration pressure and also erythrocyte sedimentation rate against time suggested visually the presence of a circadian rhythm. However, the individual differences, and hence the standard deviations of the pooled samples, were too great for evaluation of significance by variance analysis and therefore detailed documentation is omitted.

Total protein, protein-bound carbohydrate and hexosamine levels were at a minimum between midnight and 0400. Haematocrit and sialic acid (Fig. 2) showed similar minima, but the maxima occurred at 0800, a few hours before the peak in total protein, protein-bound carbohydrate and hexosamine. Protein-bound sialic acid (Fig. 2) had an overall sample mean value of 45.7 mg per 100 ml. and mean of population sample mean of 45.6 mg per 100 ml. with a range from 36.8 to 60.3 mg, *F* ratio 2.37, *P* = 0.0085. The haematocrit had an overall sample mean value of 44.2 with a mean of the population sample mean of 44.1. The values varied between 41.0 and 48.5 (Fig. 2). The *F* ratio was 2.52, *P* = 0.0053.

Plots of the relative circadian changes in viscosity of blood at all shear rates have been superimposed and graphic representation of the standard deviations omitted

for clarity. Analysis of variance gave  $P < 0.001$  in all cases. The range of percentage change from the mean decreased with increasing shear rates as follows:

Shear rate in  $\text{sec}^{-1}$ : 2.30, 5.80, 11.5, 23.0, 46.0, 115.

Range of relative percentage difference from mean: 62-136, 79-123, 86-121, 87-114, 89-116, 91-112.

Changes in the concentration of calcium, sodium and potassium ions did not show a circadian rhythm in whole blood, in plasma or in the values calculated for red cells. Any changes that might have occurred in the distribution of cations would be masked because the time required for the cations to exchange with the extravascular compartments is brief in comparison with the time required for significant circadian changes to occur in an associated parameter such as the blood volume.

The values of blood viscosity and levels of total protein, protein-bound carbohydrate, hexosamine and sialic acid in plasma follow, not unexpectedly, in view of their

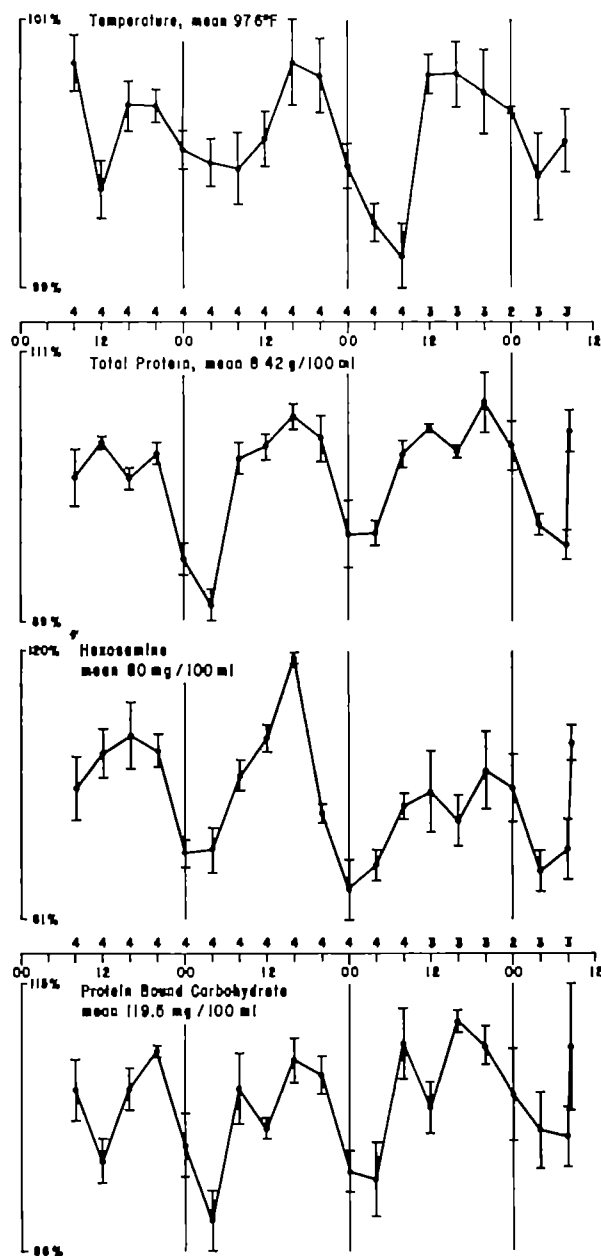


Fig. 1. A transverse profile on four subjects relating variations over a 72-h period in temperature, total plasma protein, hexosamine, and protein-bound carbohydrate. The data are plotted as relative change, per cent of mean, and the standard deviation is indicated. The mean is given in absolute values. Midnight is marked by 00 and a vertical line; noon by 12. The number of pooled samples is indicated at 4-h intervals on the time axis.

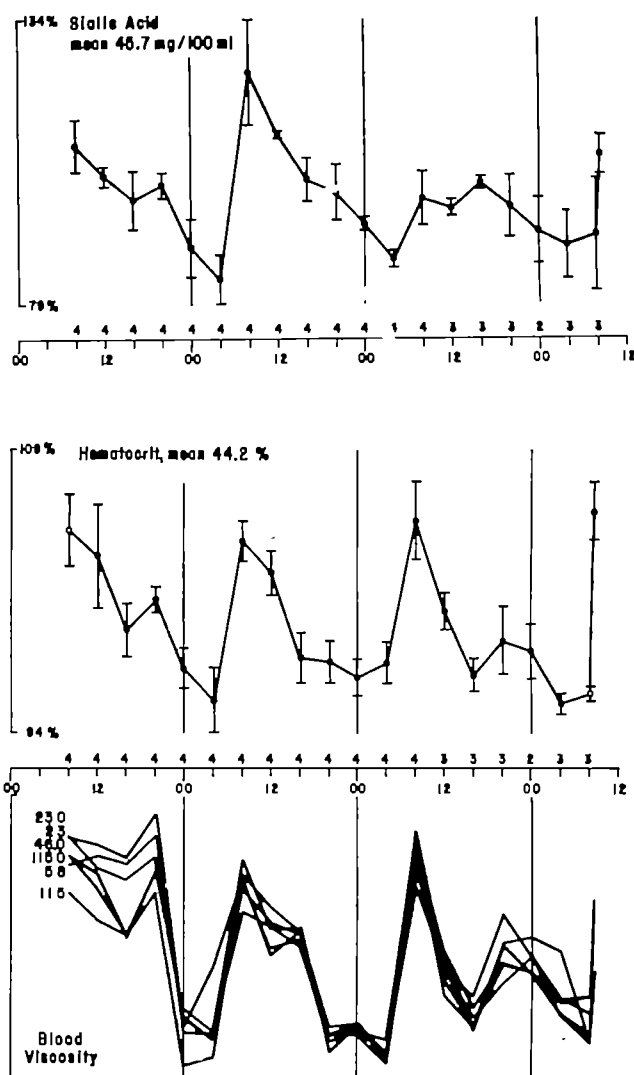


Fig. 2. A transverse profile on the same four normal adults relating variations over the 72-h period in sialic acid, haematocrit and whole blood viscosity. The data are plotted as relative change, and per cent of mean. Viscosity is measured in centipoises at six specific shear rates as indicated by the numbers ranging from 2.30 to 115  $\text{sec}^{-1}$ . In the figure for blood viscosity the plotting of standard deviations has been omitted because of overlapping.

relationship to blood volume, the known course of circadian changes in haematocrit<sup>6</sup>. It is likely that the fluctuations in the physicochemical parameters arise in part from the rapid transference of water from the peripheral circulation into other compartments as a result of the changes in posture and muscular activity which occur from day to night. Venous stasis of a limb, for example, leads to a general exchange of water in the body<sup>10</sup>. The increases observed in these parameters, following additional exercise, support the hypothesis of the rapid transfer of water.

However, there is also an underlying endogenous periodicity. Renbourn<sup>7</sup> has reported a significant decrease in plasma protein-level after 22.00 h which is independent of bed rest, and Bartter, Delea, and Halberg<sup>8</sup> have shown that the diurnal elevation of urinary aldosterone cannot be explained solely on the basis of haemodynamic changes.

The elevation in temperature and blood viscosity during the initial period of the observations is attributable to normal subjects showing a greater variation than usual because of anxiety ('novelty effect'). Once the daily routine was established even the perturbation produced by the venepuncture at 4-h intervals was normally without significant effect on the circadian rhythm. Amplification of the changes in the physicochemical parameters occurs

if an enhancing procedure such as exercise is carried out during the endogenous rises at the time of arousal.

The individual variation in the screen filtration pressure was too great to enable any quantitative assessment of circadian periodicity in this parameter. The screen filtration pressure depends on a number of variables, including concentration of blood elements, plasma constituents and, in particular, adhesiveness and aggregation of platelets, leucocytes and red cells. The white cell count is known to exhibit a circadian cycle mediated by the activity of the adrenal gland. In man the lowest white cell counts occur during the day<sup>22</sup>, which would favour low values for the screen filtration pressure, whereas the plasma protein-levels and haematocrit tend to be high at that time and thus nullify this effect.

Again, the erythrocyte sedimentation rates which depend on haematocrit, rate of aggregation of the red cells, composition of the plasma, electrical charge on the cells, etc., showed no direct circadian periodicity. It was not considered feasible to correct the erythrocyte sedimentation rates for the effects of circadian variations in the values of the associated parameters.

Maxima in the value for the viscosity of blood are better synchronized with the peaks in concentration of protein-bound sialic acid than with the peaks of total protein-bound carbohydrate. Sialic acid is largely responsible for the electrokinetic charge carried by circulating blood cells, especially erythrocytes<sup>23</sup>, and also in part for the charge and rheological behaviour of many glycoproteins. The fluctuation in levels of sialoproteins in the blood may affect the value of the electrokinetic charge borne by circulating cells and endothelial surfaces and thus influence the cycle of such parasites as microfilariae. The 24 h rhythms in *Wuchereria bancrofti* microfilariae and blood eosinophils have been compared by Engel, Halberg, Dassanayake and de Silva<sup>24</sup>. These organisms are known to accumulate in the lungs during the day, being released

again at night. If the microfilariae are constrained in the lung capillaries by electrostatic forces, physicochemical changes mediated by the circadian variation in the level of sialoproteins could lead to changes in the electrical charge carried either on the filariae or wall of the capillary and thus to the release of the microfilariae into the peripheral circulation again.

Although the fluctuations in many of the physicochemical parameters are largely attributable to 'activity potentiated' changes in haemodynamics there remain endogenous rhythms for which the nature of the mechanism of the ultimate controlling system, or 'master biological clock', is unknown at present.

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## DENSE RANDOM PACKING OF BINARY MIXTURES OF SPHERES

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A NUMBER of investigations of the properties of dense random packing of spheres have been reported recently. McGeeary<sup>1</sup> has presented experimental packing density measurements for binary mixtures and maximum packing densities for ternary and quaternary mixtures. Benenati and Brosilow<sup>2</sup> investigated the local void fraction distribution for single-size spheres over a large range of aspect ratio, that is, container-to-sphere diameter ratio. Yerazunis, Bartlett and Nissan<sup>3</sup> proposed a theory for the packing of binary mixtures based on a distortion mechanism. The present work was undertaken to obtain more definitive data with which to test this theory.

The desired amounts of the two classes of spheres, in excess of that which could be accommodated by the final container, as determined by weight, were mixed carefully and poured into a 'Lucite' cylinder, 63 mm in diameter and 63 mm in height. A second cylinder of the same diameter but of smaller height, 50 mm, was joined and sealed with tape to the first cylinder. The mixing device so formed was tumbled extensively to homogenize the mixture. With the device oriented vertically and the sphere mixture in the smaller of the cylinders, the system was vibrated to maximum density using the actuator from a door-bell mechanism. In order to avoid too intense a vibration and the concomitant low-density packing, the actuator and the impact surface were arranged to obtain a subtle, mild action. The effectiveness with which the dense random packing was achieved is suggested by the measured packing fraction for single-size spheres of

0.635–0.639, a range which corresponds rather well with the extrapolated value of 0.637 reported by Scott<sup>4</sup>. Two min vibration proved sufficient to compact mixtures of the smaller size, but vibration was extended to 5 min for mixtures involving a large-size class. After compaction the cylinders were separated and the surface of the compacted mixture in the smaller of the cylinders was levelled using a stiff sharp-edged steel rule. The excess mixture was separated and the weight of the larger spheres in the excess was determined. The packing fraction and the composition of compacted mixture in terms of fraction of occupied volume were calculated from the initial weights charged, the compacted weight and volume of the smaller cylinder, the weights of excess spheres and the densities of the two sizes of spheres involved. The diameter ratios investigated, together with the diameters of the spheres employed, are summarized in Table 1. The experimental data are shown in Fig. 1, in which are also shown the data obtained by Atkins and Barriah<sup>5</sup> for the diameter ratio of 12.

Previous investigations<sup>1,2,6</sup> have revealed that the packing fraction of mixtures of spheres is greater than

Table 1

Diameter ratio	Diameter (mm)	
	Large spheres	Small spheres
180	15.83	0.068
67.7	5.95	0.068
5.74	0.50	0.068
3.41	0.297	0.038
2.58	0.297	0.115



that of either single class and exhibits a maximum value, which is a function of diameter ratio, in the neighbourhood of 0.73 volume fraction large spheres. This general behaviour can be explained in terms of two models, one applying to the range of large-sphere concentrations up to the critical value corresponding to the maximum packing density, the other to the remaining concentration range. Consider for the moment a dense random array of small spheres. The addition of a large sphere results in the displacement of the equivalent volume of small spheres and increases the overall packing fraction by eliminating the micro-voids associated with the small-sphere régime which was replaced. This replacement process may be continued up to that point at which the large spheres are themselves arrayed in dense random packing. Starting with the opposite extreme, small spheres can be added to an existing random array of large spheres by the filling of existing voids. The procedure may be continued until the macro-voids formed by the large spheres are entirely filled by small spheres.

In the case of an infinite diameter ratio, the small-sphere régime will be characterized by a void fraction corresponding to dense random packing in a container of infinite aspect ratio and the packing fraction-composition function can be predicted theoretically. However, for finite diameter ratios, the distortion of the small-sphere régime due to the large-sphere surfaces must be taken into account for compositions of large spheres below that corresponding to maximum packing density. Beyond this point, two factors present themselves. Starting with a large-sphere array, small spheres can be introduced into sufficiently large macro-voids without disturbing the large-sphere arrangements. At some composition determined by the sphere diameter ratio, the macro-voids large enough to accommodate small spheres will become saturated. Further additions of small spheres can be effected only by expanding the large-sphere lattice.

This investigation has been concerned with the behaviour of mixtures of spheres for compositions less than

that corresponding to the maximum packing fraction. The observed behaviour can be explained as follows. In the binary mixture, the small spheres can be imagined to occupy domains of size and shape determined by the number of large spheres per unit volume and their arrangement. On average, the volume of a small-sphere domain will be given by  $(1 - V_1 N_1)/N_1$ , where  $V_1$  is the volume of a large sphere and  $N_1$  is the number of large spheres per unit volume. A length characteristic of the size of the small-sphere domain can be defined  $D_s$  equal to  $[(1 - V_1 N_1)/N_1]^{1/3}$ . Accordingly, a measure of the local aspect ratio, that is, particle-to-domain dimension, is  $[D_1/D_s]/(1 - V_1 N_1)^{1/3}$ , where  $D_1$  is the diameter of the small sphere.

Scott's investigation provides a basis on which to relate the packing fraction of the small-sphere domain,  $f_p^\infty$ , to the aspect ratio by:

$$f_p^\infty = f_{p^\infty} - K D_s [N_1 / (1 - V_1 N_1)]^{1/3} \quad (1)$$

where  $f_{p^\infty}$  is the packing fraction for dense random arrays of equal spheres in an infinite container and  $K$  is an empirically determined constant.

Since the degree of distortion of the small-sphere domains from the infinite aspect ratio configuration would be expected to be related to both the aspect ratio and the extent of 'wall' (surface of large spheres), equation (1) must be modified. On average, one large sphere is associated with each small-sphere domain. It is proposed that an effective packing fraction,  $f_{p^\infty}$ , be defined so as to reflect the area of the large sphere relative to a sphere of diameter corresponding to the characteristic domain dimension,  $D_s$ , that is, a function of  $[D_1/D_s]^2$ . The simplest assumption is to replace  $K$  in equation (1) by  $K' [D_1/D_s]^2$ , with the result that:

$$f_{p^\infty} = f_{p^\infty} - K' (D_1/D_s)^2 D_s [N_1 / (1 - V_1 N_1)]^{1/3} \quad (2)$$

Since the packing fraction for the mixture,  $f_p$ , is:

$$f_p = V_1 N_1 + (1 - V_1 N_1) f_{p^\infty} \quad (3)$$

equations (2) and (3) can be combined with the expression for domain dimension to yield:

$$f_p = V_1 N_1 + [1 - V_1 N_1] \left\{ f_{p^\infty} - K' D_s^2 \frac{N_1}{1 - V_1 N_1} \right\} \quad (4)$$

Noting that  $V_1 N_1 = f_{p^\infty} y$ , where  $y$  = fraction of large spheres in the occupied volume, equation (4) can be rewritten as:

$$f_p = \frac{f_{p^\infty}}{1 - (1 - f_{p^\infty} - \frac{6K'D_s}{\pi D_1})y} \quad (5)$$

The question which now requires resolution is whether the distortion parameter,  $K'$ , is dependent on the relative curvatures of the small and large spheres. The experimental measurements obtained in this study indicate that the distortion parameter is related to the diameter ratio through:

$$K' = 0.165 (D_1/D_s)^{-2.164} \quad (6)$$

Equation (6) is to be interpreted as an indication that the distortion of the small-sphere régime is reduced with decreasing large- to small-sphere diameter ratio. The concept is not only in keeping with one's intuition but also with the fact that in the limit of a diameter ratio of unity, where the addition of one sphere involves only a simple replacement, there is zero distortion. Unfortunately, equation (6) does not extrapolate to this limit as is noted later; however, it does describe the phenomenon over the range of diameter ratios investigated.

Substituting (6) in (5):

$$f_p = \frac{f_{p^\infty}}{1 - (1 - f_{p^\infty} - 0.315 [D_1/D_s]^{0.766})y} \quad (7)$$

Equation (7) was used with  $f_{p^\infty} = 0.638$  to obtain a comparison with the experimental data. The agreement was quite good over the range of diameter ratios studied, with the exception of some deviation for the 3.41 diameter ratio for compositions exceeding 0.5 volume fraction and more substantial disparity for the 2.58 case above a composition of 0.3.

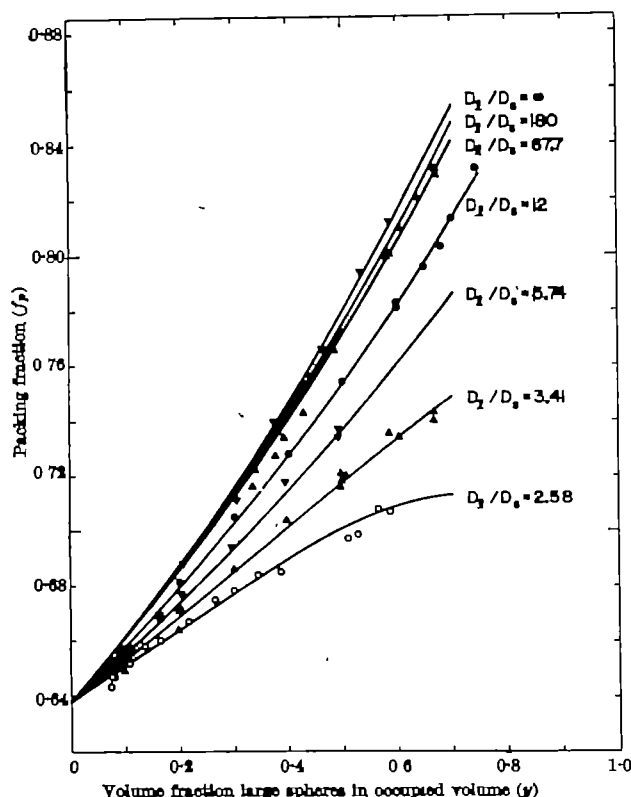


Fig. 1. Packing fractions in binary mixtures. Lines represent theoretical values derived from equation (11). Point symbols represent experimental data.

Some indication as to the reason for this behaviour is obtained by noting that the number of small spheres per domain corresponding to the composition at which the experimental results depart from the theoretical expectations is approximately 40 in both instances. The average domain dimension expressed in terms of small bead diameters is somewhat over six. As the concentration of large spheres is increased, the size of the small-sphere domain is reduced even further. In the case of the 5.74 : 1 mixtures, the 40 to 1 ratio of small to large spheres is reached only at a composition of 0.82, which is beyond the maximum packing fraction point. It is proposed that the distortion parameter defined by equation (6) is not adequate for small-sphere domains of less than 40 spheres and that the following relationship is more suitable:

$$K' = A[D_s/D_l]^m + B[N_l/N_s] \quad (8)$$

The first term on the right-hand side of (8) is the correction for the effect of curvature on the distortion parameter which was described earlier. The second term is proposed as an empirical approximation to the effect of domain size expressed in terms of number of small spheres per large sphere.

The ratio of large to small spheres can readily be expressed in terms of diameter ratio and composition as:

$$N_l/N_s = [D_s/D_l]^3[y/1-y] \quad (9)$$

Equations (5), (8) and (9) are combined to produce the final relationship between packing fraction, diameter ratio and composition:

$$f_p = \frac{f_{p\infty}}{1 - [1 - f_{p\infty} - \frac{6}{\pi} A(D_s/D_l)^{1+m}y - \frac{6}{\pi} B(D_s/D_l)^4y^3/1-y]} \quad (10)$$

The optimum values of the correlating constants were determined to be:  $A$ , 0.165,  $m$ , -0.294 and  $B$ , 0.5.

The numerical equation actually used then was:

$$f_p = \frac{0.638}{1 - [0.362 - 0.315(D_s/D_l)^{0.706}y + 0.955(D_s/D_l)^4y^3/1-y]} \quad (11)$$

At this point it should be noted that the constants  $A$  and  $m$  in the expression (8) for the distortion parameters are believed to be reasonably reliable because of the number of data points available for test. The correction for domain size is less confidently known. The form chosen in (8) satisfies the requirements that this term should be

negligible for larger diameter ratios and for low compositions. It is by no means the only formulation which could fit the available experimental data. Accordingly, the recommendation made above should be regarded with some restraint as but one possible representation. Additional experimental data will be required before a more positive conclusion can be reached. On an overall basis, equation (11) correlates the experimental findings quite adequately as shown by the comparison between theoretical and experimental results in Fig. 1.

The predicted packing fraction-composition curve for the infinite diameter ratio is obtained by letting  $[D_s/D_l] \rightarrow 0$  yielding:

$$f_p = \frac{0.638}{1 - 0.362y} \quad (12)$$

The difference between predictions for the infinite ratio and for the largest ratio studied experimentally, 180 : 1, is quite small as one might reasonably expect. It is to be noted that the model (12) predicts a concave upward curve for the infinite ratio case which is in contradiction to the linear relationship which had previously been proposed in (3) and (6). It would appear that the latter conclusion may have occurred as a consequence of oversimplification of the replacement mechanism.

Although the proposed model and the final expression, (11), fit the available data and lead to the expected results for the very high diameter ratio, they do not extrapolate effectively in the opposite direction. For the case of mixing equal-sized but otherwise distinguishable spheres, the packing fraction should be independent of composition. Equation (11) clearly does not satisfy this asymptotic requirement. The problem to be faced with small diameter ratio of the order of two or less is that the domain size expressed as the number of small spheres per large sphere reaches the critical value of the order of 40 at very low concentrations of large spheres. Under these conditions, the distortion parameter formulation defined (8) would appear to be entirely inadequate.

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## STRONTIUM ISOTOPE INVESTIGATION OF IGNEOUS ROCKS FROM ICELAND

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THE limited range of variation in the initial  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of basaltic rocks (particularly in oceanic areas) suggests that the source regions of basalts are relatively homogeneous with respect to their Rb/Sr ratios<sup>1,2</sup>. The average Rb/Sr ratio in the sialic crust is substantially higher than in the source regions of basalt<sup>3-5</sup>. On this basis, Faure and Hurley<sup>1</sup> suggested that there is sufficient enrichment of strontium-87 (resulting from the radioactive decay of rubidium-87) in crustal materials to use the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of igneous rocks, at time of crystallization, as a criterion for the origin of the material. Thus the

initial  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of an igneous rock formed by remelting of—or contamination with—ancient crustal material may be expected to be measurably higher than that of an igneous rock formed by differentiation of basaltic magma.

Strontium isotope measurements are reported in this article for a small, but reasonably representative, set of basic and acid igneous rocks of Tertiary to Recent age from a number of localities in Iceland (Fig. 1). The rock-types include gabbro, basalt, andesite, basaltic acid tuff, pitchstone, obsidian and granophyre. Most of

the specimens were collected by one of the authors (G. P. L. W.), but those from the Snafrudal intrusion were collected by A. E. Beswick.

Iceland exhibits many features of unusual geological interest, not least its situation astride the mid-Atlantic ridge, as well as the large-scale, intimate association of basic and acid igneous rocks. The problem of the origin of the acid igneous rocks is of considerable interest. Carmichael<sup>6</sup>, in his investigation of the crystallization of feldspar from volcanic acid liquids, considers those Icelandic rhyolites and pitchstones which he has examined to belong to that class produced by the fractionation of tholeiitic magma. On the other hand, Walker<sup>7</sup> states that "such large amounts of acid (and intermediate) magma seem unlikely to have originated by crystal fractionation of basaltic magma and that a sialic crust seems a more likely source. Recent seismic work in western Iceland by Tryggvason and Baath<sup>8</sup> has, however, failed to reveal a layer that can be identified as sial, and direct evidence for the existence of a sialic crust below eastern Iceland is lacking; but it may be that the great abundance of acid material in the Icelandic central volcanoes is evidence of its existence." According to Cargill *et al.*<sup>9</sup> and many later workers, no floor of sial is visible anywhere in Iceland, no 'accidental' xenoliths have been discovered in lavas or intrusions, nor blocks in the volcanic piles; nor is there any evidence in the petrological character of the rocks of the assimilation of sedimentary material.

Strontium isotope measurements were carried out with an A.E.I. MS-2 mass spectrometer with a 60°, 6-in. radius analyser tube and an electron multiplier as the ion-beam detector. A single tantalum-filament, surface ionization technique was used. Full experimental details have been described elsewhere<sup>10,11</sup>. In order to eliminate unpredictable isotopic fractionation effects, all measured  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios were normalized to  $^{87}\text{Sr}/^{86}\text{Sr}=0.1194$ , in line with the procedure adopted by other workers<sup>1,2</sup>. This also has the effect of improving the reproducibility of replicate measurements. The average  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio found in the course of the present work was 0.1183, nearly 1 per cent below the conventional value of 0.1194. It appears, however, from the work of Hedge and Walthall<sup>3</sup>, that the single filament ionization technique can yield slightly lower  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios than triple filament ionization. A check on reproducibility was provided by

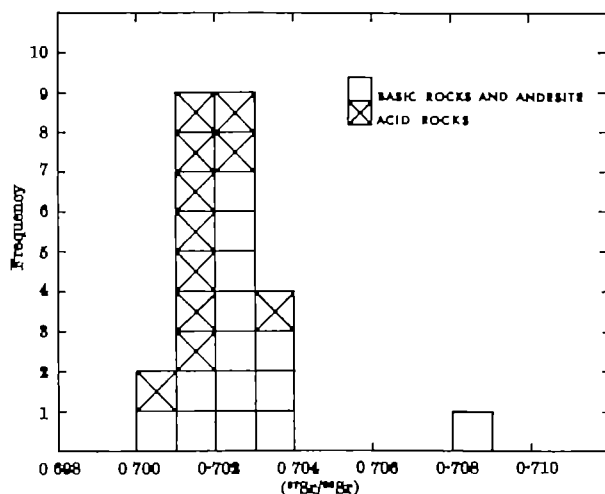


Fig. 2. Histogram of initial  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios from Iceland

frequent isotopic analysis, during the course of the work, of the Eimer and Amend strontium carbonate standard, circulated internationally by the Massachusetts Institute of Technology group. The mean of eight separate measurements of the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio was 0.7076, with a standard deviation of  $\pm 0.0010$ . In the 1964 report of the Massachusetts Institute of Technology group<sup>12</sup> it is stated that the normalized values found by several other laboratories for the Eimer and Amend  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio lie between 0.7075 and 0.7085. It is considered that in the present work the reproducibility of a single  $^{87}\text{Sr}/^{86}\text{Sr}$  measurement is  $\pm 0.0015$  at the 66 per cent confidence-level.

Rubidium and strontium determinations were made by X-ray fluorescence analysis, yielding values to at least  $\pm 10$  per cent. The lower detection limit for rubidium was about 5 p.p.m. The Rb/Sr ratio and the geological age of the rocks were too low to necessitate an age correction in the measured  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios.

Measurements were carried out either on powdered, whole rock specimens, or on plagioclase feldspar separates.

The results of strontium isotope measurements, expressed as the normalized, initial  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios, are given in Table 1. Fig. 2 is a histogram of the results. It is evident that, with one exception, there is no significant difference in the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio between any of the rocks so far measured. The mean value for twelve basic rocks (omitting P. 673, see following) is  $0.7024 \pm 0.0009$  and for eleven acid rocks  $0.7016 \pm 0.0008$ . (Six of these acid rock samples are from the same rock unit, see following.) A single andesite measurement yields  $0.7020 \pm 0.0015$ ; this sample has an appreciable Rb/Sr ratio, but even on the assumption of a maximum age of 50 million years, the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio would only decrease to 0.7022.

The basalt lava P. 673 (No. 7, Table 1) from Breiddalur gives the only 'anomalous' strontium isotope result, with an  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of  $0.7089 \pm 0.0010$ , representing the mean of two separate determinations. The rock does not appear in any way unusual, but it occurs in an unusual geological environment. It occupies the caldera or crater of the Tertiary Breiddalur central volcano, described by Walker<sup>1</sup>. Below, and separated from it by one other lava flow, is a thick hyaloclastic accumulation, the result of subaqueous eruption of basalt into the lake which occupied the caldera. It appears unlikely that the water was sea-water. (Since the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of sea-water is about 0.708, soaking of the lava in sea-water and resulting element and isotope exchange could account for the comparatively high  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of the basalt.) However, fumaroles and hot springs are thought to have contributed to the lake, and the rocks now visible beneath the basalt lava consist mainly of highly altered rhyolites and acid pyroclastics, which might have been the source of the slight excess of  $^{87}\text{Sr}$ .

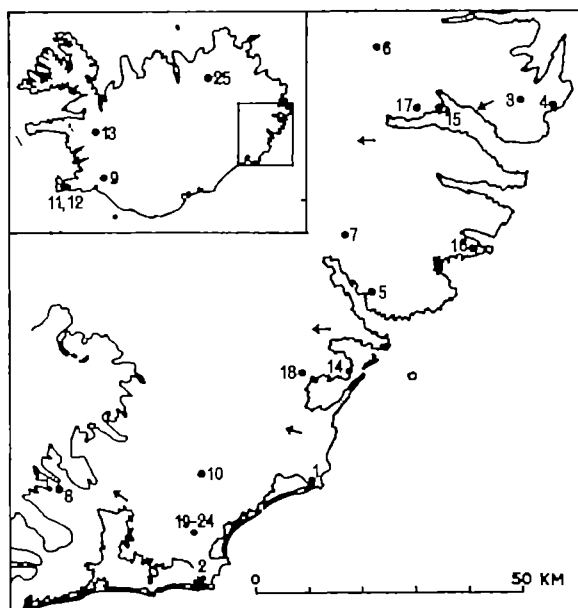


Fig. 1. Sketch map of eastern Iceland showing location of analyzed samples. The arrows indicate the direction of younging of the volcanic rocks. Inset map of Iceland showing location of analyzed samples of Quaternary volcanic rocks

Table 1. STRONTIUM ISOTOPE RESULTS FROM ICELAND

Map No. (Fig. 1)	Specimen No.	Rock type	Locality	Stratigraphic age	Sample form	Rb* (p.p.m.)	Sr* (p.p.m.)	( <sup>87</sup> Sr/ <sup>86</sup> Sr) <sup>†</sup> (initial)
1	N. 718	Gabbro	Austurhorn intrusion (ref. 9)	Tertiary	Whole rock	n.d.	393	0.7029 (1)
2	N. 787	"	Lilla-horn, Vesturhorn intrusion (ref. 9)	"	"	n.d.	450	0.7028 (1)
3	N. 469	Olivine-basalt lava	Vikurvata group (ref. 18), Vindhafstindur, Reydarfjörður	"	"	n.d.	523	0.7023 (1)
4	N. 413	Tholeiitic basalt	Lava above Bardartangur tuff (ref. 18), Gerpr, Reydarfjörður area	"	"	5	243	0.7021 (1)
5	N. 704	Feldspar-porphyrilitic basalt lava	Kollur group (ref. 18), Gautavik, Berufjörður	"	"	n.d.	290	0.7023 (1)
6	P. 701	Olivine-basalt lava	Near summit of Skagafell, N.W. of Reydarfjörður	"	"	n.d.	238	0.7021 (1)
7	P. 673	Basalt lava	North nose of Berufjardartindur, Breiddalur (ref. 7)	"	"	n.d.	187	0.7089 (2)
8	R. 314	Basalt tuff	Vidbordalur	"	Bytownite	n.d.	602	0.7016 (1)
9	R. 341	Olivine-basalt lava	460 ft. on S.W. corner of Ingolthfjall	Pleistocene (? Inter-glacial)	Whole rock	n.d.	150	0.7033 (1)
10	R. 298	Tholeiitic basalt lava	Columnar basalt at base of Dalaboldi palagonite breccia mass	Pleistocene	"	5	160	0.7033 (2)
11	R. 153	Basalt lava	Afstaþalraum, W. of Hafnarfjörður, Reykjanes peninsula	Recent	"	n.d.	118	0.7028 (1)
12	R. 162	"	6 km W.S.W. of Grindavik, Reykjanes	Recent	"	n.d.	122	0.7004 (1)
13	R. 257	Feldspar-porphyrilitic basalt dike	Older cone, Blifrost	"	Bytownite	n.d.	451	0.7012 (1)
14	P. 868	Hornblende-rich porphyritic dyke	Metrakkanes	Tertiary	Whole rock	81	238	0.7029 (1)
15	N. 461	Pitchstone top of rhyolite lava	Holmabak, Reydarfjörður	"	Oligoclase	n.d.	504	0.7015 (1)
16	N. 779	Acid tuff	500 ft. on Mestell, S. of Stodvarfjörður	"	"	n.d.	872	0.7014 (2)
17	N. 450	"	720 ft. in Ljosa, N. of Reydarfjörður	"	Andesine	n.d.	777	0.7013 (1)
18	R. 106	"	N. side Geithellnadalur	"	Oligoclase	n.d.	977	0.7007 (1)
19	A. 31a	Granophyre	Slaufudal intrusion (ref. 9), 1,560 ft. altitude, S.W. side of Skogafind	Late Tertiary	Whole rock	78	194	0.7010 (1)
20	A. 36	"	Ditto, 1,100 ft. altitude, S.W. side of Skogafind	"	"	93	40	0.7014 (1)
21	A. 39	"	Ditto, 1,430 ft. altitude, N. side of Upper Slaufudal	"	"	90	72	0.7023 (1)
22	A. 77	"	Ditto, 1,630 ft. altitude, S.W. of Bleidindur summit	"	"	83	184	0.7013 (1)
23	A. 103	"	Ditto, 190 ft. altitude. Bottom of Endalsdalur valley	"	"	104	102	0.7026 (1)
24	A. 115	"	Ditto, 1,280 ft. altitude. Bottom of Upper Slaufudal	"	"	100	50	0.7024 (1)
25	OWR-1	Obsidian	Hrafninnhryggur, near Myvatn (ref. 13-15)	Pleistocene or Recent	"	70	106	0.7017 (2)

\* Determined by X-ray fluorescence, n.d. = not detectable.

† Normalized by adjusting <sup>86</sup>Sr/<sup>86</sup>Sr to 0.1194 and the measured <sup>87</sup>Sr/<sup>86</sup>Sr ratio by half this amount. The figure in parentheses after the ratio indicates the number of analyses performed for each specimen.

K/Ar age on sample A. 39 whole rock is 6.5 ± 1.1 million years (analysis, D. C. Rex).

Of the acid rocks, the six samples from the Slaufudal granophyre stock (Nos. 19-24, Table 1) of Eastern Iceland are of particular interest. This is the largest known intrusive mass in Iceland, with an outcrop covering an area of 15 km<sup>2</sup> and with a volume of at least 10 km<sup>3</sup>. The structure and petrology of this intrusive have been described by Cargill *et al.*<sup>9</sup>, and further details will be published elsewhere by A. E. Beewick. Although many of the principal plutonic intrusions of Eastern Iceland are composite masses, mainly composed of gabbro, granite and granophyre, the Slaufudal stock consists of acid rock only. A potassium-argon determination on whole rock sample A. 39 yielded an age of 6.5 ± 1.1 million years. Even allowing for a plausible maximum of about 20 per cent loss of argon from potash feldspar, this puts the age of intrusion of the stock very late in the Tertiary. This unexpectedly young age explains why the <sup>87</sup>Sr/<sup>86</sup>Sr ratios all agree within the limits of experimental error, despite the fact that the Rb/Sr ratios of the six samples range from 0.4 to 2.3.

Samples No. 16, 17 and 18 (Table 1) are feldspar crystals obtained from interbasaltic acid tuff horizons. These tuffs are the products of explosive eruptions of acid magma, during which feldspar phenocrysts became separated from the acid magma. Being in a protected environment, surrounded by soft and loose tuffaceous material, these crystals are usually free from fractures and in a very fresh condition.

The well-known postglacial obsidian from Hrafninnhryggur (No. 25, Table 1) was first described and chemically analysed by Wright<sup>13</sup> and later by Carmichael<sup>14,15</sup>.

The strontium isotope results suggest that these relatively few acid rocks from Iceland so far investigated are ultimately derived from the same source region as the basic rocks, presumably the upper mantle. The acid rocks were formed either by the fractional crystallization

of basic magma<sup>6</sup> or by some process of partial melting of basic rocks. The possibility that they were produced by the partial or complete remelting of an ancient sialic crust beneath Iceland with anything like the average crustal Rb/Sr ratio is excluded by the isotopic data. If such a crust exists—and there is no direct evidence for it—then, either, it has not contributed any significant amounts of material to the acid rocks investigated in this study, or it is not older than about 100 million years. Clearly, many more strontium isotope measurements on Icelandic rocks are needed. However, if none of the acid rocks is found to contain a significant enrichment of strontium-87 over basalt, it would provide rather strong circumstantial evidence for the absence of ancient, sialic crust beneath Iceland. The presence or absence of such a crust would have an important bearing on reconstructions of the northern land masses prior to (hypothetical) continental drift. It is inherent in the present interpretation of the isotope results that there is some mechanism by which comparatively large volumes of acid magma can accumulate at depth by derivation from the same source regions as basalt magmas, or from basalt magma itself. This may have happened at various times during the Tertiary geological history of Iceland.

It is of interest that the average <sup>87</sup>Sr/<sup>86</sup>Sr value for the Icelandic rocks is significantly lower than for the basic rocks of at least some of the continental areas of the North Atlantic Tertiary igneous province. An average value of 0.7058 ± 0.0010 has been reported for basic rocks from the Isle of Skye, north-west Scotland<sup>11</sup>, while the average value for the basic rocks of the Skaergaard intrusion, East Greenland, is 0.7065 ± 0.002<sup>16</sup>. However, the mass spectrometer used in the present study gave an average <sup>87</sup>Sr/<sup>86</sup>Sr value for the Eimer and Amend standard which is about 0.0020 lower than that obtained with the mass spectrometer used in the Skye and East Greenland investigations. (There is something to be said for correct-

ing the measured  $^{87}\text{Sr}/^{86}\text{Sr}$  from all laboratories to an agreed value of this standard.) Nevertheless, even allowing for instrumental differences, there still appears to be a significant difference of about 0.002–0.003 between the Iceland results and those from Skye and East Greenland.

This difference is in accord with the findings of previous workers<sup>1,2</sup> that the  $^{87}\text{Sr}/^{86}\text{Sr}$  values for oceanic basalts are, on the whole, slightly lower and less variable than those of continental basalts. Hedge and Walthall<sup>3</sup> report an average of 0.703 for oceanic basalts, while two Recent basalts from Iceland measured by them gave an average of 0.7025. The values for continental basic volcanic rocks appear to lie in the range 0.702–0.710<sup>1,2</sup>.

It was mentioned above that the high  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of  $0.7089 \pm 0.0010$  for the Breiddalur basalt, P.673, might be due to some superficial process such as leaching and isotopic exchange. Nevertheless, the possibility must be kept in mind that this is a true, primary ratio, indicating local inhomogeneities in the Rb/Sr ratio of the basaltic source regions. More work is in progress on this problem. It is of interest to note that Faure and Hurley<sup>4</sup> reported a single  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of  $0.7101 \pm 0.0004$  for a Recent olivine basalt of unspecified locality from Iceland. These authors have recently revised all their originally published  $^{87}\text{Sr}/^{86}\text{Sr}$  values downwards by about 0.003, but in either case the quoted value is close to that of the Breiddalur Tertiary basalt. Clearly, the suspicion is justified that not all the basic rocks of Iceland have  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios as low as 0.702–0.703.

It is important not to carry the analogy between the acid igneous rocks of Iceland and of the Tertiary igneous centres of north-west Scotland too far. While there may be some general similarities in field relationships and mode of occurrence, the ultimate origin of the granitic material may be quite different in the two cases. This emerges already from recent investigations of the feldspars in certain volcanic acid liquids by Carmichael<sup>5</sup>, as well as from geochemical and trace element studies on natural acid glasses from the North Atlantic Tertiary province by the same author<sup>14,15</sup>. Indeed, Carmichael<sup>14</sup> considers that "the acid magma available for eruption in Iceland, as represented by the pitchstones, shows little variation

in composition throughout perhaps as much as 60 million years".

The general uniformity of the Iceland strontium isotope results contrasts strongly with the results from two large Tertiary plutonic igneous centres in the Isle of Skye, north-west Scotland, where it has been shown<sup>11</sup> that the initial  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of granitic and related rocks are significantly higher (about 0.713) than those of nearby basaltic rocks. This suggested that the granitic and related rocks were produced by partial melting of ancient Lewisian rocks (>1,600 million years) which form the underlying basement at no great depth in Skye. This interpretation was in agreement with conclusions drawn from other recent, totally independent, experimental lines of evidence<sup>6,17</sup>.

There are clearly several ways in which granitic magma can be formed, even within a single volcanic province. It is to be hoped that strontium isotope investigations, in addition to the more conventional geological, geochemical, petrological and mineralogical criteria, will provide a clue to the mode of origin in any particular case.

We thank our colleagues for their advice. The work at Oxford forms part of the programme of age and isotope studies directed by Prof. L. R. Wager.

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## FORMATION OF OCEANIC RIDGES

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OCEANIC ridges form an interconnected system of underwater mountain ranges about 80,000 km in total length (Fig. 1). They stand 2–4 km above the average depth of the oceans and vary in width from a few hundred to four thousand kilometres. They form the largest uplifted surface feature of the Earth. This article suggests a mechanism for their uplift.

It has recently been shown that the East Pacific Rise was formed by uplift of the sea-bed from normal oceanic depths during the Tertiary<sup>1</sup>. The Mid-Atlantic Ridge was also probably formed during the Tertiary<sup>2</sup>. Menard<sup>3</sup> has shown that the atolls and guyots scattered over a substantial area of the north-west Pacific were originally volcanoes reaching sea-level on the crest and flanks of a Mesozoic ocean rise (the Darwin Rise) which has since subsided to normal ocean depths.

A similar type of uplift has affected some continental regions. Much of the western United States has been uplifted nearly 2 km since the Eocene<sup>4</sup>, forming a continental extension of the uplift which formed the East Pacific Rise<sup>5,6</sup>. This uplift is particularly well displayed in the Colorado Plateau, which remained close to sea-level from before the Cambrian until the early Tertiary and was

afterwards uplifted without folding. A similar situation appears to exist where the Indian Ocean Ridge passes into the part of Africa and Arabia which shows evidence of strong epeirogenic uplift.

Thus vertical movements of 2-km amplitude have affected vast areas of ocean floor and more restricted (but still quite substantial) areas of continent in extension of the ocean ridges. It has been widely suggested that mantle convection currents upwelling beneath the ocean ridges may indirectly provide a mechanism for the formation of the ridges and for their elevation. I support this hypothesis and further suggest that dilatation accompanying partial fusion in the rising convection current provides a direct explanation of the uplift. The evidence and arguments favouring this hypothesis are stated here.

It is inconceivable that vertical movements of 2-km amplitude can have affected regions of large areal extent without a change in density in the underlying rocks adequate to maintain approximate isostatic equilibrium. Otherwise these regions would have isostatic anomalies of more than 200-mgal magnitude either now or before the uplift occurred. Nowhere are widespread isostatic

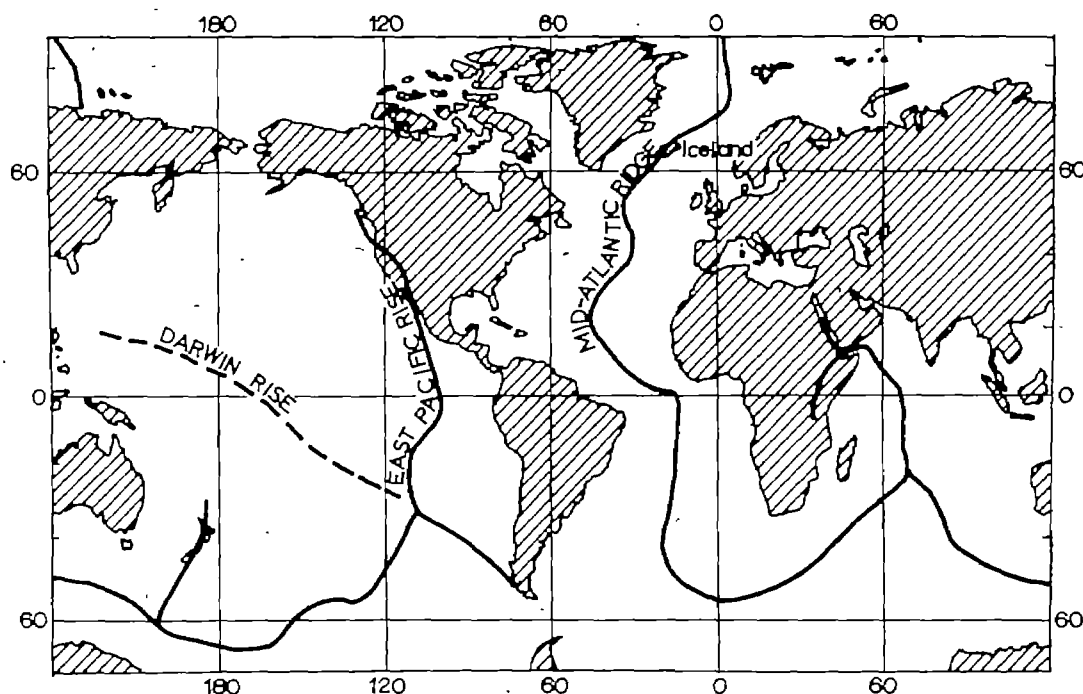


Fig. 1. System of oceanic ridges

anomalies of this magnitude known: in particular the western United States<sup>6</sup> and the oceanic ridges<sup>7,8</sup> are in approximate isostatic equilibrium despite small local departures. It also seems out of the question that regions such as the Colorado Plateau were out of isostatic equilibrium by  $-250$  mgal from the Cambrian until the Eocene unless the mantle was very much stronger then. Thus uplift and subsidence have occurred in isostatic response to substantial changes in the density of the underlying rocks.

Seismic refraction profiles show that changes in crustal thicknesses are inadequate to explain the uplift of the East Pacific Rise<sup>1</sup> (Fig. 2), or the subsidence of the Darwin Rise<sup>2</sup>, or the present elevation of the Mid-Atlantic Ridge<sup>4</sup>. The region of anomalously low density causing the elevation is therefore within the upper mantle beneath. Support for this conclusion comes from the relatively low-compression wave velocities in the topmost mantle beneath the Mid-Atlantic Ridge<sup>3,9</sup>, part of the East Pacific Rise<sup>3</sup>, Iceland<sup>9</sup> and the western United States<sup>10</sup>, since low seismic velocities normally correspond to low densities.

A region of relatively low density in the upper mantle could be attributed either to chemical inhomogeneity or to changed physical conditions causing dilatation of otherwise normal upper mantle rocks. Chemical inhomogeneity could result from a crust-mantle mix<sup>11</sup> or from variation in the proportion of minerals such as plagioclase<sup>12</sup>. Possible explanations depending on changed physical conditions include thermal expansion, silicate mineral phase changes, partial fusion and serpentinization of olivine<sup>13</sup>. Thermal expansion seems to be inadequate to produce sufficiently large changes in density<sup>13,14</sup>. If it is assumed that a single process is of dominant importance, four disconnected pieces of evidence enable a tentative choice to be made between the remaining hypotheses. These are: (1) the gravimetry and seismology of Iceland<sup>14</sup>; (2) the epeirogenic and reversible character of the uplift; (3)–(4) the inadequacy

of silicate phase changes and reactions to produce a sufficiently large density contrast. These are discussed in turn as follows:

(1) Iceland lies on the Mid-Atlantic Ridge. The average Bouguer anomaly is about  $+15$  mgal<sup>15</sup>. If the upper mantle beneath were normal, this would mean that the crust is continental (30–35 km thick). However, seismic studies in Iceland<sup>14,9</sup> suggest that the crust is only about 17 km thick and has a high average  $P$  velocity. Combining these observations, we conclude that the upper mantle beneath Iceland possesses an anomalously low density which contributes a gravity anomaly of about  $-250$  mgal. This could be caused by a reduction in density of  $0.03$  g/cm<sup>3</sup> extending over a vertical thickness of at least 225 km, or  $0.3$  g/cm<sup>3</sup> over at least 23 km. Further evidence of critical importance comes from Tryggvason's<sup>9</sup>

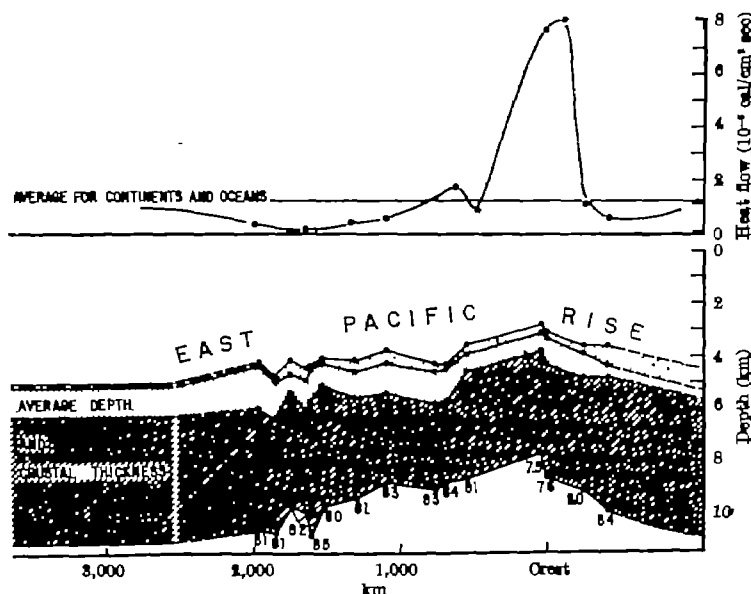


Fig. 2. Topographic and crustal structure profile across the East Pacific Rise, showing also the heat flow and the sub-Moho seismic  $P$ -velocity. Reproduced from an article by H. W. Menard<sup>1</sup> in *Science*, 128, 1741 (December 9, 1960), with permission.

discovery that compression wave arrivals from distant earthquakes arrive about 1.3 sec late at Reykjavik. Tryggvason interprets this delay as caused by an anomalously low compression wave velocity of 7.4 km/s extending to a depth of 200 km below Iceland. Most common rocks show an empirical relation between compression wave ( $P$ ) velocity and density, such that reduction in  $P$  velocity from the normal upper mantle value of 8.2 km/s to 7.4 km/s would correspond to a reduction in density of more than 0.1 g/cm<sup>3</sup>. Spread over a vertical extent of 200 km, this would cause a mass deficiency more than three times as large as the observed value. This suggests that the region of low density is not caused by common rock types with relatively low density. This rules out a crust-mantle mix, serpentine and phase changes in common silicate minerals, although it is possible that an unusual chemical composition could explain the discrepancy. The simplest explanation is that partial fusion causes the mass deficiency, since this would reduce the  $P$  velocity relatively more strongly than the density as the rigidity modulus vanishes on fusion.

(2) Menard<sup>3</sup> has suggested that the uplift of oceanic rises is reversible. Furthermore, there is no evidence to suggest that equal vertical movements in the opposite sense affect the adjacent belts of ocean floor during the uplift or subsidence of an oceanic rise. These considerations suggest that uplift occurs as a result of dilatation within the underlying upper mantle and that subsidence accompanies contraction to normal volume; this is difficult to reconcile with the hypothesis of chemical inhomogeneity.

(3) Experimental observations on solid-solid silicate phase transitions likely to be of importance in the topmost 200 km of the mantle show that the phase boundaries follow gradients within the range 17°–35° C/km (ref. 17). Yoder and Tilley show the basalt-eclogite transition with a gradient of about 50° C/km (ref. 18). Suppose the temperature of the upper mantle were raised by 100° C. The phase boundaries would move to greater depths by 2–6 km. If the aggregate change in density caused by all such phase changes were 0.3 g/cm<sup>3</sup>, this would cause a negative gravity anomaly within the range –25 to –75 mgal, which would only be capable of causing an uplift of 0.7 km at most. Thus solid-solid silicate phase transitions appear to be inadequate as a complete explanation of the cause of uplift, unless the upper mantle temperatures are raised excessively.

(4) Another possibility is that olivine is hydrated to give low-density serpentine. This reaction cannot occur much above 500° C. The geothermal gradient of Iceland<sup>10</sup> is such that temperatures of 500° C almost certainly occur at a shallower depth than 10 km. Similar considerations apply to regions of anomalously high flow of heat on the East Pacific Rise and the Mid-Atlantic Ridge. This raises a serious difficulty for the serpentinization hypothesis.

The foregoing considerations (1)–(4) together suggest that partial fusion is probably the dominant cause of the low-density region in the upper mantle beneath oceanic ridges and their continental extensions. It remains to demonstrate the feasibility of this hypothesis.

Experiments by Daly<sup>11</sup> show that basalt undergoes a reduction in density of about 10 per cent, or 0.3 g/cm<sup>3</sup>, on fusion. The fused fraction of an ultrabasic parent rock would almost certainly undergo a similar reduction in density. Suppose 10 per cent of the parent rock undergoes fusion, then the overall reduction in density is 0.03 g/cm<sup>3</sup>. Thus 10 per cent partial fusion extending over a vertical range of 150 km would explain the uplift of an oceanic ridge 2 km high, or a continental uplift of about 1.4 km. Partial fusion causes reduction in density by dilatation, causing uplift without the necessity of contemporaneous subsidence nearby. We now require a mechanism for causing partial fusion on this scale.

Partial fusion of the upper mantle can be caused by reduction in the confining pressure provided the initial

temperature is near to the melting point. Reduction in pressure can occur by: (1) stress release in a static mantle; or (2) progressive reduction of confining pressure in an uprising mantle convection current. Both processes depend critically on the fusion gradient, which is about 6.5°–10° C/kbar or 2°–3° C/km. This is an order of magnitude higher than the adiabatic gradient and an order of magnitude lower than the conduction temperature gradient observed at the Earth's surface.

I make the simplifying assumption that a two-phase boundary separates a liquid phase and a single solid phase (Fig. 3 shows a fusion zone). During reduction of pressure  $p$ , material initially on the phase boundary is constrained to remain on the boundary until fusion is complete. Consequently the temperature ( $T$ ) is reduced by  $(dT/dp)_i \cdot \Delta p$ , where  $\Delta p$  is the reduction of confining pressure. Under adiabatic conditions in the field of the solid or liquid phase the reduction in temperature is given by  $(dT/dp)_s \cdot \Delta p$ , where the suffix refers to the adiabatic gradient. The excess cooling resulting from the steeper fusion gradient provides the latent heat of fusion,  $L_f$ . If the appropriate specific heat is  $c$ , assumed the same for both phases, the fractional amount of fusion,  $f$ , is given approximately by:

$$f = \left\{ \left( \frac{dT}{dp} \right)_i - \left( \frac{dT}{dp} \right)_s \right\} \cdot c \cdot \Delta p / L_f$$

In a static mantle the vertical principal pressure is the weight of the overburden, which is fixed in value at a given depth. Release of confining pressure can only come about through reduction of horizontal pressure. The amount of pressure reduction is limited by the strength of the upper mantle which probably does not exceed 200 bars. If both horizontal principal pressures are reduced by 200 bars, the confining pressure is reduced by 133 bars. This would cause a lowering of temperature of less than 1.5° C. Taking the latent heat of fusion as 400 J/g and the specific heat as 1.2 J/g, the amount of fusion possible is only 0.45 per cent. This is inadequate to provide magma on the scale required to explain the uplift of the ocean ridges.

The alternative mechanism is that an upwelling mantle convection current causes fusion in its upper reaches (Fig. 4). Since the fusion gradient is steeper than the adiabatic gradient, fusion is restricted to the topmost section of the rising current. As material is carried upwards in the convection current, it follows a temperature gradient slightly in excess of the adiabatic gradient (Fig. 3, AB) until it reaches the fusion boundary (or zone) until fusion (or partial fusion) is complete (Fig. 3, BC). Taking the difference between the fusion and adiabatic gradient

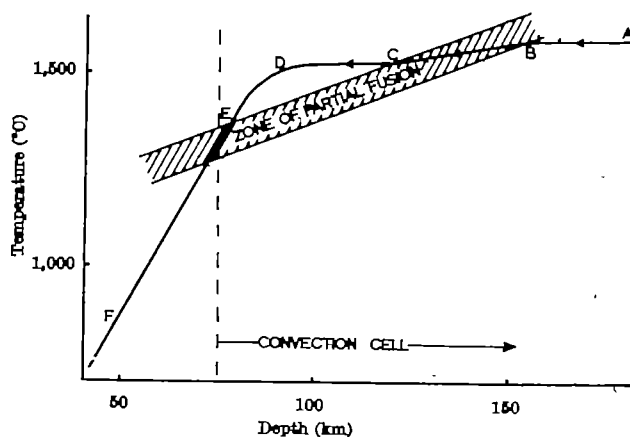


Fig. 3. Mechanism of partial fusion in a rising convection current. AB represents a temperature gradient slightly in excess of the adiabatic gradient. Partial fusion commences as the upwelling current passes B, and continues until C with a steeper temperature gradient. No further partial fusion occurs in the topmost section of the convection cell CDE. Cooling by thermal conduction affects DEF. A fusion zone is shown, since this is more realistic than a simple two-phase boundary.



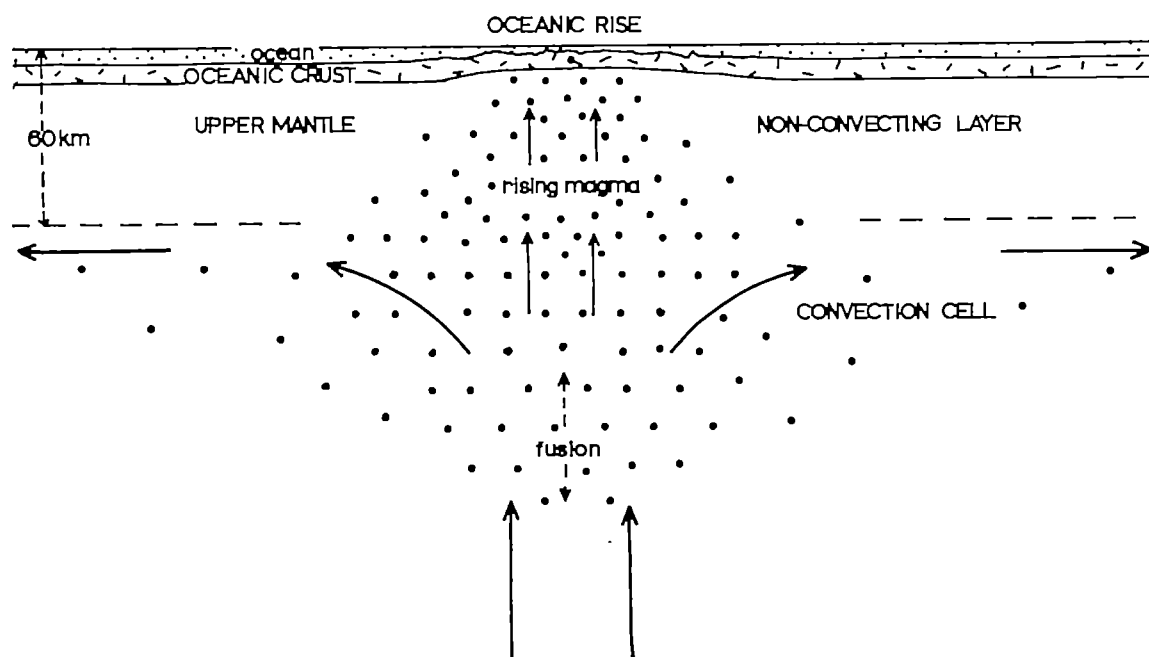


Fig. 4. Mechanism suggested for the uplift of oceanic ridges in isostatic response to a mass deficiency caused by dilatation on partial fusion. Partial fusion occurs in an upwelling mantle convection current.

to be  $1^{\circ}\text{C}/\text{km}$  and latent and specific heats as above, the amount of fusion is 10 per cent in a 33 km rise. Here we have a powerful mechanism for producing magma on a very large scale in a rising convection current. The existence of more than one solid phase complicates the argument but does not alter the conclusion. In reality, it is likely that the olivine present cannot fuse completely, and thus that there is a limit to the degree of partial fusion possible.

The magma produced in this way would tend to rise towards the Earth's surface owing to its relatively low density. It is likely to form a network of dykes and magma chambers in the layer overlying the convection cell. In this way, the mass deficiency causing the uplift is probably partly within the topmost part of the upwelling current and partly in the overlying layer, where it may build up to a substantial fraction locally.

Subsidence of an ocean ridge would occur when the underlying low-density rocks increased in density to the normal value appropriate to their depth. On the convection hypothesis, this would start happening when the convection current died out, causing the source of fresh magma to disappear. The residual magma would partly rise towards the Earth's surface to solidify rapidly, forming lava flows and near-surface intrusions: part of the magma would probably remain trapped in the upper mantle where it would solidify more slowly by cooling through the geothermal gradient. Calculations show that magma distributed uniformly between depths of about 60 km and 200 km would probably solidify in a period of about 80 my. at the most.

The uplift and subsidence of oceanic ridges have important implications for the stratigrapher, as recognized by Hallam<sup>21</sup> and Menard<sup>22</sup>. Uplift of an oceanic ridge would cause eustatic rise in sea-level of the order of 100 m, and subsidence of a ridge would cause a regression of similar magnitude. In general, this mechanism explains transgressions and regressions of world-wide incidence. In particular, it may explain the extraordinarily widespread and deep transgression at the base of the chalk and the similar regression when the chalk seas withdrew.

### Conclusions

The low-density underlying rocks causing the uplift of the oceanic ridges and the western United States lie in

the upper mantle, and substantially result from partial fusion, although other causes such as serpentinization may contribute. The evidence leading to this conclusion comes from a combination of geological, geophysical and geochemical discoveries of recent years, and particularly the present burst of oceanographical research. When pieced together, this evidence suggests rejection of other hypotheses such as crust-mantle mix and solid-solid phase changes as the major cause on the assumption that a single mechanism is of prime importance throughout the whole system of ocean ridges. The hypothesis of partial fusion, however, seems to be consistent with all the lines of evidence.

The only mechanism known for causing magma generation on such a large scale depends on the reduction of confining pressure in an upwelling convection current in the mantle. Such convection currents appear to rise beneath the oceanic ridges, causing their volcanism and uplift.

The hypothesis is equally applicable to uplifts such as the East Pacific Rise which have taken place without associated horizontal movements, and to the Mid-Atlantic Ridge where in addition to the vertical uplift new oceanic crust may be in the process of formation.

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## LETTERS TO THE EDITOR

## ASTRONOMY

## Surface Relief of Mars

THE first reports from *Mariner IV* indicate that the planet Mars possesses a negligible magnetic field when compared with that of Earth. This is strong confirmation of the view that Mars has not developed a core. In a recent communication to *Nature*<sup>1</sup> we suggested that a cyclical process which can be discerned in the Earth's history, and is responsible for its major geological structures, is related to the development of the Earth's core. If our arguments are valid, then it can be predicted that Mars will not possess any system of orogenic mountain belts comparable with those of Earth. Any relief on its surface would be of volcanic or volcano-tectonic origin as on the Moon.

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## OCEANOGRAPHY

## Morphology of North Macquarie Ridge

SOUNDINGS in the Southern Ocean between New Zealand and the Antarctic continent have been sufficient to permit definition of a general elevation of the sea floor extending from the Balleny Islands region, across the Pacific Antarctic and Indian Antarctic Ridge junction, north through Macquarie Island towards New Zealand. This elevation, the Macquarie Ridge, is commonly depicted on bathymetric charts either as a substantially continuous feature at least as far north as Macquarie Island with its crest lying in depths between 1,000 and 2,000 metres; or as in the chart utilized by Adie<sup>1</sup>, with an extensive deep water gap separating the northern end of the Ridge from New Zealand.

For a number of years the N.Z. Antarctic Research programme has included studies of the Macquarie Ridge between the Antarctic continent and New Zealand carried out by the New Zealand Oceanographic Institute and Geophysics Division, Department of Scientific and Industrial Research.

The principal geological objectives have been to define the morphology of the Ridge and to elucidate its structure. The aim of zoological work has been to throw light on the biogeographic relation of the benthic fauna of the New Zealand region with that of various areas to the south and on the ridge as a faunal dispersal route.

The question whether the elevation on which Macquarie Island lies extended north to New Zealand as a major ridge has hitherto been unresolved. Hardman stated<sup>2</sup> that "On the (sounding) evidence now available it appears . . . that the existence of a connection between New Zealand and the Macquarie Rise is doubtful".

Fell<sup>3</sup>, in discussing the biogeographic relationships of the New Zealand and Antarctic echinoderm faunas, stresses the apparent differences between the Macquarie Island and New Zealand elements, and correlates these with the circumstance that "Macquarie Island . . . stands in deep water" and there is " . . . no available shallow water route between the two regions".

In discussing the hydrology Deacon<sup>4</sup> states: "The bottom topography south of New Zealand is so irregular that the eastward current (of deep water) must be interrupted by numerous eddy movements. . . . The deep channel between the New Zealand shelf which extends as far south as the Campbell and Auckland Islands, and the Antarctic shelf, is less than 1,000 miles wide and is obstructed by several shallow banks and islands. A preliminary examination of our soundings . . . suggests that the bottom topography is even more rugged than has hitherto been supposed".

In view of the significance, in the three fields of geology, zoology and hydrology, of proper resolution of the question, numbers of echo-sounding traverses have been made in a south-east-north-west direction from the Foveaux Strait region just south of New Zealand to 58° S. of Macquarie Island. These traverses have extended from the Campbell Plateau and South-western Pacific Basin west to the Tasman Basin; each of them crossing the possible location

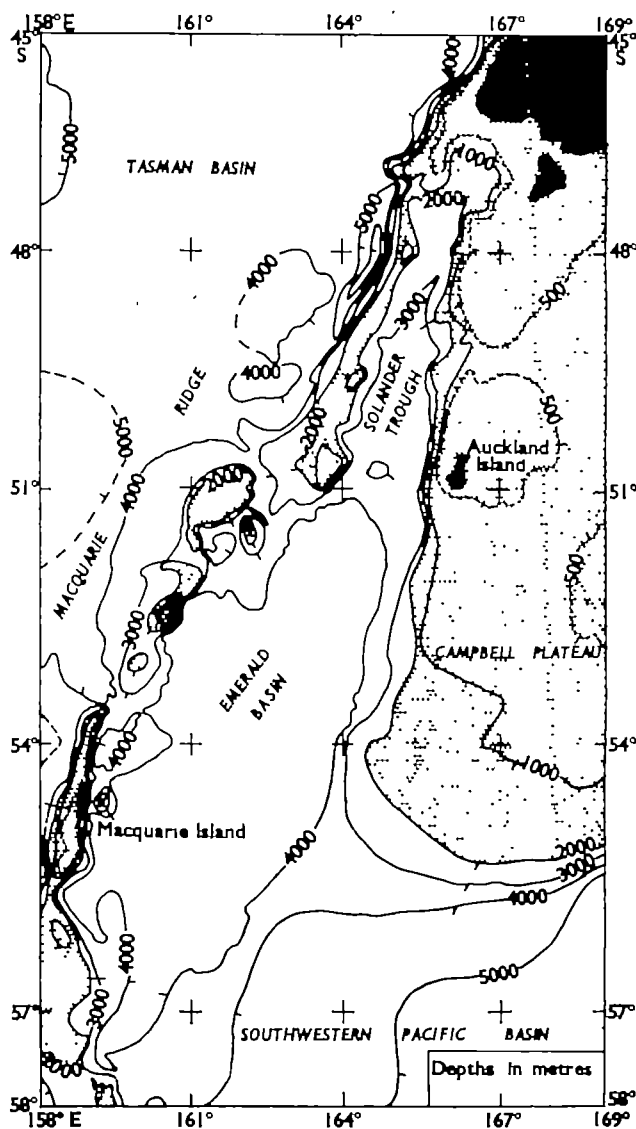


Fig 1

of the ridge: The traverses were on the average 40 nautical miles apart.

Most of this work was carried out during N.Z. Oceanographic Institute cruises on H.M.N.Z.S. *Endeavour*, but other available soundings have been utilized to construct the accompanying bathymetric chart (Fig. 1).

The principal morphological features that became apparent in the area between Macquarie Island and New Zealand are:

(i) A general ridge form extends north from Macquarie Island to the New Zealand shelf (in the Pyusegur Point area).

(ii) Much of the ridge is shallower than 2,000 metres, little of the crest is shallower than 1,000 metres.

(iii) The maximum distance between portions of the ridge shallower than 2,000 metres is 35 miles (near 53° 30' S. and 51° S.) and here the maximum depth in these intervals is less than 4,000 metres.

(iv) Individual highs top the ridge and in places form shallow banks with flat tops now at 100 metres or less.

(v) A marked east-west offset occurs at 51° S. where the northern portion of the ridge is offset 50 miles to the east.

(vi) The foot of the western slope of the Ridge is bordered by a series of partly elongated shallow basins, analogous to the Fiordland Trough at the slope-foot of south-west South Island of New Zealand<sup>4</sup>.

(vii) A low-relief sill at just less than 4,000 metres depth separates Emerald Basin from the south-western Pacific Basin. Emerald Basin floor is a little more than 4,000 metres deep.

(viii) Solander Trough extending south from the New Zealand shelf was previously conjectured to turn west and descend to the Tasman Basin near latitude 47° 30' S. Though there is some room for ambiguity the present evidence favours an interpretation of the bathymetry in which the Solander Trough descends to Emerald Basin on the eastern side of Macquarie Ridge.

While the intervals between sounding lines still admit the prospect of further revisions of the present bathymetry, yet the possibility of a substantial deep between New Zealand and Macquarie Island is removed. The physical connexion of the ridge with the south-western corner of New Zealand rather than with any element of the Campbell Plateau calls for reorientation of both structural and biogeographic generalizations for the region and for a revision of some present views on the structure of southern New Zealand.

The spread of benthic animals with restricted larval life and specific depth requirements<sup>5</sup> can now be more readily accepted as the water gaps at given depth are shown to be reduced from those of earlier bathymetric concepts.

The present definition of the bathymetry provides a firmer basis for the detailed discussions of the geological structures of the region and of the biogeographic significance of the animals obtained that will be published elsewhere.

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## GEOPHYSICS

### Sinusoidal Disturbances due to Shipping, on Seismograms at Rabaul, New Britain

UNUSUAL sinusoidal disturbances, which have been a feature of seismograms at Rabaul since continuous recording commenced in 1954, have been definitely linked with movements of shipping in the confined waters of Simpson Harbour (Fig. 1).

The disturbances, which appear to be a resonance effect, bear a marked resemblance to 'singing' events, such as are commonly noticed in offshore seismic surveys. Liu<sup>1</sup> has shown that an extended source is necessary for this phenomenon, and that it may be produced by standing waves or by multiple reflexions from a strong bottom reflector. At Rabaul, the extended source is provided by movement of a ship's propeller. It is suggested that the phenomenon, which to my knowledge has not been recognized before, should be known as 'ship-singing'.

A total of 122 disturbances has been examined, covering the periods January 1954–December 1955 and January 1960–January 1963. Of these, 59 can be correlated with the movement of overseas shipping in Simpson Harbour; in 48 cases there is no such correlation, and in 15 cases records of the movements of ships for the dates concerned are not available. Of the 48 cases for which there is no correlation, seven are known to have occurred while ships were anchored in mid-harbour, during which time they may have moved position, and five can be correlated with movement of a Diesel-powered 'workboat' which is used once weekly by Observatory staff to visit thermal areas in Blanche Bay.

Thus 71 out of 107 events have been found to correlate tolerably well with the movements of ships, while in 36 cases there is no known correlation. Since, however, movement of small inter-island ships was not recorded, it is at least possible that these remaining instances may be attributed to them. The duration of these events varied between 0.5 min and 45 min.

In 55 cases a fairly accurate estimate can be made of the position of the ship considered to have been re-

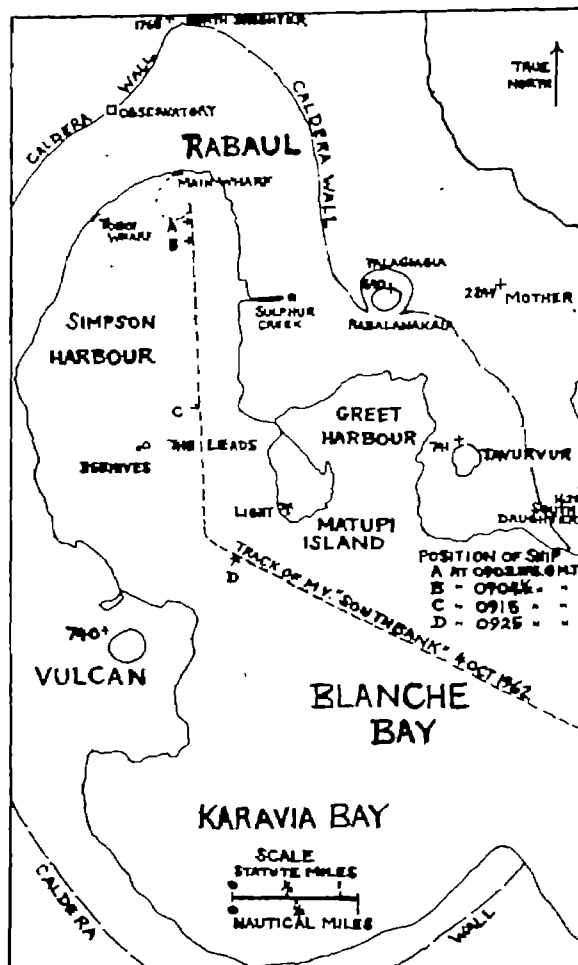


Fig. 1. Rabaul locality map

sponsible for the disturbance. In 29 of these cases ships were moving from the wharves to the 'leads' (Fig. 1), in 12 from the 'leads' to the wharves, in six from the 'leads' to the open sea, and in two from the open sea to the 'leads'. In four cases disturbances were apparently caused when the ship was moored to a wharf, usually shortly before sailing, and in two cases when ships were anchored in mid-harbour. It is evident that the phenomenon may occur at Rabaul in various positions and in various depths of water, usually 20–30 fathoms.

The size of the ship does not appear to bear a direct relationship to the amplitude of the oscillations on the seismograms. The two largest ships to have entered Simpson Harbour during the period, *Oronsey* and *Orcades* (respectively 15,122 and 15,839 registered tonnage), caused smaller sinusoidal disturbances than many which have been associated with Bank Line ships (3,000–5,000 registered tonnage). The frequency of the oscillations, which appears to be governed by the speed of revolution of the ship's propeller, varies between 0.8 and 8 c/s. Overlap of frequencies is often observed, the higher frequencies of 3–6 c/s being superimposed on lower ones which may persist for several minutes.

Fig. 2 illustrates by far the most striking example of 'ship-singing' recorded at Rabaul, in which a ground amplitude, peak to peak, of  $17\mu$  was recorded on the horizontal north-south component of the World-Wide Standardized System at the Observatory. It occurred on October 4, 1962, between 0902 and 0915 h G.M.T. (maximum at 0904.5 h G.M.T.), and is thought to have been due to M.V. *Southbank* (3,523 registered tonnage), which cast off from Main Wharf at 0850 h G.M.T. (1850 h local time).

In addition to being recorded with exceptional amplitudes on the 3-component short-period instruments of the Standardized System at the Observatory (illustrated) and on a 3-component old model Benioff seismograph at Sulphur Creek, it is the only disturbance known to have been accompanied by long-period movement. The vertical long-period component of the World-Wide Standardized System at the Observatory (also illustrated) recorded movement commencing 3 min before the short-period disturbance, at 0859 h G.M.T., and ending at 0925 h

G.M.T., 10 min after the end of the short-period disturbance at the Sulphur Creek Station. The period of this movement increased during the course of the event from 165 to 215 sec, and the peak-to-peak amplitude represented apparent ground motion of  $245\mu$ , many times greater than the maximum ground amplitude on the short-period instruments.

Movement of such a kind is unusual on the long-period vertical instrument at Rabaul. It resembles the effect due to persons in the Observatory building but is of far greater regularity. It was established that no one was in or near the building at the time, and this is confirmed by the fact that the horizontal long-period records show no trace of effects due to persons: they recorded a short-period oscillation of minute amplitude (approximately the width of the trace) such as is observed in strong motion earthquakes, but no long-period movement of any kind.

This long-period event is sufficiently unusual to suggest that it may have been connected with the exceptional disturbance on the short-period instruments, although the connexion is by no means certain. The very large apparent motion and the fact that the greater amplitudes on the short-period instruments occurred on the horizontal components suggest that the long-period disturbance may have been due to a spring-resonance effect rather than to a ground movement. It is noteworthy that it occurred well before resonance on the short-period instruments, at a time when only low-amplitude interference was being recorded.

The approximate position of M.V. *Southbank* at the time of this event was deduced from the deck and engine-room log books and is indicated in Fig. 1. The ship left the wharf at 0850 h G.M.T. and commenced to move full ahead at 0900 h G.M.T. When the maximum effect occurred the ship is estimated to have been about 2,500 yards from the Observatory, gradually picking up speed to three or four knots. 'Singing' ended on the Observatory records at 0914 h G.M.T., at which time the ship, making about eight knots, was about 5,000 yards from the Observatory. About 1 min later the event ended on the Sulphur Creek records, when the ship was approximately 2,400 yards away. Water depth along the line of origin of the disturbance is 20–30 fathoms.

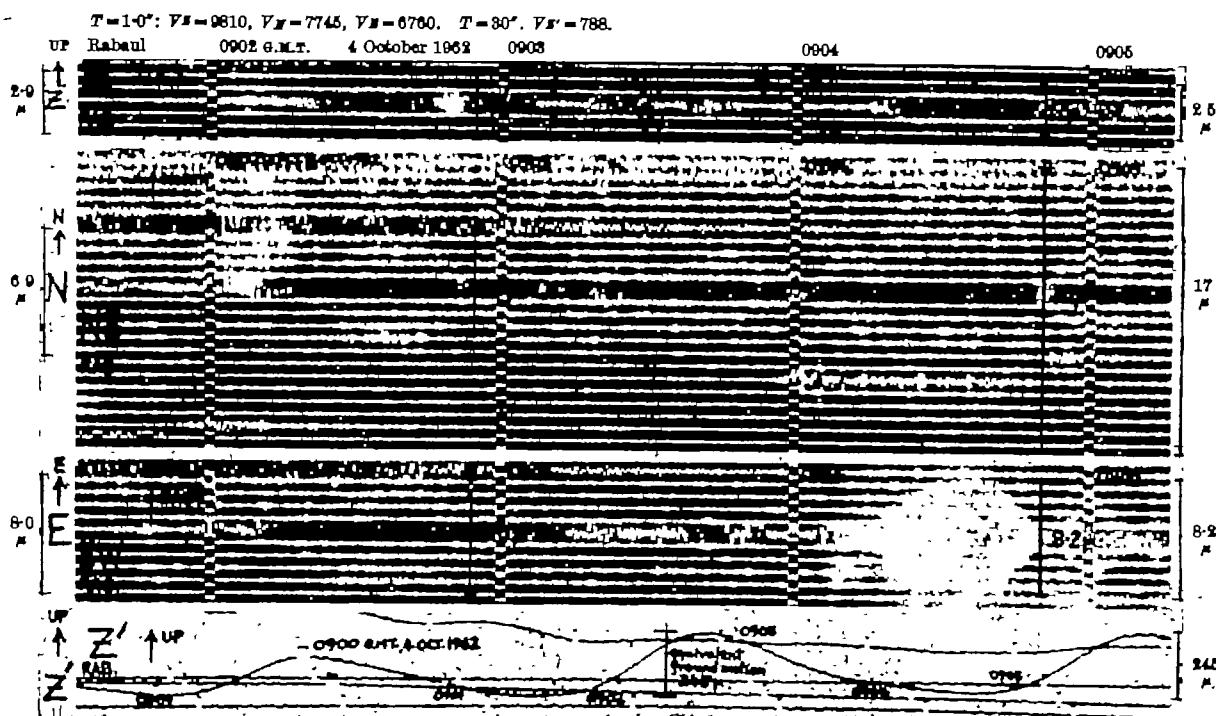


Fig. 2. Three-component short-period and  $\frac{1}{2}$  long-period recordings of 'singing' caused by M.V. *Southbank* at Rabaul

When manoeuvring full ahead the propeller of M.V. *Southbank* makes about 90 r.p.m. This is in accord with the observed frequency of the disturbance, which was about 1.4 c/s.

In common with the majority of 'singing' events observed at Rabaul, most of the energy was propagated longitudinally in a north-south plane. There have, however, been occasions on which the maximum effect has been recorded on the east-west component at the Observatory, and on the north-south at Sulphur Creek.

I thank my colleagues at the Rabaul Volcanological Observatory and Mr. James Hileman of Texas Instruments, Ltd., for their help, and the Bank Line, Ltd., and the Harbourmaster, Rabaul, for information about the movements of ships.

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### Ellipticity, Viscosity and Expansion of the Earth

It is known that the ellipticity<sup>1</sup> of an Earth in hydrostatic equilibrium  $(3.3532 \pm 0.0003) \times 10^{-3}$  is greater than that of the actual Earth  $(3.3535 \pm 0.0003) \times 10^{-3}$  derived from high-precision satellite observations<sup>2</sup>.

The purpose of this communication is to show that, if the gravity constant is inversely proportional to a time parameter comparable with the age of the Earth; that is, in the case of the Dirac-cosmology, then the observed ellipticity may be regarded as the ancient ellipticity of an Earth in hydrostatic equilibrium retained by its viscous inner part.

For the ellipticity, the Clairaut-equation is valid:

$$\epsilon = 3/2 J_2 + 1/2 m + 1/2 \epsilon^2 - 1/7 \epsilon m - 4/7 \lambda$$

where  $J_2 = \frac{C-A}{Ma^2}$  and  $m = \frac{\omega^2 r_1^3}{fM}$  ( $M$  is the mass of the Earth,  $A$  and  $C$  are the moments of inertia,  $\omega$  is the angular velocity,  $a$  the equatorial radius,  $r_1$  the mean radius, and  $\lambda$  a quantity of an order of magnitude of  $10^{-9}$ ).

The gravity constant,  $f$ , in the case of the Dirac-cosmology is  $f = \frac{x}{t}$ , where  $x$  is a constant and  $t$  a time parameter comparable with the age of the Earth. The recent value of  $t$  can be taken as  $4.5 \times 10^9$  years.

In the case of hydrostatic equilibrium:

$$C - A = \frac{8\pi}{3} \int_0^a \epsilon \rho r^4 dr$$

( $\epsilon$  is the ellipticity, and  $\rho$  the density, as a function of radius).

Therefore, in the case of an Earth in hydrostatic equilibrium the following expression is valid for unit time:

$$\frac{d\epsilon}{dt} = \frac{2Q}{\omega} \cdot \frac{d\omega}{dt} + \frac{Q}{t} + \left(5Q + \frac{3\rho_s}{\rho_e} - 2\right) \frac{\alpha}{a}$$

where  $Q = \frac{m}{2\epsilon} = 0.513$ ;  $\rho_s = 2.85$ , the surface density;  $\rho_e = 5.52$ , the mean density of the Earth;  $\alpha$  = the yearly radius increase.

The recent value of  $\frac{d\omega}{dt} = -4.81 \times 10^{-32}$  radian sec<sup>-2</sup> =  $-1.44 \times 10^{-14}$  rad sec<sup>-1</sup>/year (ref. 3).  $\frac{d\epsilon}{dt} = (2.9 \pm 0.3) \times 10^{-3}$ . In the case of  $t = 4.5 \times 10^9$  years the value  $\frac{d\epsilon}{dt}$  is positive only if  $\alpha > 0$ , that is, in the case of an expanding Earth.

It has been shown that the minimum rate of the Earth's expansion amounts to  $0.6 \pm 0.1$  mm/year<sup>4</sup>.

In this case the yearly value of:

$$\frac{d\epsilon}{dt} = 0.6 \times 10^{-10}$$

that is, the value of  $2.9 \times 10^{-3}$  may be obtained only in  $4.8 \times 10^7$  years.

The maximum rate of expansion gives the radius of the Earth divided by its age. This is 1.4 mm/year, and the foregoing relative change of ellipticity may occur in  $0.9 \times 10^7$  years.

If the difference between the actual ellipticity and the equilibrium ellipticity can be ascribed to viscosity, the time interval obtained can be regarded as the relaxation time of deformations retarded by viscosity. In the mantle the rigidity is always greater than  $1 \times 10^{11}$  dynes cm<sup>-2</sup>. Therefore, the viscosity (according to the Maxwell relation) is:

$$\eta = \mu\tau \geq 3 \times 10^{16} \text{ poise}$$

and probably:

$$> 7 \times 10^{16} \text{ poise}$$

It has been shown by Niskanen<sup>5</sup>, and recently by Crittenden<sup>6</sup>, that the viscosity of the subcrustal material is less than  $10^{21} - 2 \times 10^{22}$  poises, at least to a restricted depth. Therefore, the viscosity of the greater part of the mantle must exceed several times  $10^{24} - 10^{27}$  poises. This excludes the existence of convection currents in the greater part of the mantle.

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<sup>4</sup> Eged, L., *Nature*, 173, 534 (1956).

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### Gravity Field of the Niger Delta (West Africa)

GRAVITY data are now available for 73 land stations situated in the general area of the Niger Delta, West Africa. The Bouguer anomaly map shows a gravity low of  $-35$  mgal (the 'Niger Delta Minimum') which occupies a large part of the sub-aerial Niger Delta, and a gravity high of  $+40$  mgal (the 'Ekenie High') situated in the extreme south-western corner of the sub-aerial delta. Isostatic reduction does not very appreciably affect the anomalies, as is only to be expected in an area where the topography is low and gentle. The isostatic anomaly maps, based on various possible systems of isostatic compensation, accordingly do not differ very much one from another or from the Bouguer anomaly map, and show the same general features as the latter. However, there is some difference in the 'Ekenie High' area; isostatic anomalies there are less pronounced for a higher degree of regionality. This is interpreted as an indication that the continental slope off the Niger Delta is regionally compensated.

If the sediments constituting the Niger Delta had been laid down on a completely rigid, unyielding crust which was in isostatic equilibrium before the sediments were deposited, one would expect to find strongly positive gravity anomalies of, say,  $+125$  to  $+220$  mgal, depending on the density value assumed. In reality, gentle negative anomalies down to  $-40$  mgal are encountered. This is interpreted as meaning that the Niger Delta is practically in isostatic equilibrium. Accordingly, there must have been adjusting crustal movements (subsidence) to bring about this state of near-isostatic equilibrium and there is every reason to interpret this as subsidence under a sedimentary load.

A simple relation exists between initial water depth and the total thickness of sediment that can be deposited. If, namely,  $W$  is the initial water depth (density of sea

water is  $1.03 \text{ g/cm}^3$ ) in an area which is in isostatic equilibrium, and sediment of density  $d \text{ g/cm}^3$  is supplied until the top of the sediment reaches sea-level and the area is again in isostatic equilibrium, then the total thickness of sediment,  $X$ , is related to the other quantities by the equation:

$$1.03 W + 3.27 (X - W) = dX$$

This relation is based on the required equality of pressures at an arbitrary reference-level in the mantle, and may also be written as:

$$X = \frac{3.27 - 1.03}{3.27 - d} W \quad (1)$$

Analysis of present-day isobaths in the adjacent Gulf of Guinea indicates that the initial water depth in the area now occupied by the south-western tip of the sub-aerial Niger Delta may have been as much as 3,000 m. Accepting  $2.40 \text{ g/cm}^3$  as the most likely value for the average density of the Niger Delta sediments, the foregoing equation yields a value for  $X$  of nearly 8,000 m in conjunction with a value for  $W$  of 3,000 m. The total sediment thickness in the Niger Delta is accordingly estimated to be about 8,000 m.

A nearly identical reasoning can be applied to the case where the sea is not completely but only partially filled up with sediment. In this case, the total thickness of sediment deposited ( $X$ ) is related to the amount  $Y$  by which the sea floor has been raised after sedimentation and subsidence have again resulted in isostatic equilibrium, by the expression:

$$X = \frac{3.27 - 1.03}{3.27 - d} Y \quad (2)$$

The configuration of the sea floor before the delta was there can be inferred from the present-day isobaths by interpolation between the isobaths on either side of the delta. Everywhere,  $W$  or  $Y$ , as the case may be, can therefore be found from the difference between the inferred initial isobaths and the present-day isobaths. At all points,  $X$ , the total thickness of sediment, can hence be calculated. This simply means that the total volume of the Niger Delta can be calculated. Assuming  $d = 2.40 \text{ g/cm}^3$ , this yields a total volume of  $500,000 \text{ km}^3$  for the Niger Delta sediments.

A more detailed and complete account of this investigation will appear in the *Bulletin* of the Geological Society of America.

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### Infrasonic Waves of the Auroral Zone

INFRASONIC waves of the auroral zone have been observed<sup>1,2</sup> using a suitable microphone system. These waves are thought to originate in the joule heating by the electrojet currents<sup>1</sup> or in the corpuscular heating during ionization and excitation processes<sup>3</sup> in the auroral ionosphere—at about the 100-km level. Periodic influx of electrons at this level due to processes, which may be solar, magnetospheric or extra-magnetospheric, could cause periodic fluctuations in the current systems and the heating processes; hence the observed periods (in the range 20–80 sec and, at times, upwards of 100 sec). Time-scales of this order are known in auroral pulsations. It is suggested here that it is also possible to account for the energy fluctuations from the atmospheric dynamics, specifically turbulence near 100 km, which can generate these waves via the pressure fluctuations.

It is shown elsewhere<sup>4</sup> that the time-scales of eddy decay in turbulence near 100 km can range from less than a second to several seconds going up to a few minutes; and several geophysical events show such time scales<sup>5,6</sup>.

The energy dissipation varies from approximately low values of the order  $10^{-8} \sim 10^{-6}$  to relatively high  $10^{-1} \sim 10$  erg  $\text{cm}^{-3} \text{ sec}^{-1}$ . Some of the high values are due to the high speeds used. For example, a few km/s to a few tens of km/s are known for intense and moving aurorae<sup>1-7</sup>. Scale sizes from less than 1 km to a few km can be considered. Refraining from very-high-energy rates until their implications become fully understood, still one has to consider moderately high values of the order  $10^{-4} \sim 10^{-3}$  erg  $\text{cm}^{-3} \text{ sec}^{-1}$ . During disturbed conditions, this order of energy is certainly comparable to, or in excess of, the joule heating rates. Most of the energy of this magnitude is derived either from the auroral input during strong aurorae, or from the solar input.

We can consider a 10-km thickness of the turbulent layer in accordance with the experimental results<sup>8</sup>. An estimate of the energy flux corresponding to densities of the order  $10^{-4} \sim 10^{-3}$  erg  $\text{cm}^{-3} \text{ sec}^{-1}$  would be about  $100 \text{ erg cm}^{-2} \text{ sec}^{-1}$ , or more. This is roughly the order of energy flux due to Maeda and Watanabe<sup>9</sup> required for a surface pressure amplitude of the order of 1 dyne  $\text{cm}^{-2}$ .

One can compute the pressure fluctuation magnitudes in turbulence. The order of this fluctuation will be denoted by  $\delta P$ . The only quantity having the dimension of pressure and formed from the quantities  $\delta V$  (= velocity in fluctuation),  $\rho$  (= density of the fluid), and  $l$  (= scale size of eddy), is given by  $\delta P \approx \rho(\delta V)^2$ . For the pressure and density for the height concerned, one can get fluctuations ranging from less than 1 up to 5–10 per cent. From this, during auroral periods, one could expect the order of flux required to generate the observed pressure waves.

Fig. 1 shows some of the energy sources in the ionosphere. We can assume<sup>3</sup> that the peak of the hydromagnetic heating which occurs at about 170 km is too high for the required order of energy flux to generate the pressure

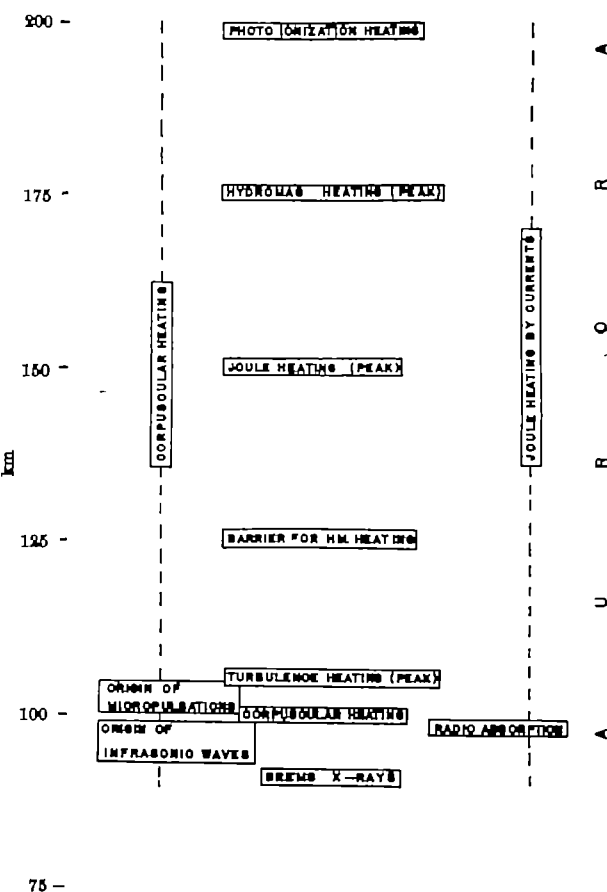


Fig. 1. Relative heights of some energy sources in the ionosphere

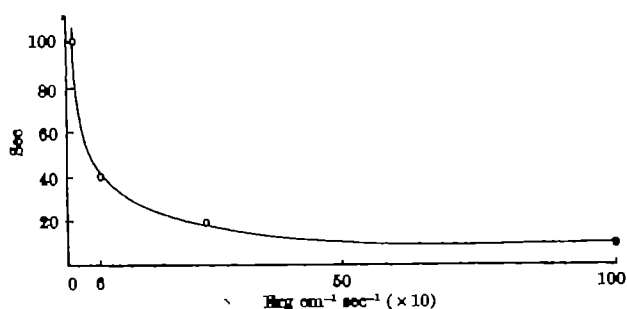


Fig. 2. Relative variation of eddy decay time with energy dissipation rate in turbulence about 100 km

waves. The peak of the relative ionization rate is about 100 km; and auroral coruscations, micropulsations of the magnetic field and related absorption effects are attributed to this level<sup>9</sup>. Turbulent mixing becomes very little above 100 km<sup>9</sup>. Transfer processes in the momentum space is large in the region 80–100 km. Autocorrelation and power spectrum methods are certainly effective<sup>10</sup> in obtaining signal periods and the discrete frequencies that are hidden in the random noise processes. The life-time of an eddy is relatively longer as the dissipation energy decreases as shown in Fig. 2. One can certainly expect relatively longer periods for smaller energy rates.

In conclusion, turbulence near the 100-km level can be easily considered as an atmospheric source of energy modulation and, thus, for the generation of infrasonic waves.

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## GEOLOGY

### Dual Classification for Point-counting Limestones

Most recent petrographic accounts of limestones utilize the classification of Folk<sup>1</sup> as a basis for quantitative description, and as a convenient shorthand for general descriptions. During studies of English Upper Jurassic limestones it has been found that point-count results using Folk's categories do not adequately convey all the characters of a particular rock specimen.

Folk divides limestone constituents into two groups: terrigenous constituents (derived from consolidated rocks outside the basin of sedimentation), and allochemical constituents (allochems) formed by physico-chemical or biological processes within the basin of sedimentation. The allochems are further divided into groups including intraclasts, oolites, pellets, and other particles of organic origin. The distinction between intraclasts and pellets is usually made by the ovoid shape of the latter, but in English Corallian (Upper Oxfordian) rocks the two particle types are of similar size and so are difficult to distinguish; therefore only one category has been used, that of intraclasts. More important in rocks of this age, however, is the admixture of allochemical and terrigenous material; thus quartz grains may form the nuclei of oolites, or possess discontinuous coats of fine-grained calcite, and hence be termed intraclasts (Fig. 1). Therefore a quantitative analysis of an oolite will include the constituent

particles, but will not detail the proportion of quartz within the sample. Similarly, the true fraction of organic carbonate material within a sample may be obscured, for, if the shell fragments are coated with fine-grained calcite, they are counted as intraclasts. Fig. 1 shows the variety of material that may constitute intraclasts or oolites.

In order to clarify the anomaly produced by point counting limestones using Folk's categories, a second count (by experience, five hundred points have been found adequate) must be made to estimate the proportion of matrix (micrite or sparite), very fine-grained allochemical material, organic and terrigenous material (not particles) present in the sample. Thus oolites possessing a quartz nucleus are registered under two categories for the purpose of the second count, as fine-grained allochemical material as well as terrigenous material.

The results obtained by the method described may readily be plotted on triangular diagrams (with end members fine-grained allochemical, terrigenous and organic; hence A:T:O) by recalculating the results to 100 per cent after subtracting the matrix total from the original result. The latter procedure is justified by the results it has produced, and also because the frequency distribution of sparite and micrite percentages (Fig. 2) is distinct in the Corallian specimens studied; however, it should be noted that the results shown in Fig. 2 are biased towards samples with micritic matrices, as extensive quantitative studies of Upper Oxfordian rocks of this

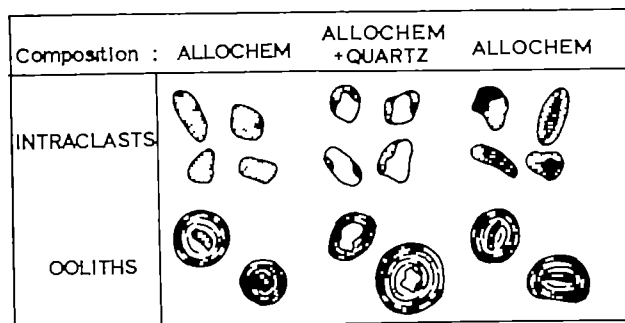


Fig. 1. Diagrammatic representation of possible compositions of intraclasts and oolites. Stippled, very fine-grained allochemical material (calcite); concentric lines, as above, but with oolite laminae; black, quartz; black and horizontal lines, fragments of organic origin.

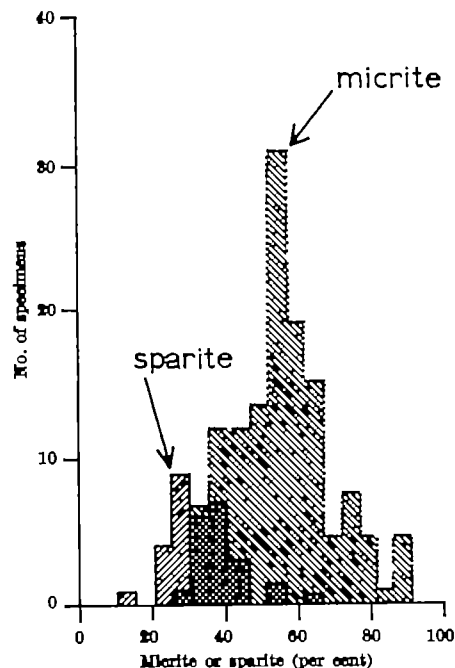


Fig. 2. Histogram of micrite and sparite percentages in Corallian rocks; 160 specimens



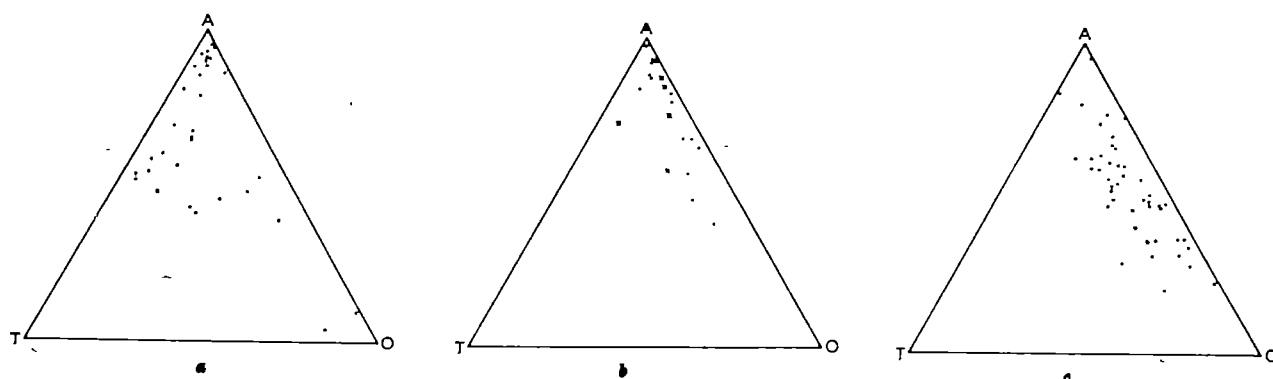


Fig. 2. Triangular diagrams, with end members 100 per cent. A, very fine-grained allochemical material; T, terrigenous material; O, organic material. (a) 12 specimens from Lower and Middle Oolites, Osmington Oolite Series, coast east of Weymouth. (b) Dots, 16 specimens from Upper Oolites, locality as A; crosses, 7 specimens from Oolite Freestones (Osmington Oolite Series) of Abbotsbury and north Dorset (Marnhull). (c) 47 specimens from the 'Trigonia' clavellata beds, coast east of Weymouth.

type have been carried out. Such a division is to be expected, as micritic rocks are produced by current régimes differing considerably from those associated with primary sparry limestones.

It has often been emphasized, particularly for sedimentary rocks, that individual quantitative analyses are of little value, except when comparative evaluations of a number of results are made; it is in the latter context that the A : T : O diagrams are particularly valuable.

Fig. 3 shows the results obtained from specimens collected from the Dorset Corallian (for descriptions of which see Arkell<sup>2,3</sup>). Fig. 3a includes specimens from the Lower and Middle Oolites of the Osmington Oolite Series exposed in cliffs to the east of Weymouth; here specimens containing less than 85 per cent fine-grained allochemical material are from micritic facies marginal to lens shaped oolite banks, whereas those with such material in excess of this figure are sparry, well-sorted oolites. Fig. 3b is plotted from results obtained from the Upper Oolites; the resulting distribution is in marked contrast to Fig. 3a, showing a distinct paucity in terrigenous material, and is paralleled by the opposing dominant current direction (from the west instead of east as in the Lower and Middle Oolites), and the lack of marginal facies showing the progressive build-up of oolitic coasts around shell fragments and quartz grains. In addition, the oolites of the Upper Oolites are extremely well sorted (there being few present with diameters less than 0.7 mm), but despite this, they are set in a micritic matrix. Results obtained from the Oolite Freestones of the same age exposed at Abbotsbury to the west of Weymouth, and at Marnhull in north Dorset, are comparable to those of the Upper Oolite (crosses in Fig. 3b); these freestones are known to be in proximity to coral limestones. Figs. 3b and 3c are very similar, the latter showing results from the 'Trigonia' clavellata beds (Arkell<sup>4</sup>) to the east of Weymouth, which are of a 'lagoonal' aspect. Therefore the Upper Oolite appears to be transitional between a phase of oolite formation and calmer sedimentation, perhaps resulting in part from the erosion of earlier oolite banks nearby.

While in no way replacing Folk's excellent concept, the method described enables various 'suites' of carbonate rocks to be compared by quantitative methods. The results obtained corroborate differences observed in the field and by qualitative thin section studies, and it is hoped that the method will find useful application in carbonate rocks of other ages and localities.

I thank Dr. G. R. Orme and Prof. L. R. Moore for their advice. This work was financed by a studentship from the Department of Scientific and Industrial Research.

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### Fossil Burrows on the Coast of Kenya

THIS group of several dozen burrows to be described was found in July 1963 in an outcrop on the shore situated on the east side of the entrance to Mida Creek, about 16 miles south-west of Malindi, in the Coast Region of Kenya. The reference on the Universal Transverse Mercator Grid used in Kenya is Zone 37, FG 077258.

The rock containing the burrows resembles the coquinas described by Thompson<sup>1</sup>. It is cream-coloured granular limestone which in thin section is seen to consist of fragments of shell, with some foraminifera and a few quartz grains, cemented together by calcite. In outcrop the bedding is picked out by weathering. The burrows are found in a part of the outcrop where flat-bedding overlies and truncates cross-bedding.

Thompson thought that the coquinas accumulated as off-shore bars on which dunes developed. He distinguished two sets, one at 100 ft. above sea-level, and a younger set approximately at sea-level. The outcrop containing the burrows belongs to the younger set, considered by Thompson to be of Gamblian (Upper Pleistocene) age.

The burrows were found on vertical rock faces where they have been etched out by weathering. Usually the outer half of the wall has weathered away, so that the burrow is exposed as a semi-circular groove on the rock-face, but sometimes they pass through projecting ribs of rock as circular tubes. In other cases the whole of the wall of the burrow has resisted weathering so that it stands out as a hollow cylinder. All the burrows are straight or gently curved, and vertical or steeply dipping; one was found as much as 43° from the vertical. Their diameter varies from  $\frac{1}{8}$  in. to  $1\frac{1}{4}$  in. Usually a part only of a burrow, between 6 in. and 18 in. long, was visible, but one burrow 28 in. long was exposed. Where the lower end of a burrow was visible it was a blind end with no trace of chamber or return shaft. The upper part of the burrow either ended abruptly in the upper flat-bedded part of the outcrop or was obviously weathered away. Fig. 1 shows a burrow with most of these features. The burrows were present on one outcrop only, although similar patterns of weathering and bedding were present on nearby outcrops. It seems unlikely that these structures are of inorganic origin or are casts of roots, but on modern beaches of this coast, burrows of similar diameter to the fossil ones are inhabited by the pink ghost crab, *Ocypode kuhlii*. This animal lives on sandy beaches (with sand of quartz or of calcareous debris) at about high-water mark, retreating into the surf for protection at high tide and into a shallow curved burrow at low tide. At neap tides some crabs dig very deep burrows between the high-water mark of neap tides and the high-water mark of spring tides. The deepest of these burrows which I dug out completely ended 26 in. vertically below the surface, but I followed other burrows to depths of over three feet before losing them. They ended abruptly without any trace of a living chamber. They descended less

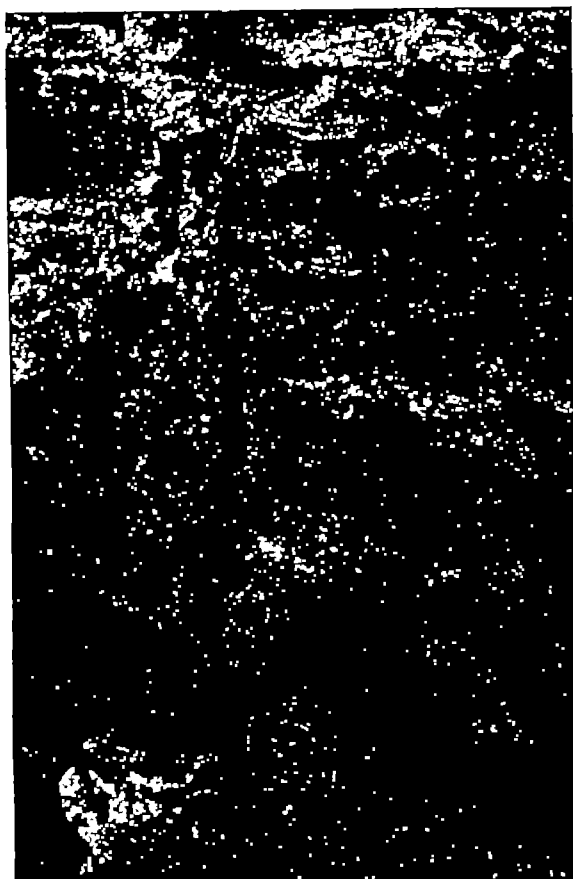


Fig. 1. One of the fossil burrows, showing the outer wall preserved in the lower part

steeply and were more curved than the fossil burrows. However, greater differences are found between burrows of different present-day species of *Ocyrops*. Kalk<sup>1</sup> describes *Ocyrops* at Inhaca Island, Mozambique, as digging to the surface from burrows destroyed by spring tides "in wide spiral tunnels", while Milne and Milne<sup>2</sup> imply that the majority of the tunnels of the North American species *O. quadrata* are U-shaped. It seems likely, therefore, that the fossil burrows were made by some species of *Ocyrops*, perhaps burrowing deeply at neap tides. The control of burrowing by the tide might account for the occurrence of the burrows in one outcrop only. If this interpretation is correct, it supports Thompson's view of the mode of accumulation of the coquinas.

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### Newly Discovered Mesosiderite containing Achondrite Fragments: the Mount Padbury Meteorite

As the result of an unusual find by the joint managers of Mount Padbury sheep station, near Meekatharra, Western Australia (latitude 25° 40' S.; longitude 118° 06' E.), a peculiar occurrence of ferruginous coated boulders has been investigated and recognized as mesosiderite material of meteoritic origin. This new find, resulting in the recovery of more than 800 lb. of material including a single 195-lb. mass, ranks only second to the recovery after an observed fall at Estherville, Iowa, in 1879, of

340 kg (750 lb.) of mesosiderite material (ref. 1, p. 119). Not more than a score of these stony irons are known (ref. 2, p. 122); they are much rarer than pallasites, and some of them are diminutive fragments or very weathered, and so are of little use for the purpose of precise petrographical investigations. This new find from Mount Padbury would be important on account of the amount of the material in itself, and the fact that, though many fragments are weathered to iron-shale, some are completely fresh and covered by only a thin iron oxide veneer. But it assumes an even greater significance with the recognition of enclaves of achondrite material up to an inch or so across and free, or nearly free, from nickel-iron, which are included within the mesosiderite matrix alongside large single crystals of olivine and lumps of solid nickel-iron. Such material must provide an opportunity to find out more about this somewhat variable group of stony-irons and even learn more concerning the wider problem of meteorite genesis.

Detailed studies now in hand will probably endorse the conclusions of Prior<sup>3</sup> and Lovering<sup>4</sup>, although the story is even more complex than those authorities suggest. Prior suggested that mesosiderites are essentially mixed meteorites of combined eucritic and pallasitic composition, the latter invading the former; but Lovering cited evidence from the Pinnaroo mesosiderite indicating that in certain cases the same two components are present but the converse is the case—the eucrite invading the pallasitic component. In the case of the Mount Padbury mesosiderite the textural evidence leaves no doubt that the relationship suggested by Prior applies, but it is even more complicated since the achondrite enclaves are highly variable and include eucrite, brecciated eucrite and diogenite, the rare hypersthene achondrite. No two thin sections of the dozen so far prepared of the abundant eucrite material are alike in texture and in many there are dissimilarities of mineralogy. The olivine did not come in with the metal but represents achondritic inclusions.

The simplest of the eucrite material shows ophitic texture and is composed of a single pigeonitic clinopyroxene, showing spectacular exsolution lamellae, plagioclase (bytownite possibly grading into labradorite and marked by conspicuous droplet inclusions, pink or dark-tinted) and a mineral of low refractive index and negative relief low birefringence giving mottled grey coloration under crossed nicols, and a biaxial positive interference figure with moderate optic angle. This last mineral is believed to be tridymite. The amount of ore mineral present is variable but it is never more than accessory. It is magnetite, not nickel-iron.

More complicated mineralogy is observed in other eucrite enclaves which show an equigranular, almost granulitic texture. The pyroxenes are in complex aggregates forming reaction rims around a central core. Several varieties of pigeonite and also hypersthene are represented in these corona assemblages.

This eucritic material recalls the Moore County eucrite<sup>5</sup>. The mineralogy and presence of droplet inclusions are comparable, but the feldspars in the Mount Padbury eucrite are well cleaved. Moore County is said to possess a fabric suggestive of crystallization in the crustal zone of an Earth-sized planet—where a similar gravitational force was operative. This new eucritic material, so varied as it is, will allow similar petrofabric studies to be carried out, to endorse this evidence or otherwise. Leaving aside this petrofabric evidence, which is difficult to reconcile with the classic theory of break-up of an asteroid-sized planet and derivation from the asteroid belt, the terrestrial appearance of this eucritic material from Mount Padbury recalls igneous rock derived from magmatic crystallization in lopoliths or other layered intrusions, and even dykes, at no great depth in the Earth's crust. This, in itself, leads one to have doubts about the possibility of asteroidal provenance (as opposed to planetary provenance). Production of such microgabbroic-

doleritic rock material even within an asteroid of the dimensions of Ceres seems difficult to envisage in the light of recent studies of basalt genesis. Moreover, beyond this anomaly there remains the anomaly of 'crustal' material trapped within denser material reasonably referred to the 'deep mantle'.

The diogenite enclaves possess granulitic texture: they are not strongly brecciated and are almost monomineralic, containing only fine specks of a more birefringent clinopyroxene, an ore mineral, apatite (?) and tridymite, of the same character as occurs within the eucrite. The presence of this tridymite and the close association of eucrite and diogenite would seem to introduce a fundamental piece of evidence to meteoritics—it suggests that calcium-rich and calcium-poor achondrites are closely related and that both probably stem from processes of magmatic crystallization.

Olivine crystals within the mesosiderite matrix are up to an inch long and have been identified (Mason, personal communication) by X-ray diffraction studies as  $Fe_{80}$ , an unusually magnesian olivine for a mesosiderite. Brecciated olivine achondrite enclaves are also present. The metallic nodules show fine-medium octahedrite etch patterns (Widmanstätten).

One of us (G. J. H. McC.) is supervising petrographic and mineralogical investigations of this new material: he thanks Dr. B. H. Mason of the American Museum of Natural History for his help, and Dr. H. B. Wiik, who is undertaking chemical analyses.

A full account of the field occurrence and external features of the meteorite is being prepared by W. H. C.

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## METEOROLOGY

### Outgoing Long-wave Radiation from Indian Stations

In recent months average values have been available to us of outgoing long-wave radiation recorded by *Tiros IV* for the equatorial region between 50° E. to 110° E. and 20° N. to 20° S. These observations were obtained through the courtesy of Mr. P. Krishna Rao, of the U.S. Weather Bureau. They represent averages for 5-day periods in the pre-monsoon months of April, May and June 1962, and are shown in Fig. 1 in langley/min.

It was felt worthwhile to compare the *Tiros* observations with estimates of outgoing radiation obtained from a radiation diagram. We computed, therefore, the total outgoing radiation from the mean aerological ascent of each radiosonde station in India. The computations were made with Elsasser's radiation diagram for the pre-monsoon period, under the assumption of a cloudless sky. It was also assumed that the water-vapour content decreased linearly from 500 mbar to the tropopause. Our estimates of outgoing radiation in langley/day are given in Table 1.

In Tables 2a and 2b we have compared our estimates with the *Tiros* observations for April and May.

The very small difference, of about 10 per cent or less, between our estimates and the *Tiros* observations is of

Table 1. ESTIMATES OF OUTGOING RADIATION FROM INDIAN STATIONS (langley day<sup>-1</sup>)

Station	March	April	May
1 Varanasi	440	450	460
2 Calcutta	440	467	446
3 Jodhpur	454	481	490
4 Madras	459	458	458
5 Nagpur	454	458	501
6 New Delhi	440	470	463
7 Bombay	467	456	463
8 Trivandrum	434	445	432
9 Vizagapatnam	431	439	442

Table 2a. COMPARISON OF *Tiros* OBSERVATIONS WITH ESTIMATES OF OUTGOING RADIATION OBTAINED FROM A RADIATION DIAGRAM (April)

Station	<i>Tiros</i> observation (langley day <sup>-1</sup> )	Estimated value from an Elsasser diagram (langley day <sup>-1</sup> )	Difference
Trivandrum	500	445	+55
Madras	502	458	+44
Vizagapatnam	490	439	+51
Bombay	487	456	+31
Nagpur	484	458	+26
		Average	+41

Table 2b (May)

Station	<i>Tiros</i> observation	Estimated value from an Elsasser diagram (langley day <sup>-1</sup> )	Difference
Trivandrum	443	452	+10
Madras	456	458	-2
Vizagapatnam	474	442	+32
Bombay	476	463	+13
Nagpur	480	501	-21
		Average	+06

some interest. It indicates that the assumption regarding the linear decrease of water vapour content from 500 mb to the tropopause is probably correct. The generally good agreement also tends to show that the effect of dust particles in the long-wave region (4–80 $\mu$ ) is small, because this was neglected in preparing our estimates. However, it is obvious that in the short-wave region (0.5–4.0 $\mu$ ) dust particles have an important effect.

Some indication of this is obtained if we compare the results of a theoretical investigation by Das<sup>1</sup> with recent radiosonde observations by Bryson<sup>2</sup>. From an examination of the mean vertical motion during the monsoon season, Das concluded that to maintain a steady circulation, non-adiabatic cooling at the rate of 2.4° C a day was required over north-west India. But the maximum cooling, estimated from an Elsasser diagram assuming water vapour and carbon dioxide as radiators, was only 1.8° C a day. Bryson's field experiments in north-west India confirmed that the observed cooling rate was indeed 2.4° C a day as predicted by Das. His estimate of cooling by outgoing radiation was, however, 1.6° C a day, which is slightly less than Das's estimate of 1.8° C a day. It is, therefore, likely that a part of the additional cooling of about 0.8° C a day is provided by the presence of dust. In the absence of clouds, the net effect of dust particles is to diminish the amount of solar radiation received at the Earth's surface. Although this depends on the dust

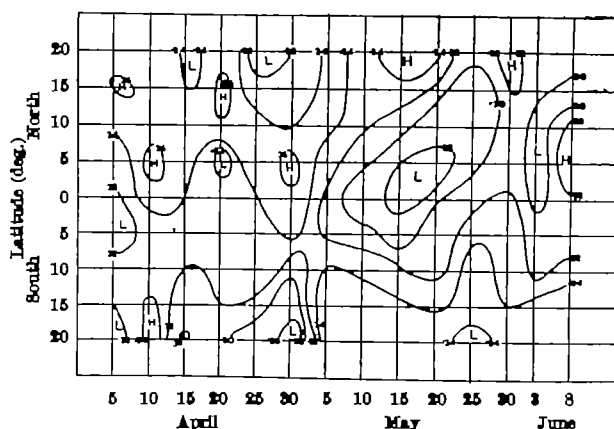


Fig. 1. *Tiros IV* long-wave radiation (langley/min) averages between 50° and 110° E.

content of the air, and is difficult to estimate with much confidence, the total depletion of the direct solar beam may amount to as much as 10 per cent.

I thank Prof. R. A. Bryson, University of Wisconsin, and Dr. P. K. Das for their advice.

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<sup>1</sup> Das, P. K., *Tellus*, 14, 2, 212 (1962).

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## PHYSICS

### Sub-resonant Response of a Mechanical System parametrically excited at its Resonant Frequency

In the course of an attempt to improve the response of an inertial seismometer to very-low-frequency frame movements, it appeared worthwhile to investigate the response of a spring-mass system subjected to parametric excitation<sup>1</sup>. The parametric excitation or 'pumping' consisted of modulating the restoring force on the mass by means of an electromechanical spring<sup>2</sup> driven from a sinusoidal current source. In the initial work the restoring force was varied at twice the resonant frequency of the system as is usually done in a degenerate parametric device. It was found both from theory<sup>3</sup> and experiment that this will increase the relative motion between mass and frame only for inputs with a frequency at or near the resonant frequency, and that the input must bear the proper phase relationship to the pump. There is no improvement for frequencies much below resonance.

Pumping at twice the resonant frequency is associated with the critical value,  $a = 1$ , in Mathieu's equation,  $\ddot{x} + (a - 2q \cos 2\alpha)x = 0$ , which governs the stability of such systems<sup>4</sup>. One would also expect to obtain gain near the other critical values,  $a = 4, 9, 16$ , etc., which correspond to pumping at the resonance frequency, two-thirds of the resonance frequency, half the resonant frequency, and so forth.

These phenomena were investigated experimentally and with a computer. Both sets of data confirmed that pumping at the resonant frequency of the system greatly increased the relative motion between mass and frame. In particular, it was found that this increase in motion can be made very large for input frequencies much lower than the resonant frequency of the device; and the increase remains constant and independent of phase relationships down to zero frequency. It is believed that this phenomenon has not been noticed before.

Table 1

	Pendulum A $T_p = 0.57$ sec	Pendulum B $T_p = 7.5$ sec
Period of input	57 sec = 100 $T_p$	6.25 min = 50 $T_p$
Motion of mass without parametric excitation	1	1
Motion of mass with parametric excitation	875	1,250

The experimental results are given in Table 1. They were obtained with two different horizontal pendulums (seismometers) with free periods,  $T_p$ , of 0.57 and 7.5 sec. These were modified to permit modulation of their period<sup>5</sup> with the spring of time-variable stiffness. Both were pumped at their free period and driven by inputs with periods many times greater than their free periods. In each case it was found that parametric excitation at the resonant frequency could produce as much as three orders of magnitude increase in the motion of the mass with respect to the frame.

An observatory-quality 'parametric seismometer' utilizing this principle is being constructed for the purpose of recording very-long-period vibrations of the Earth.

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### Effect of Magnetic Dilution on the Paramagnetic Behaviour of an $Ni^{++}$ Ion in the Crystal of $Ni(SO_4)_2 \cdot (NH_4)_2 \cdot 6H_2O$

It is well known that the symmetry and intensity of the electric field arising out of an axially distorted octahedral water cluster surrounding a paramagnetic ion ( $M^{++}$ ) determines its magnetic anisotropy. When paramagnetic Tutton salts are magnetically diluted by partially replacing  $M^{++}$  ions by  $Zn^{++}$  or  $Mg^{++}$  ions, the charge distribution is likely to remain the same for different concentrations of  $M^{++}$  ions in the mixed crystal. Consequently, the magnetic anisotropy per gram is expected to be almost independent of concentration in the mixed crystal.

Joglekar<sup>1</sup> did not detect any effect of magnetic dilution on the magnetic anisotropy of Tutton salts. Paramagnetic resonance investigations by Griffiths and Owen<sup>2</sup> on the magnetically dilute salt ( $Ni \cdot Zn$ )  $(NH_4)_2(SO_4)_2 \cdot 6H_2O$  showed that up to a dilution of  $Ni : Zn = 1 : 50$ , the crystal field axes and crystal field splitting at 90° K are very similar to those of the concentrated salt at 90° K. Bose *et al.*<sup>3</sup>, however, found that the crystal field about  $Ni^{++}$  ion in nickel Tutton salts is very sensitive to structure and temperature.

In order to test the foregoing points, we have measured the magnetic anisotropy of mixed crystal, of the type  $A + nB$  (where  $A$  is  $Ni(NH_4)_2(SO_4)_2 \cdot 6H_2O$ ,  $B$  is  $Zn(SO_4)_2 \cdot (NH_4)_2 \cdot 6H_2O$  or  $Mg(SO_4)_2 \cdot (NH_4)_2 \cdot 6H_2O$ , and  $n$  is the number of molecules of  $B$  associated with one molecule of  $A$  in the mixed crystal) by the well-known method of Krishnan and Banerji<sup>4</sup>. The temperature variation of the anisotropy of the crystal ( $A + 15.3 B_1$ ) was measured by following the method of Krishnan *et al.*<sup>5</sup>. The temperature was controlled by a cryostatic device due to Bose *et al.*<sup>6</sup>.

The crystal anisotropy so obtained is converted into ionic anisotropy ( $K_{\perp} - K_{\parallel}$ ), following Mookherji<sup>7</sup>. These are shown in Table 1. The temperature variation of ( $K_{\perp} - K_{\parallel}$ ) is shown in Table 2. Here,  $K_{\parallel}$  represents the ionic susceptibility along the axis of symmetry of the cluster and  $K_{\perp}$  that normal to it, expressed in c.g.s. electromagnetic units.

It is clear from Table 1 that with increase of dilution, that is, decrease of concentration of  $Ni^{++}$  ion, the anisotropy of the cluster ( $K_{\perp} - K_{\parallel}$ ) increases. This clearly demonstrates that the magnetic anisotropy is structure sensitive.

Following Mookherji<sup>7</sup>, we have calculated  $-(\alpha_{\perp} - \alpha_{\parallel})$ , the difference between the crystal field coefficients along the symmetry axis of the cluster and that normal to it from ( $K_{\perp} - K_{\parallel}$ ) values given in Table 2. It is observed

Table 1

Crystal	A	A + 3.896 B <sub>1</sub>	A + 15.14 B <sub>1</sub>	A + 15.3 B <sub>1</sub>	A + 25.22 B <sub>1</sub>
% of $Ni^{++}$ ion ( $K_{\perp} - K_{\parallel}$ ) $10^6$	100.00 186	3.24 220	0.91 243	0.89 349	0.56 355

Table 2

Temp. ° K	310	230	150	110	80
( $K_{\perp} - K_{\parallel}$ ) $10^6$	349	448	714	967	1,203
$-(\alpha_{\perp} - \alpha_{\parallel}) 10^4$	25.7	23.5	21.8	19.5	19.9

that unlike most of the concentrated nickel Tutton salts  $-(\alpha_1 - \alpha_1)$  decreases with fall of temperature, bringing out prominently the temperature sensitiveness of the field coefficients and thus supporting the findings of Boese *et al.*<sup>2</sup>

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### Light Scattering by the Relativistic (Non-linear) Oscillator

A SIMPLE non-linear model of the scattering particle is the relativistic oscillator (without damping), the approximate equation of motion of which<sup>1</sup> is:

$$\ddot{x} + \omega_0^2 x - \epsilon x \dot{x}^2 = (F/m_0) \cos \omega_1 t \quad (1)$$

Here  $\epsilon = (3/2) \omega_0^2/c^2$ ,  $\omega_0$  being the linear frequency and  $m_0$  the rest mass. Assuming  $F$  of the form  $\epsilon F_0$ , a solution is  $x = A \cos \omega_1 t$ , where:

$$A = \frac{F_0/m_0}{\omega_0^2 - \omega_1^2 - \frac{\epsilon \omega_1^2}{4} A^2} \quad (1.1)$$

Setting  $F_0 = eE_0$ ,  $e$  being electric charge and  $E_0$  the field strength, the electric dipole moment  $p = ex$ , the average rate of emission  $d\bar{U}/dt = \frac{\omega^4 p^2}{30c^2}$ , and the intensity  $\bar{I} =$

$(c/8\pi)E_0^2$ , we find for the ratio of incident to emitted intensity (for  $N$  oscillators):

$$\frac{\bar{I}}{\bar{I}_0} \approx \frac{8\pi N e^4}{3m_0^2 c^4} \frac{1}{\omega_1^4 \left[ 1 - \frac{3\omega_1^2 A^2}{8c^2} \right]} \quad (2)$$

For Thomson scattering,  $\omega_0 \ll \omega_1$ :

$$\bar{I}/\bar{I}_0 \approx \frac{8\pi N e^4}{3m_0^2 c^4} \quad (2.1)$$

the cross-section remains constant as in the linear case.

For Rayleigh scattering,  $\omega_0 > \omega_1$ :

$$\bar{I}/\bar{I}_0 \approx \frac{8\pi N e^4}{3m_0^2 c^4 \omega_0^4} \cdot \frac{\omega_1^4}{\left[ 1 - \frac{3\omega_1^2 A^2}{8c^2} \right]} \quad (2.2)$$

The Rayleigh scattering cross-section decreases with the amplitude of the incident radiation of frequency  $\omega_1$ .

Using a ruby-laser beam, the Rayleigh scattering cross-section has been found<sup>2</sup> to be smaller than the classical value by a factor of  $10^3$ . With the energy flux of  $8 \times 10^{12}$  ergs/cm<sup>2</sup>/sec as a typical value, the observed reduction in cross-section is of the same order of magnitude as predicted by the formula.

**Notes added in proof.** Formulae similar to (2.2) may be derived also for the types of oscillator for which the non-linear term is proportional to  $x^3$  or to  $x^5$ . However, a numerical estimate depends on the value assumed for the factor of proportionality. Thus, the reduction in Rayleigh cross-section appears to express the amplitude-frequency relationship that characterizes any non-linear oscillator.

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### METALLURGY

#### A Metastable Intermediate Phase in the System Indium-Indium Antimony

Using a technique for the ultra-fast quenching of thin metal foils from the melt by solid conduction (splat cooling)<sup>1,2</sup>, we have recently shown<sup>3</sup> that in many tin alloy systems a metastable phase of the 'HgSn<sub>1-1.5</sub>' type<sup>4</sup> ( $\gamma$ -phase, simple hexagonal, 1 atom/cell,  $a = 3.212$  Å;  $c/a = 0.9312$ ) can be obtained. This phase exists in equilibrium in the systems Sn-In, Sn-Od and Sn-Hg, and could be retained in non-equilibrium at  $-190^\circ$  C in the systems Sn + Pb, Ga, Zn, Cu, Ag, Au, Pd, Ca and Mg, at solute additions of 5-20 atom per cent, depending on the system. All these elements, in leading to the formation of  $\gamma$ , acted to reduce the valence electron concentration of tin to less than 4.0.

InSb resembles Sn in some crystal-chemical respects: identical tetrahedral bonding in  $\alpha$ -Sn (A4) and InSb (B3); existence of a metallic high-pressure modification of InSb with the  $\beta$ -Sn structure<sup>5</sup>; and identical position in the periodic table. This made it likely that a metallic phase of the  $\gamma$ -type could also be retained from a melt of InSb with a suitable addition element, if the formation of equilibrium InSb could be partly suppressed.

The eutectic system In-InSb was selected. Alloys of In + 10, 15, 20, 30 and 40 atomic per cent Sb were rapidly quenched on to Cu or Ag substrates and investigated by X-ray diffraction at  $-190^\circ$  C and  $20^\circ$  C, as described in detail in ref. 3. Especially in the alloy In<sub>75</sub>Sb<sub>25</sub>, but in lesser degree also in In<sub>65</sub>Sb<sub>35</sub> and In<sub>55</sub>Sb<sub>45</sub>, twelve additional diffraction peaks were found. These lines could be indexed with a hexagonal unit cell of the  $\gamma$ -type ( $a = 3.205 \pm 0.003$  Å;  $c = 2.981 \pm 0.006$  Å;  $c/a = 0.930 \pm 0.003$  at  $-190^\circ$  C) and correspond closely in position and intensity to the powder pattern given by Sreeraton and Ferguson<sup>6</sup> for the equilibrium  $\gamma$ -phase in In-Sn. From the relative intensities of In,  $\gamma$  and InSb lines, it was estimated that with the present cooling rates ( $10^4$ - $10^7$  °C/sec) (ref. 2) about 50 per cent of the alloy In<sub>75</sub>Sb<sub>25</sub> consisted of  $\gamma$  and that the composition of  $\gamma$  is In<sub>75</sub>Sb<sub>25</sub> with  $0.35 < x < 0.45$ . The lines observed are the only lines expected and present in the investigated  $2\theta$  range from  $20^\circ$  to  $100^\circ$  (copper K $\alpha$ ); higher angle lines are not sharp enough due to transformation and texture effects. In general, while structure determinations based on few lines are problematic, in the case of metastable alloys or high-pressure phases no other evidence is available; for the In-InSb system, the analogy of the observed diffraction lines to those of the In-Sn  $\gamma$ -phase makes the reported result certain. At room temperature, the equilibrium structure In + InSb is established. Using the  $c/a$ -ratio observed for  $\gamma$  of 0.930 and the table for valence electron concentration versus  $c/a$  values in ref. 3, a valence electron concentration of 3.80  $e/a$  is calculated for  $\gamma$ (In-InSb). This would correspond to a concentration of In<sub>65</sub>Sb<sub>35</sub> for  $\gamma$ , and would thus place  $\gamma$ (In-InSb) at approximately the same valence electron concentration as the corresponding equilibrium phase  $\gamma$ (In-Sn). The observation that a maximum amount of  $\gamma$  is obtained at In<sub>75</sub>Sb<sub>25</sub> rather than at In<sub>65</sub>Sb<sub>35</sub> can perhaps be explained on the assumption that an excess of In is necessary to prevent the formation of tetrahedrally bonded InSb nuclei on solidification, which would favour the equilibrium phases.

The formation of a second orthorhombic high-pressure phase of InSb<sup>7</sup>, in addition to the  $\beta$ -Sn type modification, with a unit cell closely related to the new  $\gamma$ -phase (distortion of the orthohexagonal basal plane of  $\gamma$  with  $b/a = \sqrt{3}$  into an orthogonal basal plane with  $b/a \neq \sqrt{3}$ ) permits the speculation that in this high-pressure phase, just as in the new  $\gamma$ -phase, the effective valence electron concentration may be less than 4.

There are also indications of the existence of a further metastable phase at the composition In<sub>35</sub>Sb<sub>65</sub> (valence

electron concentration = 4.5) in analogy with the stable phase SnSb (trigonally distorted B1); the atom sites in the new phase similarly form a rhombohedral primitive lattice. These results will be reported elsewhere.

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## CHEMISTRY

### Purification of Sodium Tungstate

SODIUM tungstate is used to provide charge compensation in neodymium-doped single crystals of calcium tungstate (scheelite) which are widely used as lasers<sup>1</sup>. These crystals are generally grown by the Czochralski technique from melts which can contain up to 10 wt. per cent sodium. It is therefore desirable to remove impurities from the sodium tungstate which could lead to the formation of light-scattering centres in laser crystals incorporating this material<sup>2</sup>. In this communication, a zone-refining method of purifying the readily available reagent grade of sodium tungstate is described.

The source material was 'AnalaR' grade material which corresponds to the composition Na<sub>2</sub>WO<sub>4</sub>·2H<sub>2</sub>O. The water of crystallization was removed by heating this material in a vitreous carbon boat at a temperature of 400° C. The charge was afterwards compacted by slowly melting at a temperature of 700° C. Solidification at this stage yields a white opaque ingot. However, the passage of two molten zones, approximately 2.5 cm wide, along a 15-cm ingot produces transparent material for three-quarters of the ingot length, provided the movement rate of the zone does not exceed 1.5 cm h<sup>-1</sup>. An atmosphere of argon, flowing at a rate of 200 c.c. min<sup>-1</sup>, was used both for the compaction and for the zone-refining. Under these conditions the carbon remains chemically unattacked. The material produced in this manner is polycrystalline as expected for a substance which exhibits trimorphism<sup>3</sup>.

Samples of the transparent sodium tungstate, the impure end of an ingot and the source material have been analysed in an 'M.S. 7' mass spectrometer. The results of the analysis (Table 1) show that all the impurity elements detected, except molybdenum, have been reduced in concentration by the zone-refining process.

Table 1

Element	Concentration (p.p.m. atoms)		
	Source material	Impure end	Pure end
Mo	1,500	1,500	1,500
As	90	230	6
Ca	165	100	20
K	250	150	10
Cl	170	240	60
P	35	170	Not detected
Si	150	230	90
B	5	15	1

The reduction in the concentration of chlorine and silicon is particularly important since these elements are known to cause scatter in calcium tungstate single crystals<sup>4</sup>. The high molybdenum concentration is

unlikely to be detrimental, as sodium tungstate and sodium molybdate are isomorphous compounds which may both be used for charge compensation in scheelite structures.

We thank Mr. C. M. Wilson (A.E.I., Rugby) for his assistance with the analysis.

### Magnetic Properties of Some N : N'-bis-Salicylidene-ethylenediamine Iron (III) Complexes

THE magnetic properties of some binuclear complexes of chromium (III) and iron (III) in which magnetic interaction was considered to occur through oxygen bridging groups were reported previously<sup>1</sup>. In the cases discussed, it was not possible to fit the magnetic data very satisfactorily to a model for spin interaction between the metal ions. We report here a complex of iron (III) with N : N'-bis-salicylidene-ethylenediamine in which we have been able to establish a binuclear formulation and account for the magnetic properties satisfactorily in terms of spin interaction between the two metal ions.

The complex N : N'-bis-salicylidene-ethylenediamine iron (III)-μ-oxy-N : N'-bis-salicylidene-ethylenediamine iron (III) was first reported by Pfeiffer<sup>2</sup>. In agreement with this formulation, the complex is a non-conductor in nitrobenzene and nitro-methane and the molecular weight in chloroform and nitrobenzene corresponds to the binuclear complex (C<sub>18</sub>H<sub>14</sub>O<sub>4</sub>N<sub>2</sub>Fe)<sub>2</sub>O. The magnetic moment at 300° K was found to be 1.87 B.M., being appreciably lower than the value of 2.4 B.M. reported previously by Klemm and Raddatz<sup>3</sup> and very dependent on temperature, falling to 0.60 B.M. at 80° K. Considerable care was taken to ensure that the susceptibility was independent of field strength.

The characteristics of the magnetic behaviour may be satisfactorily accounted for using the Hamiltonian  $H = -2JS_1 \cdot S_2$ , where  $J$  is the exchange interaction between the two ions and  $S_1$ ,  $S_2$  are the spin vectors for the two ions. For  $S = 5/2$ , the susceptibility  $\chi_A$  is given by:

$$\chi_A = \frac{3K}{T} \frac{[55 + 30 \exp(10x) + 14 \exp(18x) + 5 \exp(24x) + \exp(28x)]}{[11 + 9 \exp(10x) + 7 \exp(18x) + 5 \exp(24x) + 3 \exp(28x) + \exp(30x)]} + N(\alpha)$$

where  $K = g^2 N \beta^2 / 3k$ ,  $x = -J/kT$  and  $N(\alpha)$  is a temperature-independent paramagnetic term. The figure gives the calculated curve for  $J = -100$  cm<sup>-1</sup>,  $g = 2.07$  and  $N(\alpha) = 0$ ; the values of  $g$  and  $N(\alpha)$  are of the order anticipated for an iron (III) complex with a <sup>6</sup>S ground-state. With the exception of binuclear copper (II) complexes, this is the first example of a binuclear complex with strong spin-spin interaction for which a satisfactory interpretation of the magnetic behaviour can be given.

We have also investigated the magnetic properties of the corresponding iron (III) bromide and chloride complexes over a temperature range. At room temperature the magnetic moments of the bromide and chloride are 5.41 and 5.36 B.M. respectively. These values are lower than the spin-free value of 5.92 B.M. expected for iron (III), which suggests the possibility of magnetic exchange interactions. The crystal structure of the chloride has been reported<sup>4</sup> to consist of layers of N : N'-bis-salicylidene-ethylenediamine iron (III) groups separated by 3.4 Å. However, in adjacent layers, the iron atoms are

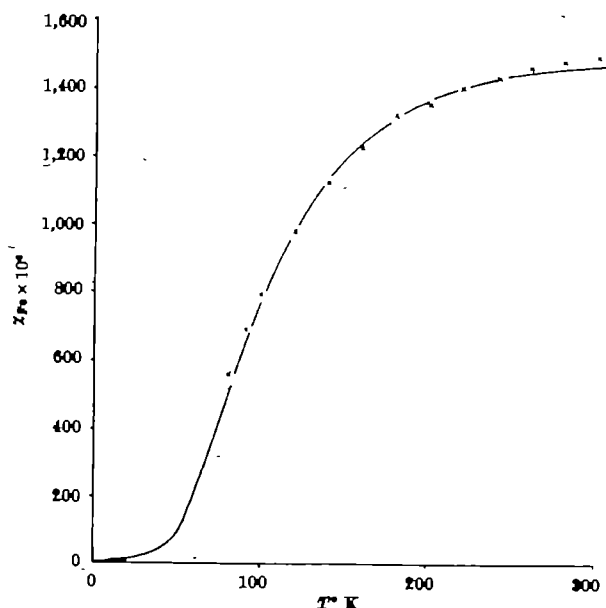


Fig. 1. —, Theoretical results; x, experimental results

laterally displaced by about 2 Å. This structure indicates that any exchange which takes place will do so between iron atoms in chains throughout the crystal. Using this model, an approximate fit for the experimental results of the chloride and bromide has been obtained, using the parameters summarized in Table 1. However, using the binuclear model we have also obtained good fits. The parameters required are also summarized in Table 1.

Table 1

Complex	$J \text{ cm}^{-2}$	$\beta$	$N \times 10^4$	Structure assumed for curve fitting
$[\text{C}_{12}\text{H}_{14}\text{O}_2\text{N}_2\text{Fe}]_2\text{O}$	-100	2.07	0	Binuclear
$[\text{C}_{12}\text{H}_{14}\text{O}_2\text{N}_2\text{Fe}]_2\text{Cl}$	-8.0	2.06	0	Binuclear
	-7.0	2.10	0	Long linear chain
$[\text{C}_{12}\text{H}_{14}\text{O}_2\text{N}_2\text{Fe}]_2\text{Br}$	-7.5	2.06	0	Binuclear
	-7.0	2.10	0	Long linear chain

In chloroform solution, the bromide and chloride complexes are monomeric and in nitro-methane they are non-conducting. It has not been possible to determine the magnetic susceptibility of any of the complexes in solution due to their limited solubility.

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### Use of Phosphoric and Sulphuric Acids in Hydrocarbon Electrode Investigations

DURING an examination of hydrocarbon electrodes, evidence was obtained which confirms that analytical reagent grade phosphoric and sulphuric acids contain an oxidizable impurity. The cell and procedures used will be described elsewhere<sup>1</sup>. In Fig. 1 is shown a plot of potential versus log apparent current density for platinized platinum immersed in nitrogen-stirred unpre-electrolysed 5M  $\text{H}_2\text{PO}_4$  at 80°C (inverted triangles). Prior to these measurements the system had not been in contact with a hydrocarbon or any other oxidizable material. The slope of the linear part of the curve is 143 mV. After an anodic pre-electrolysis (60 h at an anodic current density of 62 mA/cm<sup>2</sup>), the Tafel behaviour was no longer observed with nitrogen stirring, and a limiting current was reached (triangles, Fig. 1). When the purified acid was stirred

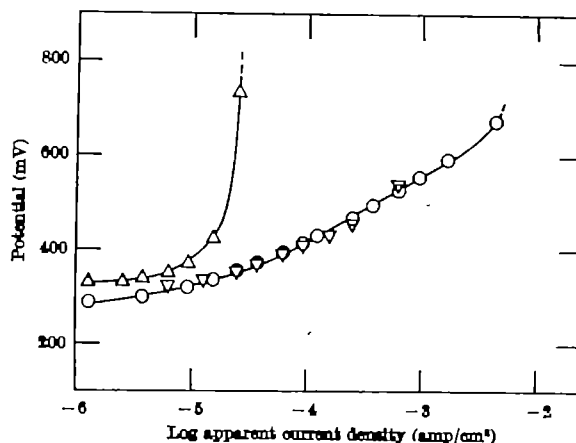


Fig. 1. Current-potential relations for electrodeposited platinum black in 5M  $\text{H}_2\text{PO}_4$  at 80°C. V, nitrogen, unpre-electrolysed acid;  $\Delta$ , nitrogen, anodically pre-electrolysed acid; O, ethylene, anodically pre-electrolysed acid

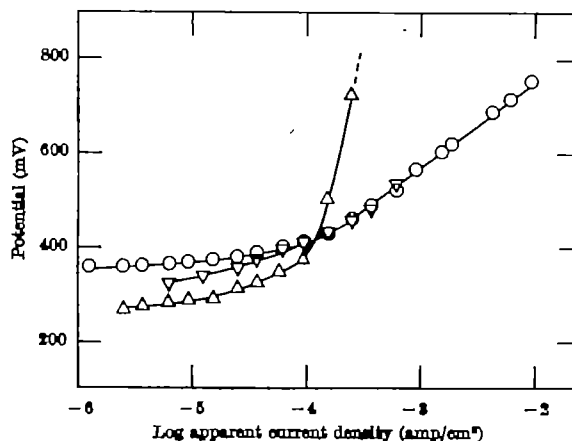


Fig. 2. Current-potential relations for electrodeposited platinum black in 3N  $\text{H}_2\text{SO}_4$  at 80°C. V, nitrogen, unpre-electrolysed acid;  $\Delta$ , nitrogen, anodically pre-electrolysed acid; O, ethylene, anodically pre-electrolysed acid

with ethylene at 80°C, the polarization curve obtained (circles, Fig. 1) was identical with that obtained with nitrogen stirring of the unpre-electrolysed acid. Similar data obtained with 3N  $\text{H}_2\text{SO}_4$  are shown in Fig. 2. The slope of the linear parts of the curves in this case is 183 mV.

The Tafel behaviour exhibited by the nitrogen-stirred unpre-electrolysed solutions, which is no longer observed after purification, strongly suggests the presence of an oxidizable impurity. The higher limiting current in 3N  $\text{H}_2\text{SO}_4$  than in 5M  $\text{H}_2\text{PO}_4$  after purification indicates that pre-electrolysis is not so effective in removing the impurity from the former. It is interesting to note that the impurity gives the same Tafel slope as ethylene<sup>3</sup> and other unsaturated hydrocarbons<sup>4</sup>. Another interesting feature is that the polarization curves for ethylene and propylene do not appear to be affected by the impurity. In the unpre-electrolysed solutions, however, the electrode reaction probably involves the simultaneous oxidation of the hydrocarbon and the impurity.

An unresolved problem concerning hydrocarbon electrode reactions is the mechanism responsible for the potentials observed at rest. Since the thermodynamically reversible values are not obtained<sup>3,4</sup>, it would seem that a complex process is involved. Four interpretations of these potentials have been suggested<sup>5</sup>; but none of them is entirely satisfactory. An interesting feature is that the potentials are only slightly different from those obtained with nitrogen before the ingress of hydrocarbon<sup>3,4</sup>. This observation was also made in the present work. Since a platinized platinum electrode, which is exhibiting an 'oxide' potential, can be depolarized by oxidizable sub-



stances such as methanol, formaldehyde, and formic acid<sup>6</sup>, the possibility that the oxidizable impurity contained in unpurified 5M  $H_3PO_4$  and 3N  $H_2SO_4$  plays a part in the establishment of the 'nitrogen potential' was investigated.

It was found that the 'nitrogen potential' initially at 80°C in unpre-electrolysed 5M  $H_3PO_4$  was  $\sim 1,000$  mV and in 2-4 h, the potential dropped to 300-400 mV. When the 5M  $H_3PO_4$  had been anodically pre-electrolysed as described, the potential dropped from 1,000 to  $\sim 850$  mV in approximately 2 h, and in four days it dropped to about 780 mV. After an additional seven days, it dropped to 300-600 mV. With both unpre-electrolysed and pre-electrolysed 3N  $H_2SO_4$ , the 'nitrogen potential' fell from an initial value of  $\sim 950$  to  $\sim 300$  mV in approximately 2h.

When a platinized platinum electrode that has been exposed to air is immersed in an acidic electrolyte, the high potential observed initially (1,060 mV) is probably a mixed potential, arising from the interaction of the 4-electron  $O_2/H_2O$  reaction and the Pt/Pt-O reaction<sup>7</sup>. In a nitrogen atmosphere, which would sweep out any entrained oxygen, the potential drops to about 850 mV, the potential of the Pt/Pt-O reaction<sup>7</sup>. Hoare showed<sup>8</sup>, using bright platinum in 2N  $H_2SO_4$  at 25°C, that the potential fell from about 880 to 770 mV in 25 h, and Schuldiner and Roe<sup>9</sup> showed that in a system in which rigorous steps had been taken to exclude oxygen, a potential of about 400 mV was obtained with helium stirring after several hundred hours. The steady drop in potential below 880 mV has been attributed to the instability of Pt-O in the absence of oxygen which chemically decomposes or dissolves in the platinum<sup>8,9</sup>. The lower potentials observed are considered to be dipole potentials<sup>10</sup>.

The results obtained here, particularly with phosphoric acid, suggest that the more rapid decline of the 'nitrogen potential' in the unpurified acid might be the result of the reduction of Pt-O by the impurity, as the removal of the impurity leads to a much lower rate of decline. In sulphuric acid, the potential decay was equally rapid before and after purification. This might mean that the impurity is not completely removed by anodic pre-electrolysis, or that the Pt-O is much more unstable in sulphuric acid than in phosphoric acid at the higher temperature. Some evidence for the first point is indicated by the higher limiting current in the pre-electrolysed sulphuric acid with nitrogen stirring than in the pre-electrolysed phosphoric acid.

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## BIOCHEMISTRY

### Presence of Maltodextrins in Potato Phosphorylase Preparations

In the presence of excess  $\alpha$ -D-glucose-1-phosphate ( $\alpha$ -D-G-1-P) potato phosphorylase catalyses the *in vitro* synthesis of amylose-like polysaccharides by 1:4 linking of glucose residues to the non-reducing ends of maltodextrin primer molecules, either added or present as

impurities in the reactants. Maltotriose is the smallest molecule which will act as a primer for the synthesis<sup>1</sup>. The action pattern of the enzyme has been shown to be of the 'multichain' type over a wide range of conditions<sup>1</sup>.

The isolation procedure described by Baum and Gilbert<sup>2</sup>, involving specific adsorption of the enzyme on to retro-graded amylose, was used to prepare phosphorylase. Traces of hydrolytic enzymes, principally  $\alpha$ -amylase, were removed by heating the phosphorylase preparation at 53.5°-54° C for 10 min<sup>3,4</sup>; their removal was confirmed by incubating the phosphorylase with a solution of natural amylose at 20.0° C at pH 7.0. None of the phosphorylase preparations examined produced a fall in the intrinsic viscosity of the amylose over a period of 24 h. Purified phosphorylase gave a single symmetrical peak in sedimentation velocity experiments (Fig. 1) and a single line in Ouchterlony agar double diffusion experiments using rabbit anti-potato proteins antiserum. The enzyme activity of the preparations was measured in terms of the Green and Stumpf unit<sup>5</sup>. Protein concentrations were determined on concentrated samples of the enzyme either colorimetrically by Folin's method or by optical density measurements at 280 m $\mu$ . The preparation had a specific activity of about 12-13 Green and Stumpf units/mg protein.

Incubation of the enzyme with commercially available dipotassium  $\alpha$ -D-G-1-P (British Drug Houses, Ltd., Poole, Dorset) at either 20° C or 38° C at pH 7.0 resulted in the synthesis of maltosaccharides in the absence of added primer. This indicated that either the enzyme preparation or its substrate, or both, were contaminated with maltodextrins. Similar observations have been made by other workers<sup>6</sup>.

Experiments were carried out which indicated that most of the primers were present as impurities in the  $\alpha$ -D-G-1-P. However, phosphorylase was found to synthesize iodine staining maltosaccharides using chemically synthesized  $\alpha$ -D-G-1-P as the substrate; indicating that the enzyme preparation also contained primers.

The maltodextrin impurities in  $\alpha$ -D-G-1-P could be removed entirely, either by preparative paper chromatography or more simply by incubating an excess of  $\alpha$ -D-G-1-P with purified phosphorylase and growing the maltodextrins to a sufficient size to be separated from  $\alpha$ -D-G-1-P by dialysis through 'Cellophane'.

The effective primer concentration present in the enzyme preparation was determined quantitatively by comparing the rates of amylose synthesis in the presence and absence of known concentrations of chromatographically pure maltopentose under standardized conditions. The amylose synthesis was followed by removing samples

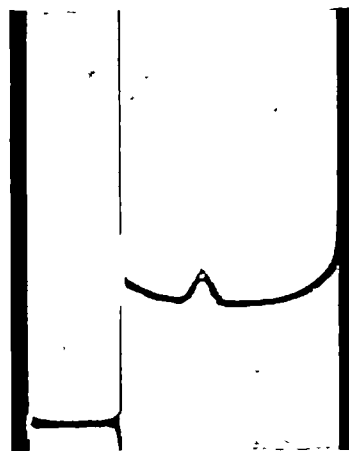


Fig. 1. Sedimentation pattern obtained from a sedimentation velocity experiment with potato phosphorylase in the Spinco model B analytical ultracentrifuge. Speed 59,780 r.p.m.; temperature 20.0° C, photograph taken 28 min after reaching set speed; phase plate angle 55°; enzyme concentration 3-4 mg/ml in 0.06 M citrate buffer, pH 7.0, containing 0.2 M NaCl.

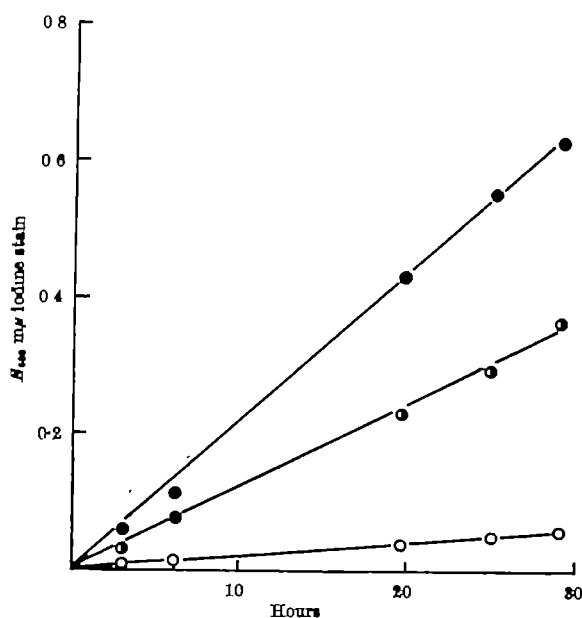


Fig. 2. Variation in rate of amylose synthesis with change in concentration of added maltopentose. Substrate: primer-free  $\alpha$ -D-G-1-P. —○—, Synthesis on enzyme primers alone. —□—, Synthesis with 1.25  $\mu$ g/ml. maltopentose added. —●—, Synthesis with 2.5  $\mu$ g/ml. maltopentose added.

from the reaction mixtures at suitable intervals of time and staining these with iodine-potassium iodide solution under standard conditions. The extinction of the stained solution was measured in a Hilger Spekker absorptiometer at 680 m $\mu$  (No. 8 filter) and this provided a measure of the amylose synthesized. The rate of amylose synthesis was shown to be proportional to the added primer concentration (Fig. 2) and thus the primer content of the enzyme preparation could be calculated in terms of maltopentose.

At first it seemed probable that the primers in the enzyme preparation were soluble maltodextrins introduced during the preparative procedure. However, it was found that all the enzyme preparations examined had primer activity of the order of 0.45  $\mu$ g maltopentose per Green and Stumpf unit of enzyme.

Several methods were used in an attempt to remove the enzyme primers. These methods were: (a) dialysis of the enzyme to remove small, non-protein molecules; (b) incubation of the enzyme in 0.2 M phosphate buffer at 38° C at pH 6.5 to digest the primers, followed by dialysis; (c) ultracentrifugation of the preparation to sediment the enzyme, leaving smaller molecules in solution; (d) treatment of the enzyme with purified  $\beta$ -amylase to degrade maltosaccharide impurities. The first three methods had no significant effect on the primer: enzyme ratio. Husemann<sup>3</sup> has recently reported the removal of enzyme primers by dialysis; but it is probable that these primers were present initially greatly in excess of the critical ratio. It was impossible to test for primer activity in the presence of  $\beta$ -amylase and attempts to remove this enzyme resulted in complete loss of phosphorylase activity. The instability of the phosphorylase after  $\beta$ -amylase treatment may be connected with the removal of primer. Preliminary experiments, in which the enzyme was subjected to equilibrium dialysis against maltotetraose primers by passage through 'Sephadex G 100' columns saturated with carbon-14 labelled maltotetraose primers in phosphate buffer at pH 7.0, indicated that the bound primers could be exchanged for primers of known structure and size.

The constant ratio of primer concentration to enzyme activity suggested that primer was reversibly bound to the enzyme molecule in some way. Assuming an equimolar association, the ratio indicated a molecular weight of the

order of 180,000 for potato phosphorylase. This was in good agreement with values of the order of 200,000 calculated from ultracentrifuge sedimentation velocity measurements<sup>4,5,7</sup>.

These investigations were made as preliminary investigations into the *in vitro* use of potato phosphorylase for the preparation of synthetic amyloses with molecular weights of the order of 1,000,000 for use in ultracentrifugal investigations. High molecular weight amyloses have been synthesized and details of their properties will be published later.

Samples of chromatographically pure maltopentose and chemically synthesized barium  $\alpha$ -D-G-1-P were kindly supplied by Dr. S. A. Barker, Chemistry Department, University of Birmingham.

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### Precipitation of Salmon Sperm Deoxyribonucleic Acid with Purine-specific Antibody

DEOXYRIBONUCLEIC acids (DNA) have been found to react with antisera prepared by immunizing animals to Gram-negative bacteria<sup>1</sup>, DNA<sup>2</sup>, nucleosides<sup>3-5</sup>, nucleotides<sup>6</sup>, purines<sup>7-9</sup> and pyrimidines<sup>10</sup>. This was first demonstrated by complement fixation<sup>1,6</sup> and then by precipitin reaction<sup>2-5</sup>. The ease and degree to which these reactions occur seem to depend on the source of the DNA. Pneumococcal and *E. coli* DNA, undenatured and denatured, fix complement with pyrimidine specific antibody; but these and calf thymus DNA are reported to require heat denaturation for complement fixation with anti-purinoyl antibody, although some complement fixation was observed with undenatured pneumococcal and *E. coli* DNA<sup>6,9</sup>. For precipitation, calf thymus DNA required heat denaturation to bring down anti-calf thymus<sup>3</sup> or anti-uridine antibody<sup>3</sup> and heat denaturation in the presence of formaldehyde to precipitate with anti-purinoyl, anti-pyrimidine or anti-nucleoside antibody<sup>4</sup>. In contrast, heat-denatured chick embryo DNA precipitated both with anti-purinoyl and anti-nucleoside antibody<sup>5</sup>. The difference in reactivity between calf thymus and chick embryo DNA was accounted for on the basis of the lack of hyperchromicity for heat-denatured chick embryo DNA and formaldehyde denatured calf thymus DNA, cycled slowly through the thermal transition temperature, compared to a hyperchromicity of 19.5 per cent for heat-denatured calf thymus DNA treated similarly<sup>4</sup>.

In the course of experiments designed to detect and isolate DNA using immuno-adsorbents, we have found that salmon sperm DNA reacts with anti-purinoyl antibody to some extent without heat denaturation and to a greater extent after heat denaturation.

Antiserum was obtained from New Zealand white rabbits immunized with 6-trichloromethylpurine coupled

to California giant keyhole limpet haemocyanin<sup>8</sup>. A stock solution of highly polymerized salmon sperm DNA (California Corporation for Biochemical Research, Los Angeles) containing 1.00 mg DNA/ml. in 1 per cent saline was prepared. A portion of this was denatured by heating at 100° C for 15 min followed by rapid chilling of the solution in an ice bath. Quantitative precipitin reactions were carried out as described by Kabat and Mayer<sup>10</sup>. Protein was determined by the method of Lowry<sup>11</sup>, using rabbit  $\gamma$ -globulin as a standard.

Quantitative precipitin curves for the reaction of heat-denatured salmon sperm DNA with the purine-specific antiserum showed an equivalence zone elongated in the region of antigen excess (Fig. 1). In control experiments using DNA and normal rabbit serum non-specific precipitation was small. The antiserum was found to contain 590  $\mu$ g of anti-purinoyl antibody per ml., by precipitation with 6-trichloromethylpurine conjugated to bovine serum albumin<sup>9</sup>. Of this antibody, approximately 32 per cent precipitated with the thermally denatured salmon sperm DNA. Typical quantitative precipitin reactions were also observed with the unheated salmon sperm DNA; however, the antigen-antibody weight ratios varied somewhat (Fig. 2). Up to 17 per cent of the antibody precipitated with the untreated DNA, about half that for the heat-denatured material.

A sample of DNA from *Olostridium* sp. (Worthington Biochemical Corporation, Freehold, New Jersey) was thermally denatured and tested with similar results. Approximately 33 per cent of the anti-purinoyl antibody precipitated at equivalence.

The denatured salmon sperm DNA shows little hyperchromicity on heating above the thermal transition temperature, followed by slow cooling, and thus resembles the

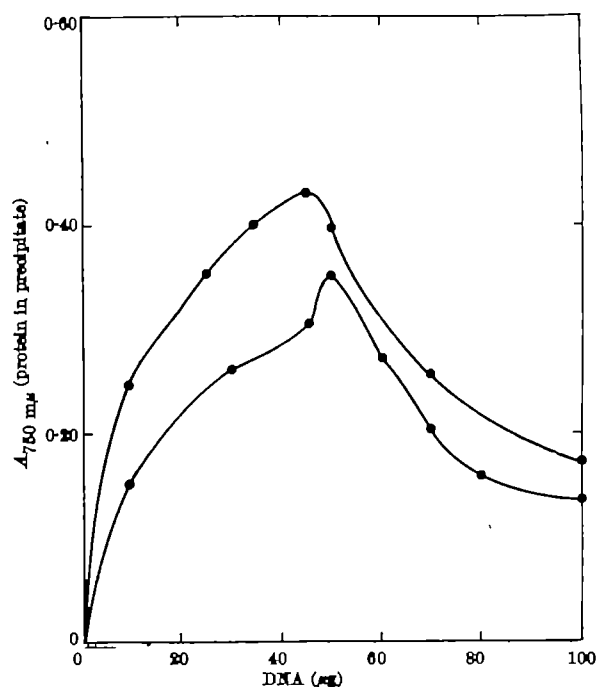


Fig. 2. Quantitative precipitin reaction curves for antiserum with unheated salmon sperm DNA

chick embryo DNA and formaldehyde denatured calf thymus DNA in thermal behaviour. It is interesting to note that Christian, DeSimone and Abruzzo<sup>1</sup> found that complement fixation for undenatured salmon sperm DNA was 22 per cent of that for the denatured DNA.

The reactivity of DNA with antibody thus seems to be dependent on the ease, degree and irreversibility of DNA denaturation. Particularly for the case of antibody to purine derivatives, the results are consistent with the DNA model wherein the bases are hydrogen bonded within the double helix and so are relatively inaccessible to antibody except at the ends or non-helical portions of the molecule. It would appear that our results are in accord with the suggestions of Butler *et al.*<sup>6</sup> that the extent of reaction to DNA with antibody to purines and pyrimidines or their derivatives may be used as a measure of the 'nativeness' of DNA.

Our observation that the incomplete precipitin reactions of salmon sperm DNA with anti-purinoyl antibody display an equivalence zone is somewhat at variance with a suggestion that DNA might react completely with this antibody if sufficient antigen were present<sup>8</sup>. The sources and treatment of the DNA, however, were different for each case. Precipitation of anti-purinoyl antibody with thermally denatured salmon sperm and *Olostridium* DNA has the characteristics of a cross-reaction between antibody and a heterologous antigen. If all the anti-purinoyl antibody could react with the DNA or even some specific fraction of the DNA, then by continued addition of antigen all the antibody would eventually be precipitated. This is not the case. As with anti-5-acetyluracil antibody<sup>8</sup>, only a portion of the antibody precipitates with the DNA. By interfacial ring test, anti-purinoyl antibody could be detected in the supernatant fluid at equivalence. Precipitation of all anti-adenosine, anti-ribosylthymine<sup>8</sup> and anti-uridine<sup>8</sup> antibodies, on the other hand, indicates that antibody to DNA components and their derivatives may react completely with DNA.

We have found that our samples of salmon sperm and *Olostridium* DNA, thermally denatured in the absence of formaldehyde, precipitated with anti-purinoyl antibody and showed only slight hyperchromicity after re-heating past the thermal transition temperature and cooling slowly, thus resembling formaldehyde denatured calf

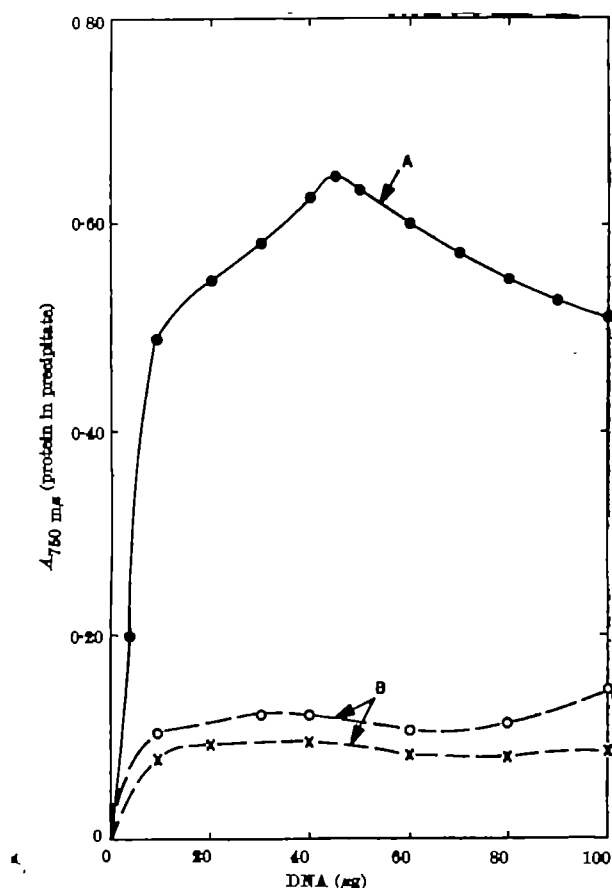


Fig. 1. A, Quantitative precipitin reaction curve for antiserum with thermally denatured salmon sperm DNA, B, quantitative precipitin reaction curve for normal rabbit serum with thermally denatured (x) and unheated (O) salmon sperm DNA (control)

thymus and chick embryo DNA<sup>8</sup> in their immunochemical reactions. Comparison of the reported base composition of salmon sperm DNA<sup>12</sup> with those of calf thymus and chick embryo DNA<sup>8</sup> disclosed little difference.

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### Serum Cholesterol Concentrations in Chicks

We were very interested in the article by De Somer *et al.*<sup>1</sup>. Their results are in good agreement with our earlier observation, where the influence of several antibiotics on the stability of fat emulsions was studied<sup>2,3</sup>. The emulsion was prepared by using a mixture of 3.0 ml. olive oil, 1.5 ml. 0.9 per cent NaCl and 1.5 ml. human duodenal juice; after 2 h of shaking the percentage of split ester bonds was estimated and the stability of emulsion was observed during the following 60 min. The addition of neomycin sulphate in concentrations (1 mg/ml., 10 mg/ml., 100 mg/ml.) corresponding to a dilution of a therapeutic dose in 10,000 and 1,000 ml. of digestive juice caused a significant decrease of lipolysis and prevented the formation of a stable emulsion. A similar effect was observed with some other antibiotics. In further experiments we observed that the addition of 30 mg neomycin sulphate to 1 ml. olive oil, administered intragastrically to rats, caused a decrease of alimentary lipaemia in the next 3 h (ref. 4).

The explanation presented by De Somer *et al.* seems very probable to us. The malabsorption syndrome following the administration of neomycin, however, cannot, in our opinion, be elucidated only by the effect of the antibiotic on the precipitation of bile acids and emulsification of fat; damage to the intestinal mucosa<sup>5</sup> and the deleterious effect on the intestinal flora should also be considered. The question arises, what will be the effect of neomycin and its derivatives on lipolysis, especially in other than biphasic systems?

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We agree with Dr. Krondl that the malabsorption syndrome following the administration of neomycin cannot be explained by precipitation of bile acids in the gut, and that disturbances in the intestinal flora, toxic effects on the mucosa and inhibition of lipase activity may play a part.

Our studies, however, were not performed in order to find an explanation for the malabsorption syndrome during neomycin therapy. The *N*-methylated derivative of neomycin described by us has lost its antibiotic properties and does not cause a malabsorption syndrome in patients taking the drug for more than 12 months at a dosage of 6 g a day. Nevertheless the compound is still an active hypocholesterolaemic agent and this effect seems to be due to an action on bile acid micelles in the gut.

We also tested the influence of neomycin, *N*-methylated neomycin and streptomycin on porcine pancreatic lipase with  $\beta$ -naphthyl stearate as a substrate in the presence of taurodeoxycholate above the critical micellar concentration (De Laey, unpublished results). In complete agreement with the studies of Krondl *et al.* we find a significant (22–48 per cent) inhibition of the lipolytic activity after addition of neomycin or *N*-methylated neomycin to the test system. However, this inhibition of lipase activity seems also to be due to an effect on the bile acid micelles because no inhibition of the residual lipolytic activity was observed when bile acids were omitted from the test system or when taurodeoxycholate was present at submicellar concentrations (0.05–0.8 mM). Furthermore, streptomycin, having only mild bile acid precipitating activity, was also significantly less active in inhibiting porcine lipase activity.

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### Hexosamine in *Bombyx mori* Silk

CONSTITUENTS other than protein in *Bombyx mori* silk have received little attention<sup>1</sup>. The significant amounts of hexosamine found in hair and wool<sup>2</sup> prompted this study to quantitate and to identify the amino-sugars in *B. mori* silk.

Raw silk was obtained from Gerli and Co., Inc., Park Ave., New York, and ground in a Wiley mill. For dry weight determinations, aliquots were dried in a vacuum drying apparatus over boiling toluene with P<sub>2</sub>O<sub>5</sub> as a desiccant. The ground silk was hydrolysed with twenty times its weight of 4 N HCl in sealed tubes overnight at 104° C. The hydrolysates were dried and purified on Dowex 50 × 8 cation exchange resin using 1 × 5 cm columns after the method of Boas<sup>3</sup> and 1 × 45 cm columns after the method of Gardell<sup>4</sup>. Hexosamine was quantitated with the Elson-Morgan reaction<sup>5</sup> using glucosamine standards. The amino-sugars were identified by chromatography of the ninhydrin degradation products from peaks derived from the long columns<sup>6</sup>.

Two Elson-Morgan reactive peaks were obtained from the Gardell columns and these were degraded with ninhydrin to arabinose and lyxose respectively. The total amounts of hexosamine derived from both the Boas and Gardell methods agreed quite well, 1.52 and 1.47  $\mu$ M/100 mg dry weight silk respectively of which slightly more than half (56 per cent—see Table 1) was glucosamine.

*B. mori* silk has significant quantities of the hexosamines, glucosamine and galactosamine present in nearly equivalent amounts.

Table 1. HEXOSAMINE IN *B. mori* SILK

Boas method Total	$\mu$ M/100 mg dry weight		Total
	Glucosamine	Galactosamine	
1.52	0.82	0.66	1.47

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### Solubility of Renal Stones

THE cause of renal stone formation is unknown. Of the many current theories, majority opinion probably favours a 'supersaturation' mechanism, in which the urine concentrations of the ions of a poorly soluble salt exceed the 'precipitation product' for that particular salt, thereby causing spontaneous nucleation and subsequent growth of salt crystals.

The chemical composition of most stones is complex, and even if one considers only the calcium phosphates found therein, it is not clear which particular stoichiometry is actually involved in the nucleation and crystallization processes leading to stone formation and growth. Although the presence of  $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$  (ref. 1),  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$  (ref. 2) and  $\text{Ca}_3(\text{HPO}_4)_2(\text{PO}_4)_4$  (ref. 3) has been claimed from the use of X-ray crystallographic techniques, and the presence of apatites and other more basic calcium phosphates suspected, we feel that identification of these compounds in renal stones may not be relevant to the actual physicochemical relationship which exists between the ions of calcium and inorganic phosphate in the stone and their concentrations in the corresponding urine. The critical feature of 'solubility' investigations is the identification of the lattice structure of the crystal surface, since it is the surface stoichiometry which determines the solubility of the solid phase in a bathing fluid, and consequently whether a stone will dissolve or grow under a given set of conditions.

Recently, MacGregor and Brown<sup>4</sup> have applied a non-prejudicial method of determining the stoichiometry of a solubility equilibrium to bone mineral, and the same technique has now been applied to renal stones.

An unselected series of renal stones obtained from a urological clinic were finely ground and equilibrated in 'Cellophane' dialysis tubing with buffers, at constant ion strength, over the pH range 7-8. The details of the equilibration technique have been published elsewhere<sup>5</sup>.

The pH and calcium and inorganic phosphate ion concentrations were determined at equilibrium, and the negative logarithm of the ion products  $[\text{Ca}^{++}][\text{OH}^-]^2$  and  $[\text{H}^+]^2[\text{PO}_4^{--}]$  calculated. The fit of  $\text{pCa}(\text{OH})_2$  ( $y$ ) against  $\text{pH}_2\text{PO}_4$  ( $x$ ) was determined by linear regression analysis, where the regression coefficient is the negative reciprocal of the Ca : P ratio of the solubility equilibrium.

The equilibration investigations with renal stones yielded a regression coefficient of  $-0.7591$  ( $S.E. \pm 0.0201$ ) which represents a Ca : P ratio of 7.90/6. The confidence limits of this ratio, using the 's'-distribution, are 7.44/6-8.42/6 (5 per cent) and 7.25/6-8.66/6 (1 per cent).

We therefore believe that these data indicate that the solid phase determining the 'solubility' of calcium phosphate stones is octo-calcium phosphate (OCP), even although it may only be present as a surface structure

and may indeed only be a small proportion of the total mineral present.

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### Stimulation of the Uptake of Water and Ions by Indolyl-3-acetic acid : Its Dependence on Nucleic Acid and Protein Synthesis

THE plant growth hormone indolyl-3-acetic acid (IAA) affects almost all aspects of growth and development, and the very remarkable promotion of growth is associated with a simultaneous stimulation of water uptake, ion uptake and several metabolic processes. We have previously presented evidence to show that the plant growth substances, IAA, gibberellic acid and kinetin, all regulate the synthesis and release of nuclear RNA and that the fraction of RNA involved has a base composition complementary to that of DNA<sup>1-4</sup>. The synthesis of nuclear protein, as well as some enzyme systems concerned with the metabolic pathways known to be affected by IAA, is also stimulated by growth promotive concentrations of IAA<sup>4,5</sup>. In this communication we report that the IAA-induced stimulation of water uptake and ion uptake also involves a nucleic acid system, that this process is inhibited by actinomycin D and that the inhibition is removed by IAA.

The materials used were disks of potato tuber 5 mm in diameter and the epidermal cells of the lower surface of the leaves of *Rhoeo discolor*, which are rich in anthocyanin and thus easy to observe under the high-power lens of a light microscope. These tissues have been used successfully by several workers in previous investigations on the effects of IAA on water uptake<sup>6</sup>. Potato disks were treated with actinomycin D, chloramphenicol, puromycin and porphyromycin 2 h after they were removed from the tuber. After a suitable incubation period IAA was added depending on experimental requirements. Thin peels of the midrib regions of *Rhoeo* were first plasmolysed in 0.5 M mannitol and deplasmolysed in water or in appropriate concentrations of the antibiotic to facilitate their uptake. This was followed by re-plasmolysis in mannitol before they were placed in IAA solution of varying concentrations. The concentrations of actinomycin D, porphyromycin, chloramphenicol and puromycin were 100  $\mu\text{g}/\text{ml}$ , 10  $\mu\text{g}/\text{ml}$ , 3.2 mg/ml, and 100  $\mu\text{g}/\text{ml}$ . Water uptake by the potato disks was determined by weighing them 22 h after incubation. Water uptake by *Rhoeo* cells was measured by noting the time taken for the cells to deplasmolyse. <sup>32</sup>P uptake was estimated by measuring the radioactivity of the dried tissue in an end-window  $\beta$ -counter. Actinomycin D, porphyromycin and puromycin were obtained through the courtesy of Dr. V. Bryson, of Rutgers University, Dr. G. Savage, of the Upjohn Co., Kalamazoo, Mich., U.S.A., and Dr. Fritz Lipmann, of the Rockefeller Institute, New York. Orthophosphate-<sup>32</sup>P was supplied by the Atomic Energy Establishment, Trombay, India.

Experiments concerning the effect of IAA on water uptake by potato disks yield a two-phase concentration curve characteristic of the effects of IAA on growth; low concentrations are promotive but higher concentrations are inhibitory. With  $10^{-4}$  M IAA water uptake is practically trebled, but when actinomycin D is also included in the incubation mixture the IAA-induced stimulation is cut down by about 50 per cent. Higher concentrations

of IAA further remove the inhibitory effect of actinomycin D and the curve continues to rise (Fig. 1). Similar results were obtained with the epidermal cells of *Rhoso*, where IAA effects on water uptake are discernible within a few minutes of application (Fig. 2).

Actinomycin D also inhibits the uptake of orthophosphate- $^{32}\text{P}$  by potato disks and here also the inhibition can

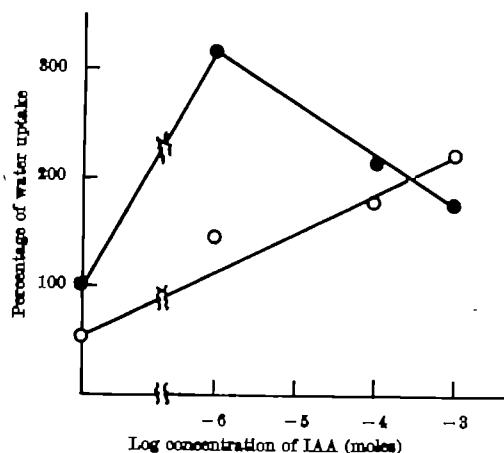


Fig. 1. Effect of actinomycin D on IAA-induced water uptake by potato disks. Potato disks treated with actinomycin D for 1 h followed by the addition of IAA. Incubation was for 22 h in darkness at 25° C

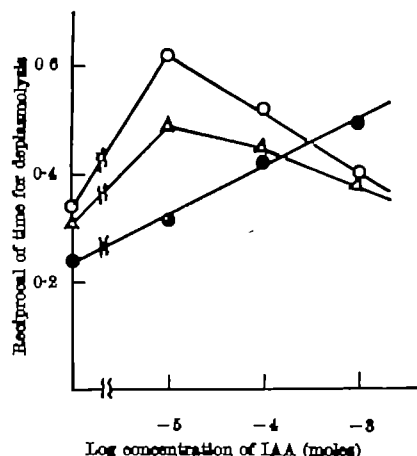


Fig. 2. Effect of actinomycin D and porphyromycin on IAA-induced water uptake by plasmolysed *Rhoso* cells. (*Rhoso* cells were treated with actinomycin or porphyromycin for 45 min, then solid mannitol was added to provide a concentration of 0.5 M. The plasmolysed cells were transferred to IAA solutions and the time taken for deplasmolysis noted)

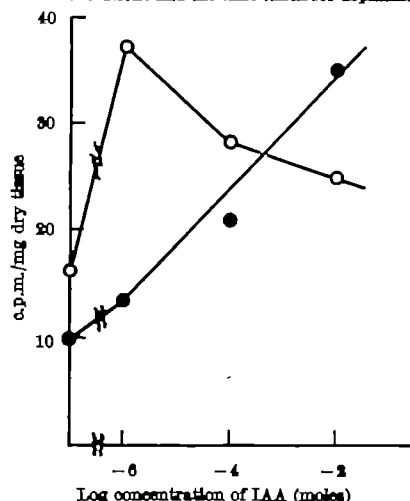


Fig. 3. Effect of actinomycin D on  $^{32}\text{P}$  uptake by potato disks. Potato disks were treated with actinomycin D in phosphate buffer of pH 6.0 for 1 h followed by the addition of  $3.5 \times 10^4$  c.p.m./ml. orthophosphate- $^{32}\text{P}$  and IAA. Incubation was for 3 h in darkness at 25° C

Table 1. EFFECT OF IAA AND CHLORAMPHENICOL ON WATER UPTAKE BY POTATO DISKS AND *Rhoso* EPIDERMAL CELLS

Treatment	Potato disks % Water uptake	<i>Rhoso</i> Reciprocal of time taken (min) for deplasmolysis
Control	100	0.38
IAA $10^{-4}$ M	152	0.52
IAA $10^{-3}$ M	100	0.43
Chloramphenicol $10^{-4}$ M	51	0.25
Chloramphenicol $10^{-4}$ M + IAA $10^{-4}$ M	97	0.34
Chloramphenicol $10^{-4}$ M + IAA $10^{-3}$ M	93	0.30

be considerably lessened by increasing concentrations of IAA (Fig. 3).

The IAA-induced stimulation of water and ion uptake thus seems to be related to DNA-dependent RNA synthesis. Porphyromycin, which inhibits nucleic acid synthesis through its effect on purine metabolism<sup>7</sup>, retards the stimulation of water uptake by IAA, but the inhibitory effect is not reversed by higher concentrations of IAA (Fig. 2). Chloramphenicol, which inhibits protein synthesis, also inhibits IAA-induced water uptake by potato disks and epidermal cells of *Rhoso*, but here also the inhibitory effect of chloramphenicol cannot be removed by IAA (Table 1). Similar results have been obtained with another inhibitor of protein synthesis, puromycin, for both water and ion uptake. The dimethylphenol inhibition of nucleotide synthesis as indicated by phosphate esterification is only partially countered by IAA, and the inhibitory effect is never completely reversed<sup>8</sup>. It has been suggested that actinomycin D binds with guanine of DNA<sup>9</sup>.

Noodén and Thimann<sup>10</sup> have reported recently that actinomycin D at a concentration of  $10^{-6}$  M brings about a 50 per cent inhibition of IAA-induced growth. We have also evidence to show that the actinomycin D inhibition of the synthesis of nuclear RNA and protein is removed by higher concentrations of IAA. The inhibition of water and ion uptake by actinomycin D is presumably due to the inhibition of the synthesis of the enzyme system and carriers required for these processes, which in turn is regulated by specific RNAs produced on a DNA template. The evidence presented here lends further support to the contention<sup>11,12</sup> that plant growth substance action is concerned, in a large measure at least, with the regulation of RNA and protein synthesis. It is of considerable interest to mention in this connexion that, as in the case of plant hormones, the steroid hormones also stimulate the synthesis of *m*-RNA, some specific proteins and ion uptake<sup>11,12</sup>.

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### Influence of Alterations in Intracellular Levels of Amino-acids on Protein-synthesizing Activity of Isolated Ribosomes

EVIDENCE has been presented which strongly suggests that the rate of protein synthesis *in vivo*<sup>1,2</sup> and *in vitro*<sup>3,4</sup> may be very dependent on intracellular concentrations of amino-acids. Of particular significance to the present

investigations was the finding that protein turn-over in surviving liver tissue was stimulated in the presence of elevated levels of a single essential amino-acid<sup>1,4</sup>. The catabolic as well as the anabolic phase of protein turn-over appeared to be augmented in these circumstances. Interpretation of the results obtained was partially obscured by the fact that intracellular compartmentation of amino-acids may exist, with the result that exogenous and endogenous amino-acids are differentially metabolized<sup>5,6</sup>. The experiments recorded here were performed in an attempt to provide definitive data on the influence of cellular levels of amino-acids on the activity of protein-synthesizing systems. The results revealed that the amino-acid incorporating activity of ribosomes isolated from liver slices incubated with a single essential amino-acid varied with the concentration of that amino-acid.

Hepatic tissue was obtained from adult male rats (160–180 g) of an inbred Sprague-Dawley strain. The animals were killed by bleeding under light 'Nembutal' (sodium pentobarbital) anaesthesia<sup>7</sup>. Liver slices were incubated in Krebs-Ringer bicarbonate buffer containing varying amounts of a single essential amino-acid. Incubation was carried out for 15 min at 37° in a Dubnoff metabolic shaker under a gas phase of 95 per cent oxygen–5 per cent carbon dioxide. After incubation, tissue and medium were separated by filtration through fine gauze. The tissue was washed once with cold *tris*-buffer solution composed of 0.25 M sucrose, 5 mM MgCl<sub>2</sub>, 80 mM KCl, and 50 mM *tris*-hydrochloride buffer, pH 7.4 (medium A).

Ribosomes were isolated from liver tissue by a modification of the methods described by Zomzely, Roberts and Rapaport<sup>8</sup>, and by Kerner<sup>9</sup>. All procedures were carried out at 0°–4°. Incubated slices or freshly minced samples of liver were homogenized in medium A (3 ml./g tissue). The homogenates were centrifuged at 10,000*g* for 10 min and the pellet discarded. The supernatant was treated with one-ninth its volume of 10 per cent sodium deoxycholate in 50 mM *tris*-hydrochloride buffer, pH 8.2, and centrifuged at 105,000*g* for 2 h. The pellet was then rinsed 3 times, re-suspended by gentle homogenization in medium A, and re-centrifuged at 105,000*g* for 1.5 h. Finally, the pellet was again rinsed 3 times with medium A and stored overnight under medium A at –60°. Amino-acid incorporating activity of 'control' ribosomes from non-incubated liver tissue was not diminished even after several weeks of storage.

Preparations of pH 5 enzymes<sup>10</sup> were obtained from the incubated liver slices and media independently, and from freshly minced liver. Tissues were homogenized in medium A. These homogenates and the incubation media were centrifuged at 10,000*g* for 10 min and the pellets were discarded. The supernatants were re-centrifuged at 105,000*g* for 2 h. The resulting supernatants were then acidified to pH 5.0 with 1 N acetic acid and centrifuged at 15,000*g* for 10 min. The precipitates were suspended in *tris*-buffer medium containing 0.25 M sucrose, 4 mM MgCl<sub>2</sub>, 25 mM KCl and 50 mM *tris*-hydrochloride buffer, pH 7.4. Finally, centrifugation of these suspensions at 300*g* for 10 min yielded supernatant fractions containing the pH 5 enzymes. These solutions were stored overnight at –60°. Activity of 'control' pH 5 enzymes from non-incubated liver tissue was unaffected by storage for several weeks.

Ribosomes and pH 5 enzymes were assayed for activity in amino-acid incorporating systems containing 0.5–1.0 mg of ribosomal protein, an equal amount of pH 5 enzyme protein<sup>8</sup>, 0.5  $\mu$ Ci uniformly labelled L-(<sup>14</sup>C)-amino-acid, 5 mM ATP, and 0.25 mM GTP in 1 ml. of medium A. After incubation for 30 min in air at 37°, the reaction was stopped by adding cold medium A containing 0.1 per cent non-labelled amino-acid. Ribosomes were recovered from the incubation mixture by centrifugation at 105,000*g* for 1.5 h. The pellet was homogenized in medium A

and treated with trichloroacetic acid (final concentration = 5 per cent). The precipitated protein was purified of free amino-acids, RNA, and lipid<sup>4</sup>, then homogenized in 70 per cent ethanol. Aliquots of this suspension were removed for determination of protein<sup>11</sup> and radioactivity<sup>12</sup>.

Prior incubation of liver slices in the presence of varying amounts of one essential amino-acid resulted in marked alterations in the amino-acid incorporating activity of ribosomes afterwards isolated from these tissues (Table 1). Thus, when the initial extracellular concentration of phenylalanine was increased from 0 to 73  $\mu$ M, incorporation of L-(<sup>14</sup>C)-leucine into protein of isolated ribosomes was elevated approximately 40 per cent. In another experiment (not shown) a further increase was noted at the highest level of phenylalanine (155  $\mu$ M). Similarly, incorporation of L-(<sup>14</sup>C)-leucine or L-(<sup>14</sup>C)-phenylalanine into ribosomal protein was enhanced when the initial extracellular concentration of threonine during prior incubation of liver slices was raised from 0 to normal plasma (228  $\mu$ M) or liver (500  $\mu$ M) levels.

Activity of the pH 5 enzymes remaining in the tissue was also altered by prior incubation of hepatic slices in media containing different concentrations of a single essential amino-acid (Table 2). Thus, incorporation of L-(<sup>14</sup>C)-leucine into protein of 'control' ribosomes was markedly increased when the pH 5 enzymes were recovered from liver slices incubated with added phenylalanine as compared with incubations in the absence of this amino-acid. Similarly, prior incubation of liver tissue in the presence of elevated levels of threonine yielded pH 5 enzymes with increased activity. No differences in activity were observed for pH 5 enzymes recovered from the medium. However, total recovery of pH 5 enzyme protein (medium plus tissue) appeared to be significantly increased when incubation was carried out in the presence of an essential amino-acid.

These and related<sup>1–4</sup> observations reveal that protein synthesis in mammalian cells is acutely responsive to variations in intracellular levels of amino-acids induced by primary alterations in the extracellular concentration of a single essential amino-acid. Thus, within 15 min after exposure of hepatic cells *in vitro* to elevated levels of one amino-acid, ribosomes and pH 5 enzyme preparations isolated from these cells exhibited increased activity in protein synthesis. The precise mechanisms underlying

Table 1. EFFECT OF INITIAL EXTRACELLULAR LEVEL OF ONE ESSENTIAL AMINO-ACID ON PROTEIN-SYNTHESIZING ACTIVITY OF LIVER RIBOSOMES

Incubation conditions for liver slices		Incubation conditions for amino-acid incorporation		Incorporation	
Amino-acid	Concentration ( $\mu$ M)	Amino-acid	Concentration ( $\mu$ M)	a.p.m./mg protein	% increase
Phe	0	Leu	100	403 $\pm$ 10	—
	73	Leu	100	575 $\pm$ 23	43
	155	Leu	100	569 $\pm$ 24	41
Thr	0	Leu	100	537; 533	—
	228	Leu	100	758; 734	37
	500	Leu	100	741; 697	32
Thr	0	Phe	25	448; 464	—
	228	Phe	25	778; 731	64
	500	Phe	25	811; 978	85

Liver slices were incubated for 15 min in the presence of varying amounts of one essential amino-acid. Normal plasma and liver concentrations for phenylalanine (Phe) were 50  $\mu$ M and 86  $\mu$ M, respectively, and for threonine (Thr) were 228  $\mu$ M and 500  $\mu$ M (ref. 4). Ribosomes recovered from the liver slices were incubated for 30 min with 'control' pH 5 enzymes and 0.5  $\mu$ Ci of uniformly labelled L-(<sup>14</sup>C)-leucine or L-(<sup>14</sup>C)-phenylalanine. Incorporation data represent individual values or averages  $\pm$  S.E.M. for three samples.

Table 2. EFFECT OF INITIAL EXTRACELLULAR LEVEL OF ONE ESSENTIAL AMINO-ACID ON ACTIVITY OF LIVER pH 5 ENZYMES IN PROTEIN SYNTHESIS

Incubation conditions for liver slices		Incubation conditions for amino-acid incorporation		Relative incorporation (%)
Amino-acid	Concentration ( $\mu$ M)	Amino-acid	Concentration ( $\mu$ M)	
Phe	0	Leu	100	100
	73	Leu	100	140
	155	Leu	100	171
Thr	0	Phe	25	100
	228	Phe	25	132
	500	Phe	25	123

Conditions were similar to those in Table 1, except that pH 5 enzymes recovered from the incubated liver slices were assayed with 'control' ribosomes. Results shown are averages calculated from three experiments with each amino-acid; 'P' values for differences at highest and lowest extracellular levels of each amino-acid were between 0.01 and 0.03.



these responses are unknown. However, in the context of presently accepted theories of the regulation of protein synthesis<sup>12</sup>, variations in the production and release of messenger RNA in response to alterations in amino-acid levels may be involved. The findings recorded here provide support for the hypothesis that primary alterations in amino-acid transport into the cell, such as those which may occur under hormonal or other metabolic influences, may be responsible for subsequent changes in protein synthesis.

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## Occurrence of Esterified Hydroxy Fatty Acids as Precursors of Lactones in Butter

It has been demonstrated by Boldingh and Taylor<sup>1</sup> that butterfat contains various  $\gamma$ - and  $\delta$ -lactones in amounts of 1-20 p.p.m. Using the isotope dilution method, 5-octanolide, 5-decanolide, 5-dodecanolide and 5-tetradecanolide could be determined quantitatively.

In addition, it was found that the free lactone content rises two- to three-fold when the butterfat is heated at about 140° C. This led us to believe that butterfat contains small amounts of hydroxy acids in esterified form, most likely in the form of monohydroxy-acyl-triglycerides. Synthetic glycerides of this type actually showed similar behaviour on heating<sup>1</sup>.

In this communication, observations are described which lend support to the aforementioned assumption. This was obtained by applying thin-layer chromatographic techniques to specific fractions derived from non-heated butterfat.

Our procedure was based on a number of considerations with regard to the  $R_F$ -values to be expected for monohydroxy-acyl-triglycerides as compared with those of mono-, di- and tri-glycerides of normal fatty acids in the systems applied. Thus:

(a) The  $R_F$ -value is about the same as that of a normal diglyceride (Fig. 1, Nos. 1 and 3; in this communication fractions are referred to in order of increasing  $R_F$ ).

(b) The presence of the hydroxy acid moiety in the 1- or in the 2-position could be expected to cause a difference in  $R_F$ -value similar to that observed between those of normal 1,2- and 1,3-diglycerides (Fig. 1, No. 1).

5-g butterfat dissolved in light petroleum/benzene (1:1) was chromatographed on a column filled with a mixture of 30 g 'Silicagel' ('Mallinckrodt', containing 6 per cent physically bound water) and 15 g 'Hyflo' (dried

for 16 h at 120° C). In this way about 80 per cent, consisting entirely of normal triglycerides, was removed. The remainder was eluted with diethyl ether and afterwards chromatographed by thin-layer chromatography on silica, which yielded three main zones, chiefly consisting of mono-, di- and tri-glycerides, respectively (Fig. 1, No. 2).

The silica between the extreme fractions was collected and extracted with ether. On re-chromatographing this fraction (200 mg) on a silica plate using the horizontal developing technique<sup>2</sup>, six fractions A-F were obtained

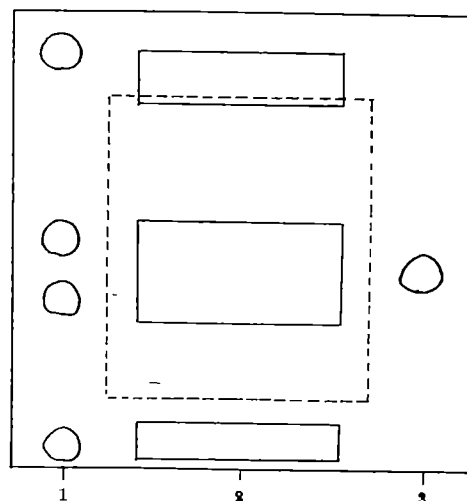


Fig. 1. Separation of column concentrate of butterfat. 1, Mixture of glyceryl monooleate, 1,2- and 1,3-dioleate and trioleate; 2, 200 mg of column concentrate; 3, 1-mono-(5-hydroxyacyl)-triglyceride. Thickness of silica layer 1 mm, 'Silicagel G' (Merck) purified by extraction with acetone/methanol 1:1 (v/v), eluant iso-octane/ether 60/40 (v/v). Detection with 'Ultraphor'.

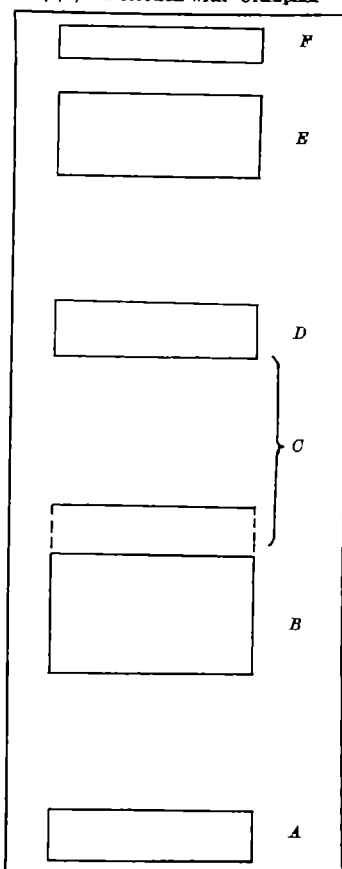


Fig. 2. Separation of second concentrate of butterfat. Thickness of silica layer 1 mm, eluant iso-octane/ether 80/20 (v/v). Detection with 'Ultraphor'.

(Fig. 2). Fractions *B* and *F* were found to consist of normal triglycerides, fraction *A* of monoglycerides. Fraction *D* was identified by infra-red spectroscopy as triglycerides containing an acetyl group.

Fraction *B* consisted mainly of 1,3-diglycerides, 1,2-diglycerides and sterols. Because of their  $R_F$ -values, free lactones should also be contained in this fraction (Fig. 3, No. 7). Removal of the lactones was performed by means of thin-layer chromatography on silica after acetylation; they were present in such small amounts, however, that they yielded no visible spot (Fig. 3, No. 8). As a check a synthetic mixture of diglyceride and labelled lactone was acetylated. In this operation, no cross-esterification between the two components was observed. Lactones remain unchanged during the acetylation treatment, as can be seen from Fig. 3, Nos. 1-4.

The acetylated glyceride fraction, now being free from lactones, was saponified, the unsaponifiable part removed and the soap converted into acids. If in this mixture the presence of lactones could be demonstrated, the presence of esterified hydroxy acids in butterfat would be established.

In this we succeeded by first esterifying the acids with diazomethane, followed by acetylation to decrease the polarity of irrelevant hydroxy compounds and afterwards, by separating the mixture by thin-layer chromatography, using a thick silica plate. Only a very weak zone was observed at the site where lactones could be expected on the plate (Fig. 4, zone 3, compare spots 1 and 2 of model 5-decanolide and 4-octanolide). The material extracted from zone 3 was then re-run as a single spot on a thin (0.25 mm) silica plate. When sprayed lightly with a 1 per cent aqueous solution of 'Ultraphor' (BASF) and viewed under ultra-violet light it showed a clear spot at the correct  $R_F$ -value. Moreover, the characteristic lactone smell was clearly noticeable, after extraction of the spot and evaporation to dryness.

Fraction *C* (Fig. 2) was analysed similarly. Free lactones were absent, but—after saponification of the acetylated glycerides—lactones could be detected in the same way as described for fraction *B*.

In both fractions  $\gamma$ - and  $\delta$ -lactones were detected by means of gas-liquid chromatography. Details will be reported elsewhere.

Small differences in  $R_F$ -value of the relevant glycerides in fractions *B* and *C* suggest that in *B* the hydroxy acids are located in the 1-position of the glycerides, whereas in *C* they might occupy the 2-position.

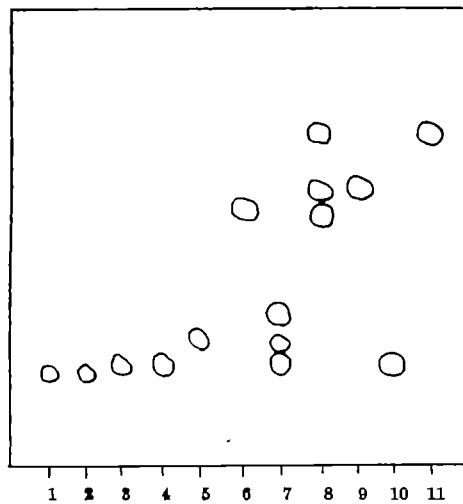


Fig. 3. Influence of acetylation on  $R_F$ -values: 1, 5-decanolide; 2, 5-decanolide after acetylation; 3, 4-nonanolide; 4, 4-nonanolide after acetylation; 5, 1-mono-(5-hydroxyacyl)-triglyceride; 6, 1-mono-(5-hydroxyacyl)-triglyceride after acetylation; 7, fraction *B*; 8, acetylated fraction *B*; 9, acetylated glyceryl 1,3-diolate; 10, cholesterol; 11, cholesterol acetate. Thickness of silica layer 0.25 mm, eluant iso-octane/ether 60/40 (v/v). Detection with molybdophosphoric acid.

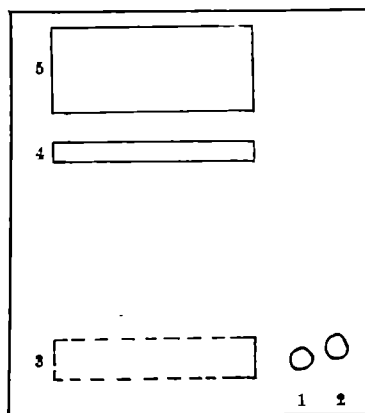


Fig. 4. Separation of methylated and acetylated fatty acids of fraction *B*: 1, 5-decanolide; 2, 4-octanolide; 3, lactones; 4, methyl esters of acetylated hydroxy fatty acids; 5, methyl esters of fatty acids. Thickness of silica layer 1 mm, eluant iso-octane/ether 60/40 (v/v). Detection with 'Ultraphor'.

In addition to the occurrence of keto-acid esters described in earlier publications<sup>3,4</sup>, evidence has now been obtained for the presence of hydroxy acid esters in butterfat. Though the overall behaviour observed during the various separation steps strongly suggests the presence of such esters as glycerides, it must be stated that definite proof for the combination with glycerol is still lacking.

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### Changes in the Quantity and Nature of Collagen in Rabbit Skin as a Function of Age

THE process of ageing is known to be accompanied by changes in the chemistry of connective tissue. In general collagen seems to accumulate with increasing age. Nevertheless, this is only true for the insoluble fraction, since the soluble collagens, which represent the newly formed molecules, decrease with age, reflecting the lowered rate of synthesis. Most of the work in this connexion has been reviewed in detail by Harkness<sup>1</sup>.

In order to explain this accumulation of collagen which occurs in spite of a decrease in synthesis, we recently examined the turnover of collagen in the skin of growing rats and found it to be an age-dependent process. During periods of rapid growth the turn-over time ( $T_{1/2}$ ) was 28 days whereas as the animal aged it reached 300 days<sup>2</sup>.

In the series of experiments recorded here we intended to evaluate quantitatively the collagen content of the rabbit skin at different stages of development as well as its distribution among different soluble fractions. It was shown by Bakerman, using human skin of different ages, that the amount of collagen extractable by citric acid increased rapidly during foetal development but began to fall exponentially shortly before birth, to reach very low levels in older individuals<sup>3</sup>. In these experiments no distinction was made between the different types of soluble collagens, nor was the total collagen measured.

For the purpose of our experiments rabbits of different ages as well as embryos obtained by Caesarean section were employed. The skin was shaved and, after killing the animals, cleaned from the underlying connective, muscular, and adipose tissues. These as well as all the following steps were performed in the cold (4° C). The skin was afterwards extracted with 0.15 M NaCl, 0.5 M

NaCl and 0.5 M citrate buffer pH 3.6. The insoluble collagen was solubilized by converting to gelatine. All fractions were dialysed as previously described<sup>3</sup>, and hydroxyproline determined in the acid hydrolysate by the method of Woessner<sup>4</sup> adapted to the 'Auto-analyser'. Collagen was calculated on the basis of the hydroxyproline content.

Fig. 1 shows the changes of the insoluble collagen content of rabbit skin as a function of the age of the animal. On the 20th day of gestation, shortly before birth, the skin contains 1.12 per cent insoluble collagen and, as the animal ages, its concentration steadily increases to reach a value of 15.5 per cent in the adult animal.

Fig. 2 summarizes our findings on the collagen distribution among the different soluble fractions. The amount

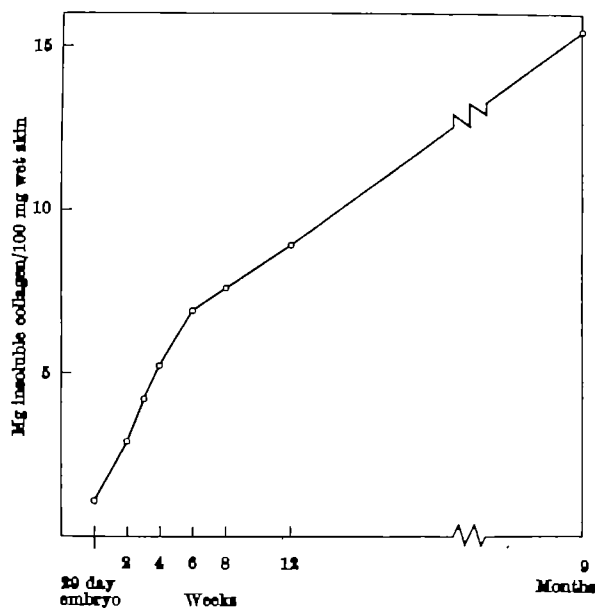


Fig. 1. Insoluble collagen of rabbit skin at different ages

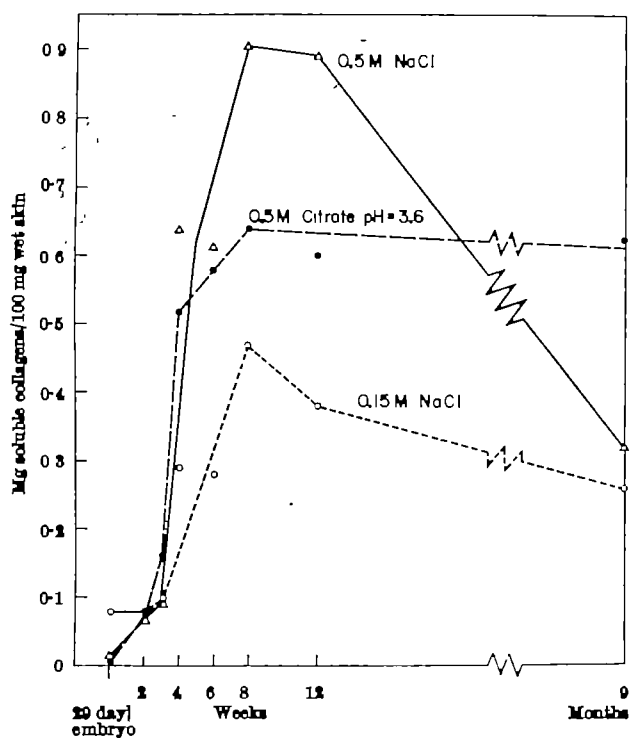


Fig. 2. Soluble collagen fractions extracted from rabbit skin at different ages

present in the three fractions studied increased with age until the 8th week after birth. At this time the amount extractable by neutral salts began to decline, whereas that soluble in citrate buffer remained constant.

These findings indicate that during the period of maximum growth and rapid rate of collagen synthesis, soluble collagens rapidly build up on their way to becoming insoluble fibrils. As the rate of synthesis of these soluble collagen precursors drops, their pool size decreases due to its rapid turn-over. In the rat we have found a  $T_{1/2}$  of 17-20 days for the 0.15 M and 0.5 M NaCl fractions. The citrate fraction does not act as an immediate precursor, since its concentration does not drop as should be expected if it were being utilized to form insoluble fibres. It seems to reach some kind of equilibrium with the insoluble fraction. This is substantiated by our previous findings using rat skin, where we observed that the turn-over time of the citrate extractable collagen was of the same low magnitude as that of the insoluble collagen.

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## PHYSIOLOGY

### Influence of Plasma Urea on Urine Concentration in the Opossum (*Didelphis marsupialis virginiana*)

INCREASING the amount of urea in the plasma has been shown to enhance urine concentration in many mammals (man, cat, dog, sheep, white rat, kangaroo rat, jerboa<sup>1,2</sup>, and rabbit<sup>3</sup>). In some mammals, however, changing the concentration of urea does not affect urine concentration; in the pig, beaver and *Aplodontia*, enhancement does not occur<sup>1,2</sup>. It was hypothesized that the inability of the latter group to raise its urine osmotic ceiling following increases in plasma urea is a primitive condition because these species are relatively primitive, phylogenetically.

The opossum was chosen to test this hypothesis because this species is considered to be one of the oldest living North American mammals and is usually considered to be the most primitive and generalized of living marsupials. With the exception of its larger size, it is apparently very similar to its early ancestors and is considered a 'living Cretaceous fossil'<sup>4</sup>.

Thirteen adult opossums were examined and two or three animals were treated simultaneously. Some animals were tested several times. Urine and plasma osmolalities were determined by a Fiske osmometer following vasopressin injection (1  $\mu$ /kg of body-wt.) during water diuresis according to the method of Dolph *et al.*<sup>5</sup>. Control animals were injected with 1.0 ml. water instead of vasopressin. The delayed response of the opossum to water diuresis and vasopressin required a slight modification of this procedure. Urine samples were taken every 30 min instead of every 20 min and vasopressin was injected when the animal exhibited a urine osmolality hypotonic to that of the plasma. This sometimes required 5 h. The water load was given in two equal portions 15 min apart to prevent regurgitation. Immediately prior to hydration, plasma urea-nitrogen was determined

according to the method of Richter and Lapointe<sup>6</sup> on a Beckman DB spectrophotometer.

Each animal was fed a high- or low-protein diet for a minimum of 14 days prior to treatment. A period of at least 7 days was allowed between treatments of the same animal. The high-protein diet consisted of 80 per cent meat (rabbit muscle and liver) and 20 per cent mixture of powdered milk, cereal, salt, alfalfa meal, and fish oil. The low-protein diet consisted of 90 per cent potatoes and carrots and a 10 per cent mixture containing cornmeal, apples, salt, and lard.

Table 1 presents the U/P ratios which were calculated from measurements taken at the time of peak urine concentration following hydration and vasopressin injection. The opossum can concentrate urine more effectively (U/P 4.75) when fed a high-protein diet than when fed a low-protein diet (U/P 2.32).

Table 1. MEAN URINE (U) AND PLASMA (P) OSMOLALITY AT THE TIME OF PEAK URINE CONCENTRATION FOLLOWING HYDRATION AND VASOPRESSIN INJECTION

Diet	Plasma (mOsm)	Urine (mOsm)	U/P
Low protein (9)	277.6	645	2.32
High protein (7)	281.2	1,354	4.75

\*P < 0.01

No. of observations in brackets.

\* Probability calculated at the 0.01 level of significance using the Mann-Whitney U test.

The urine osmolality of all control animals declined during the experimental procedures. The plasma osmolalities of the two dietary groups before the beginning of experimental procedures showed no significant difference. The plasma urea-nitrogen concentrations in the opossum fed a high-protein diet were significantly higher ( $P < 0.01$ ) than in those fed a low-protein diet (Table 2).

Table 2. MEAN PLASMA OSMOLALITY AND PLASMA UREA-NITROGEN ON DIFFERENT DIETS IMMEDIATELY PRIOR TO HYDRATION

Diet	Plasma (mOsm)	Plasma U/N (mg/100 ml.)
Low protein (11)	323.8	24.9
High protein (10)	326.6	43.2

\*P < 0.01

No. of observations in brackets.

\* Probability calculated at the 0.01 level of significance using the Mann-Whitney U test.

These results show that the maximum urine-concentrating ability of the opossum following vasopressin injection is comparable to that of higher mammals with similar kidney form (Table 3). The relative thickness of the medulla and the maximum urine concentration of the opossum lie between those of the rabbit and the rat, suggesting that the counter-current mechanism in this primitive mammal functions like that of higher mammals. The relative medullary thickness is an indication of the relative length of the loops of Henle, and the greater the relative thickness the greater should be the urine-concentrating ability according to the counter-current hypothesis.

Table 3. COMPARISON OF THE RELATIVE THICKNESS OF THE RENAL MEDULLA AND URINE CONCENTRATING ABILITY FOLLOWING VASOPRESSIN INJECTION

Mammal	Relative thickness of medulla*	Maximum urine concentration following vasopressin injection (mOsm)	Maximum U/P osmolal ratio
<i>Apodonta</i>	2.0	569	1.6
Man	3.0	850	2.9
Dog	4.8	1,875	4.9
Rabbit	5.4	1,045	3.7
Opossum	5.7	1,325	4.75
Rat	5.8	2,636	8.8

\* The medullary thickness  $\times 10$  divided by kidney size; where kidney size equals the cube root of the dimensions of the kidney.

These experiments show that the ability to raise the urine osmotic ceiling following increase in plasma urea is not restricted to higher mammals. The response of the 'primitive' opossum does not support the hypothesis that the inability of a species to raise its urine osmotic ceiling following increases in plasma urea-nitrogen is a primitive condition.

The response of the opossum to urea may be a function of certain anatomical relationships in the kidney. In the renal medulla of the opossum the nephron segments are grouped in such a manner that they form an inner and an outer zone clearly visible in fresh or fixed sections. Zonation of the vasa recta correlated with the zonation due to nephron arrangement is also present in the opossum<sup>8</sup>. Thus, the opossum exhibits characteristics of kidney form and function very similar to those of man, cat, dog, rat, sheep, kangaroo rat, jerboa<sup>1,2,8-10</sup>, and rabbit<sup>1,10</sup>. The beaver, pig, and *Aplodontia*<sup>1,8,11</sup> lack such zonation and they are incapable of raising their urine osmotic ceiling after an increase in plasma urea-nitrogen. It is, therefore, possible that the opossum's ability (and that of other species with similar kidney form) to raise this ceiling after an increase of plasma urea-nitrogen is related to the zonation of nephrons and vasa recta.

Further investigations comparing nephron functions in species showing the zonation described above with nephron function in species with no zonation may help to elucidate the way that urea is handled by the mammalian kidney.

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## Local Control of Luteal Function by the Uterus of the Guinea-pig

THERE is now no doubt that distension of the uterus of a number of species of mammal results in characteristic changes in the oestrous cycle which stem from a shortening of the life span of the corpora lutea in the ovaries<sup>1,2</sup>. Thus in the guinea-pig the presence of two glass beads in each horn of the uterus, when inserted during the first four days after oestrus, shortens the length of the cycle in progress at the time of operation and that of succeeding cycles<sup>3</sup>. The degree to which the cycle is shortened appears to be governed by the number of beads in the uterus, for when one bead is present in each horn the cycle in progress is shortened from a normal length of 16 days to 14 days, whilst when two beads are present in each horn the cycle is reduced to 12 days<sup>4</sup>. Although one bead located in one horn of the uterus of the sheep exerts a definite shortening effect on the oestrous cycle<sup>5</sup>, four beads in one horn of the guinea-pig uterus do not exert as marked an effect as when they are shared between both horns. The mechanism underlying this effect is not understood, although it has been suggested that the pituitary gland is involved in exercising a luteolytic function<sup>6</sup>. The present work has been concerned with the examination of this quantitative effect at the level of the ovaries, and attention directed to the morphological changes taking place in the corpora lutea, rather than to changes in the oestrous cycle over a longer period.

Using the technique of Donovan and Traczyk<sup>7</sup>, one glass bead was inserted into each horn of the uterus of 11 virgin guinea-pigs during one of the first 3 days after oestrus

and ovulation. The animals were killed at various intervals before the next expected oestrus and the ovaries were fixed, histologically processed and sectioned serially at  $10\mu$ . A quantity related to the volume of the corpora lutea in each ovary was estimated by measuring two diameters at right angles in the largest section of each corpus luteum and multiplying the product of these figures by the number of sections of each organ and by the thickness of the sections. No allowance was made for shrinkage during fixation. The results (in  $\text{mm}^3$ ) provided a mean value representing the volume of each corpus luteum ( $D^3$ ) and in this way the changing size of the corpora lutea during the cycle could be followed and compared with the results for normal animals presented by Rowlands<sup>7</sup>. The comparison is illustrated in Fig. 1 and reveals that, while the calculated curve for the growth of the corpora lutea up to the 9th day of the cycle is parallel to that given by Rowlands, there is a marked discontinuity evident on the 11th day. Regression of the corpora lutea appears to have taken place abruptly between the 9th and 11th days, an observation which supports earlier postulations concerning the existence of a process causing degeneration of the corpora lutea following the insertion of beads into the uterus<sup>3</sup>.

In order to examine whether the regression of the corpora lutea is determined by a local rather than by a systemic mechanism, one uterine horn alone was distended and the effect of this procedure on the ipsilateral and contralateral ovary compared. Accordingly 2 glass beads were inserted into the right uterine horn in each of a further 9 guinea-pigs on the second day after oestrus and the animals were killed at various intervals before the next expected oestrus. Histological processing of the ovaries and measurement of the corpora lutea were carried out as before. The results are presented in Table 1, and plotted in Fig. 2. It can readily be seen that the corpora

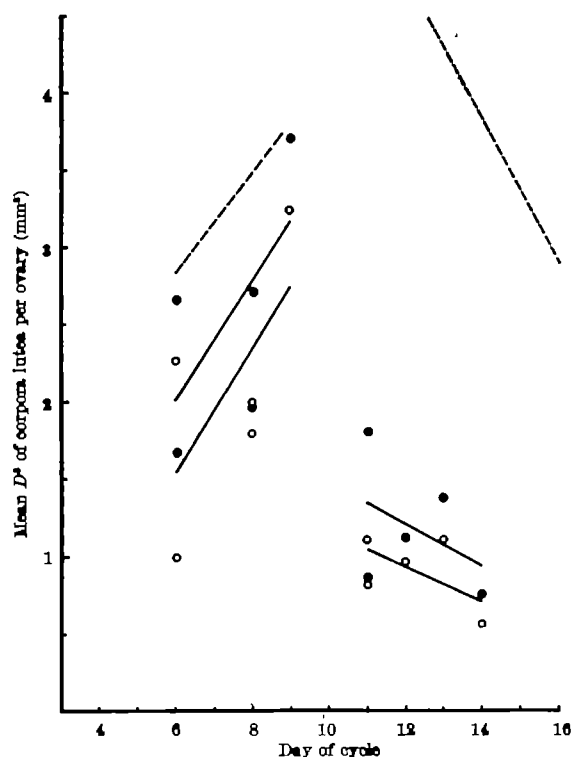


Fig. 1. The mean volume of the corpora lutea in each ovary plotted against day of cycle for animals bearing one bead in each uterine horn. As the ovaries for each animal were processed together and their left and right individually lost, the data concerning the ovary with the largest corpora lutea ( $\bullet$ ) in each pair were arbitrarily grouped together and compared with their partners ( $\circ$ ) in order to exaggerate any differences. The regression lines for each group are plotted. The broken line indicates the volume/time curve for the corpora lutea of normal animals as calculated by Rowlands<sup>7</sup>.

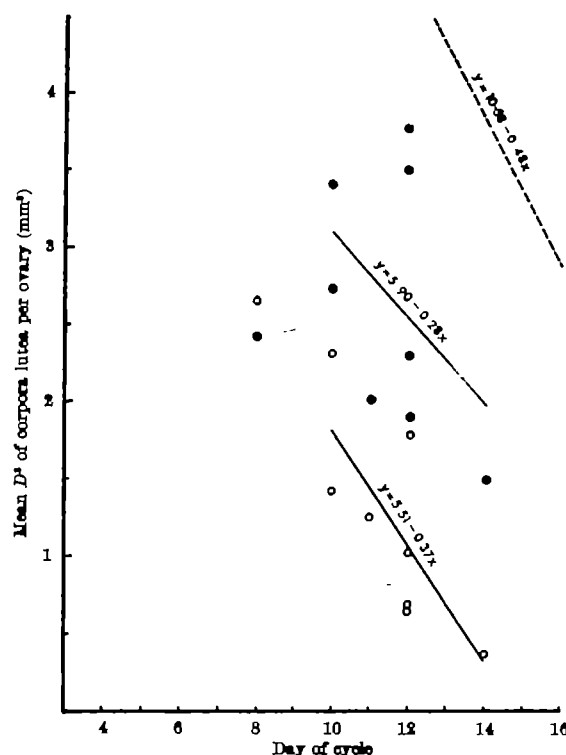


Fig. 2. The mean volume of the corpora lutea in the ovaries on the operated (—○—) and unoperated (—●—) side of animals bearing two beads in one horn of the uterus. The broken line shows the volume/time curve for corpora lutea in normal animals as calculated by Rowlands<sup>7</sup>.

lutea in the right ovary (associated with the horn of the uterus containing the beads) are significantly smaller than those of the left ( $P < 0.001$ ), indicating that the process initiating regression of the corpora lutea utilizes a local rather than a systemic mechanism. The parallelism observed between the curves for regression of the corpora lutea in each ovary implies that once regression has been initiated (to a greater or lesser degree) it thereafter proceeds at a constant rate.

Table 1. MEAN VOLUME OF CORPORA LUTEA IN INDIVIDUAL OVARIES FOLLOWING DISTENSION OF THE RIGHT UTERINE HORN WITH GLASS BEADS

Animal No.	Day of cycle at autopsy	Mean $D^3$ for corpora lutea of left ovary	Mean $D^3$ for corpora lutea of right ovary	Difference
1003*	8	2.430	2.661	-0.232
1010	10	2.739	1.438	+1.301
1007	10	3.433	2.320	+1.113
1016	11	2.014	1.303	+0.712
916	12	1.908	0.649	+1.259
911	12	2.806	1.045	+1.761
932	12	2.806	0.674	+2.132
973	12	3.779	1.799	+1.980
996	14	1.511	0.870	+0.641

\* Luteal size on day 8 has been omitted from the mathematical analysis due to uncertainty whether at this stage luteal size is increasing or decreasing.

The present findings, though of a preliminary nature, clearly imply the existence of a mechanism through which stimuli originating in one horn of the uterus can promote the regression of the corpora lutea in the ovary on that side. In this connexion it is reasonable to suppose that a luteolytic factor is produced by the uterine horn and could diffuse to the neighbouring ovary. Supporting evidence for this possibility can be drawn from work on hysterectomized animals where, for example, it has been shown that autotransplantation of uterine tissue at the time of hysterectomy prevents the expected persistence of the corpora lutea<sup>8</sup>. In this connexion it is also noteworthy that a close correlation between the presence of uterine tissue and the life of the corpora lutea in the ipsilateral ovary has been shown following sub-total hysterectomy in the pig<sup>9</sup> and guinea-pig<sup>10</sup>. However,

this is the first time that a local effect on the corpora lutea has been observed to follow distension of the uterus and casts doubt on the importance of the pituitary gland in this regard.

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## PHARMACOLOGY

### Effect of Dihydrostreptomycin on Tissue Lipid of Guinea-pigs

ANTIBIOTICS are known to have profound effects on nucleic acids and protein biosynthesis. Coniglio and Bell<sup>1</sup> reported that when antibiotics are administered orally to rats there is a significant increase in the incorporation of <sup>14</sup>C-acetate into hepatic fatty acids. Chatterjee *et al.*<sup>2</sup> observed that dihydrostreptomycin significantly influences the lipid metabolism of intestine and whole body of rats. The neurotoxic effects of dihydrostreptomycin on prolonged use in tuberculosis treatment and other infections are well known<sup>3</sup>. The present work was undertaken to study the effects of DHS on lipid changes in various tissues of guinea-pigs.

Female guinea-pigs in groups of four, weighing 350-380 g, were intramuscularly given saline solution of dihydrostreptomycin sulphate (Pfizer) (0.1 g/kg body weight). Guinea-pigs without dihydrostreptomycin served as the controls. After ten days of administration of the drug a saline solution of NaH<sub>2</sub>PO<sub>4</sub> (400 µc/kg body-weight) was given intraperitoneally. The guinea-pigs were killed 6 h after

<sup>32</sup>P administration. Brain, lung, heart, liver, kidney, spleen and adrenals were quickly removed, cleaned, weighed and transferred to a volume of chloroform:methanol (2:1, v/v). The tissues were homogenized in a Potter-Elvehjem homogenizer with 5 volumes of chloroform:methanol (2:1, v/v). Heart and lung tissues were ground with a pestle in a mortar. The lipids from the tissues were extracted with 20 volumes of chloroform:methanol (2:1, v/v) for 4 h at room temperature by the method of Folch *et al.*<sup>4</sup>. The lipid extract was evaporated to dryness *in vacuo* and freed of proteo lipids according to Folch *et al.*<sup>4</sup>. The final lipid residue was dissolved in 5.0 ml. chloroform and used immediately for analysis or stored at -8° in glass-stoppered tubes. All solvents used were of analytical grade and were re-distilled and degassed with nitrogen before use.

The lipid solutions were analysed for cholesterol<sup>5</sup> and phospholipid phosphorus<sup>6</sup>. <sup>32</sup>P activity (c.p.m.) was determined in a TCG-14 Tracer Lab Geiger-Müller tube. The weights of the lipids were determined by weighing 1.0-ml. aliquots to a constant weight. Free and esterified cholesterol were estimated by the digitonin method<sup>7</sup>. The cholesterol on the digitonide was estimated by a method<sup>8</sup> modified from that of Hanel and Dam<sup>9</sup>. The results are shown in Table 1.

From the results (Table 1) it is seen that dihydrostreptomycin does not bring about significant changes in the total lipid contents of any tissues studied. Similar observations are noted in total cholesterol contents of these tissues except in kidney, where dihydrostreptomycin lowers the kidney total cholesterol. An insignificant increase in the free cholesterol of kidney is seen as a result of dihydrostreptomycin treatment, and this finding is in agreement with those of Chatterjee *et al.*<sup>2</sup>. Brain showed a significant decrease in free cholesterol on dihydrostreptomycin treatment, though total cholesterol is not significantly affected. This may possibly be due to some inhibition of cholesterol esterase in brain. Phospholipid phosphorus in brain is significantly reduced in dihydrostreptomycin-treated animals, but it is not affected in any other tissues. Though a decrease in phospholipid phosphorus in brain is seen as a result of dihydrostreptomycin administration, the specific activities (c.p.m./mg P) of brain phospholipids are not significantly affected. The phospholipid phosphorus of heart is not reduced on dihydrostreptomycin treatment, but the specific activities are significantly reduced, indicating a slow turn-over rate of

Table 1. EFFECTS OF DIHYDROSTREPTOMYCIN ON TISSUE LIPIDS OF GUINEA-PIGS

Tissue	Cholesterol (mg/g wet tissue)				Phospholipid phosphorus (mg/g wet tissue)		Specific activity (c.p.m./mg PLP)		Lipids (mg/g wet tissue)	
	O	H	O	H	O	H	O	H	O	H
Brain	7.53 ±0.370 P>0.10	6.25 ±0.719 P>0.10	3.39 ±0.599 P>0.50	3.15 ±0.788 P>0.50	4.24 ±0.248 P<0.05	3.10 ±0.376 P>0.05	0.453 ±0.0192 P<0.05	0.288 ±0.0158 P>0.10	945 ±144 P>0.10	700 ±56 P>0.10
Lung	3.22 0.403 P>0.50	2.90 0.578 P>0.50	1.23 0.245 P>0.50	1.15 0.251 P>0.50	1.90 0.225 P>0.50	1.65 0.317 P>0.50	0.425 0.0700 P>0.40	0.232 0.1140 P>0.40	18,000 2,042 P>0.90	18,250 2,117 P>0.90
Liver	2.27 0.076 P>0.80	2.44 0.191 P>0.80	1.29 0.467 P>0.90	1.24 0.309 P>0.90	0.98 0.251 P>0.50	1.30 0.150 P>0.50	0.875 0.0631 P>0.40	0.995 0.1223 P>0.40	25,400 3,361 P>0.40	28,000 1,154 P>0.40
Heart	1.54 0.301 P>0.70	1.41 0.164 P>0.70	1.12 0.207 P>0.60	1.01 0.049 P>0.60	0.41 0.112 P>1.0	0.41 0.062 P>1.0	0.516 0.0216 P>0.60	0.602 0.1330 P>0.60	5,500 499 P<0.05	3,000 1,000 P<0.05
Spleen	3.23 0.322 P>0.70	4.10 0.500 P>0.70	1.25 0.301 P>0.50	0.85 0.450 P>0.50	2.57 0.439 P>0.50	3.25 0.949 P>0.50	0.429 0.0387 P>0.70	0.490 0.0623 P>0.70	26,750 1,654 P>0.20	21,670 3,168 P>0.20
Kidney	2.90 0.178 P<0.05	2.03 0.182 P>0.05	1.07 0.259 P>0.10	0.72 0.194 P>0.10	1.62 0.027 P>0.60	1.31 0.244 P>0.60	0.548 0.0500 P>0.20	0.477 0.0520 P>0.20	22,500 1,709 P>0.20	20,000 818 P>0.20
Adrenals	60.30 0.577 P>0.50	54.85 0.606 P>0.50	51.00 1.196 P>0.50	44.00 6.189 P>0.50	10.97 2.967 P>0.90	10.85 3.940 P>0.90	1.189 0.0610 P>0.50	1.225 0.2152 P>0.50	78,750 2,401 P>0.90	52,500 453 P>0.90

The results are expressed as the mean ± S.E. of four experiments.  
P values below 0.05 are significant.  
O = Control group.

H = Experimental group.  
PLP = Phospholipid phosphorus.

heart phospholipids. An insignificant increase in total lipid contents and an insignificant decrease in specific activities of phospholipids of adrenals is seen as a result of dihydrostreptomycin treatment. Neither group of animals showed any change in their daily food consumption and body weights.

The results show that brain is the only tissue, where dihydrostreptomycin treatment has significantly lowered both the phospholipid phosphorus and free cholesterol. Whether these observations have any direct or indirect relationship to the neurotoxic effect<sup>3</sup> of dihydrostreptomycin is not certain at present.

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### Chronic Toxicity Testing

CERTAIN types of hazard consequent on the administration of chemical substances are estimated by the performance of chronic toxicity tests. Such tests are usually designed according to certain empirical rules. Groups of animals (commonly three) are given a test substance in regularly repeated doses of constant size, each group receiving a different dose; the material can be given either by individual dosing to each animal or by administration in the diet. The interval between doses in the case of individual doses may vary according to the properties of the substance; the most generally used interval is 24 h. The experiment continues for an arbitrarily chosen period of time since no general principle enables the duration of such tests to be determined more rationally.

The results of such tests may be death of the dosed animals or some lesser toxic effect which will occur earlier, and more severely, in the group treated with the highest dose. When toxic effects occur in such experiments certain regularities may be expected; this is because each toxic effect may be considered to have two principal phases—development and restitution. If the interval between two successive doses is too short to permit complete restitution of the previous injury, persistent lesions will develop, accumulate and grow worse. The magnitude of such effects will be related to the size of the individual dose, to the interval between successive doses and to the period over which dosing continues; they will also be modified by such factors as increasing resistance, or sensitivity, to the action of the compound and to the development of secondary factors not primarily related to the injury caused by the compound.

It is well known that the acute biological effects of a single dose of a biologically active substance are related to the logarithm of the dose. It is reasonable to enquire whether, in the circumstances of properly conducted chronic toxicity tests, biological effects are related to time in the same way. It is certainly true that lower doses of a compound will be tolerated for longer periods than larger doses and that some doses can be found, even of very toxic materials, which do not cause overt toxic action in any period of time that can be examined. Such

observations might be expected if effects were related to log time as well as to log dose. If this generalization were true, then there should be a straight-line relationship between the effect and the duration of an experiment when both co-ordinates are logarithmic.

Because chronic toxicity studies are usually terminated at an arbitrary point, it is not easy to obtain sufficient data to support this suggestion. Nevertheless, it is possible to find some examples, two of which are shown in Figs. 1 and 2. Fig. 1 is taken from literature using the data of Hawkins and O'Shanny<sup>4,5</sup> on ataxia occurring in cats

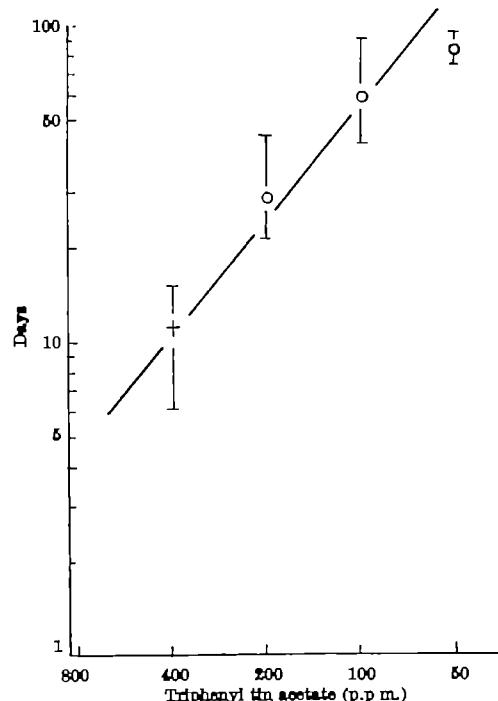
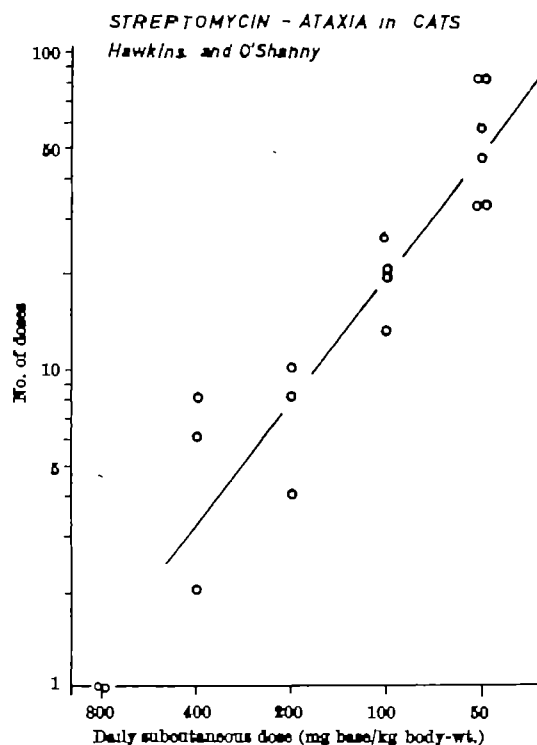


Fig. 2. Time of death in guinea-pigs receiving triphenyl tin acetate in the diet. +, Experiment started March 1962; O, experiment started November 1962



after daily subcutaneous doses of streptomycin. Fig. 2 presents death in guinea-pigs receiving triphenyl tin acetate in the diet.

Other experimental data on chronic toxicity studies with acrylamide (J. M. Barnes, private communication) tend to show the same relationship. Although these experiments were performed simultaneously under different conditions, namely, administration of the substance in daily oral doses, as an ingredient in the diet or in adequate high doses in intervals of a week, the toxic effect, which was weakness of the hind legs, and all the related data fit very well with the foregoing hypothesis.

The principle of accumulation in pharmacology has been examined mathematically by A. J. Clark<sup>1</sup>. Further development of the theoretical background of the kinetics of chronic toxicity by Druckrey<sup>2,3</sup> led to his interpretation of the special conditions in carcinogenicity as the 'summation action' and the 'intensifier action'. In his experiments he could demonstrate an analogous connexion of dose-response and time to that discussed here. In the case of carcinogenic action, however, the conditions are more particular than in the case of the chronic toxicity experiment because there appears to be no repair, the effects of a single action being summated without loss.

If the hypothesis that drug effects are related to the logarithm of the duration of chronic administration as well as to the logarithm of the dose given, the problem of choosing the best duration of a chronic experiment is simplified, and it may also be possible to estimate safe levels of a substance more effectively.

The hypothesis enunciated here suggests several possible experimental tests; since each test is elaborate, any one laboratory can only undertake a very small number.

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## HAEMATOLOGY

### Activity of Nicotine and Inactivity of Kallikrein and Kallidin in Aggregation of Blood Platelets

In 1961, Gaarder *et al.*<sup>1</sup> reported that adenosine diphosphate (ADP) causes human blood platelets to aggregate. Born *et al.*<sup>2,3</sup> described a method by which the aggregation of platelets may be followed quantitatively. They confirmed the results of Gaarder and described inhibition of the ADP-effect by 2-chloroadenosine and other substituted nucleosides<sup>4</sup>. Lately, some more results on this subject have been published<sup>5-7</sup>, but the mechanism of platelet aggregation is still unknown.

Recently we described release of 5-hydroxytryptamine (5-HT) and other biogenic amines from isolated rabbit blood platelets by nicotine<sup>8,9</sup>; Singh and Oester<sup>10</sup> reported that nicotine lowered coagulation time *in vivo* with concomitant release of epinephrine, and Wenzel and Singh<sup>11</sup> showed that intravenous injection of nicotine or epinephrine reduces coagulation time in rabbits. Damage of arterial walls and formation of thrombi in rabbits brought about by epinephrine, acetylcholine, 5-HT, several polypeptides and inhalation of cigarette smoke can be prevented by nialamide<sup>12</sup>. Investigations on the influence of these substances on platelet aggregation *in vitro* should therefore contribute to knowledge of the mechanism involved.

We therefore tested nicotine, 5-HT and iproniazid with respect to an eventual effect on platelet aggregation using the slightly modified method of Born and Cross<sup>3</sup>.

Gokeen and Yunis<sup>13</sup> presented strong evidence that fibrinogen is involved in platelet structure, using trypsin and other proteolytic enzymes. Kallikrein, which resembles trypsin in many respects, was not studied. Kallikrein has proteolytic and esterolytic properties and produces the polypeptide kallidin, which is strongly hypotensive<sup>14-16</sup> and enhances the permeability of membranes and capillaries.

To investigate the possibility that kallikrein may influence platelet aggregation by an effect on platelet membrane structures, we tested kallidin and kallikrein. The latter was investigated with special regard to the fact that inability of coagulation due to fibrinolysinaemia may be treated successfully with a kallikrein inhibitor<sup>17-19</sup>.

By measuring the optical density at 600 mμ of platelet-rich mechanically stirred rabbit blood plasma we tested the platelet aggregation with results shown in Fig. 1.

Under our conditions ADP causes the platelets rapidly to aggregate. Nicotine causes aggregation of platelets dependent on the concentrations used, but relatively high nicotine concentrations are needed. When nicotine and ADP were added simultaneously the effects of both enhanced each other. Kallikrein (up to 10 units/3 ml. plasma) and kallidin (15 and 90 units/3 ml. plasma) had no effect and did not influence platelet aggregation elicited by ADP. Iproniazid  $5 \times 10^{-3}$  M inhibited the action of ADP and nicotine. 5-HT had no measurable effect, either alone or in combination with ADP. Thioglycolic acid (0.05 ml.), which prevents breakdown of kallidin by kallidinase<sup>20</sup>, had no influence on the negative results obtained with kallikrein and kallidin, but totally prevented the aggregation induced by ADP. (After completion of this investigation Jokay *et al.*<sup>21</sup> have reported thioglycolic acid and other thiol compounds to inhibit the endotoxin induced platelet aggregation and the simultaneous release of histamine and 5-HT.)

Our results confirm the findings of Gaarder *et al.*<sup>1</sup>, Born *et al.*<sup>2,4</sup> and Shimamoto<sup>18</sup>. The aggregation of platelets by nicotine may be caused by a change of the electrical charge of the thrombocyte membrane, because there is

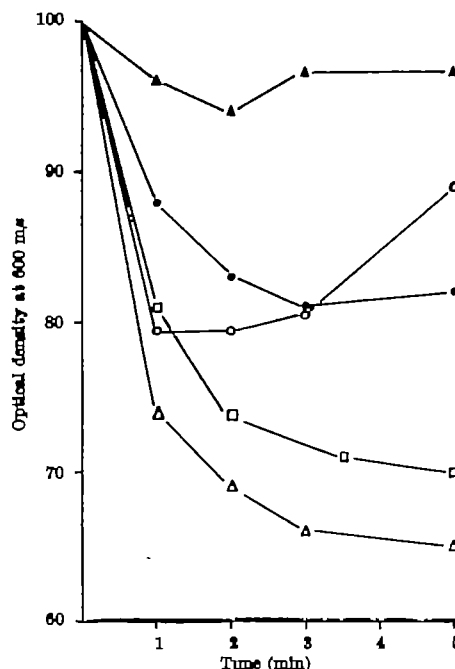


Fig. 1. Effect of ADP, nicotine and iproniazid on the aggregation of rabbit blood platelets as measured by optical density of platelet-rich rabbit blood plasma at 600 mμ.  $\Delta$ , ADP  $1 \times 10^{-3}$  M + nicotine  $5 \times 10^{-3}$  M;  $\bullet$ , nicotine  $5 \times 10^{-3}$  M;  $\square$ , ADP  $1 \times 10^{-3}$  M;  $\triangle$ , nicotine  $1 \times 10^{-3}$  M after iproniazid  $5 \times 10^{-3}$  M;  $\circ$ , ADP  $1 \times 10^{-3}$  M after iproniazid  $5 \times 10^{-3}$  M.

strong evidence that nicotine may enhance the permeability of the platelet membrane by changing its charge. Nicotine could cause aggregation by releasing ADP; this, however, seems unlikely, because a very similar compound ATP is not released from platelets by nicotine<sup>21</sup>.

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### Iron Studies of Thalassemic Erythrocytes after Splenectomy

DURING the past few years, the uptake of iron by immature red cells *in vitro* has been studied extensively. Studies have included the kinetics of stromal iron as well as of intracellular iron. Thus Tishkoff<sup>1</sup> measured stromal iron after intravenous injection of <sup>59</sup>Fe in thalassemic patients and also in patients with other diseases. Allen and Jandl<sup>2</sup> studied the kinetics of intracellular iron in an *in vitro* system, using reticulocyte-rich rabbit red cells incubated with <sup>59</sup>Fe-labelled plasma, and measured the <sup>59</sup>Fe in the stroma, mitochondria, and microsomes. Clark<sup>3</sup> used radioactive iron *in vivo* and *in vitro* to measure the stromal iron in rat red cells. Falbe-Hansen and Lothe<sup>4</sup> studied the stromal iron in rabbit red cells after intravenous injection of <sup>59</sup>Fe.

In a previous study from this laboratory, it was found that after incubation of thalassemic blood with <sup>59</sup>Fe, the radioactivity associated with the stroma was greater than in normal blood<sup>5</sup>. The difference was statistically significant after 6 h incubation, but was not statistically significant after 1 h. This finding was considered to indicate some retention of iron by the stroma of the thalassemic red cells. The beneficial results of splenectomy in thalassemia led us to extend our studies to the red cells of splenectomized thalassemic patients. The uptake of iron by the stromal fraction after incubation of blood with <sup>59</sup>Fe has been compared with that observed in thalassemic patients without splenectomy.

Fourteen thalassemic subjects with splenectomy were studied. The haematological and other data concerning the patients are shown in Table 1.

The method used for the iron uptake by immature red cells was similar to that described previously<sup>6</sup>. The method of Tishkoff *et al.*<sup>1</sup> was used for the separation of the stroma of the red cells.

The <sup>59</sup>Fe content of the stromal fraction is expressed as a percentage of the total <sup>59</sup>Fe taken up by the immature red cells. The results are given in Table 2. In Table 3 the

Table 1. HAEMATOLOGICAL DATA OF PATIENTS STUDIED

Case No.	Age	Sex	Diagnosis	Time after splenectomy	Haemoglobin concentration (g/100 ml)	Immature red cells (%)	Alkali-resistant haemoglobin (%)
1	6	F	Thalassemia	12 days	10.4	1.0	15.2
2	15	F	"	16	7.6		57.0
3	5	F	"	28	14.6	0.6	9.8
4	28	M	"	86	10.7	4.4	67.8
5	24	M	"	42	7.2	4.3	44.0
6	21	F	"	9 months	8.0	11.7	18.0
7	9	M	"	10	8.0		14.1
8	3	M	"	1 year	9.0	3.9	45.0
9	3	M	"	1	6.4	6.5	75.0
10	11	M	"	2 years	7.0	8.0	50.0
11	28	M	"	4	10.6	8.4	66.9
12	14	F	"	10	8.6	5.9	70.0
13	25	F	"	9	7.2	5.5	50.0
14	35	F	"	14	7.2		7.0

Table 2. PERCENTAGE OF <sup>59</sup>Fe IN STROMA

Case No.	Incubation time		
	1 h	4 h	6 h
1	39.1	43.1	43.6
2	35.3	39.4	39.2
3	51.2	36.0	37.3
4	49.3	49.3	51.0
5	44.1	43.6	48.2
6	52.3	66.2	54.4
7	50.4	53.7	—
8	35.7	40.8	43.0
9	43.7	45.4	46.9
10	56.7	54.9	52.1
11	43.2	46.0	44.7
12	47.8	44.8	50.2
13	67.6	63.8	63.9
14	47.1	44.5	45.2

Table 3. MEAN VALUES OF THE PERCENTAGE OF <sup>59</sup>Fe IN THE STROMA ± ONE STANDARD DEVIATION IN THALASSEMIC PATIENTS WITHOUT AND WITH SPLENECTOMY

Incubation time	Percentage <sup>59</sup> Fe in stroma		
	1 h	4 h	6 h
Thalassemic patients without splenectomy	25.9 ± 9.2	29.3 ± 8.3	29.1 ± 8.2
Thalassemic patients with splenectomy	47.4 ± 8.3	46.7 ± 9.3	47.7 ± 6.9

Table 4. PERCENTAGE OF <sup>59</sup>Fe IN STROMA AFTER INTRAVENOUS INJECTION OF <sup>59</sup>Fe *in vivo*

Case No.	Days after injection of <sup>59</sup> Fe							
	1	2	3	4	5	6	7	8
6	57.2		26.7					24.4
13	56.7	47.3				20.5		
14			46.2		30.3			28.9

mean values of the stromal <sup>59</sup>Fe are compared for thalassemic patients with and without splenectomy. The values from the thalassemic patients without splenectomy are taken from the published data of Malamos *et al.*<sup>4</sup>. Three of these cases were studied also *in vivo*. The results are summarized in Table 4.

The results summarized in Table 3 show that the <sup>59</sup>Fe associated with the stromal fraction is greater in erythrocytes from splenectomized thalassemic patients than in those from thalassemic patients without splenectomy. This difference is statistically significant ( $P < 0.001$ ).

It is known that after splenectomy there are morphological changes in the blood elements, such as the appearance or the increase of nucleated red cells in the blood stream, the increase of reticulocytes, the appearance of siderocytes, Howell-Jolly bodies and Hainz bodies, as well as changes in the surface area, which becomes larger than before without any change in the cellular volume<sup>7,8</sup>.

It is possible that the increase in the number of circulating nucleated red cells after splenectomy, as well as the change in their shape, may affect the <sup>59</sup>Fe content of the stromal fraction. Another possible explanation is provided by the appearance in the blood stream of large numbers of siderocytes, in which the incorporated iron is not used for haemoglobin synthesis, but is thought to be deposited in the stroma or in the mitochondria. Thus Hoffman *et al.*<sup>9</sup> have described a new high-density particle in the stroma of thalassemic erythrocytes. Beasis and Breton-Gorins<sup>10,11</sup> have shown by electron microscopy that iron-containing granules are present in normal erythroblasts, normoblasts

and reticulocytes and also in certain pathological erythrocytes. They found that in thalassaemia, 80 per cent of the erythrocytes had iron-containing granules. In thalassaemia also large quantities of iron in the form of ferritin were seen in the mitochondria of nearly all polychromatophilic and acidophilic erythroblasts. Larizza and Orlandi<sup>12</sup> have also found by electron microscopy that marrow siderocytes show accumulation of iron in the mitochondria. Tishkoff<sup>1</sup> found that the stromal iron of erythrocytes was moderately increased in two patients after splenectomy and he considered it a reflexion of the siderocytosis.

It is considered that the appearance of siderocytes in the blood after splenectomy is due to the absence of the "pitting function" of the spleen<sup>7,13</sup>. An increase of stromal <sup>59</sup>Fe after intravenous injection of <sup>59</sup>Fe in splenectomized thalassaemic patients could possibly be explained by the absence of the "pitting function" of the spleen. This opinion is supported by the fact that after intravenous injection of <sup>59</sup>Fe the radioactivity associated with the stromal fraction in thalassaemic patients without splenectomy is low, amounting to only about 2-5 per cent of the radioactivity of the erythrocytes (our unpublished data). On the other hand, in thalassaemic patients with splenectomy the values are high, as illustrated by the three cases of the present study (Table 4).

However, absence of the "pitting function" of the spleen cannot explain the increased <sup>59</sup>Fe in the stromal fraction after incubation with <sup>59</sup>Fe of blood from splenectomized patients. In one case (No. 4 of Table 2) the values for stromal <sup>59</sup>Fe (studied *in vitro*) before and after splenectomy were 21 per cent and 51 per cent respectively.

The observed increase of stromal <sup>59</sup>Fe after *in vitro* incubation is not specific for splenectomized thalassaemic patients, but is a phenomenon observed also in other splenectomized subjects. In pyridoxine deficiency anaemia, sickle cell thalassaemia, Hb E/thalassaemia and myelodysplasia we have observed values of 55, 59.6, 72.2 and 43.9 per cent, respectively, for <sup>59</sup>Fe in the stromal fraction.

It is suggested by Dameshek<sup>14</sup> that the spleen acts as an endocrine organ and releases a humoral factor which affects erythropoiesis. Such a humoral factor has not been isolated. However, whether or not there is a humoral factor released by the spleen affecting erythropoiesis, it is probable that the role of the spleen in erythropoiesis is fulfilled not only by its pitting function but also in some other way.

We thank Dr. E. Harries for her advice.

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## HISTOLOGY

### Argentaffin Cell Incidence in the Rectal Mucosa of Man, Mouse, and Hamster

THE often quoted standard work on the argentaffin cell by Jacobson in 1939<sup>1</sup> is doubtless the source cited by many authors on the distribution of argentaffin cells. From it we quote, "In man and all animals investigated, there appears to be a steady decrease in the number of (argentaffin) cells towards the caudal end of the intestine. The rectum of cattle seems to be an exception to this rule".

This communication reports quantitative work on the frequency of argentaffin cells within human rectal mucosa as compared with mouse and hamster rectal mucosa. The rule set out by Jacobson was not borne out and there were exceptions not only for cattle but, to a greater degree, for man. In addition, data for two animal species have been quantitated to provide an accurate index of the low incidence of these cells per crypt rather than per unit area as reported by investigators prior to Jacobson.

Eleven biopsies were performed on patients at Bellevue Hospital with no illness related to the gastrointestinal tract. Three one-year-old female Swiss hybrid mice from the Charles River animal farm and four Syrian hamsters, two years of age, from the Lakeview hamster colony in New Jersey were killed and the rectum was dissected out for histological study. All material was fixed in 10 per cent formalin and stained using the Schmorl and Diazo technique as described by Pearce<sup>2</sup>. Sections were cut at 5μ for the human material and at 5 or 10μ for the mouse and hamster material. Insufficient material from one patient made it impossible to obtain a Schmorl reading.

As many crypts as possible were scored for the number of argentaffin cells present in each crypt in the human material. As few as 61 crypts were scored for a given stain reaction because of the scarcity of the biopsy material, but at least 50 per cent of the human data is based on more than 100 crypts. In the case of hamster and mouse material, at least 200 crypts were scored (Table 1). The argentaffin cell values are expressed as the number of cells per crypt for each individual sampled; the mean argentaffin cell value is given with the standard error of the mean.

As seen from Table 1, the mean number of argentaffin cells for human rectal material was approximately two per crypt although the average per person varied between one and three per crypt. Age did not appear to account for the variability in argentaffin cell number between adults tested.

Table 1. ARGENTAFFIN CELL INCIDENCE IN MAN, MOUSE AND HAMSTER

Normal man		Schmorl		Diazo	
Patient	Age	Crypts	Arg. cells/ crypt	Crypts	Arg. cells/ crypt
1	66	70	91 1.30	100	162 1.62
2	61	163	257 1.58	130	146 1.12
3	68	101	254 2.61	125	332 2.66
4	23	177	552 3.12	160	566 3.54
5		61	116 1.90	91	318 3.49
6	78	96	130 1.35	61	90 1.48
7	64	75	206 2.75	70	175 2.50
8	27	—	—	110	202 1.84
9	32	115	218 1.90	180	194 1.49
10	67	64	173 2.70	69	133 1.93
11	38	106	115 1.06	130	159 1.23
		Mean 2.02 ± 0.23 (S.E.)		Mean 2.08 ± 0.26 (S.E.)	
Mouse					
Female					
1 yr. Swiss					
1		400	186 0.47	200	30 0.15
2		200	86 0.43	200	73 0.37
3		200	73 0.37	200	55 0.28
		Mean 0.43 ± 0.03		Mean 0.27 ± 0.20	
Hamster:					
Male					
2 yr. Syrian					
1		200	20 0.10	200	10 0.05
2		200	4 0.02	200	6 0.03
3		200	9 0.05	200	20 0.10
4		200	2 0.01	400	0 0
		Mean 0.06 ± 0.02		Mean 0.06 ± 0.0	

While the average was found to be between one and three cells per crypt, it was not very unusual to see as many as ten or more argentaffin cells present in one crypt (Fig. 1). It is true that while many of these cells are found at the base of the crypt, they are frequently seen at some distance from the base, migrating to the surface. In fact, they are occasionally seen close to the surface and lumen of the crypt (Fig. 2). Although these cells are described as seldom found together and occurring singly between the other cells<sup>2</sup>, in human rectal mucosa, it was not uncommon to find large clusters of these cells at the base of the crypts, lying one beside the other (Fig. 1).

The results of the analysis of the frequency of argentaffin cells in mouse and hamster rectum appear in Table 1. An argentaffin cell may be seen in approximately one out of three rectal crypts in the mouse, and more nearly one for every 20 crypts in the hamster. Although quantitation of the argentaffin cells in the rectum of mouse and hamster was difficult because they were not numerous,



Fig. 1. Base of several rectal crypts in a human biopsy specimen, with ten argentaffin cells in one crypt and many of these cells in one cluster (top left). Bohm method ( $\times 700$ )



Fig. 2. Three argentaffin cells at the upper portion and near the lumen of a human rectal crypt. Diano method ( $\times 700$ )

Table 2. DISTRIBUTION OF ARGENTAFFIN CELLS IN 5 CM OF RECTUM FROM VARIOUS SPECIES (according to Tehver<sup>4</sup>)

Species	Samples	Argentaffin cells
Dog	5	0
	1	0
Cat	2	0
	1	20
	1	24
Cow	1	120
	1	210
	1	165
Calf	1	0
	1	140
	1	70
Horse	1	0
	1	9
Pig	1	0
	1	43

the figures measure their relatively low incidence, which was reproducible in both 5- $\mu$  and 10- $\mu$  sections.

There are enough data to demonstrate that argentaffin cells are not rare in human rectal mucosa. They are at least five times as numerous as in the mouse and forty times more frequent than in the hamster. The figures reported by Tehver in 1930<sup>4</sup> for cattle seem large when compared with the figures obtained in dog, cat, horse and pig (Table 2), but he examined 5 cm of rectum in 10- $\mu$  sections. Our data are derived from specimens of human rectal mucosa not more than 1-2 mm long. 5 cm of bovine rectum or 1-2 mm of human rectum contained 120-210 argentaffin cells.

Throughout the course of this study the nucleus of only one argentaffin cell was thought to be in mitosis. The loss of these cells due to their migration up the crypt must require them to be replaced in a manner similar to the epithelial cell. Their relative frequency in human rectal mucosa suggests that this tissue, obtainable at biopsy, would provide suitable material for investigation of their renewal.

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## PATHOLOGY

### Haemolytic Activity of Suspensions of Different Silica Modifications and Inert Dusts

RECENTLY, Harley and Margolis<sup>1</sup> reported haemolytic activity of amorphous silica particles of sizes larger than 5  $\mu$ . In the course of an investigation on the possible role of complement (C') in the cytotoxicity of quartz, our experiments revealed a specific haemolytic effect of buffered saline suspensions of this mineral. Using corundum ( $\alpha$ - $\text{Al}_2\text{O}_3$ ) and anatase ( $\text{TiO}_2$ ) of the same surface area as quartz, significantly less haemolysis was found<sup>2</sup>.

Since haemolysis is a simple model for the damage of the membranes of cells and sub-cellular particles, several mineral dusts which had been checked previously for fibrogenic effects in animal experiments<sup>3,4</sup> were tested for their haemolytic activities.

Six different modifications of silica were used in these tests: quartz prepared by grinding pure rock crystal from Brazil to a respirable size less than 2  $\mu$  (corresponding to a specific surface area of 6.5  $\text{m}^2/\text{g}$  of the final powder); vitreous silica prepared by grinding pure fused silica to

8.8 m<sup>2</sup>/g; cristobalite prepared by sintering vitreous silica in presence of a mineralizing reagent and afterwards grinding to 9.7 m<sup>2</sup>/g; tridymite prepared as in the case of cristobalite, specific surface area 7.2 m<sup>2</sup>/g; coesite (14.1 m<sup>2</sup>/g) and stishovite (21 m<sup>2</sup>/g) isolated from Coconino sandstone of Meteor Crater near Winslow, Arizona<sup>5</sup>. Two commercial types of alumina ('Degussa Al 23 VT',  $\alpha$ -Al<sub>2</sub>O<sub>3</sub>, 13.8 m<sup>2</sup>/g, and 'Degussa Aluminiumoxid P',  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>, 86 m<sup>2</sup>/g) and one of TiO<sub>2</sub> (anatase, 'Kronos A', Titangeellschaft Leverkusen, 8.6 m<sup>2</sup>/g) were used as inert dusts, that is, dusts showing no significant fibrogenic effects.

Specific surfaces were determined by means of argon adsorption (Brunauer-Emmett-Teller method).

Human and sheep red cells were washed three times in isotonic saline (pH 7.4, barbital buffer). Samples were prepared from the aforementioned dusts, each with a surface area of 0.02 m<sup>2</sup>. To 0.5 ml. saline suspensions of each of these samples were added 0.5 ml. of 3 per cent red cell suspensions. These mixtures were incubated each for 1 h at 37° C. Cells and dust particles were kept in suspension by gently agitating the test-tubes every 5–10 min. Four ml. amounts of saline were added to every tube, and the optical density measured at 550 m $\mu$  after centrifuging (2,000 r.p.m., 5 min).

As shown in Table 1, all silica modifications except stishovite have a much higher haemolytic activity than the pathologically inert dusts, based on the surface area of the dust particles in suspension. The haemolytic activity fairly parallels the fibrogenic effect in animal experiments; this is especially clear-cut in the case of stishovite, the only non-silicogenic crystalline silica modification<sup>6</sup>.

The haemolytic action of quartz is suppressed by preincubation of mineral powder in a solution of polyvinylpyridine-*N*-oxide (P 204), a compound that shows an antisilicogenic effect in animal experiments<sup>7</sup>. Haemolysis is diminished to varying degrees by coating the quartz with trimethylsilyl-groups<sup>8</sup>, alumina<sup>9</sup>, gelatine, cholesterol, and a lipid extract from erythrocyte stroma<sup>3</sup>.

In another series of experiments the samples of dust were increased with respect to surface area. The results (Fig. 1) demonstrate that haemolysis increases with the surface of the dust.

In experiments fully reported elsewhere<sup>3</sup> practically no additional haemolysis was observed if dilutions of homologous serum (1 ml.) with complement titres up to 30 C' H<sub>50</sub> units were added to red cell suspensions (0.5 ml.) that had been in contact with a limited amount of quartz that lysed less than 50 per cent of the cells.

It can be assumed that the mechanism of the lysis of erythrocytes by the crystalline silica modifications tested involves the adsorption of lipids from the erythrocyte

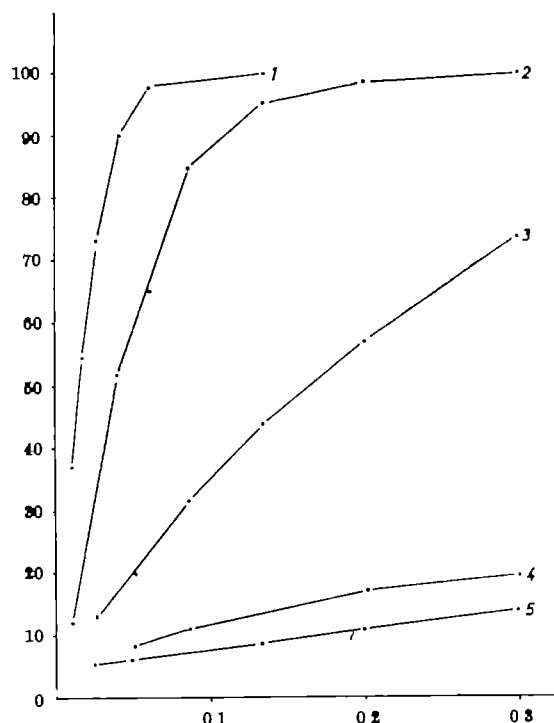


Fig. 1. Abscissa, m<sup>2</sup> total surface area of dust particles brought in contact with 1 ml. 3 per cent human red cell suspension in buffered saline; ordinate, percentage haemolysis. 1, Quartz; 2, coesite; 3, corundum; 4, stishovite; 5, anatase

membrane similar to what Brown<sup>9</sup> has reported for the amorphous silica preparation, 'Hyflo Super Cel'. The composition of erythrocyte lipids adsorbed by these crystalline silica modifications is under study.

The agreement of the results from the model of haemolysis *in vitro* with those from animal experiments<sup>3,4</sup> indicates that damage of subcellular structures is the specific step in the silicotic process. All membranes including those of mitochondria are composed of substantial amounts of lipids<sup>10</sup>, as are microsomes.

Damage of these structures in the phagocyte would inhibit simultaneously the oxidation processes as well as protein synthesis and result in necrobiosis, and in the increased turn-over of macrophages characteristic for early stages of silicotic lesions, as released dust particles are newly phagocytosed.

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Table 1. HAEMOLYSIS BY SALINE SUSPENSIONS OF DIFFERENT CRYSTALLINE SILICA MODIFICATIONS, DIFFERENTLY COATED QUARTZ POWDERS, AND SOME NON-FIBROGENIC DUSTS

Mineral*	Coating	Percentage haemolysis	
		Sheep erythrocytes	Human erythrocytes
Quartz	None	92.0	90.0
Tridymite	None	100.0	91.0
Vitreous silica	None	68.5	62.5
Cristobalite	None	85.0	63.5
Coesite	None	55.0	53.5
Stishovite	None	7.0	8.0
Corundum	None	17.0	17.5
$\gamma$ -Al <sub>2</sub> O <sub>3</sub>	None	11.5	21.0
Anatase	None	8.5	6.5
Quartz	Alumina†	42.5	47.5
Quartz	Trimethylsilyl-groups†	36.0	35.0
Quartz	P 204‡	10.5	7.0
Quartz	Stroma lipid§	86.0	72.5
Quartz	Cholesterol§		74.5

\* Particles with 0.02 m<sup>2</sup> total surface area per 0.5 ml. 3 per cent red cells.

† Coated with monomolecular layer<sup>8,9</sup>.

‡ Suspended (10 min) in a saline solution of poly-2-vinyl-pyridine-*N*-oxide\* (0.1 per cent) and washed once with saline.

§ Methanol-chloroform (1:1) extract from horse erythrocyte stroma, 1 per cent emulsion in saline; 0.5 per cent emulsion of cholesterol obtained by mixing acetone solution with saline and evaporating the organic solvent, treatment similar to ‡.

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## Time-course Concentration of N-Acetylneuraminase Lyase from Rat Granulation Tissue

The time sequence of enzymatic and metabolic alterations during inflammatory response and repair by connective tissue has been inadequately studied. Enzymatic changes related to connective tissue and the inflammatory process are virtually unknown. However, observations during the development of the chick embryo<sup>1</sup> and of

biological development of tissue-specific proteins and metabolic adaptations in response to environmental effects or with ageing<sup>4</sup> suggest that similar events are operative when injury occurs and inflammation and repair are initiated by connective tissue.

The metabolism of *N*-acetylneuraminic acid (*N*-AN) is presumably of importance in inflammation. This was suggested by the finding of a *N*-AN-containing glycoprotein fraction in an inflammatory tissue<sup>5</sup>. Lyases which cleave *N*-AN aldolytically have been isolated from *Cl. perfringens*<sup>6,7</sup> and from rat kidney cortex, and in addition demonstrated in a variety of other animal tissues<sup>8</sup>. Pathways for the biosynthesis of *N*-AN have also been clearly established<sup>9-11</sup>. The purpose of this communication is to demonstrate an enzyme system from experimental inflammatory tissue which yields pyruvic acid from *N*-AN and which varies in activity within the tissue with time.

Air-turpentine pouches were prepared in male albino rats, weighing approximately 200 g, of the Sprague-Dawley strain<sup>12</sup>. Granulation tissue was allowed to develop for periods of 3-16 days. A complete series of tissues at different phases of development could not be run simultaneously, but various stages were studied in duplicate, and replicate observations were obtained at various time intervals. For each analysis, the granulomata from 2 to 4 rats were dissected from the skin and the finely minced tissue was washed twice with a buffered salt solution (100 volumes 0.15 M KCl, 5 volumes 0.15 M MgSO<sub>4</sub>, 20 volumes 0.1 M potassium phosphate buffer, pH 7.4 with cysteine. HCl was added to a concentration of 10<sup>-3</sup> M). The washed mince, averaging approximately 7 g per rat, was weighed and homogenized in a 'Virtis 45' apparatus with 2 volumes of the buffered salt solution for 30 sec. The brei was centrifuged at 2,200g for 15 min and the supernatant decanted through double-thickness gauze after removal of the lipid pellet. All preparations were maintained at 0° C. Samples containing 0.7 ml. of the resulting homogenate were incubated with shaking with 3 μmoles of *N*-AN (isolated from human meconium<sup>13</sup>) and 15 μmoles potassium phosphate buffer, pH 7.2 in a final volume of 1 ml. 37° for 30 min. After incubation inactivation was accomplished by heating in a rapidly boiling water bath. Appropriate aliquots of the incubation mixture, cleared by centrifugation at 16,000g after addition of 'Celite', were analysed for pyruvic acid spectrophotometrically using lactate dehydrogenase (Boehringer) and DPNH (Pabst). The methods employed were those of Comb and Roseman<sup>8</sup> with minor modification. The protein concentration of each homogenate was determined by a biuret method<sup>14</sup>. Each series of incubations was run in duplicate with appropriate controls. The average variation of enzyme activity for multiple extractions was ± 6.3 per cent with a reproducibility of multiple determinations from a single extract of ± 14.1 per cent. The production of pyruvate from *N*-AN was linear with respect to protein concentration.

The observed data suggest that the enzyme activity of 7-day granuloma tissue is greater than that obtained from tissues of other ages. Although certain overlaps in the ranges of activity occurred it was clear that the maximal enzyme activities with respect to tissue weight and maximal specific activity were observed at 7 days. Fig. 1 graphically illustrates the results.

The accounts of enzyme variation or adaptation, which have been referred to, demonstrate some correlation between the differentiation of tissues and their specific function. Quite often, however, changes in enzyme patterns could only be related to the age of the tissue. Even in the face of rapid structural and morphologic changes in the granulation tissue<sup>15,16</sup> the factors controlling *N*-AN lyase activity remain obscure. Time-course studies of guinea-pig granulomata concerning changes in DNA, collagen and ascorbic acid indicate that on or about the seventh day of induction there is a rise in DNA and collagen content<sup>17</sup>. A fucose-containing glycoprotein fraction also

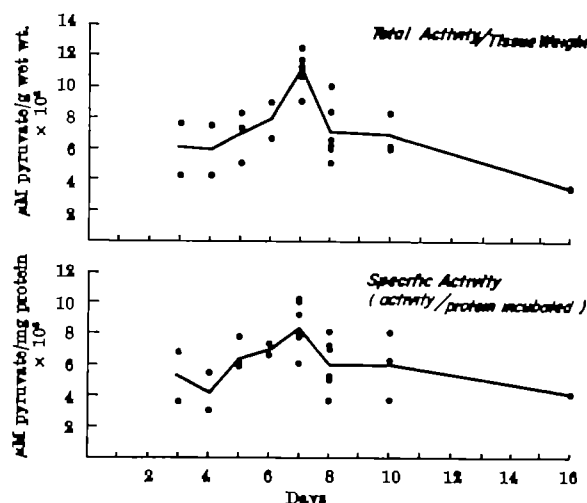


Fig. 1. *N*-Acetylneuraminidase lyase activity in granulation tissue of varying ages. The top graph represents changes in total activity of the enzyme per g of tissue expressed as the amount of pyruvate formed in 30 min. The lower graph represents changes in enzyme activity per mg of protein incubated with age of granulation tissue. Mean values are indicated by continuous traces.

changes in concentration with respect to time<sup>18</sup>. Seven-day rat granulomata contain significant concentrations of an *N*-AN-containing glycoprotein material<sup>5</sup> as well as a number of acid mucopolysaccharides<sup>19</sup>. The metabolism of these biopolymers is very likely controlled by an intricate series of reactions as suggested by other experiments<sup>17-19</sup>. *N*-AN lyase is probably an active step in the catabolism of *N*-AN rather than its biosynthesis<sup>4-9</sup>. This report is a further demonstration of the complex and rapid but defined biochemical changes occurring in inflammatory tissue.

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## RADIOBIOLOGY

### Tritium-labelled Peptide Hormones with High Specific Radioactivity

PRESENT interest in the assay of peptide hormones has led to the development of radio-immunoassay methods. These are based on the competition for antibody by labelled and unlabelled hormones, and the subsequent separation of antibody-bound from 'free' radioactivity<sup>1</sup>.

<sup>125</sup>I-labelled hormones have the practical disadvantage of the short eight-day half-life of <sup>125</sup>I, and the theoretical disadvantage that iodination tends to destroy the biological potency of the hormones. With these considerations in mind, we have prepared tritium-labelled hormones of high specific activity using the technique of acetylation. In general, between 0.2 and 1.0  $\mu$ moles of hormone are mixed with 25 mc. (7  $\mu$ moles) tritiated acetic anhydride (3,570 mc./mmole from Nuclear Chicago Corporation) in 0.25 ml. water saturated with sodium acetate and adjusted to pH 8.0–8.5 with 0.1 N NaOH. The acetylated hormone is then separated from the free acetate by passing the mixture through a 2  $\times$  15 cm 'Sephadex G-25' column. With this technique we have prepared tritium-labelled human growth hormone (3.2 acetyl/mole, 275  $\mu$ c./mg) tritium-labelled bovine growth hormone (12 acetyl/mole, 570  $\mu$ c./mg), and tritium-labelled pork insulin (127  $\mu$ c./mg, 0.5 acetyl/mole). Human and bovine growth hormone acetylated to this extent with <sup>14</sup>C-labelled acetic anhydride have previously been demonstrated to be biologically and antigenically comparable with unlabelled hormone<sup>1,2</sup>.

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## BIOLOGY

### Rate of Greying of Human Hair

GREYING of hair is probably the most obvious sign of ageing in man. To determine whether or not its onset and progress are influenced by hair colour we have examined a sample of Australians.

Blood donors were readily available to us. Their use had, however, a disadvantage, since we had to restrict their examination to simple inspection. More accurate methods, involving the taking of hair samples, were impracticable. In these conditions, assessment of degrees of greying presented problems which were not satisfactorily solved. We first prepared for use as standards a series of coloured photographs, illustrating hair of different shades with increasing degrees of greying. But matching the photographs with living subjects was unsatisfactory. We then mixed samples of cut hair of different colours with increasing proportions of white hair, but again matching was difficult. Finally, we decided to rely on the observers' judgments in recording only two categories of greying. Those subjects with any visible grey or white hair were designated 'any greying'. Those with no discernible hair of the original hue were classed 'complete greying'.

The total number examined was 8,720, comprising 6,653 men and 2,067 women. Colour of the hair was judged by simple inspection. If the hair was completely grey or white, or suspected of being tinted or dyed, the subject's own statement regarding the true colour and the degree of greyness was accepted. Preliminary trials showed the observers to be in substantial agreement and their findings concerning the distribution of hair colour agree closely with those in a recent survey in Victoria, Australia<sup>1</sup>.

Only subjects aged twenty-five years or more are included in this report. Younger persons were excluded for these reasons. Few young Australians of either sex

wear hats. All are heavily exposed to direct sunlight. Many also use bleaches to obtain the desired combination of heavily tanned skin and very fair hair. It was therefore often difficult in the young to determine the true hair colour or to distinguish between naturally and artificially produced loss of pigmentation.

The age incidence of greying in men and women, judged by these criteria, is shown in Table 1. The minor differences between the sexes are not significant. For any greying,  $P < 0.30 > 0.20$ ; for complete greying,  $P < 0.50 > 0.30$ . In considering the possible influence of hair colour on greying the data for both sexes have therefore been combined.

Table 1. GREYING AS RELATED TO SEX AND AGE

Age	No.		Any greying (%)		Complete greying (%)	
	M	F	M	F	M	F
25+	2,574	698	23.29	23.85	0.23	0.14
35+	2,157	627	61.38	66.35	2.18	1.59
45+	1,493	553	88.68	88.25	12.50	9.95
55+	429	159	94.16	96.20	22.91	23.57

The age incidence of greying as related to hair colour is shown in Table 2. The data for fair and red hair have been pooled, as there was no significant difference between them. Inspection of Table 2 shows a paradoxical situation. If the first indication of greying is the criterion, greying appears sooner in dark than in fair hair and more dark than fair-haired subjects showed some greying. But more fair than dark-haired subjects were classed as completely grey.

Table 2. AGE INCIDENCE OF GREYING IN RELATION TO HAIR COLOUR

Age	At risk			Any greying (%)			Complete greying (%)		
	Fair	Med- lum	Dark	Fair	Med- lum	Dark	Fair	Med- lum	Dark
25+	502	839	454	4.6	10.4	25.4	0.4	0.0	0.0
30+	345	674	459	18.0	23.3	40.5	0.6	0.3	0.2
35+	333	666	444	37.2	53.2	64.0	0.9	1.2	1.1
40+	303	604	474	60.5	71.4	74.9	4.6	3.3	1.9
45+	283	510	406	84.0	83.3	90.4	8.9	8.6	8.4
50+	205	331	313	92.7	91.2	93.3	22.9	15.4	12.8
55+	137	222	198	96.4	93.2	94.9	43.3	31.1	19.2
60+	16	26	18	100.0	100.0	100.0	50.0	23.1	16.7
Totals	2,063	3,972	2,765	45.8	52.9	68.1	7.6	5.2	3.3

The explanation for this apparent contradiction may be found in the relatively crude method of observation. A few white hairs will stand out more distinctly against a background of dark than of fair hair. Conversely, a few dark hairs will be more noticeable against a grey than a dark background. The first signs of greying will therefore be seen most readily in dark hair; but fair hair will appear to have turned completely grey sooner than dark.

If this be so, one might question whether these results have any objective value. However, further analysis provides an interesting result. The age-incidence data in each colour-class can be satisfactorily fitted to straight lines, using the log-probit transformation (Table 3). The regression lines are parallel within each colour-class, but the slope is steeper for fair than for dark hair.

Table 3. GREYING IN RELATION TO HAIR COLOUR (Log-probit regressions)

Colour	Degree of greying	Probit	$\chi^2$	d.f.	P
Fair	Any	4.753 (in age)—12.439	5.093	6	< 0.70 > 0.50
	Complete	4.753 (in age)—14.489	6.171	4	< 0.20 > 0.10
Fair	Any	3.667 (in age)—8.910	8.539	6	< 0.30 > 0.20
	Complete	3.667 (in age)—11.243	6.144	6	< 0.50 > 0.30
Medium	Any	3.351 (in age)—6.659	7.280	6	< 0.30 > 0.20
	Complete	3.351 (in age)—9.397	5.667	5	< 0.50 > 0.30
Dark	Any	3.351 (in age)—6.659	7.280	6	< 0.30 > 0.20
	Complete	3.351 (in age)—9.397	5.667	5	< 0.50 > 0.30

Since the regression lines for minimal and maximal greying within each colour-class are parallel, let us assume that a parallel line midway between them represents the proportion of subjects in whom at least half the hair has turned white or grey. This class may be conveniently designated 'at least 50 per cent greying'. The three mid-lines so constructed have a common intersection. The calculated ages at which 50 per cent of the population at risk are 'at least 50 per cent grey' are 48.6 years for fair



hair, 49.4 years for medium hair, and 49.1 years for dark hair. For this sample it seems a fair approximation to say that at 50 years of age 50 per cent of people are at least 50 per cent grey, irrespective of sex or hair colour. The good fits in the log-probit transformations indicate that susceptibility to greying is normally distributed.

It seems probable that the rate at which the pigmentary content of human hair diminishes is independent of the initial concentration, but that visual impressions of degrees of greying are strongly influenced by the contrast between the original colour and white.

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### Strange Male Block to Pregnancy: its Absence in Inbred Mouse Strains

A SERIES of papers by Bruce and colleagues<sup>1-4</sup> has described a pre-implantation block to pregnancy in non-inbred laboratory mice which is evoked by the presence of strange males of the same strain or of a different strain ('alien' males). This effect is olfactory-mediated. The phenomenon has also been reported in another species, non-inbred prairie deer mice (*Peromyscus maniculatus bairdii*)<sup>5</sup>.

We felt that the physiological mechanisms involved in this phenomenon could be studied most effectively using highly inbred strains of mice, both because of a high degree of genetic control and because strains might differ in manifestation of the pregnancy block. Therefore a preliminary survey was made of the capacity for implantation to be blocked by strange or alien males in a number of inbred strains.

Experimental procedure for blocking implantation was as follows: females were paired individually with stud males of the same strain in the morning. All females were examined for the presence of a vaginal plug on the following four mornings. The stud male was removed from the home cage of a female after she showed a vaginal plug, and a 'strange' male of the same strain or an 'alien' male of a different strain was introduced. The strange or alien male was removed after 48 h, and all inseminated females were autopsied seven days after insemination to determine if implantation had occurred. In controls the stud male was removed, and the female remained isolated for the entire 7-day period until autopsy.

Five strains were tested. Although the data are not exhaustive with respect either to experimental design or to sample size, there was no indication that the pregnancy block was operating in any of the strains, in females exposed either to strange or to alien males (Table 1). Probability testing was by  $\chi^2$  and there were no significant differences between controls and experimentals within any of the strains.

It is perhaps premature to speculate extensively as to why the inbred strains that were tested failed to show the

pregnancy block. An inability of the females to discriminate between strange and stud males of such close genetic similarity could explain the results of within-strain testing. However, attempts to prevent implantation with alien males of a strain different from the stud male were equally unsuccessful. It is impossible to determine from the data whether blocking capacity is absent in males, females, or both.

Because of the many problems in reproduction that usually arise during intensive inbreeding, naturally occurring mechanisms that interfere with reproduction might be selected against in efforts to maintain a productive inbred line.

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### A New Record for *Leptopenus*, a Rare Deep-water Coral

AMONG the rarest of the deep-water corals are specimens of *Leptopenus* (family Microbaciidae). At present the only records of this delicate coral I have verified are those of the *Challenger Expedition*<sup>1</sup>, which took 5 specimens from 4 localities, all from depths in excess of 2,700 m. Reported occurrences of the genus off the South Australian coast<sup>2</sup> are probably incorrect and are more possibly worn specimens of *Leptosammia*, another microbaciid coral. The Vero Collection examined by Dennant cannot now be located (F. McNeill, *in lit.*), but specimens of *Leptosammia* have been taken from South Australia in comparable depths<sup>3</sup>.

The rarity of this coral and the great depths from which it has been recorded make additional specimens very significant. Only one coral, *Fungiacyathus marenzelleri*<sup>4</sup>, has been recorded from greater depths (5,868 m). The family Microbaciidae, in its evolution, shows progressive adaptation to life in deeper waters from *Microbacia*, through *Stephanophyllia* and *Leptosammia* to *Leptopenus*, at the end of the series. In this genus skeletal structures are greatly reduced with the result that a striking corallum is formed.

The specimen described here was taken from greenish clay at 2,000 m in Makassar Strait (*Galathea* Station 453, 3° 58' S., 118° 28' E.). The specimen is the central part of the corallum and was dead when collected. Maximum diameter of the fragment is 6.0 mm and its height is 2.5 mm. Septa are reduced to spines, with particularly stout and elongate spines developed over the delta formed by the union of septa of the first, second and third cycles (Fig. 1).

*Leptopenus discus* is the best known of the two species of *Leptopenus* described by Moseley<sup>5</sup>, being represented by four specimens taken from three localities:

*Challenger* Station 147: 46° 16' S., 48° 27' E., 80 miles west of Hog Island, Crozet Islands, 2,926 m.

*Challenger* Station 157: 53° 55' S., 108° 35' E., southern Indian Ocean, 3,566 m.

*Challenger* Station 323: 35° 39' S., 50° 47' W., east of Rio de la Plata, 3,475 m.

*L. hypocoelus* is known from a single specimen from *Challenger* Station 299; 33° 31' S., 74° 43' W., off Valparaiso, Chile, at 3,950 m.

Table 1. THE PERCENTAGE OF PREGNANCIES ON DAY 7 POST INSEMINATION IN SELECTED STRAINS OF INBRED HOUSE MICE EXPOSED TO A STRANGE OR ALIEN MALE FOR TWO DAYS AFTER INSEMINATION OR TO ISOLATION

Strain	Control	Strange male	Alien male
057BL/6J	86 (56)	89 (72)	90(41) CBA/J
CBA/J	89 (9)	100 (21)	—
CBA/Os	100 (23)	96 (26)	—
SWR/J	93 (27)	93 (27)	—
129/J	71 (33)	61 (52)	68(25) CBA/J or 057BL/6J

Figures in parenthesis give the numbers of mice in the sample.

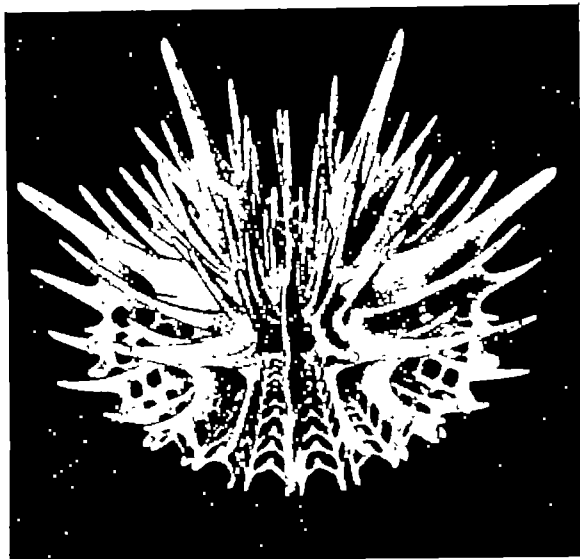


Fig. 1. A reconstruction of the central portion of the corallum of *Leptopenus* taken by the *Galathea* collection, x c. 7. Drawing by Miss Caroline Bartlett

The *Galathea* specimen is probably the central part of the disk-like corallum of *L. discois*. This species has a recorded diameter of 25 mm; but the central spinose portion of the corallum appears to be about 5 mm in diameter, judging from Moseley's illustrations. The maximum height of the *Challenger* specimens is stated to be 2 mm. In the *Challenger* specimens, the remainder of the corallum is formed by a broad, nearly flat structure which is exceedingly fragile and would easily be broken in life or in the process of collection.

This new record is an extension of the known range considerably to the north, and is the shallowest occurrence of the species. Neither the location nor the depth is surprising for a poorly known organism and, of course, the depth records of the *Challenger* in deeper waters are questionable because they were wire soundings.

I thank Drs. Torben Wolff and Jensenius Madsen for permitting me to examine the important collection of deep-water corals made by the *Galathea* Expedition. This work is part of a larger investigation of the corals of the Southern Ocean, supported by National Science Foundation grant GB-353.

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### Toxicity of Dieldrin and Endrin to Bats

IN the course of our investigation of the storage and mobilization of lipids and pesticides in Chiroptera, it became necessary to obtain some data regarding the toxicity of various insecticides to bats. In a previous paper we reported that the big brown bat, *Eptesicus fuscus*, was extremely sensitive to DDT<sup>1</sup>. This communication reports the responses of this animal to two additional chlorinated hydrocarbon pesticides.

The bats were captured during August 1964 in attics of houses in Georgetown and St. Helens, Kentucky. On arrival at the laboratory they were weighed, banded and put into individual cages consisting of wide-mouthed gallon jars containing a bedding of wood shavings covered with filter paper. A small dish of water was provided. Each animal was fed about 3-5 g of bat 'glop', a blend of

equal parts of ripe banana, cream cheese, canned dog food and adult meal worms, *Tenebrio molitor*, a day. A period of 24 h was allowed for acclimatization to laboratory conditions. Technical-grade dieldrin and endrin were dissolved in corn oil and the calculated quantities administered in single dosages directly on to the food as the bats ate while being held in the hand. Bats took both glop and corn oil avidly. Controls were fed corn oil in a quantity equivalent to the largest dosage of oil given the experimental animals. The bats were observed at hourly intervals during the first day and at intervals of 24 h or less thereafter. The experiment was terminated after 28 days.

Table 1. TOXICITY OF DIELDRIN TO THE BAT *Eptesicus fuscus*

Sex	Wt. (g)	Dosage (mg/kg)	Time after being dosed		Mortality		
			Until tremors	Until death	♂	♀	Total
♀	15.4	200	3 h	21 h	—	1/1	1/1
♀	14.6	100	3 h	23 h	2/2	1/1	3/3
♂ juv.	10.8	100	5 h	32 h			
♀ juv.	12.4	100	*	41 h			
♂ juv.	13.5	80	3.5 h	17 h	2/2	1/1	3/3
♂	14.6	80	3.25 h	41 h			
♀	15.8	80	18 h	19 h			
♀ juv.	13.8	60	*	20 h	1/2	1/1	2/3
♀	15.0	60	*	†			
♂ juv.	10.8	60	3.75 h	17 h			
♀	18.1	40	*	†	2/2	0/1	2/3
♂	13.5	40	*	12 days			
♂	14.3	40	*	24 days			
♂	14.4	20	*	†	0/2	1/1	1/3
♂	12.2	20	*	†			
♀	16.7	20	4 h	15 days			
♀	13.6	10	*	†	0/1	0/2	0/3
♀	13.0	10	*	†			
♀	15.4	10	*	†			
♂ juv.	12.8	0	*	†	0/2	1/1	1/3
♀ juv.	14.4	0	*	23 days			
♂ juv.	14.6	0	*	†			

\* Tremors never observed.

† Released in apparent good health at end of experiment.

Table 2. TOXICITY OF ENDRIN TO THE BAT *Eptesicus fuscus*

Sex	Wt. (g)	Dosage (mg/kg)	Time after being dosed		Mortality		
			Until tremors	Until death	♂	♀	Total
♀	20.0	50	1 h	2-2.5 h	—	1/1	1/1
♀	15.9	20	32 h	†	—	2/3	2/3
♀	16.9	20	19 h	118 h			
♀	16.7	20	*	17 h			
♂ juv.	13.0	15	17 h	31 h	1/1	2/2	3/3
♀ juv.	15.4	15	19 h	23 h			
♀	15.9	15	*	32 h			
♂ juv.	13.8	12	*	42 h	2/2	1/1	3/3
♂ juv.	12.7	12	*	17 h			
♀	15.7	12	*	42 h			
♀	15.3	10	*	†	1/1	3/4	4/5
♀	16.3	10	*	66 h			
♂	14.4	10	41 h	†			
♀	17.0	10	*	24 h			
♀	20.5	10	*	24 h			
♂	16.8	8	7 days	†	1/2	1/3	2/5
♂	14.2	8	*	43 h			
♀	13.1	8	*	†			
♀	16.9	8	12 days	†			
♀	17.0	8	7 days	20 days			
♀	18.6	6	*	†	—	2/6	2/6
♀	17.9	6	*	†			
♀	18.4	6	*	†			
♀	16.3	6	*	21 h			
♀	17.6	6	40 h	22 days			
♀	18.0	6	40 h	†			
♀	17.0	5	7 days	14 days	—	2/2	2/2
♀	20.3	5	19 h	6 days			
♀	17.9	4	*	†	—	1/3	1/3
♀	15.6	4	12 days	†			
♀	16.7	4	*	16 days			
♂	17.3	2	*	†	0/3	—	0/3
♂ juv.	12.3	2	*	†			
♂ juv.	13.0	2	*	†			
♀	21.7	1	13 days	†	—	0/1	0/1
♀	25.3	0	*	†			
♀	19.2	0	*	†	1/1	0/5	1/6
♀	15.2	0	*	†			
♀	16.8	0	*	†			
♀	14.4	0	*	†			
♂	15.2	0	*	32 h			

\* Tremors never observed.

† Released in apparent good health at end of experiment.

Results of the experiment are shown in Tables 1 and 2. In the case of dieldrin, deaths occurred at all doses of 20 mg/kg and above. The apparent  $LD_{50}$  is of the order of 70 mg/kg. The approximate  $LD_{50}$  seems to be in the range 20–40 mg/kg. This is not greatly different from the toxicity of dieldrin to the white rat for which the  $LD_{50}$  is reported as 46 mg/kg (ref. 2).

With endrin mortality was noted at doses as low as 4 mg/kg. The  $LD_{50}$  as seen in this experiment was 12 mg/kg, although there was one survivor at 20 mg/kg. The results indicate an approximate  $LD_{50}$  in the range of 5–8 mg/kg. Hayes<sup>3</sup> reported the  $LD_{50}$  as 7.8 mg/kg and 17.8 mg/kg for the female and male white rat, respectively.

Thus the toxicities of dieldrin and endrin are rather comparable in bats and rats. These pesticides do not show the relatively high toxicity to big brown bats that was demonstrated with DDT. This is of interest in that it suggests that the response of bats to DDT is not simply due to their unique heterothermic metabolism.

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### Recording Leaf Movements with a Strain Gauge

In our pilot studies concerning the effect of space environments on the circadian leaf movements, we have devised a small lightweight leaf movement recording system using a strain gauge coupled to an amplifier and a recorder. The need for such a system was dictated by the physical limitations of an orbiting capsule. We have also found that such a system is useful for earthbound experiments. The small size of the sensing unit makes it possible to place several units on a single plant. The monitored plant can easily be placed in a small control chamber and continuous records of several weeks' duration can be obtained when the movements are recorded on a strip chart recorder.

The active element of the leaf movement sensing unit is a strain gauge  $\frac{1}{8}$  in. wide and  $\frac{1}{4}$  in. long. The gauge (EA-09-031DE-120) is made by Micro-Measurements, Inc., Romulus, Michigan. In our experiments, the strain gauge was glued with Eastman '910' adhesive to the centre of a brass shim stock, 0.001 in. thick,  $\frac{1}{8}$  in. wide and  $1\frac{1}{2}$  in. long. The brass shim is formed into a half loop with  $\frac{1}{4}$ -in. tabs at the ends of the half loop. One of the tabs is glued to the leaf petiole and the other to the midrib of the leaf blade with polyvinylacetate ('white glue') (Fig. 1). In the amounts used, the glue was not toxic to the plant organs. The strain gauge is part of the bridge circuit that is supplied with d.c. voltage. The signal generated by the movements of the leaf blade is sent to the amplifier of the Minneapolis-Honeywell Brown recorder and the amplified signal is recorded on the strip chart.

The recording system proposed for the orbiting system has the same bridge configuration but has a Philbrick Researches solid-state amplifier 'PP85A' instead of the recorder amplifier (see Fig. 2). The voltage supply to the amplifier derives its power from a 22.5–45.0 V portable 'B' battery and the voltage is regulated at 15.0 V by the 'IN965' Zener diodes. The differential signal output of this smaller system is at least 0.2 volts with a leaf de-



Fig. 1. Leaf movement sensing unit. The unit is glued to petiole and midrib of a pinto bean leaf.

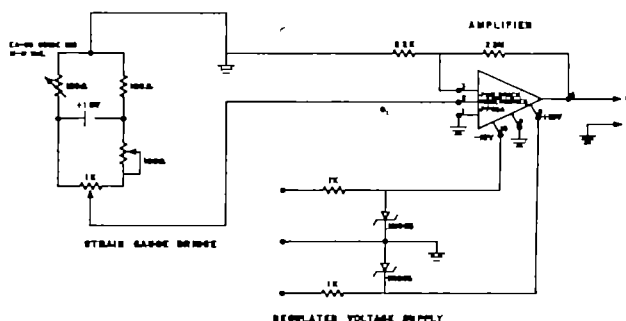


Fig. 2. Leaf movement recording circuitry. The system has 3 sections: (1) Bridge circuit with a strain gauge as the leaf movement sensing unit. (2) Amplifier unit with a Philbrick P85A or PP85A unit wired for 1,000 × amplified output. (3) Regulated voltage supply limited by IN965 Zener diodes.

flexion of around 30°. The weight of this system less power supply is about one ounce and the dimensions are  $\frac{1}{2}$  in. by 1 in. by 2 in.

The sensing unit was tested for temperature effects and for voltage change effects. When the ambient temperature was varied from 27° to 45° C, the leaf angle readings on the Brown recorder varied 2°. Thus a 1° C change in ambient temperature resulted in an error of 0.11° in the leaf angle reading. This error is considered negligible for our work since the temperature variation on our growth chamber can be kept down to  $\pm 0.2^\circ$  C. As a further refinement, the substitution of another strain gauge for the 120-ohm resistor in the bridge will eliminate this error. The inert substituted gauge will act as a standard control. A voltage variation of 1.0 V supplied to the strain gauge resulted in an error of 10.0° in leaf angle reading. However, the voltage supply can be regulated to within  $\pm 0.02$  V under most conditions and the resulting leaf angle error will only be  $\pm 0.2^\circ$ . This error is considered negligible for our experiments.

To test the reliability and accuracy of the system, the strain gauge unit was attached to a primary leaf of a pinto

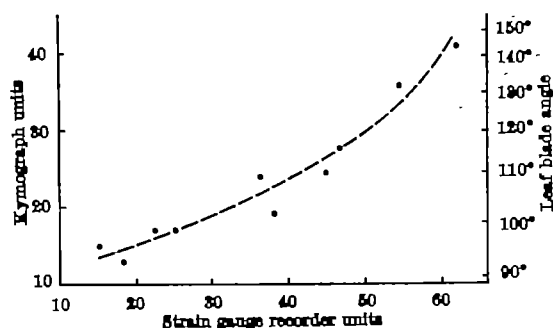


Fig. 3. The curve shows the relation between strain gauge record and kymograph record taken simultaneously from same leaf. Actual leaf angle is given at the right. Horizontal position is given a value of 90° and vertical down position is given a value of 180°. Dotted line depicts the ideal correlation curve.

been. A kymograph was also attached to the same leaf and four days of records were obtained. The kymograph recorded the leaf movements by means of a leverage system that had the leaf attached by means of a thread to one end and a pen to the other end. The pen produced a permanent record on a chart calibrated to read out the leaf angles directly. The chart was wrapped around a drum that turned once a week. The strain gauge signals were recorded on the M. H. Brown strip chart recorder. The results of the strain gauge records were compared with the records of the kymograph. The time sequence of the peaks and dips of the two records were identical. On further examination it was found that the leaf angle values were not directly correlated between the strain gauge records and the kymograph records. On plotting the corresponding points of the kymograph and the strip chart records, it was found that the maximum deviation of the strain gauge records was not more than 5° from the values of the kymograph records (see Fig. 3). Since the peaks and dips on the curves are not displaced in time sequence we feel that the deviation is not large enough to invalidate the strain gauge records for cycle-length studies.

This unit is being used in experiments testing the effects of light-dark cycles on the leaf movements of pinto beans.

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### *Bathycorixa thalassina* (Herrich-Schaeffer), (Hemiptera: Pentatomidae); a Pest of *Theobroma* *cacao* L.

LARGE nymphs and adult *Bathycorixa thalassina* (Herrich-Schaeffer) have recently been found to feed on developing cocoa pods, causing damage to the beans; the small nymphs feed mainly on the leaves and do little damage. The adults have long feeding stylets (about 2.2 cm) which can penetrate the pod cortex (see Fig. 1) and suck liquid from the developing beans; this results in malformed or atrophied beans. In ripe pods the malformed beans are brown instead of pink, and dry, lacking the sugary mucilage which normally covers beans.

The damage is often not evident externally in harvested pods apart from small black spots formed at the feeding

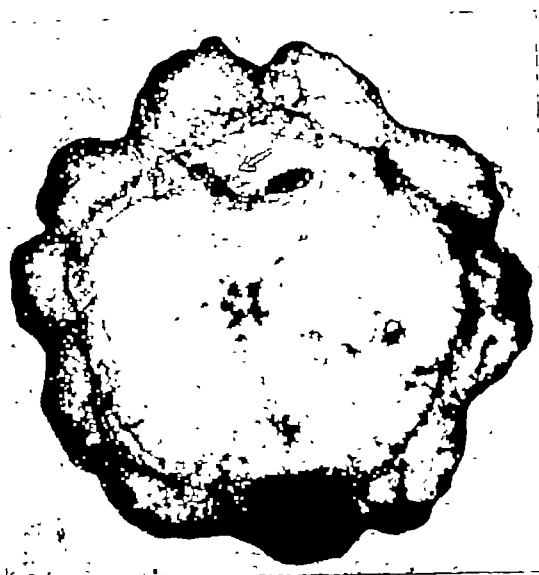


Fig. 1. Sectioned cocoa pod: damaged tissue (top left) shows the path of feeding stylets of *B. thalassina*. Pod diameter about 6 cm.

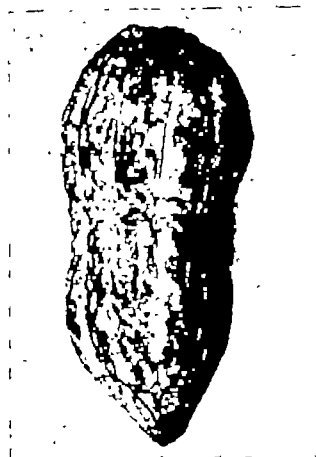


Fig. 2. Damage to young pods results in distorted mature pods often with a 'waist'. Amazon pod, 12 cm long.

lesions. But pods damaged at an early stage fail to swell where the beans have stopped developing, and show a characteristic distortion when fully developed, often in the form of a constriction or 'waist' in the middle of the pods (see Fig. 2).

In some harvests in early 1964 from plots at the Cocoa Research Institute of Ghana more than 20 per cent of the pods were damaged, and all the beans in some pods were imperfect. Damage to the total crop from the Institute was small, but *B. thalassina* populations were first noticed to be high in 1962 and may still be increasing. This species was found on cocoa in several parts of Ghana and at Ibadan in Nigeria in March, 1964.

*B. thalassina* has been reported to feed on cocoa pods in the Congo<sup>1</sup> and Cameroons<sup>2</sup>, causing very small pods to shrivel and fall, but causing negligible damage to full-sized pods; the beans were not affected.

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## ENTOMOLOGY

Hatching Pattern of *Aedes aegypti* Eggs

DURING their work on the effect of ultrasonic waves on the hatching of *Aedes aegypti* eggs Quraishi *et al.*<sup>1</sup> found an interesting pattern of hatching in the controls. *Aedes aegypti* eggs laid overnight were kept in water in Petri dishes and the percentage of larvae hatching out each day was recorded for 20 days. On the first day 2.5 per cent of the eggs hatched out; on the second day this percentage was 18, the highest recorded for any single day. The percentage of eggs hatching out gradually decreased after the second day, until after the 18th day, when there was an upward trend in hatching, and a small peak was discernible between the 14th and 16th days. There was a sudden fall in the number of eggs hatching out after the 16th day and by the 20th day only 0.2 per cent of the eggs hatched out. Thus 37.5 per cent of apparently normal eggs were left unhatched. We therefore decided to follow the hatching pattern of *Aedes aegypti* eggs for 50 days.

Fresh and well-formed eggs laid during the previous night were selected early in the morning and were divided into batches of 50 each and kept in Petri dishes, 13.5 cm in diameter, containing 75 ml. tap water. A few mg of yeast in tablet form was added to each dish and the dishes were kept in incubators at  $88^{\circ} \pm 2^{\circ}$  F. They were taken out for half an hour every 24 h for observation. The first, second, and subsequent days were counted as 24, 48, etc., hours after the eggs had been selected; since at the time of selection the age of these eggs was already 0-16 h, the time lapse will be number of days plus 0-16 h. Fifty replicates were done and the data are thus based on 2,500 eggs.

The pattern of hatching is shown in Fig. 1. Four peaks in the pattern of hatching are easily discernible; the first peak, which is also the highest, appears on the second day and is followed by a much smaller peak between the 14th and 16th days. Very few larvae hatch out after the 18th day, though some larvae come out each day. An upward trend in hatching is again discernible between the 20th and 23rd days representing a third peak which is smaller than the second. Between the 23rd and 43rd days only a few eggs hatch out, not more than 0.2 per cent of the total on any one day. On the 44th and 45th days, however, 0.2 per cent and 0.76 per cent of the eggs hatch out, representing the fourth and smallest peak.

After the completion of this work in Karachi, Pakistan, I worked in Tehran, Iran, and maintained another colony of *Aedes aegypti* from a local strain obtained from the Institute of Parasitology and Malariology, University of Tehran. Although only a few observations were made, a similar pattern of hatching with four peaks was observed. The last peak in this case was also observed on the 45th day.

The occurrence of four hatching cycles, each succeeding one smaller than the previous one, in the eggs of an insect

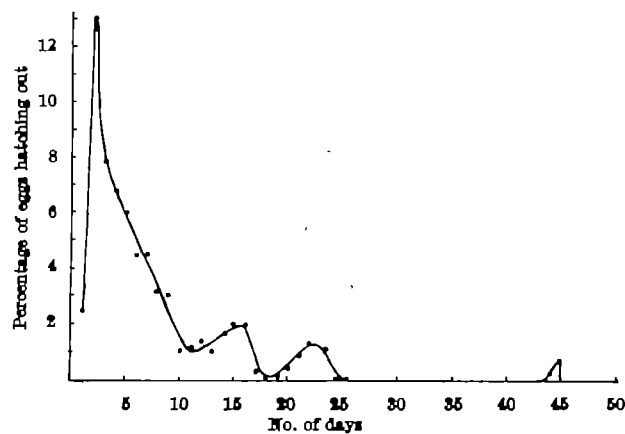


Fig. 1. Hatching pattern of *Aedes aegypti*

the larvae and pupae of which are entirely aquatic, is evidently an adaptation to meet the exigencies of a situation where the first few showers may not provide enough water for the completion of the entire aquatic cycle.

This work was done while I was working as senior scientist in the Pakistan Council of Scientific and Industrial Research and in the CENTO Institute of Nuclear Science, Tehran. I thank Dr. S. Siddiqui, Dr. I. H. Usmami and Dr. M. L. Smith for advice, and Mr. Wahajuddin Roomi and Mr. A. B. M. Lutful Kabir for their help. Dr. Ch. M. H. Mofidi provided the eggs of *Aedes aegypti* in Tehran.

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Rearing *Pieris brassicae* L. Larvae on a Semi-synthetic Diet

A METHOD for breeding *Pieris brassicae* L., the large white butterfly, all the year round has been developed<sup>1,2</sup>, and the larvae have proved to be convenient insects to use for insecticidal tests and other investigations. However, in severe winters cabbage and kale leaves are killed by frost and it becomes difficult to provide food for large numbers of larvae. One possibility, namely using the heart leaves of cabbage from clamps<sup>3</sup>, has not proved to be satisfactory. Furthermore, for investigations on pathogens, it is desirable to have a food which can be sterilized.

Recently Ignoffo<sup>4</sup> described a medium for the mass rearing of *Trichoplusia ni* Hübner, the cabbage looper, which is a modification of that developed by Vandervant and Reiser<sup>5,6</sup>. We have slightly modified this medium and have shown it to be satisfactory for rearing *P. brassicae*:

Formula of medium.

(a) Distilled water	110 ml.
Potassium hydroxide 4 molar	1.6 ml.
Oasein (light white soluble)	12.6 g
(b) Sucrose	12.6 g
Wheat germ ('Bemax')	10.8 g
Cabbage (dried powder)	5.4 g
Salt mixture	3.6 g
Whatman, chromedia cellulose powder, CF11 grade	1.8 g
(c) Choline chloride (10% soln.)	3.6 ml.
Methyl parahydroxy-benzoate (15% in 95% EtOH)	3.6 ml.
Formaldehyde soln. (10% w/v soln.)	1.5 ml.
Vitamin stock	0.8 ml.
(d) Distilled water	200 ml.
Agar (fine Japanese powder)	9 g
(e) L-Ascorbic acid	1.5 g
Aureomycin (veterinary grade)	0.8 g

The composition of the salt mixture (in g) is:  $\text{CaCO}_3$ , 120;  $\text{K}_2\text{HPO}_4$ , 120;  $\text{CaH}_2\text{PO}_4$ , 20;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 40.8;  $\text{NaCl}$ , 67;  $\text{FeSO}_4 \cdot 6\text{H}_2\text{O}$ , 11;  $\text{KI}$ , 0.32;  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ , 2.0;  $\text{ZnCl}_2$ , 0.10;  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.12.

The composition of the vitamin stock (in mg) is: nicotinic acid 600; calcium pantothenate, 600; riboflavin ( $\text{B}_2$ ), 800; ascorbic hydrochloride ( $\text{B}_3$ ), 150; pyridoxine hydrochloride ( $\text{B}_6$ ), 150; folic acid, 150; D-biotin, 12; cyanocobalamin ( $\text{B}_{12}$ ), 1.2; 100 ml. water.

The dried cotton leaf in the original medium (about 1.4 per cent wt.) was replaced by dried cabbage leaf powder. This was prepared by drying cabbage leaves in thin layers in a ventilated oven at  $105^{\circ}\text{C}$  for 15-20 min. The leaves can then be ground by hand with a roller and sieved through a 60-mesh sieve, but it is much quicker, of course, to have them ground in a small Christy-Norris mill fitted with a 0.5-mm mesh screen. The veterinary grade aureomycin soluble powder (Cyanamid of Great Britain) contained 25 g chlortetracycline hydrochloride per pound.

The ingredients listed in (a) are placed in a blender with a capacity of 800 ml. and thoroughly mixed together. The mixed solids (b) are then added with further blending. The solutions (c) are next added, separately, while the blender is running. Meanwhile, the agar solution (d) has been prepared in a water bath. It is cooled to  $70^{\circ}\text{C}$  and added to the mixture. Finally, the ingredients (e) are added and the whole medium is thoroughly blended.

The warm medium was poured into sterilized 1 lb. jam jars to a depth of about 0.5 in. and while still warm each jar was tipped and twisted so as to coat some of the sides. As soon as the medium was cool the jars were turned upside down to prevent unnecessary contamination. They can be conveniently stored at about 12° C. After the larvae were introduced the jars were kept on their sides so that comparatively little frass fell on the medium. Whatman No. 1 filter paper was first used to close the jars as this reduced evaporation and prevented the medium drying out too rapidly, but when the larvae reached the fifth instar the paper was changed for a piece of 'Terylene' gauze as more ventilation was now necessary to prevent conditions in the jars becoming too humid and sticky. About 15 fifth instar larvae can be kept in a jar and of course more younger larvae.

For smaller numbers of young larvae it saves medium to use 2 in. x 1 in. specimen tubes containing about half-an-inch of medium. These tubes can be filled rapidly from a warm water-jacketed dispenser that has been developed. After covering with filter paper the tubes may be stood upside down, either directly on a tray or on gauze, if more ventilation seems necessary. The small, light, plugs of medium seldom fall away from the bottoms of the tubes as is very liable to happen if the 1 lb. jars are kept upside down.

In order to obtain a good rate of growth and normal adults we have found it advisable to supply freshly made medium at about weekly intervals. This was necessary because the medium dried out and possibly because of the growth of contaminating organisms.

On medium (changed weekly) the development from hatching to pupation took about 1-2 days longer than on good cabbage leaves. The yield of pupae, based on the number of first instar larvae, has reached 100 per cent and is usually above 80 per cent. Of these pupae, up to 100 per cent have given normal adults. Pupal weights of larvae bred on medium (average 0.37 g) tend to be just below those of larvae fed on cabbage (average 0.38 g). The adults mated normally and the eggs were fully fertile.

Besides being useful for rearing larvae in winter the medium has other advantages. It provides a more standard diet, it is less likely to be contaminated by pathogens since during the drying process virus and other organisms on the cabbage will be destroyed, it greatly facilitates the counting and examination of young larvae since they are much more easily seen than among leaves, and, finally, it would be more convenient for use by laboratories in cities.

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### Development and Characterization of Resistance to o-Isopropoxyphenyl Methylcarbamate in the Mosquito *Culex pipiens quinquefasciatus* Say

THE possibility of development of resistance to carbamate insecticides in mosquitoes, and the character of such resistance, are questions of profound importance in the vector control and eradication programmes of public health agencies. With the gradual elimination of organochlorine insecticides in many parts of the world due to

development of resistance, attention has been directed to substitute insecticides, particularly in the organophosphorous and carbamate groups<sup>1,2</sup>. Resistance to certain organophosphorous insecticides has already appeared in strains of *Culex* and *Aedes*, especially in areas where these compounds have been used routinely as larvicides<sup>1,3</sup>. This phenomenon has created even greater urgency for research into the resistance potential of mosquitoes to the carbamate insecticides.

For the past four years we have been investigating the development of resistance in *Anopheles albimanus* and *Culex pipiens quinquefasciatus*, through selection pressure with m-isopropylphenyl methylcarbamate ('Heracles' AC-5727). Interim progress reports on this work have been published<sup>4,5</sup>. Selection pressure on the above species for 21 and 50 generations, respectively, did not materially enhance their tolerance to the selective agent. The observed shift in the dosage-mortality regression lines represents an increase in tolerance of only 2.7 and 2-fold, respectively. Although very encouraging, these results were only of academic interest since the carbamate used appears to have no commercial possibilities due to its high mammalian toxicity.

More recently our attention has been directed to a related carbamate, 'Bayer 89007' (o-isopropoxyphenyl methylcarbamate), which is of considerably lower mammalian toxicity (oral, rats, 175-200 mg/kg). A strain of *Culex pipiens quinquefasciatus*, containing both DDT- and dieldrin-resistant individuals was colonized from Southern California, and subjected to larval selection pressure with the above carbamate at approximately the  $LC_{50}$  level. Resistance increased rapidly in the first selected generation and more slowly thereafter, reaching a 10.75-fold level in the 17th generation. The  $LC_{50}$  values of the parental and  $F_{17}$  generations were 0.29 and 3.12 p.p.m., respectively ( $b$  values 3.42 and 9.04, respectively).

The cross-resistance characteristics of the selected strain toward various carbamate and organophosphorous insecticides are shown in Table 1. It is evident that certain specificity of the resistance mechanism exists for the *ortho* substituted phenyl methylcarbamates (compounds VII, VIII, XI); this specificity appears to be of low level, however, since cross-resistance extends also to a variety of other carbamates as well as to the organophosphorous insecticides malathion and fenitrothion. Similarly there was a small intensification of the initial DDT and dieldrin resistances of the parental strain.

Table 1. RESPONSE TO VARIOUS INSECTICIDES IN SUSCEPTIBLE AND RESISTANT<sup>a</sup> LARVAE OF *Culex pipiens quinquefasciatus*

Compound	Susceptible strain $LC_{50}$ (p.p.m.)	Resistant strain Resistance ratio†
Methylcarbamate		
I m-sec-butylphenyl	0.024	4.6
II m-sec-amylphenyl	0.046	5.3
III m-isopropylphenyl	0.05	5.7
IV 2-chloro-3-isopropylphenyl	0.10	4.0
V m-tert-butylphenyl	0.11	6.4
VI 4-methylthio-3,5-xylyl	0.13	3.8
VII o-isopropoxyphenyl	0.29	10.8
VIII o-isopropoxyphenyl	0.41	8.3
IX 4-dimethylamino-3,5-xylyl	0.42	5.2
X 6-chloro-3,4-xylyl	0.59	4.8
XI o-propargyloxyphenyl	0.76	3.6
XII 1-naphthyl	0.77	5.2
XIII m-propargyloxyphenyl	1.17	1.9
XIV fenitrothion	0.0045	4.2
XV malathion	0.018	1.6
XVI malathion	0.06	3.1

<sup>a</sup> Selected with o-isopropoxyphenyl methylcarbamate

†  $LC_{50}$  Resistant strain

$LC_{50}$  Susceptible strain

A substrain separated from the  $F_7$  selected generation and reared without pressure for an additional 10 generations revealed the stability of the character of resistance. The  $LC_{50}$  values of the  $F_7$  and the  $F_{17+10}$  (unselected) generations were 2.16 and 1.50 p.p.m., respectively ( $b$  values 8.61 and 8.46, respectively).

The metabolic efficiency of the resistant and susceptible strains was compared by the use of <sup>14</sup>C-labelled o-isopropoxyphenyl methylcarbamate. Groups of 30 fourth-

instar larvae were exposed for 2 h to a concentration of 10 p.p.m. of the labelled carbamate; they were afterwards homogenized, extracted with acetonitrile, chromatographed on propylene glycol paper, and tested for radioactivity on an automatic scanning device ('Vanguard Autoscaner 880'). The relative proportion of metabolites recorded was 12.4 per cent in the susceptible strain and 30.4 per cent in the resistant; this amounts to an increase of 2.5-fold in carbamate detoxication efficiency of the resistant strain.

The genetic basis of resistance to *o*-isopropoxyphenyl methylcarbamate was investigated by testing the offspring of reciprocal crosses between the susceptible and resistant strains. Resistance in the  $F_1$  generation was intermediate. The observed responses of the  $F_1$  and back-cross generations were significantly different from those which could be expected on the basis of monofactorial inheritance. The proportion of fully susceptible and fully resistant individuals segregating in the  $F_2$  was consistently below expectation. It was, therefore, concluded that resistance to *o*-isopropoxyphenyl methylcarbamate in our strain of *Culex*, at its present level of resistance, is controlled by a polygenic system.

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## MICROBIOLOGY

### Functional Homology of the Sex-Factor and Resistance Transfer Factors

DRUG-RESISTANT bacteria can emerge from sensitive populations by acquiring a 'resistance factor' ( $R$ -factor)<sup>1,2</sup> from another strain by conjugation. Genetically, an  $R$ -factor behaves as an extrachromosomal structure<sup>3</sup> belonging to the same class of elements as the transmissible sex-factor ( $F$ )<sup>3,4</sup> of *Escherichia coli*, and its derivatives (designated  $F'$ ) in which a fragment of bacterial chromosome has become associated with  $F$  (ref. 5). In an  $R$ -factor a collection of genes determining resistance to one or as many as six different drugs is transferred as a single unit of inheritance linked to a resistance transfer factor ( $RTF$ ) responsible for the transmission<sup>6</sup>.

Cells carrying  $F$  or  $F'$  factors are recognized by their ability to conjugate and donate chromosome to female ( $F^-$ ) cells, in which genetic recombination may then occur. Bacteria with  $F$  or  $F'$  produce a specific antigen absent from  $F^-$  cells<sup>7</sup> and are sensitive to a group of  $F$ -specific RNA phages which do not attack  $F^-$  cells<sup>8</sup>. These properties suggest that  $F$  determines the formation of a surface structure responsible at the same time for (a) conjugation, (b) serological specificity, and (c) uptake of specific phage; the phage receptor has in fact been identified as a specialized pilus<sup>9</sup>. Despite the overall similarity of the  $F'$  and  $R$  factors, they differ in certain important respects which has generally been taken to mean that the respective fertility factors,  $F$  and  $RTF$ , are not the same: (1) conjugation occurs much less frequently with  $R$  than with  $F$ , as measured by the frequency with which each is transmitted; (2) chromosomal transfer with  $R$  occurs at a very low frequency, if at all<sup>6</sup>; (3) the  $F$  antigen is apparently absent from  $R^+$  cultures<sup>10</sup>; and (4) such cultures are apparently insensitive to the  $F$ -specific phage<sup>11</sup>. Moreover,

(5) with some exceptions<sup>12</sup>, the  $R$ -factor inhibits the expression of  $F$ : when  $R$  is introduced into a cell which is already  $F^+$ , the properties of the cell dependent on  $F$  disappear, and only reappear when the  $R$ -factor is lost<sup>11,12</sup>.

The apparent distinctions between  $RTF$  and  $F$  could alternatively be explained by supposing that the two factors are really identical except in one respect—that whereas the fertility functions of  $F$  are normally expressed in the majority of an  $F^+$  population, those of the  $RTF$  are expressed in only a small minority of  $R^+$  bacteria. Furthermore, it can be postulated that this restriction of  $RTF$  function is due to a repressor, made by  $RTF$  but not by  $F$ , but which can also act on  $F$  when both factors are present in the same cell. Evidence that there is repression of this sort comes from the behaviour of cells which have only recently acquired an  $R$ -factor<sup>14</sup>. Such cells transmit the factor with much greater frequency than cells which have carried it for many generations, indicating that there is a lag between the cell acquiring the  $RTF$  and the development of its repressor.

Even if the bacterial surface characters determined by  $RTF$  and  $F$  were identical, the fact that  $RTF$  is normally only rarely expressed would be enough to account for the reported failure of the  $F$ -phage to lyse or to adsorb to  $R^+$  cultures. Two sorts of experiment were therefore done: (1) to look for a minute proportion of cells sensitive to the  $F$ -phage in established  $R^+$  cultures; and (2) to find out if this proportion is greatly increased in a high-frequency resistance transfer ( $HFR$ ) culture which has just received the  $R$ -factor by conjugation. An  $HFR$  system consists of broth inoculated with a small number of established  $R^+$  cells and a much larger number of  $R^-$  cells through which the factor can spread.

The presence of small numbers of cells capable of propagating a phage can be detected by adding a known quantity of the phage and looking for a subsequent increase in titre<sup>15</sup>.  $F$ -specific phage was added to  $F$ -cultures of *E. coli* K12, infected and uninfected with  $R$ -factor, and assayed at intervals. From preliminary experiments without antiphage serum, we concluded that sensitive cells, if present, must be in such small numbers that they could only be detected if residual unadsorbed phage was eliminated. To achieve this, and at the same time to maintain a high bacterial concentration, the bacteria were mixed with a 20–100 fold excess of phage for a short time (10–15 min at 37° C) in tryptone broth containing M/500 calcium chloride, which these phages require for optimal infection of  $F^+$  bacteria<sup>16</sup>. The mixture was then diluted into antiphage serum from which the antibacterial antibodies had been absorbed, and after a further 10 min, the bacteria were collected and washed on a membrane filter which was then rinsed in a flask of broth. (Control counts showed that virtually all the bacteria were recovered by this technique.) The flask was assayed for phage immediately and again after 2-h incubation, using a standard indicator strain (*E. coli* K12  $HfrB1$ ), which gave entirely reproducible plaque counts on plates incubated at 42° C (ref. 17). The small proportion of residual unneutralized phage particles not eliminated by antiserum and filtration was measured by including a control suspension of  $F^-R^-$  bacteria which had been boiled for 10 min. After allowing for this, the number of plaques in the initial count should measure the number of infectable bacteria. This proved to be negligible in some cultures, which could mean that sensitive bacteria were either totally absent or present only in very small numbers. These two possibilities were distinguished by the second assay made 2 h after infection: in the absence of sensitive bacteria, the plaque count should not increase, but, since these phages have a very large burst size of  $10^3$ – $10^4$ , the presence of even a few sensitive cells should be detected. The results of the second assay showed that phage always increased in the  $R^+$  cultures, but never in  $R^-F^-$  strains (see Table 1).

The same experiment made with  $HFR$  mixtures gave spectacular results: it was clear that these unrepressed



Table 1. REACTIONS OF  $R^+$  CULTURES WITH  $F$ -SPECIFIC PHAGE

Bacteria	Initial plaque count	Plaque count after 2 h	Increase $\times$
711 ( $K12 F^-$ ) budded	$1.7 \times 10^6$	$1.9 \times 10^6$	—
711 ( $K12 F^-$ )	$8.9 \times 10^5$	$7.0 \times 10^5$	—
711 + $R_{H_1}$	$1.4 \times 10^6$	$1.5 \times 10^6$	$1.1 \times 10^6$
711 + $R_{H_2}$	$1.2 \times 10^6$	$1.9 \times 10^6$	$1.5 \times 10^6$
J8 ( $K12 F^-$ )	$7.7 \times 10^5$	$7.5 \times 10^5$	—
J8 + $R_{H_1}$	$1.6 \times 10^6$	$2.4 \times 10^6$	$1.5 \times 10^6$
J8 + $R_{H_2}$	$1.7 \times 10^6$	$1.9 \times 10^6$	$1.1 \times 10^6$
HFR $\pi$ mixture*	$3.1 \times 10^6$	$5 \times 10^6$	$1.7 \times 10^6$
711 $R_{H_1}$ + 711			
HFR $\pi$ mixture†	$3.4 \times 10^6$	$2.9 \times 10^6$	$8.5 \times 10^4$
711 $R_{H_2}$ + 711			
$K12 HfrB1$ ( $F^+$ indicator)	$8.4 \times 10^5$	$1.0 \times 10^{11}$	$1.2 \times 10^5$

The bacterial strains were all derivatives of *E. coli* K12. The  $R$ -factors,  $R_{H_1}$  and  $R_{H_2}$ , found in naturally-occurring drug-resistant salmonellae, were transferred directly to these strains by mixed culture.  $F$ -specific phages  $\pi$ 52 and  $\pi$ 2 were used, with an antiserum prepared against  $\pi$ 2 which neutralised both phages at the same rate ( $K=4,500$ ).

\*10 times and † $1.3 \times 10^6$  times as many donor cells transmitted resistance as in the corresponding established  $R^+$  cultures.

cultures contained a vastly greater proportion of infectable bacteria than the established  $R^+$  cultures, and that the proportion occasionally approached that in cultures carrying  $F$ .

The surface structure, which mediates conjugation by  $R^+$  strains, is determined by  $RTF$ , but has been thought of as distinct from that determined by  $F^+$ . The disappearance of  $F$  function in  $R^+F^+$  bacteria has consequently been attributed to replacement or alteration of the  $F$ -specific surface structure through an epistatic effect of the  $RTF$  suppressing the expression of  $F$  (ref. 13). Our results, on the other hand, suggest that the surface structure determined by  $RTF$  is simply the  $F$ -specific structure expressed in only a few cells, whether the culture is  $R^+F^+$  or  $R^+F^-$ . This view is strengthened by the finding that in cultures which have just become  $R^+$ , and in which repression has evidently not had time to be established, the  $F$ -phage infects a much greater proportion of the cells—almost as many as in an  $R^+$  culture. The unrepressed state of the  $RTF$  in newly-infected cells is only transient<sup>14</sup>, and it is for this reason that plaques are not seen when such cells are plated with  $F$ -specific phage; for by the time the indicator lawn has grown, the normal repressed state of the factor has supervened, and the bacteria are thus phenotypically  $F^-$ .

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'pure conditions' was used. There was no growth of the endophyte outside surface-sterilized root-nodule slices incubated for several weeks on agar media of widely differing nutritional composition, including those used for the cultivation of plant tissues *in vitro*. However, when these tissues were crushed in sterile water, a suspension, which although sterile according to the usual microbiological criteria, still produced normal root nodules in sterile alder seedlings growing in test-tubes on a low-nitrogen medium. This would imply that the *Alnus* endophyte is an obligate symbiont and could perhaps be propagated inside alder tissue or root-nodule tissue grown *in vitro*.

Tissue cultures of *Alnus glutinosa* from normal root and root-nodule tissue were started in 1962. Preliminary tests with a number of nutrient media showed that Hildebrandt's<sup>1,2</sup> basal mineral salts-sucrose-agar medium supplemented with coconut milk (150 ml./l.),  $\alpha$ -naphthaleneacetic acid (0.1 mg./l.) and calcium pantothenate (2.5 mg./l.), that is, *O*-medium, gave the best growth *in vitro*. Additional supplements of 'Edamin' (casein hydrolysate), glycine, kinetin, 2,4-dichlorophenoxyacetic acid (2,4-D), etc., did not stimulate this growth.

Small nodules (2–3 mm) from plants grown in test-tubes were surface-sterilized by consecutive application of 96 per cent ethanol for 1 min, 0.5–0.6 per cent sodium hypochlorite and detergent ('Teepol') for 15 min, followed by seven rinsings with sterile water. The nodules were then pre-incubated for several weeks on nutrient agar or *O*-medium for tests of visible 'sterility'. Contamination was usually heavy.

Tissue cultures of root nodules were obtained by placing the surface-sterilized whole nodules or nodule slices on *O*-medium and incubating these in the dark at 27° C. Tissue series exposed to light did not show better growth. The callus tissues produced—usually by bursting of the nodule cortex—were transferred to a new medium of the same composition. Callus tissue of normal roots was obtained from root tips of germinating seeds growing on *O*-medium agar enriched with 2,4-dichlorophenoxyacetic acid (*D*-medium). For maintenance of the root-callus cultures, however, *O*-medium was more favourable.

The tissues were re-transferred to new media about once in 3–4 months, showing the very slow growth rate of alder tissue. After the original isolation of the explants the tissues were transferred 9 times to new media without any reduction in growth. The initial tissues were about 2 mm in diameter and these gave rise to callus tissues of sometimes 25–40 mm in diameter, which represents a volume increase of 2,000–8,000 times the original tissue size. Fig. 1a and b shows a 4-month-old root-nodule callus of *Alnus*.

Most of the root-nodule tissues isolated produced no visible growth in the medium in subsequent transfers. A few, however, did produce actinomyce growth, while the host tissue still continued to propagate (Fig. 2). Sometimes such tissues produced better growth than the apparently 'sterile' tissues. However, ultimately the former tissues produced less growth and finally were overgrown by the actinomyce and died. Since in this way several actinomyce species were isolated, it was doubted that these constituted the true alder endophyte, although one of them may have been the actual endophyte.

To establish whether the *Alnus* endophyte invaded newly-formed host cells in the growing callus tissue, morphological observations of paraffin-wax sections of the tissue were made. In addition the nodule-producing capacity ('virulence') of the tissue was ascertained in inoculation tests with sterile alder seedlings grown in test tubes.

These experiments gave the following results. Host tissue was propagated by transferring a locally fast-developing tissue part to a new medium. This procedure caused a certain selection in cells, which resulted in the

## **In vitro Cultivation of Alder Root-nodule Tissue containing the Endophyte**

ONE of the main problems in non-leguminous nitrogen fixation is the isolation of the endophyte.

In attempts to isolate the symbiont of *Alnus* root nodules, Quispel's method<sup>1</sup> of obtaining the endophyte in

isolation of endophyte-free cells from root-nodule tissue containing the endophyte: apparently host-cell propagation was faster than endophyte transmission. But other transfers did not lose the endophyte. Hyphal structures were observed in the large vacuolated cells typical of callus-tissue cultures, and in the intercellular spaces between the host cells (Fig. 3). However, these tissues when minced and added to sterile alder plants did not



Fig. 1. Callus of root-nodule tissue grown for 4 months *in vitro* in the dark at 27° on C-medium. In some cases the tissue growth is compact and white (a), in other instances more loose and tends to darken with time (b). The differences are, however, not strict; many intermediate growth types are found ( $\times 10$ )



Fig. 2. Root-nodule callus proliferating in spite of actinomyces growth outside the tissue in the agar. Apparently, this actinomyces prefers to grow at lower oxygen tension, as it penetrates the agar forming a broad ring at its surface. ( $\times 10$ )



Fig. 3. Intracellular actinomyces growth in root-nodule callus tissue grown *in vitro*. Only hyphae are observed; note the plant-cell nucleus in the centre of the host cell. Methylene-blue preparation. ( $\times 950$ )



Fig. 4. Sterile alder seedling showing an ineffective root nodule produced by inoculation of root-nodule callus tissue containing the endophyte. Note the pale (yellow) colour of the leaves due to nitrogen deficiency. ( $\times 1.0$ )

produce normal nodulation. In most cases no nodules were formed and within a few months these plants showed severe nitrogen-deficiency symptoms. In a number of plants, however, nodules were produced, but these nodules were ineffective (Fig. 4). Microscopical investigation of the latter nodules showed that they contained the endophyte intracellularly as very fine hyphae, but 'vesicles', or 'bacteria-like cells' typical for normal *Alnus* root nodules, were not observed. It is remarkable that ineffective root nodules occasionally produced by suspensions of normal effective root nodules showed the same internal features.

'Vesicle' formation, therefore, seems to be particularly associated with nitrogen-fixation. As shown by us in electron microscopic work<sup>4</sup>, the 'vesicles' contain many internal cytoplasmic membranes. An analogous observation was made by Dart and Mercer<sup>5</sup> in rhizobial cells of legume nodules. Hence, it may be that these internal cytoplasmic membranes have a special function in nitrogen fixation. The idea that certain membranes play a part in the incorporation of molecular nitrogen is not new. Bergersen<sup>6</sup> has already suggested this in soybean root nodules for the membrane envelopes; however, outside the bacterial cell, enclosing a number of bacteroids.

Root-nodule tissue *in vitro* produced large iso-diametric callus cells, occasionally with clusters of smaller cells due to internal sub-division. Rarely, some tracheal elements with scalariform thickenings of the cell wall were observed. No endophyte differentiation in the callus tissue was found. Only hyphae occurred (Fig. 3); structures like 'vesicles' and 'bacteria-like cells' characteristic for normal root-nodule tissue were entirely lacking.

This investigation shows that its ultimate purpose—the effective nodule-endophyte of *Alnus* in symbiosis with its host tissue *in vitro* culture—has not yet been fully achieved. Alder root-nodule tissue grown *in vitro* and containing its endophyte is a more simple system than that obtained in leguminous symbiosis where, so far, only nodulated isolated roots have been obtained. Direct cultivation of root-nodule tissue containing the endophyte in 'pure' culture will contribute considerably to the study of the physiology of the symbiosis and nitrogen fixation, since substances normally produced by the shoot or root can be excluded and substances, the effects of which are to be tested, can be added to the medium in known amounts.

This communication is a continuation of earlier investigations<sup>1,2</sup> of the physiology of the alder root-nodule symbiotic association.

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## RADIOBIOLOGY

### Assimilation and Fermentation Patterns of Osmophilic Yeasts in Sugar Broths at Two Concentrations

Osmophilic yeasts commonly found in intermediate sugar refinery products are strains of *Saccharomyces rouxii* and *S. mellis* with *Torulopsis* spp. which normally grow more slowly under laboratory conditions.

Recent isolates of *S. rouxii*, *T. dothila*, *T. globosa* and *T. apicola* showed differences in reaction where: (1) The ability to ferment various sugars was compared at concentrations of 2 and 10 per cent w/v in yeast extract broth; sterilized in McCartney bottles with Durham tubes at 5 lb./in.<sup>2</sup> for 15 min. Results are summarized in Table 1. (2) The ability to assimilate various sugars was compared at concentrations of 1 and 10 per cent w/v in Difco 'YNB' broth (filter-sterilized and dispensed as 5 ml. vol. into pre-sterilized, capped tubes). Results are summarized in Table 2.

Inocula were taken from fresh slants on osmophilic agar<sup>1</sup>, incubated for 72 h at 30° C, washed and centrifuged in sterile Ringer's solution and, finally, re-suspended in 10 ml. sterile Ringer's solution. One drop of suspension was used per tube.

The variation in reaction does not appear to be due simply to osmotic pressure; as if 9 per cent of the sugar is replaced by an equivalent amount of lactose (negative in every case) the stimulation of the higher concentration was not demonstrated.

Anomalous fermentation patterns were reported last year by J. Santa Maria<sup>2</sup> for *S. rouxii* isolated from sugar cane molasses, but tested at standard sugar concentrations.

All the yeasts were isolated from situations of very high sucrose concentrations. They sub-cultured more readily

Table 1. FERMENTATION TESTS

Yeast % sugar	<i>S. rouxii</i>		<i>T. dothila</i>		<i>T. globosa</i>		<i>T. apicola</i>	
	2%	10%	2%	10%	2%	10%	2%	10%
Glucose	++	+	++	++	±	++	-	++
Sucrose	-	+	+	++	±	++	-	++
Maltose	-	++	-	-	-	-	-	-
Galactose	-	-	-	-	-	-	-	-
Raffinose	-	-	-	++	±	++	-	-
Lactose	-	-	-	-	-	-	-	-

Table 2. ASSIMILATION TESTS ON *S. rouxii* AND *T. dothila* ONLY  
(the other two species showed less obvious differences)

Yeast % sugar	<i>S. rouxii</i>		<i>T. dothila</i>	
	1%	10%	1%	10%
Glucose	++	++	++	++
Sucrose	-	-	++	++
Maltose	++	++	-	++
Galactose	-	+	-	+
Raffinose	-	-	++	++
Lactose	-	-	±	-

++ = positive; - = negative; + = weakly positive, ± = variable.

on an osmophilic or semi-osmophilic medium; growth on wort agar or in wort broth is usually very poor. The use of broths with 10 per cent sugar was adopted rather than a higher value because of the low solubility of some sugars such as lactose.

Similar tests on cultures from the Centraalbureau voor Schimmelcultures Delft do not show these variations. The problem arises, therefore, as to whether the incorporation of what are really ecological variations in a test is justified (a parallel case is the use of Koji extract by Onishi<sup>3</sup>). Yeast identification is essentially the 'building-up' of a picture by a number of tests, so ecological modifications must surely be considered. In the case of the osmophilic yeasts, this character may even be lost after growing in media of lower concentrations of sugar for a period<sup>4</sup>.

We consider, therefore, that this group of yeasts should: (a) always be stored in an osmophilic medium which retains these typical properties; (b) biochemical tests should, so far as possible, be adapted to the ecology of the organisms and be carried out in broths containing a minimum of 10 per cent sugar w/v as this produces more consistent results.

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## VIROLOGY

### Resistance to Sigma Virus Infection in *Drosophila*

*Drosophila melanogaster* is subject to infection by the sigma virus, either by virus carried in the fly gametes or by extracts from infected flies. Infected flies somehow become 'CO<sub>2</sub>-sensitive' and thereafter are fatally poisoned if subjected to a dose of CO<sub>2</sub>, which is merely anaesthetic to virus-free flies. Plus<sup>1</sup> showed that the time lag (incubation period) between a sigma injection and the onset of CO<sub>2</sub>-sensitivity is a measure of the injected dose, and sigma extracts are now usually titred by using them as inocula and measuring the resultant incubation period in recipient adult flies.

Brun<sup>2</sup> recently reported, for a certain dose, that the incubation time increases in direct proportion to the age of the recipient fly at injection. Further information concerning this age effect is important to the standardization of the assay for sigma virus and to understanding the course of the infection. In addition, the age effect may be related to the poorly understood general phenomenon whereby virus grows slowly or is less pathogenic in aged host tissue, an effect reported for plant<sup>3</sup> and animal<sup>4</sup> viruses.

The influence of imaginal age on the incubation time was tested in the present study by injecting virus extracted from the *Lw Drosophila* strain into reference-strain flies according to procedures previously described<sup>5</sup>. Twenty-five to 30 flies of each age were injected and the average incubation times calculated. The results obtained were similar to Brun's except that, under these conditions, the age-dependence of the incubation time was not linear. As in Brun's study, females required longer incubation times than did males (Fig. 1).

When incubation period is used to estimate inoculum titre, age differences among recipient flies can lead to significant discrepancies. For example, a 17-h difference

in incubation time such as that displayed between newly emerged males and males 12 h old (Fig. 1) leads to a two-fold difference in titre estimate. The standard curve for converting incubation time to inoculum titre has been published elsewhere<sup>4</sup>.

A control experiment was performed in order to ensure that all flies received the same amount of inoculum. There is negligible bleeding following an injection, but because the fly exoskeleton hardens with age, old flies might have accepted less inoculum fluid than young ones. Therefore, at the same time that the flies were injected with virus, an aliquot of five males of each age was injected with randomly labelled leucine-<sup>14</sup>C (0.25  $\mu$ l. of specific activity 0.49  $\mu$ c./ml.). These were squashed immediately on to planchets and then counted in a Nuclear Chicago gas-flow counter.

The average number of counts recovered per individual fly for each age is shown in Fig. 1. The variation in inoculum size as measured by recovered counts is too small and random to account for the observed changes in incubation time and, therefore, indicates that the age effect depends on the *Drosophila* physiology.

A consideration of the sequence of events following the injection of sigma into imagoes suggested that the increase in incubation time might be related to the rate of virus multiplication. It is known that the injection of strain-*La* virus into young imagoes is followed by the multiplication of the virus to a plateau level of  $10^4$ – $10^5$  infectious units per fly. The recipient fly generally becomes CO<sub>2</sub>-sensitive at the time that the virus titre reaches the plateau level<sup>1,2</sup>. The fact that the incubation period was increased in old flies suggested that the virus multiplied more slowly in old flies and reached the plateau value later.

Table 1. TIME REQUIRED FOR INFECTED FLIES TO BECOME CO<sub>2</sub>-SENSITIVE (INCUBATION TIME)

Ageing temperature (°C)	Imaginal age at injection (days $\pm$ 0.063)	Incubation time (days)
25	0.21	7.9
25	13.00	12.1
20	0.17	7.8
20	15.00	12.6

In order to test this hypothesis, virus was injected into young and old flies and the rate of virus multiplication was measured. The procedures used were the same as reported previously<sup>4</sup> except that the reference-strain flies used to assay the virus were 3–7 h old. The results are presented in Table 1 and Fig. 2. The virus doubling time during log phase was indeed increased in old imagoes as compared with young ones. This was true whether the flies

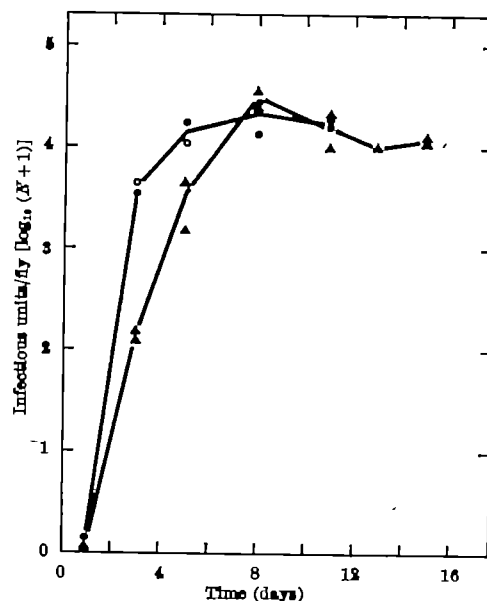


Fig. 2. Growth curves for injected virus.  $X$  is the average number of infectious units/fly. Virus was injected at zero time into flies aged 2 h after eclosion (circles) and into flies aged 13–15 days after eclosion (triangles). Flies had been raised and aged at 25° C (solid symbols) or at 20° C (open symbols).

had been raised and aged at 25° or 20° C. However, the increase in incubation time was greater than expected from the observed lag in virus multiplication. The virus titre plateau in old imagoes was reached about two days later than in young ones, but CO<sub>2</sub>-sensitivity was delayed for two days longer. Therefore, under these conditions, the appearance of CO<sub>2</sub>-sensitivity is dependent on factors in addition to the virus content of the injected fly, and the absence of CO<sub>2</sub>-sensitivity does not preclude a high virus titre in the fly.

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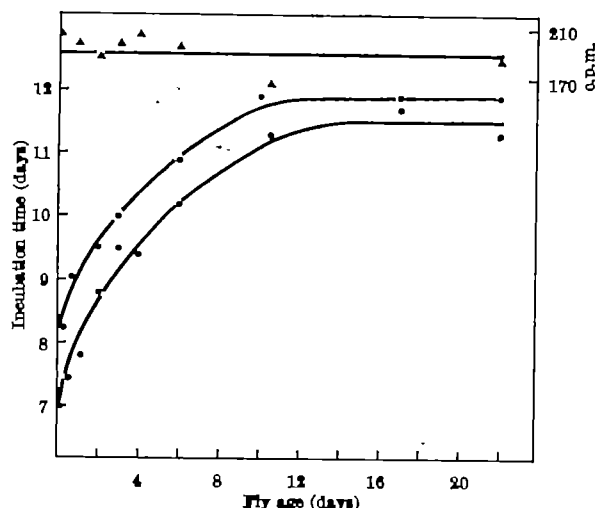


Fig. 1. The time required (incubation time) for flies of various ages to reach CO<sub>2</sub>-sensitivity after inoculation with virus. All males (○) and females (●) received identical inocula. Fly age is measured from eclosion. Labelled leucine was injected into aliquots of males at the same time. The average number of recovered counts/fly is shown above (▲).

### Inhibition of Encephalomyocarditis Virus Replication by Simple Phosphonic and Carboxylic Acids

In view of our recent finding<sup>1</sup> that multiplication of encephalomyocarditis virus (EMC) can be inhibited by non-cytotoxic concentrations of *p*-nitrobenzylphosphonic acid, it appeared worthwhile to investigate the antiviral effect of other phosphonic acids and their carboxylic analogues. It was our hope that the investigation might give some information about the relationship between the chemical structures of the compounds and their antiviral activity, and facilitate the screening of drugs which may be useful in the chemotherapy of viral diseases. In this communication we report the effect of several simple compounds of diverse structure on the replication of EMC virus in the mouse embryo tissue culture.

The EMC virus was maintained by passage in mouse brain and tested in primary mouse embryo (ME) tissue cultures. The commercial carboxylic acids and phosphonic compounds, synthesized by one of us (P. M.) according to the methods indicated in references to Table 1, were used in this work.

Table 1. THE EFFECT OF VARIOUS COMPOUNDS ON THE MULTIPLICATION OF HMO VIRUS IN MOUSE TISSUE CULTURE

No.	Compound* (acid)	Ref. No.	Formula	Concentration ( $\times 10^{-5}$ M)	Antiviral activity				Cytotoxic concentration ( $\times 10^{-5}$ M) <sup>‡</sup>	No. of experi- ments
					Protection against OPH†		% Inhibition of virus multiplication‡			
					Mean	Range	Mean	Range		
1	2	3	4	5	6	7	8	9	10	11
1	Salicylic	—		2	> 2	> 2	99	99-99.9	> 10	10
2	Acetylsalicylic	—		2	> 2	> 2	94	85-99	> 10	5
3	p-Aminosalicylic	—		2	> 2	> 2	94	90-99.7	> 10	5
4	Dibenzylphosphinic	5		2	> 2	> 2	98	96-99.5	> 10	10
5	p-Nitrophenylacetic	—		1	> 2	> 2	97	94-99.8	2.5	5
6	p-Nitrobenzyl- phosphonic	3		2	1.5	1-2	90	90-98	> 20	30
7	o-Nitrobenzyl- phosphonic	4		2	1.5	1-2	90	85-92	> 20	3
8	Benzylphosphonic	6		5	1	0.5-1.5	88	80-96	> 20	4
9	Phenylacetic	—		5	0.5	0.5	85	80-90	> 10	2
10	bis(β-Phenyl-ethyl) phosphonic	4		2	1	0.5-1	78	40-95	> 10	8
11	p-Aminobenzyl- phosphonic	7		5	0.5	0-0.5	70	60-80	> 20	4
12	β-Phenyl- ethylphosphonic	8		5	0.25	0-0.5	40	0-80	> 20	4
13	p-Nitrobenzyl- phosphonic	9		5	0.5	0-1	60	50-70	> 20	8
14	p-Aminophenyl- methylphosphinic	10		5	0	0	0	0	> 20	8
15	p-Aminophenyl- propylphosphinic	10		5	0	0	0	0	> 20	6
16	p-Nitrophenyl- methylphosphinic	10		5	0	0	80	10-80	> 20	6
17	Phenylphosphonic	11		5	0	0-0.5	16	0-50	> 20	7
18	Ethylphosphonic	12		5	0	0-0.5	23	0-70	> 20	6
19	2,3-Dihydroxy- propylphosphonic	13		5	0	0-0.5	80	80	> 20	5
20	n-Butylphosphonic	12		5	0	0-0.5	70	60-80	> 20	5
21	α-Amino γ-phos- phonobutyric	14		0.5	0	0	0	0	1	4
22	Benzoic	—		5	0	0-0.5	70	70-75	> 10	4
23	p-Nitrobenzoic	—		2	0.25	0-0.5	80	70-90	10	2
24	p-Aminobenzoic	—		5	0.25	0-0.5	60	40-80	> 10	2
25	Phenylpropionic	—		5	0.5	0.5-1	80	80	> 10	3
26	2,4-Dinitrophenylpro- pionic	—		2	0	0	0	0	5	2
27	p-Aminophenyl- phosphonic	9		5	0.25	0-0.5	75	75	> 10	2

\* Sodium salts, pH 7.3-7.4.

<sup>†</sup> Expressed as delay (in days) of degeneration of the cell population infected with virus treated with compound in question in comparison with untreated culture.<sup>‡</sup> Expressed as per cent inhibition of virus yield in the medium measured in plaque-forming units/ml. 20 h after infection.<sup>§</sup> Minimal toxic concentrations of the compounds leading to the microscopically visible degenerative changes of cells during 48 h observation period.

The compounds prepared as 0.1 M solutions of sodium salts, pH 7.3-7.4, were incorporated into the fluid maintenance medium. The medium contained 2 per cent horse serum and 0.5 per cent lactalbumin hydrolysate in Hanks's saline with bicarbonate and antibiotics. The ME cultures (about  $2 \times 10^6$  cells/ml.) were infected with approximately 100 plaque-forming units of virus. The antiviral activity of the compounds was determined by observing the protection of tissue cultures against the cytopathogenic effect (CPE) of the virus and by measurement of the virus yield in the medium 20 and/or 48 h after infection. For the virus titration plaque assays in serological tubes with 1 per cent methylcellulose overlay were used. The details of the methods used were the same as previously described<sup>1</sup>.

The antiviral effects of 27 compounds tested so far are summarized in Table 1.

The results presented indicate that marked antiviral activity (more than 90 per cent inhibition of virus yield and protection against CPE for at least one day) is exhibited by two types of compounds—compounds related to salicylic acid (Nos. 1-3) and certain phosphonic and carboxylic acids with benzyl groups (Nos. 4-9). To our knowledge the antiviral activity of salicylates *in vitro* has not previously been noticed. For this reason their mode of action was investigated more in detail and the results are described in a separate communication<sup>2</sup>.

The significance of the benzyl grouping associated with different structures for virus inhibition emerges from the results of several investigators. Tamm *et al.*<sup>12</sup>, who have examined the inhibition of virus replication by numerous derivatives of benzimidazole, suggested that hydroxybenzyl grouping at position 2 in the imidazole ring was of importance for the selective antiviral activity of 2-( $\alpha$ -hydroxybenzyl)-benzimidazole (HBB). O'Sullivan *et al.*<sup>13</sup> synthesized several substituted HBB derivatives with high inhibitory action on poliovirus multiplication and concluded that the 1-benzyl derivative of HBB is among the most active compounds. Loddo and Gessa<sup>17</sup> have recently reported that benthanidine and *o*-chlorobenthanidine, which are benzyl derivatives of guanidine, have a considerable inhibitory action on polio and vaccinia virus growth although presumably exerting their effects through a different mechanism from that of guanidine.

The work recorded here has revealed that certain simple compounds with the benzyl grouping attached to electro-negative, electrophilic structures (carboxylic or phosphonic) also suppress the replication of EMC virus, whereas the compounds with the phenyl,  $\beta$ -phenylethyl and some other groups show little, if any, virus inhibitory action. Moreover, the dibenzyl structure was found to be far more active than mono-benzyl structure. It was also observed that the nitro group increased, whereas the amino group reduced, the antiviral effect of the compounds.

Even the most active of the compounds tested so far require a rather high concentration of  $2 \times 10^{-3}$  M/l. for activity and their effect diminishes rapidly at lower concentrations. On the other side, the increase of concentration from  $2 \times 10^{-3}$  to  $5 \times 10^{-3}$  was not followed by significant increase of percentage inhibition of virus yield. In case of phosphonic acids and of salicylic acid and its derivatives the high concentration necessary is counterbalanced, however, by low toxicity. For this reason the active doses were always several times lower than the toxic doses. Among the highly active compounds only *p*-nitrophenylacetic acid was found to be cytotoxic at a concentration of  $2.5 \times 10^{-3}$  M and slightly inhibited oxygen consumption and lactic acid production by mouse embryo tissue. It was, however, interesting to note that its phosphonic analogue, *p*-nitrobenzylphosphonic acid, conferred to cells rather high protection against virus infection although it was several times less toxic and did not significantly affect the tissue respiration.

In preliminary experiments *p*-nitrophenylacetic acid and dibenzylphosphonic acid gave distinct protection of

mice infected with lethal doses of EMC virus. The *in vivo* activity of *p*-nitrobenzylphosphonic acid was described previously<sup>1</sup>.

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## GENETICS

### Genetic Recombination with Ethyl-methanesulphonate-Induced Waxy Mutants in Maize

INTRACISTRON mapping in maize has been demonstrated by Nelson<sup>1,2</sup> with a technique based on differential staining reactions of starch in pollen of different genotypes. Those with the *waxy* (*wx*) locus stain reddish brown in iodine-potassium iodide solutions; whereas *non-waxy* (*Wx*) pollen stains dark blue. Therefore, in *waxy* plants genetic recombination or back-mutation at this locus is manifested by the appearance of dark blue staining pollen. The technique has a distinct advantage in genetic investigations with higher plants in that large populations of hundreds of thousands of genotypes (pollen grains) can be scored with ease.

As part of a programme in progress here on chemical mutagenesis in higher plants, the *wx* locus in maize is being used as experimental material and ethyl-methanesulphonate (EMS) as one of the chemical mutagens<sup>3</sup>. This communication is a preliminary report of results obtained with EMS on the induction of *wx* mutations at independent sites and the ordering of these sites within the locus. So far, nearly 50 *wx* mutations have been induced in the programme. Although each appeared independently, some of the sites may be identical, and this will be investigated in subsequent research. A number of mutants other than *waxy* have also been produced with EMS (ref. 3). Results with these, together with the data on *waxy* mutations, are providing preliminary evidence that EMS produces 'point' mutations in maize.

Results are reported here for four of the EMS-induced *waxy* mutants. These were obtained from seed treatments. Disinfected seeds were first soaked in deionized water at 27° C and bubbled continuously with oxygen for 24 h. They were then soaked in 0.05 M or 0.025 M aqueous solutions of EMS for either 2 or 3 days at 3° C. The rationale for this

Table 1. GENETIC RECOMBINATION BETWEEN A *waxy* TESTER AND *wx* SITES INDUCED WITH ETHYL-METHANE SULPHONATE IN MAIZE

Mutant	Seed treatment conditions			Recombination data	
	Molarity of EMS	Days at 3° C	24-h post-treatment (° C)	Ret. No. microspores ( $\times 10^4$ )	$\bar{X}$ No. $Wx \times 10^{-3} \times 2$
BNL-85	0.05	2	27	85	25.6
BNL-87	0.025	3	3	376	44.2
BNL-88	0.025	3	3	409	76.2
BNL-88	0.025	3	18	603	86.0

treatment was to ensure thorough penetration without chemical disintegration of the mutagen. This was followed by post-incubation in water at different temperatures (Table 1).

The plants grown from treated seeds were crossed to a tester stock that was recessive at the *waxy* locus. The occurrence of *waxy* kernels in the  $F_1$  indicated a mutation. The presence of  $Wx$  pollen in the  $F_1$  plants, in excess of back-mutation frequency, was evidence of recombination between the tester and mutant *wx* site.

Recombination results on the four sites are shown in Table 1. These were selected for reporting in this preliminary article because closely comparable results were obtained by two observers (R. B. and E. A.), working completely independently of each other. The figures shown in Table 1 are averages computed from the two sources of data. The number of  $Wx$  grains has been multiplied by two to be comparable with other recombination maps. The mutant sites are arranged in Table 1 in order of increasing amount of recombination with the tester.

In so far as the  $Wx$  pollen in  $F_1$  plants heteroallelic for tester and mutant *wx* sites arises from genetic recombination, this result, in itself, is an indication that the mutant sites are positioned differently from the tester site in the *wx* locus. The results in Table 1 have not been corrected for the spontaneous back-mutation rate. Evidence from other research<sup>4</sup> with standard *waxy* sites shows that back-mutation rates range from 0.60 to  $2.42 \times 10^{-4}$ , which is about equivalent to a map distance of 0.0012–0.0048. The range of recombination of the mutants reported was from 0.0256 to 0.086. Therefore, the recombination figures shown in Table 1 are considerably in excess of a back-mutation rate from other work. Furthermore, the results presented here indicate a back-mutation frequency of zero for the homoallelic tester used in this research.

Mapping by the described procedures gives the relative recombination distances from one site. However, if a map is constructed by this method it may not give the true spatial relationships since at present all mutants are mapped to one side of the tester site.

Future mapping will be done by first obtaining stocks which are homoallelic for the induced site. These will then be intercrossed in all possible combinations so that the actual recombination distances among induced sites can be determined, rather than only the distance from the tester site. This conventional mapping method should show whether induced sites are distal or proximal to the tester site and, hence, their true spatial relationships. Furthermore, by following such a procedure insight should be gained on whether EMS-induced sites are 'point' mutations or minor deletions. The *ad hoc* mapping procedure used here is not capable of distinguishing small deletions from 'point' mutations since recombination would be expected in both. However, by the conventional mapping procedure, minor deletions should be distinguishable by non-additivity of recombination distances when all the combinations of a diallel system of crosses are tested. In the course of the analysis evidence for major chromosomal damage should also be detectable.

In summary, the evidence for intracistron recombination reported here indicates that EMS induces independent mutations at sites within the *waxy* locus in maize. The occurrence of recombination between mutant and tester *wx* sites is further indication that 'point' mutations, or at least minor deletions, have been induced by this mutagen.

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## PSYCHOLOGY

### Inappropriate Constancy Explanation of Spatial Distortions

THE perception of two- and three-dimensional space has for long been one of the central issues in the experimental study of sensory and perceptual processes. An aspect of this problem is the apparent distortions of shape, size and direction which occur when the elements of a stimulus pattern (lines, angles, forms, etc.) are juxtaposed in certain spatial relationships. Such spatial illusions, which can be defined as discrepancies between the judged and true physical properties of the stimulus, have not yet been explained satisfactorily. It is clear, however, that their explanation would constitute a considerable advance in our understanding of the perceptual processes involved in space perception.

Interest in illusory patterns has been revived recently by a further attempt at their explanation by Gregory<sup>1-3</sup>, who has extended and tested a theory originally proposed by Tausch<sup>4</sup>. Although this theory has the virtue of simplicity in addition to that of interpreting illusory phenomena in the context of the established principle of perceptual constancy, it can be seriously questioned on several grounds. Some criticism has already been raised by Brown and Housiadas<sup>5</sup>.

Gregory argues that the classical spatial illusions are two-dimensional projections of three-dimensional objects such that those elements normally further away in three-dimensional space appear larger. The principle of 'misapplied' or 'inappropriate' constancy can be illustrated in Hering's illusion shown in Fig. 1. The two vertical lines in this pattern are parallel. The radiating lines give a perspective effect; the centre of the pattern represents a point more distant than points around the margin. Since the distance between the two vertical lines is constant throughout, the visual sub-tense is also constant. But the central region of the pattern contains information for greater distance than the margin. In order to subtend the same visual angle, therefore, the separation between the parallels in the centre must be perceived as greater than at the ends, hence the outward bowing effect of the parallels. The principle of inappropriate constancy is also illustrated in the variants of Ponzo's illusion from Teuber<sup>6</sup> also shown in Fig. 1. In summary, information or cues for greater or less distance contained in the background pattern will determine the apparent size of elements in a two-dimensional display.

The principle involved is precisely that invoked by Ptolemy to explain the Moon illusion, that is, the greater apparent size of the Moon at the horizon as compared with its size at zenith. In each location the Moon subtends much the same visual angle, but the horizon is judged further than the vertical distance. Thus the Moon must be judged larger at the horizon. This apparent distance theory of the Moon illusion has been strongly supported by data from a series of recent experiments<sup>7</sup>.

A first point of criticism which has already been raised<sup>8</sup> concerns the occurrence of spatial illusions in the tactile modality. It has for long been known that spatial illusions similar to those in vision occur when the same



line patterns are impressed on the skin. A number of early investigators and, more recently, Reeves<sup>8</sup> and Rudel and Teuber<sup>9</sup> have demonstrated the occurrence of numerous spatial illusions for both active and passive touch. Further, Reeves has ruled out a dependence of these tactile illusions on vision by observing their occurrence among subjects blind from birth.

Since the touch receptors of the skin constitute a 'contact' sense which is not adapted for three-dimensional spatial discrimination, it is difficult to argue that distance information in the surrounding pattern determines the illusory effect through inappropriate constancy. Both the visual and tactile sense organs are constituted of a spatially extended receptor mosaic and the occurrence of similar illusory effects for each modality strongly suggests similar processes. A parsimonious theory would be expected to account for each. This criticism is reinforced by the recent finding that the receptor mosaics of both the skin and retina contain mechanisms which enhance borders and edges to give the Mach effect<sup>10</sup>.

A second point involves numerous variants of the Zöllner illusion. In Fig. 2A are shown the Zöllner, Wundt and Orbison illusions, all of which derive from and are complex versions of the intersection of one line with another as shown in Fig. 2B. If the line to be judged is vertical its apparent slant in the opposite direction to the slant of the intersecting line is a function of the angle of intersect as shown in Fig. 2C. The illusory effect is minimal at angles of approximately 22° and 67°. Thus, if line patterns are of variable angle and direction, as in the three patterns in Fig. 2A, the illusory effect will vary

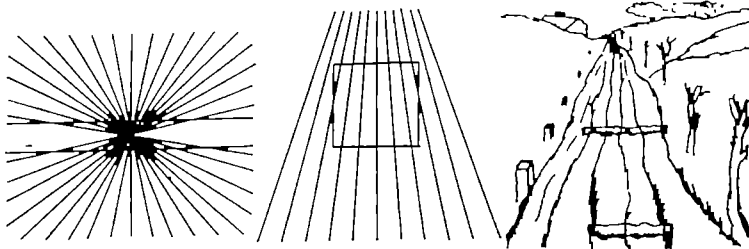


Fig. 1. The Hering illusion (left) and two versions of the Ponzo illusion (right) demonstrating the misapplied constancy principle

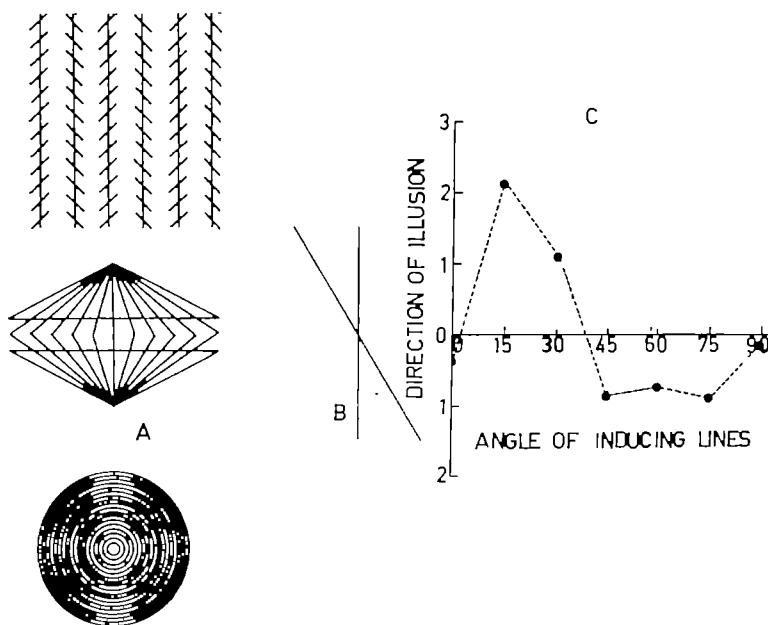


Fig. 2. A, Illusions deriving from the intersection of two lines; B, the simple case, C, variation in the direction of an illusion of slant as a function of the angle of intersection

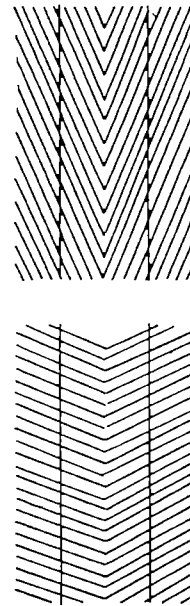


Fig. 3. Illusion of slant as a function of angles of intersection of 22.5° (upper) and 67.5° (lower)

according to the particular locus of the verticals on the background. Although the multilined backgrounds of the patterns in Fig. 2A contain perspective information for distance, the same can scarcely be said of the simpler and more basic pattern of Fig. 2B. That is, the well-known illusions of Zöllner, Wundt and Orbison are each complex versions of the relatively simple case shown in Fig. 2B in which it is extremely difficult to conceive of an apparent distance determinant.

A further point arises out of this variation in the magnitude of 'directional' illusions as a function of intersect angle as shown in Fig. 2C. At intersect angles of about 22° the two parallels appear closer in the lower part of the pattern and at angles of about 67° farther apart. But the distance information from the background perspective is the same in each case. This difficulty for the constancy interpretation is illustrated in Fig. 3. It can be noted also that a number of directional and size illusions change, often exhibiting the reverse effect, when the background pattern is systematically varied with respect to certain properties.

The Müller-Lyer illusion is an example of an illusion which can be seen in a variety of patterns. The 'dumbbell' illusion<sup>11</sup> shown in Fig. 4 is one such variant. The attached circles have the effect of lengthening or shortening the horizontal lines. Now, although the angles attached to the lines in the classical Müller-Lyer figure can be thought of as containing perspective information for distance, the same can scarcely be said of the circles in Fig. 4, all of which are the same diameter and vary only in the distance apart of their centres. To attribute distance information to equal circles would seem to be stretching the misapplied constancy hypothesis to breaking point, especially as the two circles suggesting greater distance (those closer together) produce an illusion of being shorter.

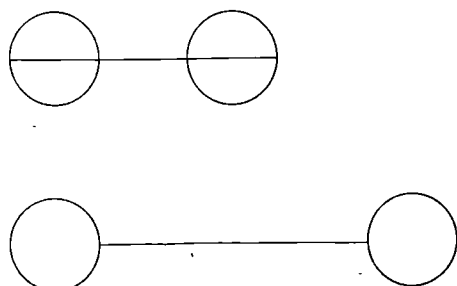


Fig. 4. The 'dumb-bell' illusion. The horizontal lines are equal in length

Gregory has also attempted to explain spatial after-effects in terms of inappropriate constancy. These effects occur in judgments of visual patterns following prolonged stimulation by another. To attribute these effects to the same processes as those responsible for spatial illusions fails to take into account that in using the same patterns to generate an illusion and an after-effect the directions of distortion are frequently opposite. This opposition between illusion and spatial after-effect is illustrated in Fig. 5. The surrounded dotted circle appears larger than the objectively equal but non-surrounded circle. If, however, the surrounding circle is fixated for a minute and then the dotted circle compared, the circle falling within the hitherto stimulated region appears smaller. In any event, it has now been shown that there is no necessary relation between spatial illusions and spatial after-effects<sup>1</sup>.



Fig. 5. Figures for showing the opposition between illusion and after-effect

There now seems to be little doubt that certain illusory phenomena derive from an apparent distance-apparent size invariance. The Moon illusion is one such case<sup>2</sup> and is probably a special case of Emmert's law as demonstrated by King and Gruber<sup>3</sup>. The argument that the classical spatial illusions and spatial after-effects derive from an essentially similar size-distance invariance is, to say the least, questionable in view of the contrary evidence presented here. In point of fact there is evidence which strongly suggests that the apparent distortions of illusory figures derive from neural interactions between the processes induced by the judged and background elements of the pattern<sup>4</sup>. If this explanation can be sustained then apparent distance would be a consequence, not a cause, of spatial distortions.

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PROF. DAY omits the major feature of my theory. The omission is evident in his reference to the Moon illusion. What is interesting about the Moon illusion is that its

apparent size is not a simple function of its apparent distance. On the horizon it appears large and near. Ptolemy was not correct in attributing its apparent size simply to its apparent distance, and the effect is not a straightforward example of Emmert's law. For this and other reasons I suggested that there is more to constancy than apparent distance: that constancy can be set directly by depth cues which are not always appropriate. Prof. Day disregards what I have called "primary constancy scaling" without which I believe we cannot hope to develop a consistent theory of these distortions in terms of depth perception.

The reported illusions in the tactile modality are certainly interesting, but should not be regarded as a straightforward "criticism", or objection, to a theory of the visual illusions in terms of depth. It seems much more to the point to discover more about these tactile illusions—to discover how they are related to the visual ones. The fact is we know very little about them. Further, it is not at all clear why this "criticism" is reinforced by the fact that borders and edges are neurally enhanced in both touch and vision. Why should the Mach effect be relevant to distortion illusions?

The discussion of the Zöllner, Wundt and Orbison illusions is not aimed at my theory, because the essential point of primary scaling is omitted. In each case the depth features of these figures can be related, by isolating the features and measuring the perceived depth of each, with the technique described briefly in my reply to Brown and Housiadas<sup>5</sup>.

Regarding figural after-effects: the notion that they may be due to constancy scaling taking some time to recover after prolonged fixation was put forward as a suggestion which seems worth following up. It is probably consistent with the known facts; but again it must be discussed in terms of 'primary' scaling. This is set by local depth features which may be opposed by other features or countermanded by the texture of the background. It is not synonymous with apparent distance except in the simplest cases, but in all cases it is possible to isolate the depth features and measure the primary constancy. This can then be directly related to the distortion of visual space in the X- and Y-co-ordinates.

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<sup>1</sup> Gregory, R. L., *Nature*, 199, 678 (1963).

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## SOIL SCIENCE

### Tracer Technique used in Examination of Activity of Roots of Grass Swards

THE interpretation of measurements of the uptake by plants of tracer isotopes from the soil is complicated by at least three factors. First, exchange takes place between the added labelled ions and the isotope ions present in the soil; the rate of this exchange has been shown to be affected by many factors<sup>1,2</sup>. Secondly, continuous exchange occurs between the nutrient ions in the roots and those in the soil<sup>3-5</sup>. The factors controlling this exchange are unknown. Thirdly, there is the possibility of damage to plant tissues, resulting from accumulation of the tracer. Although a number of experiments have shown no appreciable effects of radiation in terms of yield of dry matter<sup>6-12</sup> and uptake<sup>6,13</sup>, the question of radiation damage to plant tissues in long-term uptake experiments remains unsettled. Critical studies<sup>13,14</sup> have shown that even very low doses of radiation may produce some physiological changes in plant cells.

The problems of isotopic exchange make it impossible to compare quantitatively the amounts of tracer found in plants, at different sampling dates, in experiments in

which labelled isotopes have been placed in the soil on a single occasion. Radiation damage to absorbing cells can also invalidate such results, so that this method cannot be used to follow changes with time in the uptake of particular ions by the roots. These difficulties are minimized if uptake can be measured after a few hours only.

Changes in uptake during a given time period can be examined by repeated placement of a standard dose of tracer and measurements of the uptake by the plants over a fixed short period. Plants to which tracer has not been previously supplied are used on each occasion. This technique was considered for use in an examination of rooting differences in field swards subjected to progressively differing soil moisture conditions. Two aspects only of the technique are presented here, namely, the time interval required following placement of  $^{32}\text{P}$ , for measurable amounts to appear in the herbage, and the effect of soil moisture content, at the time of placement, on the distribution of  $^{32}\text{P}$  in the soil. In both cases, Italian ryegrass (*Lolium multiflorum*) growing on a coarse sandy loam was used.

In examining the first question,  $^{32}\text{P}$  was placed at different depths on July 19, 1963, when the sward had been cut once in the spring and the soil had received no fertilizer since the preceding barley crop. A  $\frac{1}{4}$ -in. steel rod was driven into the soil and a polythene tube liner was inserted into the hole to reach about  $\frac{1}{2}$  in. from the bottom. The  $^{32}\text{P}$  solution (25  $\mu\text{C}$ ,  $^{32}\text{P}$  carrier free as orthophosphate, in 0.4 ml. water) was then placed at the bottom of the hole with a surgical syringe fitted with a fine delivery tube. The parts of the plants above ground were sampled at intervals ranging from 6 to 72 h after placement of the tracer. Each sample consisted of the grass on a roughly circular area (4 in. diameter), cut at ground level around a single placement point. The dried (100° C, 12 hours) plant samples were ashed by ignition (450° C), and taken up in 2 N nitric acid after the method described by McAuliffe<sup>7</sup>; a sample of the solution was counted in a Geiger-Müller solution counting tube.

Sufficient  $^{32}\text{P}$  was taken up in the first 6 h after placement, even from a depth of 6 in., to allow satisfactory measurement of radioactivity (Table 1). Variability was high, but was considerably reduced in subsequent work by multiple placement.

The effect of soil moisture content, at the time of placement, on the distribution of the tracer was examined on a similar sward. In one treatment (dry), the 0-2 in. soil horizon contained 5.1 per cent moisture (moisture content at 15 atmospheres  $\approx$  3 per cent). During the 6 h before the tracer was placed, the second treatment (wetted) was watered to field capacity ( $\approx$  13 per cent moisture). On each plot,  $^{32}\text{P}$  (25  $\mu\text{C}$ , in 0.4 ml. water) was placed at a depth of 1 in. at two points spaced 6 in. apart. In order to deliver the tracer in the soil at as uniform a rate as possible, a standard time of 15 sec was taken to complete placement at each point. Each dry matter sample for radio-assay was a strip of grass 3 in. wide out at ground level between the two points of placement. Sampling was done exactly 4 h after the tracer had been applied. Identical experiments were carried out on two dates—August 31 and September 5, 1963, each being repeated five times.

Autoradiographs of vertical cross-sections from duplicate soil monoliths show that  $^{32}\text{P}$  became distributed over

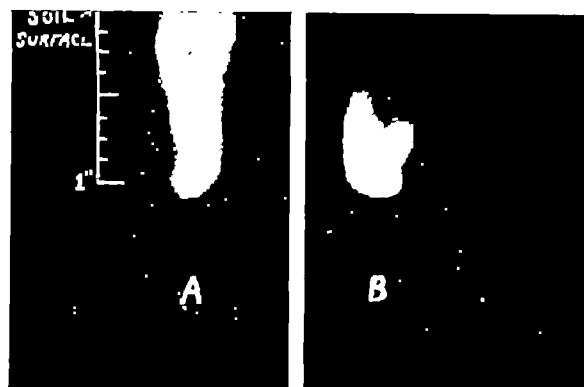


Fig. 1. Autoradiographs of soil monolith vertical cross-sections, showing the distribution of  $^{32}\text{P}$  (25  $\mu\text{C}$ , in 0.4 c.c. water) 4 h after placement. A, Soil at field capacity ( $\approx$  13 per cent moisture) at time of placement; B, Soil containing 5.1 per cent moisture at time of placement.

a much greater vertical range, up the sides of the hole, in the wetter soil (Fig. 1). Moisture content of the soil had therefore a very pronounced effect on the distribution of the  $^{32}\text{P}$  tracer solution. The effect of the treatments on the uptake of  $^{32}\text{P}$  by the grass is shown in Table 2.

The result of wetting immediately before placement is a very large increase in tracer uptake. This uptake is greater than the increased soil distribution of the tracer would suggest. However, the amount of root material under a sward declines rapidly with increasing depth from the surface and, as Fig. 1 shows, the effect of wetting before placement is to raise the upper margin of tracer-affected soil. It is not surprising that uptake values differed so greatly at the two levels of moisture content; widely differing amounts of root surface must have been exposed to the tracer in the two treatments. In using a tracer-solution placement technique to compare uptakes by plants which have been grown under different moisture regimes, it is therefore necessary to standardize the moisture content of the soil under the different treatments before the tracer is placed; in the field this can be done only by bringing the soil to field capacity on each sampling occasion.

Increasing the moisture content of the soil on plots which have previously been submitted to progressive drying may enhance tracer uptake, because of a probable increase in the diffusion of ions to absorbing cells, and because of a possible increase, even in the few hours following re-wetting, in the capacity of the roots for ion uptake. However, if the soil moisture condition in the preceding period has had pronounced effects on the amount and distribution of absorbing roots or on the rate at which the root surfaces take up the ion concerned, these will be reflected in the uptake recorded. Thus the use of this technique in the field should demonstrate relative differences in ion uptake of root systems at particular depths and as affected by different moisture regimes.

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Table 1. MEAN COUNT/MINUTE/GRAM TOTAL HERBAGE DRY MATTER (MINUS ASH), SIX HOURS AFTER PLACEMENT OF  $^{32}\text{P}$  IN SOIL, CORRECTED FOR BACKGROUND

Depth of $^{32}\text{P}$ placement in profile (in.)	Count (mean of two)
1	174
2	30
6	45
12	16

Table 2. GEOMETRICAL MEANS OF COUNTS/MINUTE/GRAM TOTAL HERBAGE DRY MATTER (MINUS ASH); CORRECTED FOR BACKGROUND

Experiment	(Dry)	(Wetted)	S.E.
Aug. 31	15	265	1.2
Sept. 5	15	167	1.3

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## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

**RESEARCH ASSISTANT** (biologist or veterinarian) in the DEPARTMENT OF VETERINARY ANATOMY, for locational studies—The Registrar, The University of Liverpool, Liverpool 3, quoting Ref. OV/228/N (August 27).

**SENIOR TECHNICIAN** (experienced in biological techniques, and an interest and ability in laboratory administration); and **TECHNICIAN (2)** (experienced in biological techniques) in the DEPARTMENT OF ZOOLOGY—The Deputy Secretary, The University, Southampton (August 27).

**LECTURER IN HUMAN GENETICS**—The Registrar, The University, Newcastle upon Tyne, 2 (August 28).

**RESEARCH ASSISTANT** (with a first degree in chemistry, biochemistry or zoology) in BIOCHEMISTRY, to join a research group carrying out research into the biochemical and nutritional properties of food after processing—The Deputy Secretary, The University, Southampton (August 30).

**RESEARCH FELLOW** (with experience in computer programming and data processing) in MEDICAL DATA PROCESSING in the SUB-DEPARTMENT OF NUMERICAL ANALYSIS—The Registrar, The University, Liverpool 3, quoting Ref. OV/237 (August 30).

**ASSISTANT STATISTICIAN** for a diverse range of work at the Authority's statistical unit at Letchworth, Hertfordshire—The Establishment Officer, Pig Industry Development Authority, 15 Ridgmount Street, London, W.C.1 (August 31).

**EXPERIMENTAL OFFICER** (young graduate interested in the design and construction of electronics and electro-acoustic equipment) in the DEPARTMENT OF AUDIOLOGY AND EDUCATION OF THE DEAF—The Registrar, The University, Manchester, 13, quoting Ref. 161/65/Wa (August 31).

**STATISTICIAN** (Assistant Lecturer/Lecturer or Experimental Officer level) in the DEPARTMENT OF PREVENTIVE MEDICINE AND PUBLIC HEALTH—The Registrar, University of Sheffield, Sheffield (August 31).

**TECHNICIAN** for a research laboratory, to assist in low temperature experiments and also to assume responsibility for the organization of teaching experiments—The Registrar, University of Warwick, Coventry, quoting Ref. 2/65 (August 31).

**PART-TIME ASSISTANT LECTURER or DEMONSTRATOR in ANATOMY**—The Registrar, The University, Manchester 13, quoting Ref. 166/65 (September 3).

**SENIOR LECTURERS (2)**, and **LECTURERS/ASSISTANT LECTURERS** (with at least a good honours degree with suitable teaching and research experience) in the DEPARTMENT OF MATHEMATICS, University of Malaya—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Kuala Lumpur and London, September 6).

**TECHNICAL OFFICER** (preferably with qualifications in science or engineering) in the DEPARTMENT OF PHYSICS, to assist the Departmental Secretary in the administration of laboratories—Dr. P. Matland, Secretary, Faculty Board of Physics and Chemistry, Chemical Laboratory, Lensfield Road, Cambridge (September 8).

**RESEARCH SCIENTIST or SENIOR RESEARCH SCIENTIST** (Organic Chemist) (with a Ph.D. degree in chemistry, or with postgraduate research experience of equivalent standard and duration, supported by satisfactory evidence of research ability) in the DIVISION OF ORGANIC CHEMISTRY, Commonwealth Scientific and Industrial Research Organization, Chemical Research Laboratories, Fishermen's Bend, Melbourne, Victoria, Australia, to participate in chemical studies of biologically active plant metabolites—The Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 606/66 (September 11).

**ASSISTANT LECTURER in ZOOLOGY**—The Registrar, The University, Manchester, 13, quoting Ref. 165/65 (September 15).

**DEMONSTRATOR/SENIOR DEMONSTRATOR in the DEPARTMENT OF ORGANIC CHEMISTRY**—The Registrar, The University, Newcastle upon Tyne, 2 (September 15).

**LECTURER or ASSISTANT LECTURER in the SCHOOL OF PHARMACY, University of Singapore**—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (September 16).

**NUFFIELD POST-DOCTORAL FELLOW** (with experience in metabolic aspects of cell metabolism) in BIOCHEMISTRY in the DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, Waite Agricultural Research Institute, to assist in a programme of work on the biochemistry of nitro fixation in bacteria—The Registrar, The University of Adelaide, Adelaide, South Australia (September 17).

**PROFESSOR, and a SENIOR LECTURER or LECTURER in STATISTICS** at the University of Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (September 18).

**LECTURER in HISTORICAL or MECHANICAL ENGINEERING** at Fourah Bay College, The University College of Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (September 20).

**LECTURER** (with an honours degree in chemistry and preferably the degree of Doctor of Philosophy) in INORGANIC CHEMISTRY at the University College of Townsville (University of Queensland), Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (September 24).

**LECTURER in the DEPARTMENT OF ORGANIC CHEMISTRY, University of New South Wales, Australia**—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 30).

**LECTURER** (qualified in the field of palaeontology) in GEOLOGY at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 30).

**SENIOR CHAIR in ZOOLOGY** at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 30).

**SENIOR LECTURER and a LECTURER in BIOCHEMISTRY** at the University of Adelaide, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, September 30).

**SENIOR LECTURER** (with a degree in science or engineering, preferably a doctorate, experience in the maintenance and operation of electron microscopes, and a wide knowledge of electron microscopy techniques, including application to biological, mineral and metallurgical materials) in ELECTRON MICROSCOPY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 1).

**LECTURER or SENIOR LECTURER** (medical graduate with postgraduate experience in pathology) in the DEPARTMENT OF PATHOLOGY, University of Otago, Dunedin, New Zealand—The Professor of Pathology, University of Otago Medical School, P.O. Box 918, Dunedin, New Zealand; or The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, November 30).

**AGRICULTURAL OFFICERS** (nationals of the United Kingdom or the Republic of Ireland, with a degree in agriculture or natural science, plus at least two years experience in tropical agriculture) in Sarawak, for the administration of an Agricultural Division, comprehensive advisory and supervisory duties, and supervision of experimental stations and staff training—The Appointments Officer, Ministry of Overseas Development, Room 301, Bland House, Stag Place, London, S.W.1, quoting Ref. 219/185/04.

**ANIMAL TECHNICIAN** (male, with at least five years experience) in the BIOCHEMICAL LABORATORY, to take charge of department concerned mainly with nutritional research on small animals—The Director of Research, Cereals Research Station, Old London Road, St. Albans, Herts.

**ASSISTANT LECTURER in CHEMICAL METALLURGY**—Prof. F. D. Richardson, Department of Metallurgy, Imperial College, London, S.W.7.

**ASSISTANT LECTURER** (interested in a research career) in the DEPARTMENT OF HUMAN BIOLOGY AND ANATOMY, for duties which will include the teaching of gross anatomy—Prof. B. Barer, Department of Human Biology and Anatomy, The University, Sheffield, 10.

**BACTERIOLOGIST** (with a first or good second-class honours degree, or equivalent qualification, in a biological science, and research experience in bacteriology) in the ISOTOPE RESEARCH DIVISION'S MICROBIOLOGY SECTION, Vantage Research Laboratory, A.M.R.B., to study the lethal effect of radiation on bacteria, particularly in relation to the influence of various environmental factors—Personnel Department (A. 5116/84), United Kingdom Atomic Energy Authority, Atomic Energy Research Establishment, Harwell, Didcot, Berkshire.

**BIOCHEMIST** (Basic Grade) to work with and under the direction of a Chemical Pathologist and the Group Biochemist—The Director of Pathology, Paddington General Hospital, Harrow Road, London, W.9.

**BIOCHEMIST in the MEDICAL UNIT** as research assistant on the isolation of peptide hormones—Prof. J. Anderson, Medical Unit, King's College Hospital, Denmark Hill, London, S.E.5.

**BIOCHEMIST** (probationary or basic grade)—The Group Secretary, Napsbury Hospital, near St. Albans, Herts.

**CHEMIST, PHYSICIST or BIOCHEMIST** (graduate) for work on the application of gas chromatography for the determination of steroid progesterone—Dr. A. B. Kellie, Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, W.1.

**COMPUTER PROGRAMMER** (recent graduate with appropriate qualifications or experience in computing techniques) for a research group in astrophysics at the Cambridge University Observatories—The Director, Observatories, Madingley Road, Cambridge.

**ENTOMOLOGIST** (male, normally national of the United Kingdom or the Republic of Ireland, with an honours degree in entomology, biology or zoology with an emphasis on entomology) in Tanzania, to carry out major surveys of tsetse infested country, to plan and estimate the cost of control measures and to finally evaluate the results—The Appointments Officer, Ministry of Overseas Development, Room 301, Bland House, Stag Place, London, S.W.1, quoting Ref. RO 217/178/07.

**EXPERIMENTAL OFFICER or SENIOR EXPERIMENTAL OFFICER** (man with an agricultural degree or N.D.B.) to conduct field experiments in new techniques in farm mechanization—The Secretary, National Institute of Agricultural Engineering, Scottish Station, Bush Estate, Pentonik, Midlothian.

**FRESHWATER RESEARCH OFFICER** (national of the United Kingdom or the Republic of Ireland, with an honours degree in natural science, with at least two years postgraduate research experience in freshwater biology, fisheries research or limnology) with the East African Common Services Organization, to investigate various problems of the freshwater fisheries in East Africa, commencing with those encountered in Lake Victoria—The Appointments Officer, Ministry of Overseas Development, Room 301, Bland House, Stag Place, London, S.W.1, quoting Ref. RO 236/214/03.

**FOREST ENTOMOLOGIST** (under 45, national of the United Kingdom or the Republic of Ireland, with a degree in zoology or entomology, with postgraduate experience in some aspect of tropical forest entomology) with the East African Common Services Organization, to work under the general direction of the Head of the Forest Entomology Division and the Director of the East African Agriculture and Forestry Research Organization on extensive field studies of tree-bored and defoliators, to assist with the East African Forest Insect Survey and with the expansion and upkeep of the I.A.A.F.R.O. reference collection of forest insects—The Appointments Officer, Room 301, Ministry of Overseas Development, Bland House, Stag Place, London, S.W.1, quoting Ref. RO 224/214/02.

**HIGHER TECHNICAL ASSISTANT** (with wide experience of laboratory techniques with special reference to physiological problems) in the PHYSIOLOGY LABORATORY—The Secretary, School of Agriculture, University of Cambridge, Downing Street, Cambridge.

**LABORATORY TECHNICIAN** for routine steroid hormone estimations—The Assistant Secretary, University College Hospital, Gower Street, London, W.C.1.

**LECTURER in PSYCHIATRY** at the University of the West Indies, Kingston, Jamaica—The Secretary, Senate Committee on Higher Education Overseas, University of London, Senate House, London, W.C.1.

**PHYSICIST FOR THE RADIOTHERAPY SECTION OF THE HOSPITAL'S PHYSICS DEPARTMENT**—The Clerk to the Governors, St. Bartholomew's Hospital, London, E.C.1, quoting Ref. No. A80/468.

**PHYSICISTS** (Auroral Spectroscopists) at the Churchill Research Range, Fort Churchill, Manitoba, Canada, for a programme of research in auroral spectroscopy—The Employment Officer, National Research Council of Canada, Ottawa, 7, Canada, quoting Ref. ORH-1.

**RESEARCH ASSISTANT** (graduate) in the DEPARTMENT OF MICROBIOLOGY, for studies of invasiveness of cancer cells—Prof. H. Smith, The University, Birmingham, 16.

**RESEARCH ASSISTANT** (graduate or G.R.I.O.) in the DEPARTMENT OF CHEMISTRY, to work on the far infrared spectra of compounds—Head of the Department of Chemistry, Northampton College of Advanced Technology, St. John Street, London, E.C.1, quoting Ref. O/N.

**RESEARCH ASSISTANT** (honours graduate in biological science) in the DEPARTMENT OF EXPERIMENTAL PATHOLOGY AND CANCER RESEARCH, for an investigation of biological aspects of carcinogenesis—Dr. J. O. Laws, Department of Experimental Pathology and Cancer Research, School of Medicine, The University, Leeds, 2.

**RESEARCH ASSISTANT in MYCOLOGY**—The Secretary, Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, Paddington, London, W.2.

**RESEARCH ASSISTANT** (with a B.Sc. in microbiology or similar qualification) for the HOSPITAL INFECTION UNIT in the DEPARTMENT OF BACTERIOLOGY—Clark to the Governor, St. Bartholomew's Hospital, London, E.C.1, quoting Project No. 109/2, reference No. ASO/465.

**RESEARCH ASSISTANT** (with a degree in chemistry and some experience in a field of polymer research) in POLYMER CHEMISTRY—The Registrar, Ref. 118Y/X, Bradford Institute of Technology, Bradford, 7.

**RESEARCH BIOCHEMIST** (under 35, biochemist or biologist with a Ph.D.) for biochemical and immunochemical research on lipocarbohydrates—The Personnel Director, Evanston Hospital Association, 2650 Ridge Avenue, Evanston, Illinois, U.S.A.

**RESEARCH SCIENTISTS** (graduates or equivalent in chemistry, physics or wood science) for investigations into (a) gluing of timber and composite materials; and (b) wood finishes: paints, varnishes, stains, water repellents, etc.—The Secretary, Timber Research and Development Association, 81, John's Road, Tysons Green, High Wycombe, Bucks.

**SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER** (honorary graduate in bacteriology or microbiology) in the BACTERIOLOGY DEPARTMENT, for work concerned with the role of micro-organisms in the nutrition of the ruminant—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, Berkshire, quoting Ref. 65/8.

**SENIOR OR CHIEF TECHNICIAN** (with a good knowledge of workshop practice and some experience of vacuum techniques and electronics) to work with a research group on atomic beam research projects, concerned with gas scattering, optical and electron excitation and radio-frequency resonance—The Laboratory Superintendent, Physics Laboratory, University of Sussex, Falmer, Brighton, Sussex.

**TECHNICIAN OR JUNIOR TECHNICIAN** for research work in the Pathology Department—The Secretary, St. Mary's Hospital Medical School, Paddington, London, W.2.

**VIROLOGIST** (or a Biologist with some virological experience) to work on a joint research enterprise between the National Institute for Medical Research and the Department of Biochemistry, Imperial College. The work will involve study of antiviral substances in animals and in tissue culture—The Director, National Institute for Medical Research, Mill Hill, London, N.W.7.

## REPORTS and other PUBLICATIONS

### Great Britain and Ireland

Joint Mathematical Council of the United Kingdom. Report on In-Service Training for Teachers of Mathematics. Pp. 12. (London: Prof. J. G. Semple, Hon. Secretary, Joint Mathematical Council of the United Kingdom, c/o King's College, 1965.) [127]

International Nickel, Ltd. Ni-Resist Irons for Field Handling in the Chemical Industry. Pp. 8. (London: International Nickel, Ltd., 1965.) [127]

Agricultural Research Council. Slatted Floor Systems for Pigs: A Review of Research Literature to 31st December 1964. By P. H. Baston and C. N. Harvey. Pp. 24. (London: Agricultural Research Council, 1965. Obtainable from H.M. Stationery Office.) 2s. 6d. net. [127]

British Empire Cancer Campaign for Research. Forty-Second Annual Report covering the year 1964. Part 1: The Chairman's Statement and the Accounts of the Central Organisation. Pp. xxxvi. Part 2: The Scientific Report of the Research Undertaken by the Central Organisation and its Autonomous Councils in the United Kingdom, and by some of its Affiliated Organisations Overseas. Pp. xxxvii+706. (London: British Empire Cancer Campaign for Research, 1965.) [127]

University College of Wales, Aberystwyth. Memorandum No. 8 (1965): Synopsis of Discussions held on March 1st, 1965, on the subject of Climatic Change, with special reference to Wales and its Agriculture. Edited by James A. Taylor. Pp. iv+104. (Aberystwyth: University College of Wales, 1965.) [127]

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University of Oxford. Changes in Regulations made by Boards of Faculties. Pp. 24. (Supplement No. 8 to the *University Gazette*, June 1965.) (Oxford: The University, 1965.) 2s. 6d. [127]

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 61: The Care and Management of Breeding Bulls. Pp. 8. Leaflet No. 67: The Cultivation of Vegetables. Pp. 19. Leaflet No. 78: Potatoes. Pp. 16. Leaflet No. 102: Mistle in Oak. Pp. 4. Leaflet No. 125: Cancers in Agriculture. Pp. 6. (Belfast: Ministry of Agriculture, 1965.) [127]

Bulletins of Marine Biology, Vol. 6, Part 4: Continuous Plankton Records—Contribution Towards a Plankton Atlas of the North Atlantic and the North Sea. Part 9: Seasonal Cycles of Phytoplankton. By G. A. Robinson. Pp. 104-123+plates 23-49. (Edinburgh: The Scottish Marine Biological Association, 1965.) [127]

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Sheffield City Libraries. *Bulletin of the Libraries of Commerce, Science and Technology*, No. 1, July, 1965. Pp. 18. (Sheffield: Sheffield City Libraries, 1965.) [127]

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The British Scientific Instrument Research Association. Annual Report 1964/5. Pp. 59. (Chislehurst, Kent: The British Scientific Instrument Research Association, 1965.) [127]

### Other Countries

Proceedings of the United States National Museum, Smithsonian Institution. No. 3506, Vol. 116: Review of the Genus *Cercaria* in America North of Mexico (Hymenoptera: Sphoecidae). By Herman A. Soullien. Pp. 333-543. No. 3507, Vol. 116: North American Moths of the Genus *Stenocryptus* (Lepidoptera: Yponomeutidae). By W. Donald Duckworth. Pp. 549-556. (Washington, D.C.: Government Printing Office, 1965.) [147]

Suomen Geodettien Laitos. Julkaisuja—Vierosmittölkönnösten des Finnischen Geodätischen Instituts. No. 60. General List of Astronomical Astrinthe Observed in 1920-1959 in the Primary Triangulation Net. By V. B. Olander. Pp. 48. (Helsinki: Geodettien Laitos, 1965.) [147]

Annals of the New York Academy of Sciences, Vol. 123, Article 3: The Laser. By Leon Goldman, Joseph Weber and 53 other authors. Pp. 571-834. (New York: New York Academy of Sciences, 1965.) 7 dollars. [147]

Institut für Atomenergie, Kjeller. Survey of Activities 1964. Pp. 27. (Kjeller: Institut für Atomenergie, 1965.) [147]

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Vierundswanzigster Jahresbericht der Schweizerischen Gesellschaft für Vererbungsforschung—Société Suisse de Génétique (S.S.G.) 1964. Mit Unterstützung der Julius Klaus-Stiftung für Vererbungsforschung, Sozialanthropologie und Rassenhygiene in Zürich. Herausgegeben von Marthe Ernst-Schwarmbach. (Separatdruck aus Archiv der Julius Klaus-Stiftung für Vererbungsforschung, Sozialanthropologie und Rassenhygiene, Band XXXIX, 1964, Heft 1/4.) Pp. 181. (Zürich: Art. Institut Orell Füssli, A.G., 1965.) [147]

Geological Survey of Tanzania. Bulletin No. 36: Microflora from the Ketswaka-Mohnehuma Coalfield, Tanganyika. By Dr. George F. Hart. Pp. 27+1 plate. 8hs. 55/-, Bulletin No. 37: The Geology of the Uluwuru Mountains. By D. N. Sampson and A. B. Wright. Pp. v+69+7 plates. 8hs. 15/-. Explanatory Notes on the Geological Map of Kilimanjaro (Covering Quarter Degree Sheets 42, 56 and 57). Pp. 9. 8hs. 6/-. (Dodoma: Geological Survey of Tanzania, 1964 and 1965.) [147]

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United States Department of the Interior: Geological Survey. Bulletin 1193: Bibliography of Reports Resulting from U.S. Geological Survey Participation in the United States Technical Assistance Program, 1940-65. By Jo Ann Heath. Pp. vi+51. 25 cents. Bulletin 1201-D: Chemical Comparison of Glassy and Crystalline Volcanic Rocks. By P. W. Lipman. Pp. iii+24. 15 cents. Bulletin 1201-E: Paleosols Stratigraphy of the Southern Part of the Mule Mountains, Arizona. By Philip T. Hayes and Edwin R. Landis. Pp. iii+43+plate 1. 45 cents. Water-Supply Paper 1791: Hydrologic Conditions Near Glendo, Platte County, Wyoming. By George H. Welder and Edwin P. Weeks. Pp. v+23+plates 1-3. Water-Supply Paper 1793: Ground Water in the East Portland Area, Oregon. By G. M. Henson and B. L. Foxworthy. Pp. iv+78+plates 1 and 2. (Washington, D.C.: Government Printing Office, 1965.) [147]

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## APPLICATIONS OF SCIENCE AND TECHNOLOGY TO DEVELOPMENT

THE Advisory Committee on the Application of Science and Technology to Development was established by the Economic and Social Council of the United Nations in January 1964 to follow up the work of the conference on the "Application of Science and Technology for the Benefit of the Less Developed Countries", which was held in Geneva early in 1963. Its eighteen members, appointed in their personal capacity, include Prof. S. A. Anderson of Denmark (chairman), Dr. P. A. Auger (France), Sir Ronald Walker (Australia), Prof. O. L. Wilson (United States) and Sir Norman Wright (Great Britain). A preliminary report was issued last year, but its second report, which represents the main outcome of the Committee's work so far, was issued in May 1965. It calls for a concerted attack, using the latest techniques, on selected problems which impede social and economic problems in the developing countries. Although subsequent sections of the report deal with international co-operation in science and technology generally, the outstanding section is that which outlines in some detail major scientific and technical problems in eight selected fields which, the Committee suggests, merit special attention and a concerted attack.

In these eight fields, the acquisition of new knowledge is of primary importance, in contrast to the wider and more intensive application of existing knowledge, suitably adapted to local conditions, which, in the Committee's view, provides the best prospect of securing rapid advance in the developing countries. They are: (1) the provision of adequate food supplies; (2) the improvement of health; (3) the fuller understanding of population problems; (4) the most effective exploration and utilization of natural resources; (5) industrialization; (6) housing and urban planning; (7) improvements in transport; (8) education, including new educational techniques. These problems are, for the most part, of great interest and importance to the developed countries also, and this is particularly true of the group of problems bearing on food supplies, to which about a third of this whole chapter of the report is devoted.

The Committee considers that major improvements in the food supplies of the developing countries could be made by a concerted attack on four broad groups of problems: improvements in water resources; improvements in some applications of meteorology to agricultural production; improvements in the supplies of edible proteins; and the prevention of losses of food, as well as on one more specialized problem—the control of the tsetse fly and trypanosomiasis in Africa. While these are not all of particular interest to Britain, those concerning water supplies and conservation should be noted. The report points out, for example, that not only are geological appraisals necessary, but also the means of determining the velocity and direction of the flow of groundwater and of establishing underground connexions between different sources of water need further elaboration, including geophysical and electrical conductivity methods, and the extremely useful application of radioactive and stable tracers. Complementary to such surveys and investigations, the collection of hydrological data and their processes-

ing by the co-operative use of digital computers would be of the greatest value. Construction of underground barrages is also a potentially important sequel to such surveys, and the importance of the Geological Survey in this connexion is not always appreciated.

With regard to the prevention of water losses, the report points out that the efficiency of the control of evaporation by using monomolecular films is still only at the early experimental stage, and seepage control by cheap lining is, as yet, imperfect. Both these problems merit much more intensive study—the former in particular being of importance in conservation of what now in many countries is already a limited resource. While the need for more intensive investigations of the possibilities of breeding improved varieties of plants with minimal requirements for water—such as drought-resistant varieties, and of salt-resistant varieties capable of growth in brackish water or in brackish water partly diluted with salt-free water—may not be directly relevant to problems in Britain, it does serve to emphasize the importance of more efficient utilization of natural resources.

Desalination, however, is another matter. The Economic and Social Council has particularly stressed the importance of desalinating brackish and sea-water. The real problem still to be faced, and with which the report is specially concerned, is the possible use of such water for agricultural production. While the Committee recognizes that the first priority in this field must continue to be the provision of fresh water for direct human needs, the major problem is to find desalination processes capable of application in agricultural production and particularly, in the longer term, for irrigating staple field crops. The Committee suggests that special attention should be directed to this problem.

It should be noted that, speaking in the House of Commons on July 14 in the debate on the Ministry of Technology, the Minister, Mr. F. Cousins, referred particularly to the programme of desalination research on which the Atomic Energy Authority was now working in collaboration with industry. The programme is expected to cost some £1.5 million in the next three years and the Minister of Land and Natural Resources, Mr. F. T. Willey, has already directed attention to the necessity of considering the use of desalinated water to meet some industrial demands. The Water Research Association, moreover, is expected to submit an interim report on the potentialities of desalination to the Water Resources Board some time this year, and the Association's staff is already working with Government support on a three-year feasibility study.

As regards improvements in the application of meteorology, the Committee directed special attention to improved weather forecasting and welcomes the initiation by the World Meteorological Organization of the World Weather Watch as likely to be of outstanding value to the developing and developed countries and meriting the fullest support. If the full co-operation of all countries could be assured, the Committee considers that the World Weather Watch would be a most valuable example of the benefits to be achieved by a world-wide application of



science and technology for the common good. The hope is expressed that those countries possessing weather satellites will consider favourably the great benefits which they might confer on the developing countries by extending and adapting their observations and computations to the tropical areas. The Committee recommends that training facilities for scientists in both developed and developing countries should be substantially strengthened and that the provision of more adequate incentives to potential trainees in this field should receive high priority.

Concerning the future development of ways of modifying and controlling weather, the Committee agrees broadly with the recommendation put forward in a report from the National Science Foundation, that there are three main goals for this purpose: (1) the attraction of new creative talent to research in this field and to the atmospheric sciences as a whole; (2) a wider attack, by means of greater support for theoretical and field research, and with fresh emphasis on the long-range investigation of the modification of climate and weather, on both regional and continental scales; (3) a shortening of the time gap between the development of theory and the start of field research so that new techniques can be tested as soon as a sound theoretical basis is established. A wise reservation was expressed, however, that in this field particularly it is essential that the most careful study should always be made by international study and agreement of the balance between possible risks and benefits: in this the Committee is fully supported by the whole trend of the Commoner Report on the integrity of science (*Nature*, 206, 1277; 1965).

So far as edible proteins are concerned, one aspect of the improvement of supplies appears to be attracting attention in Britain, namely, the production of protein from fisheries. While this is even more important in tropical countries, the Committee suggests that the first and basic need is to initiate a world-wide co-ordinated survey of fisheries resources. It suggests that, in view of the great importance of increasing the supplies of edible fish, it is now essential to make such a world-wide effort to locate and estimate those resources by international co-operation. It also directs attention to the importance of the production by industrial processing techniques of foods rich in protein. While the technical problems in the production of such foods have largely been solved, there are two major obstacles: first, a marked resistance on the part of consumers to protein-rich foods in the forms in which they are at present produced from unconventional sources, and, secondly, the reluctance of manufacturers to venture into the commercial production of such foods. The Committee suggests, therefore, that there is room for imaginative developments in producing industrially processed protein-rich foods, and designing them to meet the existing food habits of the developing countries where they are to be marketed. The urgency of this need, in the Committee's view, demands that methods in which success has already been achieved should be closely studied and, if possible, applied on a much larger scale.

As regards prevention of food losses, what is required, the Committee believes, is not a concerted attack in the search for basic new scientific principles but rather a study of the means by which such principles can be applied to particular circumstances in different countries. There is a special urgency to determine the best means of adapting existing knowledge to small-scale methods of storage and preservation, using facilities which can be made available within a village community or on an

intermediate scale in rural collecting and distributing centres. It is suggested that the use of radiation in the preservation of food and disinfection of grain has shown sufficient promise to justify very careful examination.

With regard to the control of the tsetse fly, the Committee urges that high priority should be given to scientific studies of trypanosomiasis in Africa, and to the measures afterwards needed to apply the results on a sufficient scale. Much closer integration of the different approaches to reclamation and rehabilitation of affected areas is essential if they are to be applied. Further research on both the basic and applied aspects of science relevant to trypanosomiasis, on the improvement of methods of control by drugs and insecticides, as well as on the ecology of the vector itself, is essential if new and more effective techniques of controlling the disease are to be achieved. The Committee hopes that adequate academic and financial support will be given to universities and research organizations in Africa and elsewhere to encourage them to develop training facilities for African workers in this field.

This leads the Committee to pass naturally from questions of food supply to the consideration of measures for the improvement of health. It is here that it emphasizes the relation between pure-water supply and water-borne disease, and points out that provision of an adequate and pure-water supply in the developing countries is a matter of the first priority. It does not appear possible, however, to define any one water-borne disease on which a breakthrough might be envisaged by concerted attack; the Committee suggests that such diseases should be left, as hitherto, to the normal programme of the World Health Organization.

On population problems, the Committee's examination of available evidence leads it to the opinion that further research on a world-wide scale is desirable on demographic, social and economic interrelations; on human reproduction and means for its regulation; and on communications in relation to problems of population. The Committee recognizes that the intensity of such problems varies from country to country and that ultimate solutions involve considerations that go far outside the scope of science. It welcomes the arrangements being made for a World Population Conference under the sponsorship of the United Nations in Belgrade this year, and endorses the proposals for co-operation in research in this field made in a recent memorandum from the Secretary-General to the *ad hoc* Committee of Experts.

As regards the exploration and utilization of natural resources, it is recommended that more attention should be directed to the natural resources of the developing countries, especially by scientific methods; this is regarded as a research area calling for some priority. The Committee also considers that more effective measures are still required to implement the findings of the United Nations conference on "New Sources of Energy", held in August 1961, both by the countries concerned and by the United Nations itself. Besides nuclear power, these possibilities include solar energy and geothermal energy, as well as tidal energy. While the Committee fully appreciates the importance of industrialization in development and has sought the advice of the United Nations Centre for Industrial Development, it is concerned more especially with developments appropriate to the particular conditions of the developing countries, through the use, for example, of local materials and of an appropriate relative input of



labour and capital. The Centre suggested that the main steps to be taken included the strengthening or establishment of appropriate research institutions, together with the development of suitable procedures for referring problems between industry and such institutions, including appropriate government policies. More attention should also be directed to the special requirements of the developing countries for machinery and equipment. The indigenous raw materials of such countries also present special technological problems in their processing for domestic and export markets.

In the field of housing and urban planning, the Committee suggests that the migration in the developing countries from rural to urban areas offers a problem of outstanding importance in settlement as well as an ideal opportunity for applying the techniques of operational research. The industrialization of building presents a further opportunity, calling both for more rapid techniques of building than at present exist in the developing countries and for further research, not only in science and technology but also in design and methods of production. Coordinated research on a world-wide basis is also recommended into needs, materials, design and mass production and distribution of roofing in tropical areas. With respect to transport, the Committee was concerned with the construction of roads and of vehicles suitable for use in the rural areas, though, in view of the increasing importance of sea transport to the developing countries, it suggests an enquiry into the turn-round of shipping at ports to determine more fully the nature of the problem and the possibility that research within the Committee's field of competence might assist. So far as roads are concerned, research is urgently needed to improve methods of soil stabilization and to determine the relative effectiveness of different stabilizing aspects with different varieties of soil. Better means for undertaking accelerated tests on such methods of stabilization would greatly assist such investigations. Further investigation of methods of devising vehicles specially suitable for use in rural areas where road surfaces make transport particularly difficult is also regarded as worth while.

Finally, the Committee recognizes the great obstacle to development which adult illiteracy presents in the developing countries and the urgent need for rapid adoption of means to reduce illiteracy. It suggests that the initial step for such countries should be a survey of existing needs and resources and the development of methods and media for adapting and using available resources to meet local conditions and needs. It welcomes the plans of Unesco to expand projects in teaching physics into those of chemistry and biological science, though it believes the scale of expansion is inadequate. It places the highest priority on measures designed to raise educational levels throughout the developing countries, and particularly on the initiation, development and application of new educational techniques, however novel in their conception.

Although the proposals for new work are concerned essentially with the developing countries, it is manifest that at several points they are in line with developments which are already taking place in some developed countries. That in itself could facilitate co-operation, and beyond this the Committee presents its proposals as essentially a programme of international co-operation, fully in line with the present-day scientific and technological activities of the United Nations and its related agencies. It believes that a new programme of this

magnitude is not only possible but also highly desirable. The programme should embrace co-operation in promoting education in science and technology and training scientific and technical personnel in the developing countries; in strengthening the links between universities, research institutes and laboratories in developed and in developing countries; in studies and research on priority problems; and in providing technical assistance to the developing countries.

The Committee considers that more information regarding particular institutions and forms of co-operation is needed as a basis for planning new activities; however, it does not think that a new organization for the purpose of gaining such information is necessary or desirable. The Economic and Social Council would itself be the appropriate body, under the authority of the General Assembly, to initiate and guide the programme. Nevertheless, in a reference to the availability of manpower and resources the Committee puts its finger on the weak spot. Apart from the financial instability or weakness of the United Nations, the proposals put a severe burden on the highly skilled manpower which is already severely strained in the developed countries. The former might be eased—given goodwill and acceptance of their full responsibility by members of the United Nations. The latter is a problem that is not easily removed without much closer attention being paid to the use of scientific and technical manpower as well as to its training.

Nevertheless, in this report the Committee sounds a confident note. It welcomes the constructive self-examination already being undertaken by the organizations of the United Nations. This is an essential first step to expansion in fields where activity is still low, and the Committee also emphasizes that evaluation should cover failures as well as successes. It notes the central place which scientific policy on the part of Governments must take, and, while it welcomes particularly the survey of co-operation between universities and scientific and technological institutions in different countries which Unesco has undertaken, it considers that co-operative links should be encouraged and extended to other fields. Such fields should include, for example, engineering and public utilities, and liaison between semi-government and non-government agencies concerned with science and technology. It notes that even between universities a long-term rather than a short-term approach is desirable, and it suggests that Unesco should examine the desirability and practicability of establishing liaison bureaux to facilitate contact between institutions in developing and developed countries.

Given the funds and scientific staff, as well as sufficient authority, the Committee believes that it can move ahead with confidence along the lines indicated in this report and it is proposed that a series of working groups will enable the Committee to keep progress under review, handle the problem of information, and accelerate the critical programme of education, as well as keeping in touch with whatever action its individual members may take. The report is now, it is understood, being considered by the Economic and Social Council and it is clearly important that the Committee's conclusions and recommendations should be widely discussed by scientists and technologists throughout the world. Only if such professional opinion is wholeheartedly behind the programme can its proposals have any hope of real success; but when all allowance is made for the present difficulties of the United Nations the report presents a challenge which deserves a strong and imaginative response.

## ENRICO FERMI

Enrico Fermi

*The Man and his Theories.* By Pierre de Latil. Translated by Len Ortzen. (Profiles in Science.) Pp. 178+Illustrations. (London: Souvenir Press, Ltd., 1965.) 21s.

THIS is another book in a series of concise works each of which deals with the life and work of a scientist. Pierre de Latil tells the story of Fermi's life, with ample detail to bring the man to life without swamping the explanations of the scientist's achievements.

As a young Roman student, Fermi began studying when the first explanations of the new physics had gained acceptance. He was able to build on this work to such an extent that he participated in most of the following stages of the discovery of atomic energy. In particular, he was responsible for the design and construction of the first self-sustaining uranium-graphite pile set up underneath the grandstand of the squash court at Stagg Field, the University of Chicago Stadium, in 1942, and he was one of the chief figures in the making of the first atomic bomb.

In the first place he worked as a theoretician, but this led him to spend an ever-increasing time on experimental work. The lack of technical means for continuing his experiments forced him to become his own engineer. His ability in all three fields enabled him to tackle the vast problems which he considered. He could have re-organized the whole of Italian physics teaching, but the Dictatorship in his own country was no more tolerant and enlightened than that in Germany, and in 1938 he accepted an appointment at Columbia University, immediately after his award of a Nobel Prize, which actually assisted his escape from his native land. The United States, with its vast resources, suited his genius, but for a time he was regarded as an enemy alien and subjected to security control. Indeed, although he entered the United States with a Nobel Prize, he was subjected to an infantile examination in arithmetic. Fermi died philosophically and stoically from internal cancer at the age of fifty-three.

The translation of the book, though it projects the spirit of the original, is sometimes defective; for example, fluorine and nitrogen appear as fluor and azote, respectively. Some surnames, such as those of Wigner and Bethe, are misspelt.

The chronology of some of the events in the history of atomic physics is open to question. For example, the discovery of artificial radioactivity in 1934 is described as the "first time that man had provoked activities within the nucleus of the atom". Cockcroft and Walton (1932) are not even mentioned, but the transmutation of the lithium atom might be regarded as a man-made nuclear activity. The discovery of radioactivity by Becquerel early in 1896 is described as "through pure instinct as an experimental physicist". It is surely well known that Becquerel read Röntgen's X-ray paper of December 1895, and this suggested to him an experiment of a complementary nature, which, while it did not give the expected result, quickly led to the discovery of the phenomenon of radioactivity.

This useful book is well produced and contains many good illustrations.

W. L. SUMNER

## WAVE PROPAGATION THROUGH THE IONOSPHERE

*Lectures on Modern Magneto-Ionic Theory*

By K. G. Budden. (Documents on Modern Physics.) Pp. 82. (London and Glasgow: Blackie and Son, Ltd., 1964.) 30s.

THE purpose of this book is to introduce advanced students to the theory of wave propagation through the ionosphere. This transmitting medium is both

absorbing and doubly refracting, and it often varies with distance neither slowly nor quickly compared with a wavelength; an understanding of how waves travel through it therefore involves consideration of several fundamental ideas of considerable complexity. The book includes, for example, discussion of wave packets, group velocity, signal velocity and energy velocity, resolution into angular spectra, the relation between ray surfaces and refractive index surfaces, the impulse response of transfer functions, Sommerfeld precursor waves, reflexion coefficients and their relation to characteristic admittances, WKB solutions, phase integral methods, and the methods of steepest descent. Although the topics discussed are of immediate importance for ionospheric theory they are treated so generally that they can be applied immediately to the propagation of other kinds of waves.

Dr. Budden has not attempted to write a mathematical text-book; to some extent he assumes that the basic theory has been studied elsewhere and his aim is to discuss it for the purpose of understanding the physical behaviour of the medium and of the waves. His book will help the advanced student, for whom it is intended, to realize that mathematics is not just a substitute for an automatic computer which provides the 'answer', but if it is followed intelligently step by step it also provides a precise description of the physical processes concerned. It is a guide rather than a text-book and, because it is based on a lecture course, it is ideally suited for its purpose. Dr. Budden, well known as a lecturer and a director of research students, has performed a considerable service in making some of his best lectures available more widely in this way.

The book can be recommended to all those who work in the complicated subject of wave propagation through magneto-plasma. Beginners will use it to help them to understand text-books and papers; established workers will use it to clarify their ideas; and experts will read it to admire the way in which the essentials of a problem are exposed in all their simplicity. Those who believe they have fully understood a problem when they have discussed it with the help of a complicated piece of mathematics will learn from this book how it is possible to acquire an even deeper understanding through a detailed discussion of what the analysis means.

J. A. RATCLIFFE

## CHEMICAL REACTIONS OF POLYMERS

*Chemical Reactions of Polymers*

Edited by E. M. Fettes. (High Polymers: a Series of Monographs on the Chemistry, Physics, and Technology of High Polymeric Substances, Vol. 19.) Pp. xxii+1304. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1964.) 300s.

CHEMICAL reactions of high polymers are of considerable scientific and technological importance. Knowledge of reactions that may occur during processing, exposure to heat, light and other forms of radiation, and to oxygen or ozone, is essential to an understanding of the factors governing polymer stability in different environments. Technological applications of high polymers have been greatly extended by the use of such reactions as those causing cross-linking, the combination of different polymers and modification of structure and therefore of properties. Although many of these reactions have been known for a long time and, with others, have been extensively studied, the general subject of chemical reactions of polymers has not previously received separate treatment. This book, intended for graduates in chemistry and in which a knowledge of the structure and properties of polymers is assumed, is designed to give a critical survey of the various types of reaction in which at least one re-

actant is a high polymer. Forty-seven contributors, drawn from the United States, Great Britain and France, provide sixteen chapters, each of the latter generally covering either a type of reaction common to many polymers or reactions of a particular polymer or group of polymers.

The first chapter provides a general discussion of reactions and reactivities of polymer functional groups and of some of the factors affecting reactivity. The second deals with reactions of unsaturated polymeric hydrocarbons and is, perhaps inevitably, largely concerned with reactions of rubber. Isomerization, cyclization, addition of thiols, halogenation, epoxidation and hydrogenation are among the topics covered. A shorter chapter on reactions of saturated polymeric hydrocarbons includes poly-olefine substitution, reactions involving halogens and side-chain and nuclear substitution in polyvinyl aromatics. A chapter concerned with addition polymers containing reactive terminal groups deals separately with the production and reactions of such polymers produced by free radical and ionic mechanisms, consideration of the latter including 'living' terminal groups.

Two chapters are devoted to reactions of natural polymers. One, dealing with cellulose, gives a useful review of degradative and substitution reactions and a brief account of cellulose as a substrate for vinyl graft polymerization. The other is longer; it is concerned with proteins, and deals in some detail with protein structure, reactions of side-chain and main-chain groups, cross-linking, grafting and the effects of high-energy radiation on proteins.

Different types of interchange reactions of polyesters, silicones and polysulphides form the subject-matter of one chapter, and another, on cleavage reactions, deals with hydrolysis of polyesters and polyamides, the thermal degradation of polymers generally, and degradation resulting from irradiation and ozonization. Cross-linking is considered in a chapter on intermolecular reactions which includes cross-linking by irradiation and by monomers, their effects on structure and properties and cross-linking of elastomers. An account of branching reactions covers self-branching in the polymerization of ethylene, vinyl monomers and dienes and also graft copolymerization in some detail. Coupling reactions form the subject of another chapter in which polysulphides, epoxides, polyurethanes and related isocyanate polymers are considered. A chapter on surface reactions, largely concerned with surface grafting, includes a brief account of chemical treatments of polymer surfaces. A short account of oxidative reactions deals with polymeric hydrocarbons, including consideration of antioxidants, halogen-containing polymers, vinyl and condensation polymers. Mechanochemical reactions are reviewed in a short chapter. The last two chapters, dealing with fibre-reactive dyes and chemical finishing of cellulosic fibres, are more technological in nature. Extensive bibliographies at the end of each chapter generally cover journals, books and patents up to 1963 and also provide references to *Chemical Abstracts* for abstracted patents, for most non-American references and for other less readily accessible ones.

Among the factors to be considered in assessing the value of a book of this type are the arrangement, balance and possible overlapping of contributions and, in these days of an information explosion, the availability of the information from other sources. The scope of the book and the need of groupings for which suitable contributors could be found have led, as the editor points out, to a hybrid system of arrangement. There are inevitable differences in style and approach, but variations in level and character between different contributions are not usually very great. The last two chapters seem rather out of keeping with the rest of the book and might perhaps have been omitted. Overlapping is not serious and where certain topics, such as hydrolysis and grafting, appear in more than one chapter they usually refer to different polymers. A more serious criticism, perhaps, is that some

topics, for example the reactions of cellulose and of rubber, thermal degradation and mechanochemical reactions, are well covered in other books. Their inclusion, in some cases as relatively short reviews, may perhaps be justified in that it enables the book to provide a useful survey of the principal reactions of high polymers. It should be of considerable value to both academic and industrial polymer chemists although its high price makes it unlikely to be included in their personal libraries. W. R. MOORE

## A CLASSICAL BOOK ON ANALYTICAL DYNAMICS

### A Treatise on Analytical Dynamics

By L. A. Pars. Pp. xxi + 641. (London: Heinemann Educational Books, Ltd., 1965.) 147s.

THIS volume represents the distillation of the life work of a Cambridge mathematician of the old school who devoted himself mainly to that realm of science that has engaged the attention of great applied mathematicians down the ages ever since its inception by the supreme master of them all. The author can well be proud of the beautiful achievement that is this book. L. A. Pars spent his working years as a genuine full-time don, undistracted by extramural activities and the anarchic scramble for 'recognition'; he remained cloisteredly devoted to College and Faculty, the latter so much so that he retired from it when there began the end of the great traditional standards so carefully built up and maintained this century past.

In these days of speculative discovery it is sometimes almost forgotten that gravitation remains the principal governing force throughout the universe, and that accordingly dynamics as such can never lose its importance. As I write these lines a space-ship is hurtling towards Mars guided on an astronomical course of unerring precision by methods that no one would dream of querying, so secure is the basis in Newtonian mechanics. That dynamics has largely been superseded by relativity as an academic subject for study in no way lessens its importance, and it is far from generally appreciated how little is known and how numerous are the unsolved problems in the great field of celestial mechanics. Moreover, relativity has yet to provide explicit workable rules remotely comparable with those of classical mechanics that can actually be applied directly to real problems.

The present volume is a masterpiece of scholarship and erudition in which the structural elegance of pure mathematics is brought to the presentation of the whole realm of classical dynamics. It eminently succeeds in its intended objective, which is to give a compact, consistent, and reasonably complete account of analytical dynamics as that subject now stands. The treatment adopts as its fundamental basis the virtual-work equation:

$$\sum (m_r \ddot{x}_r - X_r) \delta x_r = 0$$

of Lagrange, and developing this into various equivalent forms shows how these have their own special utility and suitability for particular domains of the theory. Pars develops each of these areas with sufficient completeness to equip the reader with the fundamental ideas and relevant techniques and enable him to proceed to further study and research.

The net result is a massive tome of transcendent worth, and though it is so much more than a text-book one must hope that present-day students will put themselves in the way of benefiting from it. One is reluctant to think that it may perhaps be too voluminous to appeal to students to-day, when the brief pocket-volume is so much more in keeping with the lines along which they are now being encouraged in their headlong demand for short-cut education. For the same reason, it is perhaps to be regretted that sets of illustrative examples do not round off the end of each chapter and that comparatively few

worked examples figure in the text, for surely it is only by such means that a reader can make certain that he understands how to apply the theory, however seemingly perfectly and extensively he may be able to reproduce it. The book can be unhesitatingly recommended to all—student, teacher, research-worker, professors, and others—and in the hope that a second edition is called for soon perhaps Mr. Pars could meanwhile collect and supply some examples further to enhance the value of this remarkable book.

R. A. LITTLETON

## THE BRITISH ISLES

### Field Studies In the British Isles

Edited by Prof. J. A. Steers. Pp. xxiii + 528. (London: Thomas Nelson and Sons, Ltd., 1964.) 70s. net.

### The British Isles

A Systematic Geography. Edited by Prof. J. Wreford Watson and Dr. J. B. Sissons. Pp. xii + 452. (London: Thomas Nelson and Sons, Ltd., 1964.) 45s. net.

WHEN the twentieth International Geographical Congress was held in the United Kingdom during July–August 1964 and attended by some 2,200 delegates, it followed the pattern laid down by the preceding Congress held in the five countries of Norden in 1960. The week of main meetings, opened by Her Majesty The Queen, accompanied by His Royal Highness Prince Philip, in the Albert Hall on July 21, was preceded and followed by specialist symposia in different parts of the United Kingdom. In this way every university and many other institutions in the country had an opportunity of playing a part. There were no less than 33 such symposia; some were essentially regional—such as the meetings in the Channel Isles, the Outer Hebrides, or Ulster—others concerned themselves with a definite topic, such as the agricultural geography of Ireland, the geomorphology of Snowdonia, or urban growth in Yorkshire and Lancashire, while still others were wide-ranging, such as geography in the National Parks, or the eight-day study tours of Ireland and Scotland.

*Field Studies* is a substantial well-produced volume of 528 pages, fully illustrated with maps and diagrams, which provides the essential background material to these symposia and study tours. Whereas previous Congresses have issued 'excursion guides' of a more or less ephemeral nature, this volume has both a permanent and topical value not only because it affords regional descriptions by the experts on the spot, but also because the itineraries followed could scarcely be bettered by anyone wishing to see on the ground the various aspects of Britain's geography. There is one chapter only on London, but the varied aspects of London's geography, seen by many during the main Congress, are covered in *Guide to London Excursions*, edited by K. M. Clayton (obtainable from the London School of Economics, 21s.).

All full members of the Congress were also presented with a volume of *Abstract Papers* (with supplement) edited by F. E. Ian Hamilton (now obtainable from Geographical Publications, Bude. 7s.) and in due course will receive the *Proceedings*, but they also received on registration *The British Isles*, a finely produced volume to which 23 leading British geographers have contributed. Among the standard works already in existence, *The British Isles*, by L. D. Stamp and S. H. Beaver, now in its fifth edition (Longmans, 1963), covers in sequence the varied aspects of the geography of the British Isles, and *Great Britain: Geographical Essays*, edited by Jean Mitchell (Cambridge, 1962), deals with the country by regions. Consequently, the Editorial Committee felt that the Congress volume should take up topics in sequence. Thus, the senior editor contributes a first chapter on the "Individuality of Britain", and is followed by R. W. Steel on "British Geographers", G. R. Crone on the "Mapping of

Britain", S. Gregory on "Climate", W. G. V. Balchin on "Hydrology", and so on. While there are chapters which cover the usual—such as "Relief and Structure" (G. T. Warwick) or "Agriculture" (W. R. Mead)—all have a freshness of approach and presentation; other contributions reflect new and developing aspects of study, such as Emrys Jones on "Cultural Geography".

Modern geographers commonly accept that their study is essentially that of the relation between man and his environment, a relationship which has undergone a complex evolution through the ages and which projects itself into the future in the realm of physical planning. Those geographers who concern themselves with the physical background, whether the relief of the land or the vagaries of climate, have become increasingly aware of the importance of minutiae. This is well brought out in the three chapters: "Tertiary Landscape Evolution" (D. L. Linton), "The Glacial Epoch" (J. B. Sissons) and "The Evolution of the Climatic Environment" (G. Manley). The essays which follow are nearly all heavily weighted on the historical side—from prehistoric (E. E. Evans), pre- and post-industrial revolution (H. C. Darby and R. Lawton) to the present-day problems of land use (J. W. Birch and J. T. Coppock) or the mineral industry (S. H. Beaver) and industry (E. M. Rawston).

As with all such collections of essays, the specialists expand on their own interests—A. E. Smailes on towns, A. O. O'Dell on transportation, H. Thorpe on rural settlement, and R. H. Osborne on population, but, thanks to skilful editing, obvious gaps are few.

The Congress has thus made a notable contribution to geographical thought: it has adequately supplemented, rather than replaced, existing works, and Britain is now well served by the range of books available.

L. DUDLEY STAMP

## THE FUNGI

### Die Pilze

Grundzüge ihrer Entwicklungsgeschichte und Morphologie. Zweite, umgearbeitete und erweiterte Auflage. Von Prof. E. Gellmann. (Reihe der Experimentellen Biologie, Band IV.) Pp. 541. (Basel und Stuttgart: Birkhäuser Verlag, 1964.) 66 Sw. francs.

THIS is the second edition of *Die Pilze*, first published in 1949 and translated into English by Wynd in 1952. The second edition is some 120 pages longer than the first and contains 170 more illustrations. In content the book is modelled closely on its predecessor, but there has been considerable revision of the classification of Ascomycetes. The emphasis throughout is on morphology and life-cycles. Attempts to interpret the probable course of evolution to elucidate phylogenetic relationships are frequently represented in the form of phylogenetic trees. The evidence of such interrelationships is often very slight, and although the suggested relationships are qualified as conjectural, there is a danger that they will become accepted as established.

Apart from the notable omission of the Fungi Imperfecti, dismissed in little more than a page, and the lichens, most groups of fungi have been dealt with. The classification of the 'Phycomycetes' does not reflect recent thinking (see, for example, the work of Sparrow), and the group Archmycetes is retained for holocarpic zoospore forms. This has the unfortunate effect of separating *Olpidium* and *Synchytrium* from the Chytridiales. The Saprolegniaceae, Peronosporaceae and Leptomitaceae are treated as families of the Oomycetes, while the Mucoraceae, Endogonaceae and Entomophthoraceae are treated as families of the Zygomycetes, instead of being accorded original rank.

The classification of Ascomycetes has been radically revised, with the structure of the ascus wall emphasized as a primary criterion of separation. Three sub-classes

are proposed: protunicate, unitunicate and bitunicate Ascomycetes. The protunicate forms are those in which the ascospores are not violently projected, and the ascus walls of which break down when the spores are ripe. Although this novel proposal has some merit in that it directs attention to the ascus wall, it is doubtful if the protunicate Ascomycetes represent a natural assemblage, because such diverse groups as the Endomycetales, Aspergillales, Microascales, Coronophorales, Onygenales, Meliolales and Laboulbeniales are included.

Some modification to the classification of Basidiomycetes has also been made. There are two sub-classes, the Holobasidiomycetes and Phragmobasidiomycetes. Within the Holobasidiomycetes the following groups are included: Aphyllophorales, Agaricales, Agaricogastreales, Gastreales, Phallales. The account of the Agaricogastreales is especially useful. The arrangement of the Phragmobasidiomycetes is more conventional, embracing Tremellales (inclusive of the Dacrymycetaceae), Auriculariales, Uredinales (with a masterly account of variation in life-cycles) and Ustilaginales.

Although the second edition is considerably longer than the first it is unfortunate that no space has been devoted to some recent developments. For example, there is no reference to the role of *Olpidium* as a virus vector, and the demonstration of heterothallism in *O. brassicae*. The appearance of new biotypes of *Synchytrium endobioticum* is ignored. The whole question of physiological specialization of pathogenic fungi is scarcely mentioned. Very little notice is given to the fine structure of fungi apart from figures of flagella of *Allomyces* and *Saprolegnia*. No details are given of fine structure of cell walls, septa, haustoria, or of yeast cells. Sansome's claims that some Saprolegniaceae and some Pythiaceae are diploid are not discussed. Sexual reproduction in the Mucorales is dealt with very briefly, and without reference to the work of Banbury and Plampel, or to the variation in content of germ sporangia in relation to mating type. The relationship of *Endogone* to phycomycetoid mycorrhiza is not mentioned. In the account of the Entomophthorales there is no mention of the variety of methods of conidial propulsion, and only the secondary adhesive conidia of *Basidiobolus* are figured. The relationship of certain Ascomycetes to their conidial states is treated very briefly. Although *Cephalosporium* and *Gliocladium* are mentioned as conidial states of *Hypocrea*, there is no mention of *Trichoderma*. It is not made clear that the macroconidia of the Hypocreaceae are phialospores, and while it is true to say that some *Ophiobolus* and *Leptospharia* species have *Phoma*-type pycnidia there are at least five other form-genera known. The genetical treatment is weak. There is no mention of heterothallism in yeast, of heterokaryosis and parasexuality in *Aspergillus* and *Penicillium*, of the tetrapolar condition or multiple alleles in Basidiomycetes. The function of the clamp connexion in ensuring redistribution of two compatible nuclei in a hyphal tip is not explained. Alternative suggestions for the mechanism of basidiospore discharge are not discussed, nor are the observations that basidiospores often carry an electrostatic charge. The significance of the divided gill of *Schizophyllum* is not given. There is no account of hyphal structure of polypore fruit bodies.

Many of the illustrations appeared in the earlier edition, copied from other authors. The best of the new figures are from the pen of E. Müller illustrating Ascomycetes. Although it is liberally illustrated, more half-tone photographs, especially of pathogenic fungi, would have further improved the text. A student would, for example, learn little of the habit of *Plasmidiophora basicae*, *Phytophthora infestans*, *Albugo candida*, *Entomophthora*, *Taphrina* or the Ustilaginales. An irritating feature is the occasional use of different names for the same organism in figures and text, for example, *Pyronema omphalodes* and *P. confluens*, and *Rhizina inflata* and *R. undulata*.

There is a similar inconsistency in the use of *Agaricus* and *Psalliota*.

To sum up, this is not a book for a beginner. It lacks excitement and neglects many features of the 'biology' of fungi. As a source of ideas on phylogeny it is valuable provided that the ideas are treated with due caution. The style is terse and authoritative, and apart from a few minor errors and misprints, its chief defects are in its omissions. It is well produced, but at 66 Swiss francs (£5 7s. 3d.) it seems rather expensive. JOHN WEBSTER

## GERMAN AND ENGLISH DICTIONARY OF PHYSICS

Dictionary of Pure and Applied Physics

Compiled by Dr. Louis de Vries and W. E. Clason. Vol. 1: German-English. Pp. 367. 1963. Vol. 2: English-German. Pp. 341. 1964. (Amsterdam, London and New York: Elsevier Publishing Company.) 55s. each volume.

In these two volumes, two of the leading scientific lexicographers of the present day have combined their efforts and produced a compact work of reference giving a good coverage of the field of pure and applied physics (a field which certainly lacked such a dictionary hitherto). The number of terms on each page is 80-100, and so it can be seen that in more than 300 pages a very considerable vocabulary is presented. Although this includes words such as *stehen* and *stellen*, and phrases such as *wie immer*, the very great majority of the terms belong specifically to the field of physics. Adjective-noun and most other phrases are entered under the first word, which is convenient, although cross-references from the other words might have been worth the extra space needed. Entering *stark gedämpft* under *stark* is not likely to be useful (and there is no corresponding entry at *schoach*); is there not a case for an indexed thesaurus arrangement rather than an alphabetical arrangement in dictionaries such as these?

Dr. de Vries's habit of omitting any distinction of meanings, though mitigated by the restriction to physics, is still in evidence ("*line*, Leitung, *f*, Stromkreis, *m*, Zeile, *f*, Linie, *f*, Schirmschrift, *f*"), although oddly enough there is some attempt to make the distinction with non-technical words: "*serious* (earnest), ernst"—*serious* (grave) presumably not being considered worth including, nor is it even given as an equivalent of *schwer* ('heavy, stout, strong, difficult').

The spellings are not always correct; one page contains "hydroxyle ions" (the same in the other volume) and "shell spektrum" (which becomes "shell spectrum" in the other volume). American spellings appear to be used throughout, with no mention of British forms. But British terminology (for example, *valve* rather than *tube*) appears more or less randomly; of the relevant entries beginning with *Röhre*, thirteen use *tube*, ten *valve*, six both (and three neither). The compilers do not name their sources, but the material thus seems to have been incorporated somewhat uncritically. Moreover, a check of one page taken at random in Volume 1 showed that about three-quarters of the entries are essentially identical with entries in de Vries's *German-English Science Dictionary*, third edition, which costs about the same, and so anyone who already possesses the latter book will probably not be getting much for his money if he buys Volume 1 of the *Dictionary of Pure and Applied Physics*.

I am myself concerned almost exclusively with translation into English, and the German-English volume has been mainly considered. The English-German volume is by no means a mere inversion of the other, and should be equally useful to translators from English into German; but let us hope that these do not include many of the "students in science and technology in the United States" for whom (according to the preface printed in both volumes) the dictionary is also intended. J. B. SYKES

## A SCIENTIST'S PHILOSOPHY

## The Philosophy of Science

A Systematic Account. By Peter Caws. Pp. xii+354. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1965.) 52s. 6d.

THIS book, as well as being attractively produced and clearly written, is full of suggestive material for instruction within the general range indicated by the title. It is indeed 'a systematic account', but it is a good deal more than that, and it is therefore on those additional features that the present review will focus attention.

The author divides his work into four parts: 1, "The Discovery of Theory"; 2, "The Structure of Theory"; 3, "The Validation of Theory"; and 4, "The Spectrum of Theory". From this plan, the reader learns quite naturally what science is, and to what extent it is conformable with the remainder of normal living and experiencing. Then, the pattern of exact thought emerges, with the consequent background of organization. After that, anybody who asks why it is that scientific method engenders confidence will find a good answer, and lastly (and more strictly philosophically) what problems have been solved, and what remains to be done. All this is extremely readable, presented with common sense, but without being particularly noteworthy.

Different in calibre, however, are Chapters 43 and 44, which cause the reader to become air-borne, and well away to a flying start on themes that really matter, especially in the present climate of epistemology. Thus, "The Unity and Diversity of Science" (43) and "Science and the Humanities" (44) are first-class contributions to what may still be called the theory of knowledge. The questions which they raise are so important as to warrant comment in some detail.

The initial tenet is that there must be a perpetual 'in-phase' relationship between logical and experimental frontiers—a species of mutual feed-back, which is in harmony with much of general system theory, as commonly understood. The next step is to realize that the logical and rational element needs a principle of economy rather than one of simplicity, leading to a degree of austerity closely linked with aesthetic satisfaction. It is only too true, as Dr. Caws remarks, that some scientific theories give an impression of the baroque (they have, one might add, occasionally become almost rococo). But this is largely a matter of touch, itself in need of prudent discipline. Practically, however, we must 'save the appearances', as the Greeks maintained. It is impossible to avoid some growth in complexity as knowledge advances, or more exactly, as new evidence accumulates. The time arrives when expansion becomes coercive. Thus, the shift from the simple gas laws to Van der Waals's equation is mentioned. This is a classic instance, and much was gained in the process (a refined version of the critical state, for example), but it is well known that a whole host of other formulae—one of them at least being a transcendental function—were propounded at about the same time, to improve on the unhappy plight of  $PV = RT$ . That 'Van der Waals' survived is as much as to say that it was the best 'fit', *ceteris paribus*. Nevertheless, it implied a lowering of standards of elegance.

The quotation from Pascal's *Pensées* is apt, namely, the difference between '*esprit de géométrie*' and '*esprit de finesse*'. Adherents to the former are in safe company with the logicians and Cartesians, whereas supporters of the latter approach very closely to toying with metaphysics, when it comes to the point. But "other ways of knowing the world", tiresome as they may be to the formalist, have satisfied axiological elements all through the centuries: indeed, they watch with eternal vigilance for any vestige of arrogance in scientific claims in regions wherein other kinds of awareness illuminate the part played by value in human life and relationships.

Chapter 44 goes to the root of contemporary discomforts in regard to liberal studies. Historically, one may still marvel at the virility of Magna Graecia; localized seats of learning throwing up important advances all along the Mediterranean shores. Concerning experimental techniques, it is certain that these were frowned on by the scholars, but in a rather subtle way. Seemingly, the objection was to application, in contradistinction to the careful questioning of Nature, with the sole object of revealing her secrets.

The particular power of Aristotelianism—at its best—is well displayed as a unity of knowledge, be it metaphysics, drama, or anything in between. The evils, as well as the benefits, of specialization were yet unknown. But one development, mentioned earlier in the book, namely, the philosophical system of St. Thomas Aquinas, did much more than merely re-edit Aristotle. It largely created natural theology, thus adding its own quota to the growing body of erudition, culminating in the renaissance and the gradual acceptance of a worthy standard of 'polite learning'. Dr. Caws is doubtless aware of the revival of Thomism, in twentieth-century form, which provides such a marked, if surprising, characteristic of much modern philosophy, both in Europe and in the United States.

These pages end on exactly the right note. Skills, be they scientific, philosophical, artistic and all the rest, are priceless possessions, and there is no need to set up rivalries between them. Time was when *parti pris* generated considerable heat between the devotees of Newton and Goethe. Luckily—and on the whole—charity, like entropy, tends to increase. F. I. G. RAWLINS

## CULTURAL DEVELOPMENT OF MAN

## History of Mankind

Cultural and Scientific Development. Vol. 2, Part 1, 1200 B.C. to 500 B.C.: Pp. xxxiv+313; Part 2, From about 500 B.C. to the Christian Era: Pp. xiii+314-664; Part 3, From the Beginnings of the Christian Era to About A.D. 500: Pp. xii+655-1048, maps and illustrations. By Prof. L. Pareti, P. Brezzi and L. Petech. (Published for the International Commission for a History of the Scientific and Cultural Development of Mankind by George Allen and Unwin Ltd., London, 1965.) 126s. per set of 3 volumes.

VOLUME 2 of *History of Mankind* takes the story of man's cultural development from 1,200 B.C. down to A.D. 500, 1,700 years that witnessed the fragmentation of the widespread cultural complexes of the second millennium B.C. into small regional cultural groups, the domination of certain political systems, and the emergence of great religious beliefs. To understand why this volume achieves a measure of both success and failure in its aims, we must examine its own story. The history of the writing of this volume is almost as complex as the historical events that it documents. Prof. Pareti was initially appointed as author-editor, with Prof. Brezzi and Prof. Petech as assistants. The draft text was finished in 1960, and after revision on recommendations from nominated specialists, the second draft was again circulated and additional material incorporated as editorial notes. Later six other authors contributed supplementary matter which appears in the text. Although considerable care seems to have been taken to preserve the continuity in content and style, it has clearly been impossible to eliminate all the interruptions where different authors are juxtaposed. This presents certain difficulties, but is at the same time the main reason for the success of the book because here, in one volume, we have the consensus of opinion about many problems of long standing, for example, the origin of the Etruscans. It is perhaps a comment on the state of our knowledge and understanding of pre- and early



historic events that such opinions are often so widely divergent.

The volume is divided into three parts: from 1,200 to 500 B.C., thence to the Christian Era, thence to A.D. 500. Each part is treated in similar vein, beginning with historical events, and followed by language, technology, organization, religion and literature with their related topics. From some aspects this arrangement is to be disputed, apart from the artificial separation of such subjects as politics and economy, because it is difficult to trace the cultural and scientific developments in any one chosen area. Certain aspects of certain areas cannot be considered, and America and the Pacific are badly neglected, but perhaps inevitably when they must compete with Greece and Roman Italy, from where much of the evidence treated here has been derived.

The aim of the volume is to present a general impression of the development of technology, political organization, religion and so on, over widespread areas. As a result, the facts that can be given to describe a specific situation in a certain region cannot be more than an abbreviated summary, and will not be of much use to one who is looking for basic factual material, although an extensive bibliography of Greek, Roman and Near Eastern literature is provided. Nevertheless, it is of the greatest interest and value to have certain aspects of cultural development brought together from different areas for comparison; the emergence and dispersal of Indo-European languages is reasonably well known, if not agreed on, but less so the complex evolution of writing systems. Of particular interest, too, may be the evidence presented here for the discovery of scientific principles and allied philosophies.

In my opinion, the volume falls between two stools; on one hand, the purely objective and factual presentation with full bibliographical references; on the other, the theory of developmental processes throughout the world. The book scarcely aims as high as the first, and the nature of its production, with many differing opinions and editorial comments added, interrupts the flow essential to the second. Nevertheless, it probably comes as close as any book could to success in both fields. J. M. COLLIS

## SOIL FABRICS

### Fabric and Mineral Analysis of Soils

By Dr. Roy Brewer. Pp. xiii+470. (New York and London: John Wiley and Sons, Inc., 1964.) 113s.

SOILS are identified and classified to a great extent by characters which are essentially morphological and can be observed in the field. The most important morphological characteristic is of course the division of the soil profile into horizons. Field observations of soil morphology even when supplemented by physical and chemical analyses do not, however, permit a sufficiently detailed and certain explanation of the processes involved in soil formation.

The need for a more precise analytical approach to pedology is expressed in the introduction to this book. Dr. Brewer directs attention to the soil material rather than the soil profile as the unit of study. The purpose of the book is to provide a system of description and classification of the phenomena observed in soils and to demonstrate the application of mineral analysis to studies of soil genesis.

Mineral analysis, dealt with in the first section of the book, is presented with an intentional geological bias and much of the information relating to mineral grains, for example the analysis of size and shape, can be found in standard text-books of sedimentary petrology.

Calculations of soil formation are discussed, with emphasis on the use of a stable mineral to estimate gains and losses of weight and volume and of a particular constituent

during soil formation. The equations proposed to calculate these changes in the soil profile seem unnecessarily complicated and could easily be worked out from first principles. The author argues convincingly in favour of using parent rock selected on the basis of mineral analysis of the profile as the parent material for evaluating profile development. There is no doubt that the present arbitrary recognition of weathered rock as parent material leads to serious errors.

The subject of mineral stability and weathering is examined on a somewhat empirical basis. Considering the significance of plasma in soil fabrics, it is a pity that the relations between mineral stability and atomic structure were not explored further. One surprising error in this chapter is the assertion that "the rate of weathering of mineral grains is directly related to surface area of the grains, that is, inversely related to size of grains" (p. 74). Specific surface must of course be intended. A chapter on the classification of rocks as the parent materials of soils, completing the section on mineral analysis, is of limited value and in some particulars inaccurate.

The valuable section of this book, making up more than three-quarters of the whole, deals with the structure and fabric analysis of soils with special emphasis on the description, classification and, to some extent, the interpretation of pedological features. Most of the phenomena described are best seen in thin section and they are particularly well illustrated with more than one hundred photomicrographs.

Much of the recent research in this field has been promoted by Dr. Brewer and his colleagues at the Soils Division, C.S.I.R.O., Australia. It is not surprising to find that this section is very largely based on their work and that several important papers have been included with little amendment. As the question might arise as to whether specialists would be prepared to pay over £5 for the convenience of having these papers in book form it is only fair to add that they make up only about one-quarter of the section.

The concepts of structure and fabric are clearly discussed with new definitions proposed for terms in common, but sometimes vague, use among pedologists. The following new definition of soil structure is essentially the theme of the book: "Soil structure. The physical constitution of a soil material as expressed by the size, shape and arrangement of the solid particles and voids, including both the primary particles to form compound particles and the compound particles themselves; fabric is the element of structure which deals with arrangement".

The main components of soil structure, voids, pedological features such as cutans and crystallaria, the soil matrix and peds are clearly described and carefully classified.

It is worth mentioning the author's proposal that the term 'ped' be restricted to those aggregates entirely enclosed by natural surfaces. Peds are thus recognized by their surface characters rather than by the mere presence of a population of units of similar size and shape. Most field workers would probably support this suggestion.

A chapter is devoted to a discussion of the role of fabric and mineral analysis in soil science followed by an appendix describing the techniques involved.

This book does not, as suggested on the publishers' jacket, provide a thorough understanding of soil genesis and this could not have been the author's intention. It cannot be recommended, therefore, to students with only a general interest in soil science. The book is the most comprehensive treatment of soil fabric analysis at present available and, with the reservations expressed here, it will be welcomed by pedologists. The fact that the soil fabric must be significant in soil/plant relationships will mean that reference to this work will benefit research workers in a much wider field. The book is well bound and printed. J. L. M. LAMBERT



## Societas Scientiarum Fennica

Commentationes Humanarum Litterarum, XXXIV, 1: *Muslim Death and Burial: Arab Customs and Traditions, Studies in a Village in Jordan*. By Dr. Hilma Granqvist. Pp. 287. (Helsinki: Societas Scientiarum Fennica, 1965.) 14.80 F. Marks.

ARAB customs in connexion with death and burial is the third work of a trilogy by Dr. Hilma Granqvist. The other volumes have dealt with *Marriage Conditions in a Palestinian Village* and *Childhood Among the Arabs*. It is important that the folklore connected with these matters should be collected now before it is too late. The unchanging East is changing rapidly and the old customs and rituals are being forgotten.

*Muslim Death and Burial* is divided into three sections: (1) mortality, death, burial, after the burial, and unnatural deaths; (2) burial and mourning; (3) songs at death and burial. Dr. Granqvist lived for various periods in Palestine, especially at Artas, a village to the south of Bethlehem, and there made friends with the people and gradually collected the ancient folklore from them. While the book is full of stories and customs, it lacks any connective thread. However, to collect and preserve the folklore has been Dr. Granqvist's purpose, and in this she has succeeded.

Perhaps one or two examples will illustrate the matter. From the Earth is obtained the dust of which man is created. But it is only a loan from the Earth and repayment must be made at the place whence came the dust. Difficulties thus arise when a man dies far away from where he was born. As a man is one of a family, his relations must see that his dust is duly collected and returned. Again, a woman who has lived for a long time in a far-away village, having come there perhaps as a stranger to many, may die in a far country. This is explained by the saying, "Her dust drew her"—implying that somehow or other the dust from which she was originally made must have come from this place. Again, a doctor cannot treat a woman until a formula has been pronounced which makes her into his sister. For if the man sees merely up to her knees sin has been committed. If she is his sister this does not matter.

The account of a burial is particularly interesting. No unclean woman must take part; indeed, everyone at the burial must be ritually clean. Those who have to touch the body must have made their ablutions before the prayer over the deceased is given. When a dervish goes into seclusion he takes with him seven barley loaves containing bran, water in a dark blue Gaza jar, and a rattle of dates. This is because the demon fears a jar of this sort and demons never eat bran. They also fear a white cock with ten toes.

The foregoing short extracts will give an idea as to what the work is about. One can only be thankful that someone has collected these customs and written them up before it is too late. M. C. BURKITT

## Physikalische Begriffe in der Klinischen Biochemie

Von Dr. Hans Koblet. Pp. xii+274. (Stuttgart: Georg Thieme Verlag, 1964.) 30 D.M.

MEDICAL students are on the whole notable for their aversion to anything faintly mathematical. Yet all lines of research require mathematics as a scientific tool and medical science is no exception, and *Physikalische Begriffe in der Klinischen Biochemie* would scarcely have been written had Dr. Koblet not felt that the medical research worker needed some special help to overcome his difficulties.

The first part of the book deals with basic principles, the definition of a molecule, of specific weight and density, principles of solutions, titrations, law of mass action and chemical equilibrium. Other parts deal with acids and bases and the laws of thermodynamics. Particular attention is directed to enzyme kinetics and the calculation

of the Michaelis constant of enzymes. Attention is also given to pools and turnover rates, to diffusion and the connexion between clearance and elimination constants in the blood stream. There is a mathematical appendix in which briefly but very clearly differential calculus and integrals are explained. No one can protect the medical research worker from the hard work which is required if he is to make up for omissions of his student days, but this book will help him to fill some of the gaps because everything is derived from first principles and no previous knowledge is expected. H. LEHMANN

## Annual Review of Nuclear Sciences

Vol. 14. Edited by Emilio Segre. Pp. vii+510. (Palo Alto, California: Annual Reviews, Inc., 1964.) 8.50 dollars.

THE volume under review consists, as is usual in this series, of a large number of articles on a wide variety of topics. Ten articles are devoted to nuclear and particle physics, and to their associated experimental techniques. Two articles discuss "Breeder Reactors" and "Modern Techniques in Reactor Design", one the "Chemistry of the Actinide Elements" and one the "Quantitation of Cellular Radiobiological Responses". This volume therefore follows its predecessors in its rather curious mixture of contents.

The nuclear physics articles are concerned with "Alpha Decay", "Electromagnetic Moments of Excited Nuclear States", "Recent Progress in the Theory of Nuclear Matter" and "Nucleon-Two Nucleon Reactions above 100 MeV". The first of these concentrates on nuclei near lead and those heavier than thorium, with particular reference to calculations based on nuclear shell models. The article on "Electromagnetic Moments" is the longest in the volume and aims to be a complete review. That on "Nucleon-Two Nucleon Processes" summarizes in rather gloomy fashion the present state of our phenomenology and understanding and makes a number of useful suggestions for further work.

Short articles on "Spark Chambers", "Dynamic Orientation of Nuclei" and "Data Systems for Multiparameter Analysis" are well written as introductions to their respective subjects. That on "Data Analysis in Particle Physics", however, is aimed at the experimentalist who, though already adept at the manipulation of his data, has little or no formal training in probability.

The article on the "Structure of the Proton" is written from the experimentalist's point of view. Though directed at the expert, much of it can be read with profit by the tyro. That on "Symmetries among the strongly interacting Particles" appears to me to be strictly for the highbrows.

The fraction of nuclear and particle physics articles in this volume is close to the five-year average for this Review. One must therefore wonder what is the virtue in the editorial board's eyes of a policy of including regular articles on such topics as chemistry, reactors, biology, etc. They cannot serve as a means of broadening the nuclear physicists' interests, for they are written on too high a plane and surely are quite likely to be missed by the people most likely to benefit from them. B. ROSS

## Yearbook of Astronomy, 1965

Edited by Patrick Moore. Pp. vi+203. (London: Eyre and Spottiswoode (Publishers), Ltd., 1964.) 25s. net.

THE *Yearbook of Astronomy* continues its well-established system of providing month-by-month information about the night sky. Some of the articles are rather interesting and one of the most pleasant is that of James Muirhead, "A Cycle of Clusters and Nebulae", which could be sub-titled "star gazing for pleasure". However, there seems to be a decline in the quality of the articles generally which it is hoped will be corrected when the next *Yearbook* is produced. D. McNALLY

## SUBMARINE FRACTURE ZONES, ASEISMIC RIDGES AND THE INTERNATIONAL COUNCIL OF SCIENTIFIC UNIONS LINE: PROPOSED WESTERN MARGIN OF THE EAST PACIFIC RIDGE

By PROF. J. TUZO WILSON, O.B.E.  
Department of Physics, University of Toronto

THIS is the fourth of a series of articles advancing the hypothesis that another class of strike-slip faults can exist besides transcurrent faults<sup>1-3</sup>. Transform faults, as the class has been called, can only exist, and must be expected, if areas of the Earth's crust are being absorbed into the interior in some places and freshly formed elsewhere. If they have not attracted much attention heretofore, it is because they are largely a feature of the ocean floors and most examples are partly or wholly submarine. The fracture zones which B. C. Heezen, E. T. Bunce, J. B. Hersey and M. Tharp<sup>4</sup> have recently described in the equatorial Atlantic have been cited as examples of dextral, ridge-ridge, transform faults related to the growth of the Atlantic Ocean, and it has been suggested that these fracture zones join points on opposite coasts which were once in contact. Such points may be called conjugate.

### Three Guides to Reconstructing Continents

If this is so, three different guides have been proposed for fitting together the opposite sides of the Atlantic Ocean. The first method is well known and was used by A. Wegener, A. L. Du Toit and E. C. Bullard, J. E. Everett and A. G. Smith<sup>5</sup>. It depends on fitting the topography and matching the geology of opposite coasts. In Figs. 1 and 2 this is illustrated diagrammatically by the fit in shape of regions A and A'.

A second proposal<sup>6</sup> pointed out that, where a pair of aseismic ridges lead from an active volcanic island on the mid-ocean ridge to opposite coasts, the ridges join the coasts at conjugate points. For example, the Walvis and Rio Grande aseismic ridges join Tristan da Cunha Island to conjugate points on the coasts of Africa and South America. The ridges from Iceland to Greenland and Europe are another example. In Figs. 1 and 2 this case is illustrated by the lines Bb and bB'.

The third method suggested that at least some great submarine fracture zones are transform faults and that their ends join conjugate points<sup>1</sup>. In Figs. 1 and 2 this is illustrated by the transform fault and fracture zone C c c' O'.

In the South Atlantic all three methods lead to the same result, but at first sight one aspect is puzzling. Both the aseismic ridges and the fracture zones are the loci of past motions of the crust, but they are not parallel. Since a crustal plate cannot move in two directions at once, this seems to imply a contradiction.

### Possible Origin of Aseismic Ridges

One possible explanation is that different motions have been recorded. The fracture zones represent the motion of the plates in the crust relative to one another with no reference to the deeper mantle. It has been suggested elsewhere<sup>6,7</sup> that the pairs of aseismic ridges perhaps represent the movements of two crustal plates relative to a source in the mantle. Thus the aseismic ridges are chains of volcanoes, now inactive, which grew at a single place on the mid-ocean ridge and were then carried away, some to one side, some to the other, by streaming motion.

Their creation has been presumably due to unusually large and continuing source of lava in the mantle beneath the mid-ocean ridge (for example, Iceland and Tristan).

This pushing away of older volcanoes from a continuing source on a central rift zone was essentially the explanation for the geology of Iceland arrived at by G. Bodvarsson and G. P. L. Walker<sup>8</sup> as a result of field mapping. This explanation may also be extended to include the pair of ridges from Iceland to Greenland and Europe. These ridges lie in the same line, although they get older in opposite directions, are normal to the mid-ocean ridge and are parallel to the De Geer fracture zone, a transform fault lying between Greenland, Spitzbergen and Norway<sup>1</sup>.

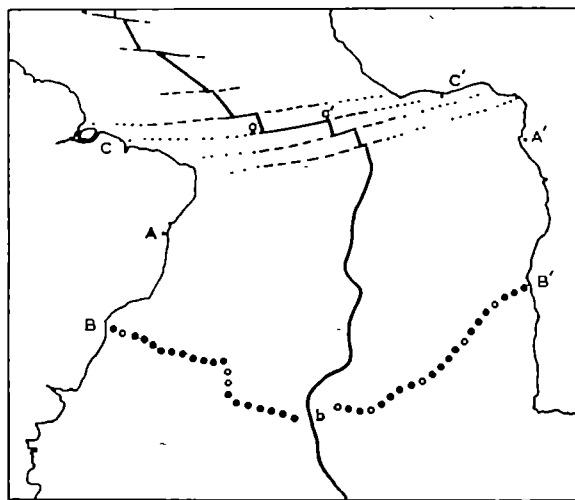


Fig. 1. Sketch map of the South Atlantic. A and A' are conjugate points which fit together. Bb and bB' are the Rio Grande and Walvis ridges and C c c' O' is a fracture zone.

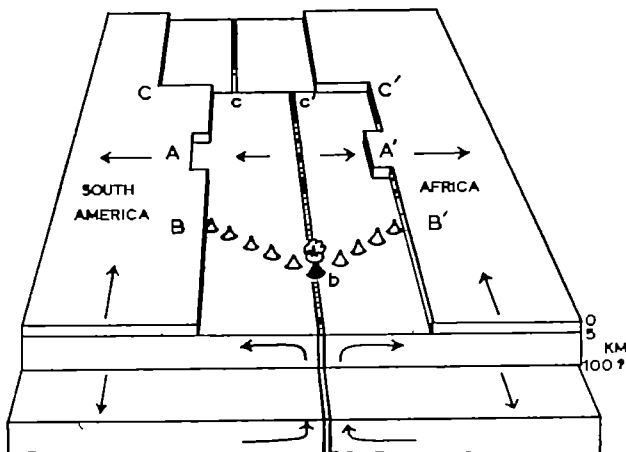


Fig. 2. Three-dimensional diagram illustrating how the two crustal plates on either side of the South Atlantic may be moving relative to each other and to a layer 100 km or more deep within the mantle.

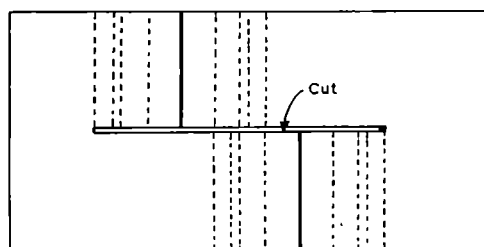


Fig. 3. Diagram showing how to cut and fold a cardboard and paper model to illustrate a fracture zone intersecting two lengths of mid-ocean ridge which are separating with the formation of new crust. —, Hinge to fold sides up, ---, hinge to fold sides down

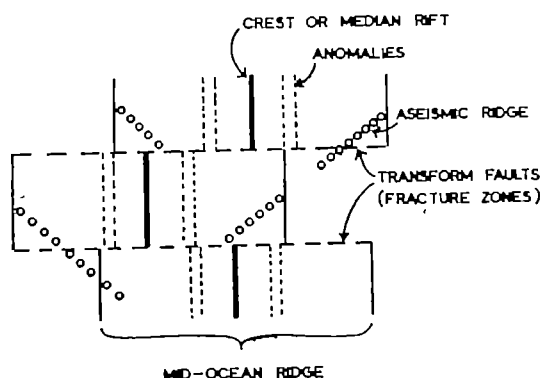


Fig. 4. Diagram showing the relationship of aseismic ridges to fracture zones which they intersect. Note that of each pair one has to terminate at a fracture zone

This arrangement of features may be interpreted to mean that the directions of motion of two crustal plates and of flow in the underlying mantle are all parallel with each other and normal to the axis of the mid-ocean ridge.

In the South Atlantic, on the other hand, the aseismic ridges form a chevron and are not parallel with the fracture zones of the equatorial Atlantic as Figs. 1 and 2 show. This has been interpreted to mean that the crustal plates are not moving in the same direction as that part of the mantle in which the source of the volcano lies. Since no method is known for measuring the direction of flow in the mantle directly, this interpretation is hypothetical, but, as will be shown towards the end of this article, it is an interpretation capable of further development.

In Figs. 1 and 2 the fracture zone  $O c c' O'$  is considered to represent the loci of relative motion of two crustal plates, whereas the two aseismic ridges  $Bb$  and  $bB'$  each represent the loci of motion of one of the plates relative to the mantle beneath. The crustal plates are not considered to be bounded by the Mohorovičić discontinuity, but by a zone of mobility in the upper mantle.

#### Relationships between Aseismic Ridges and Fracture Zones

This led me to speculate on what might be expected to happen if, during their growth, a pair of aseismic ridges came to intersect a fracture zone. This is hard to visualize, and indeed most of us are unaccustomed to thinking of geology in terms of growing oceans and continental drift so that this introduces new and difficult ideas. Simple paper and cardboard models help, and the construction of one which enables the properties of ridge-ridge transform faults to be studied is shown in Fig. 3.

With the aid of such a model and that of Fig. 4 it can be appreciated that on intersecting a transform fault one of a pair of aseismic ridges (that one which intersects the active part of the fault) is immediately cut off and removed laterally away from the source. That aseismic ridge must therefore come to an abrupt end against

the fault. The other aseismic ridge of any pair is not moved laterally, but the central rift in the mid-ocean ridge is moved away from it. It is therefore uncertain whether this aseismic ridge will end on the fault or continue. If it continues it will no longer touch the mid-ocean rift but it will seem to start at a random point. Only this member of each pair should still be active.

Some of the chains of islands in the Pacific Ocean seem to meet well the patterns predicted for pairs of aseismic ridges intersected by fracture zones as is shown in Fig. 5. In part, this is copied from Figs. 5.7 and 6.6 in H. W. Menard's book<sup>8</sup>, which shows three straight and parallel chains of volcanic islands (the Nasos, Cocos and Tehuantepec Ridges) each intersecting and apparently terminating against a fracture zone. It is particularly significant that on the other side of the East Pacific Rise in an approximately mirror-image position to the Nasos Ridge lie the Austral, Society and Tuamotu-Gambier ridges. This suggests that many chains of islands in the Eastern Pacific which are parallel either with the Nasos aseismic ridge or the Tuamotu aseismic ridge are associated in origin with the East Pacific mid-ocean ridge and that the great east-west fracture zones are too. If this is so and if the theory of transform faults is correct, then some very specific relationships can be predicted that should hold between the East Pacific Ridge, fracture zones, aseismic ridges and the patterns of magnetic anomalies.

The East Pacific Ridge has been well described by H. W. Menard<sup>8</sup> and the following account owes much to him and especially to his Fig. 6.12. This has been redrawn and modified in Fig. 5, which shows the general shape of the ridge. The eastern margin and northern crest have been overridden by the Americas. H. H. Hess has given me much advice, especially about the western Pacific. It would be interesting to identify the remaining, western margin of this ridge and important for the present discussion.

#### The ICSU Line, Possible Margin of the East Pacific Ridge

Menard shows the western margin of the East Pacific Ridge crossing the Pacific Basin. This margin should surely be discernible and, in seeking to identify it, other known rift margins provide the best possible guide. Heezen and Ewing<sup>10</sup> have implied that the Lomonosov Ridge is such a submarine margin. It may be partly volcanic according to Gakkell and partly sedimentary according to Ostenso. Elsewhere the Atlantic is bordered by continental blocks. Along the coasts are sporadic, large outpourings of basalt as in Greenland<sup>11</sup>. The only features which lie in the expected location in the Pacific Basin and which appear to have the required properties are, from north to south, the Emperor Seamounts, the group of very large seamounts at the north-western end of the Hawaiian chain, part of the western end of the Hawaiian Islands, part of the Mid-Pacific Mountains, the Line Islands Ridge and the eastern edge of the New Zealand submarine plateau. Between the last two features charting is inadequate, but a few elevations recorded as seamounts may represent crossings of a marginal ridge. No other group of features crossing the Pacific is so prominent.

Because many nations have contributed to knowledge of this feature and because great progress in oceanography resulted from the International Geophysical Year, it is proposed to call this feature the ICSU Line after the International Council of Scientific Unions which sponsored the International Geophysical Year.

#### Relationships of Ridges to Other Features

There has already been considerable debate about whether the fracture zones are genetically related to the



Island) with the north end of the Line Island ridge (near Johnston Island), but the distance cannot be precisely measured. According to this interpretation the Hawaiian Islands have a composite structure.

H. H. Hess assures me that there are offsets at the places where the Clarion and Clipperton fracture zones probably reach the Line Island ridge. They seem to have appropriate directions and lengths. The charting is too uncertain to draw any conclusions about fracture zones farther south. Again the displacements of the margin appear to agree well with theory.

(c) Transform faults should terminate against margins. The fracture zones become harder to trace beyond the ICSU Line, but the data are insufficient to make this a good test.

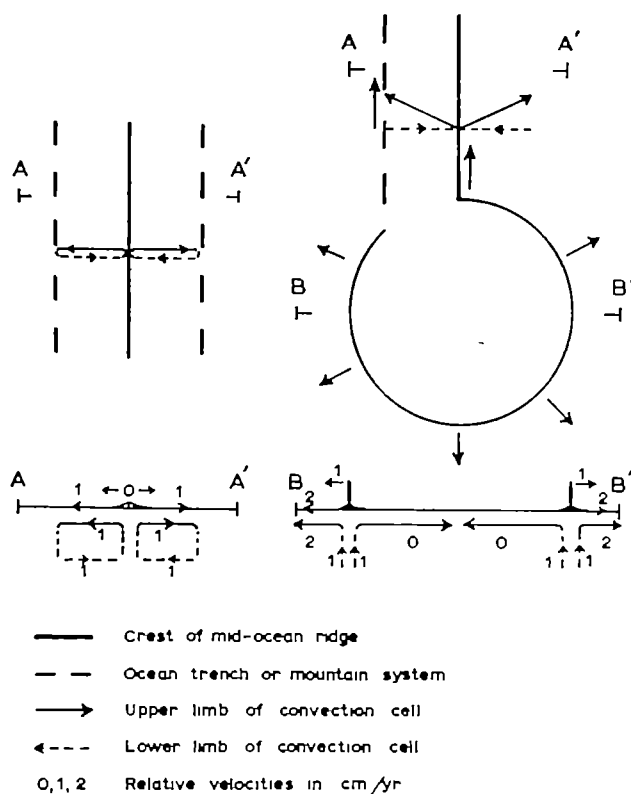


Fig. 6. Left, diagrammatic sketch of an unconstrained pattern of possible crustal and sub-crustal flow; right, the same for a pattern representing in simplified fashion the Earth's mid-ocean ridges, showing how the loop of ridge in the southern oceans could explain the directions of aseismic ridges, if the sources of their volcanoes lay below the crustal motions.

(d) All the aseismic ridges in the same crustal plate and on one side of a mid-ocean ridge should be parallel. On the east side of the East Pacific Ridge this is true of the Tehuantepec, Cocos and Nasca ridges and of the small companion ridge to the East (Nasca Two). On the west side it is true of the Gulf of Alaska Seamounts, the Hawaiian, Tuamotu-Gambier, Society and Austral Islands<sup>7</sup>.

(e) All the aseismic ridges should get younger from the margin towards the mid-ocean ridge with which they are connected, and so far as is known this is true of the East Pacific Ridge.

(f) Aseismic ridges should generally begin at a margin. The Hawaiian Tuamotu-Gambier, Society and Austral Islands all seem to start on or near the ICSU Line; the Marquesas do not and the older ends of others have been over-ridden.

(g) One should expect to find pairs of aseismic ridges symmetrically arranged in chevron pattern about the crest. The Tuamotu-Gambier, Society and Austral chains are so arranged relative to the Nasca and Nasca

Two ridges (to the extent that they have not been over-ridden).

(h) The theory predicts that in the Pacific aseismic ridges should terminate against the eastern end of dextral transform faults (that is, those which paradoxically show apparent sinistral offsets) and against the western end of sinistral faults. Aseismic ridges may cross and be active at the other ends of fracture zones. According to Menard<sup>8</sup>, the Mendocino, Pioneer, Molokai, Clipperton and Easter fracture zones show apparent sinistral offsets. As predicted the Tehuantepec, Nasca and Nasca Two ridges all stop against their eastern ends, and according to Hess<sup>10</sup> the Hawaiian Islands cross the western end of the Molokai fracture zone and are still active while the Austral Islands cross the Easter fracture zone.

For the other fracture zones the relationships should be the reverse, and it is true that the Galapagos Islands are still active, even if they scarcely cross the Galapagos zone. The Hawaiian Islands should not cross the Murray zone but appear to do so. This is the only exception, but may be due to a greater complexity in the structure of the Hawaiian chain than has heretofore been suspected.

The conclusion is that the fracture zones and aseismic ridges are associated with the East Pacific Ridge. The oldest rocks reported are Eocene in cores<sup>13</sup> and from Makatea Island in the Tuamotu group near the western margin<sup>10</sup>. Rapid spreading and rapid rates of sedimentation are indicated. The orientation of the aseismic ridges suggests that the floor of the Pacific is moving northwards like that of the Atlantic Ocean and several southern continents.

#### Possible Causes of Northward Spreading and Orientation of Aseismic Ridges

An explanation can readily be offered in terms of the possible sub-crustal currents mentioned earlier. On an infinite body they might flow as in Fig. 6 (left), but on a sphere they might be constrained to flow as in Fig. 6 (right). This pattern, like a J folded about the Earth, can be taken to be a diagrammatic simplification of the real mid-ocean ridge system and can be compared with Fig. 10 in my previous article<sup>1</sup>. The stem represents the Mid-Atlantic Ridge, the loop the mid-ocean ridge system through the Southern Ocean to the West Chile Ridge. Antarctica would be in the centre of the loop.

Because there is no sink within the looped source the diameter of the loop itself increases and the surface currents move outwards with twice the velocity of the deeper counter-currents. If the sources of volcanoes lie in the counter currents (if the convecting system is shallow), or within the stagnant cores of convection cells (if the system is deep), then the differential in rates of flow will produce chevron patterns in the aseismic ridges.

The northward motion is absorbed in the Alpine Himalayan and Circum-Pacific Mountains and hence has not affected the Icelandic ridges.

Besides Antarctica, another continent, Africa, is ringed by mid-ocean ridges on all but the northern side and corresponding effects can be detected in the Indian Ocean ridges.

I should like to acknowledge that this article could not have been written if the oceans had not been so well charted by others. I have particularly used maps by H. W. Menard<sup>8</sup> and H. H. Hess<sup>11</sup>, and a globe showing bathymetry<sup>12</sup>, but I first noticed the ICSU Line on a large new Soviet chart<sup>13</sup> which shows it clearly.

This article was written while I was a guest in the Departments of Geodesy and Geophysics and of Geology and in Churchill College, University of Cambridge; I thank them and particularly B. C. Browne, Sir Edward Bullard, H. H. Hess, M. N. Hill, R. G. Mason, D. H. Matthews and F. J. Vine for discussions which have been invaluable in arriving at these conclusions, though they

may not agree with all of them; and I also thank Sue Chappell and Sue Vine for their assistance.

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## THE LONDON UNIVERSITY NUCLEAR REACTOR CONSORT

By Dr. P. J. GRANT

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ON June 22 the London University nuclear reactor *Consort* was formally opened by Lord Sherfield, chairman of the Governors of the Imperial College of Science and Technology and former chairman of the Atomic Energy Authority. The reactor was first made critical on April 9 and full power was reached on May 18. Commissioning was completed by the beginning of June.

*Consort* is a water-cooled and moderated reactor fuelled with enriched uranium (80 per cent <sup>235</sup>U) and has a power of 100 kW (thermal). It was designed jointly by the Nuclear Power group of Imperial College and by the General Electric Co., Ltd. The instrumentation was designed and supplied by Elliott Nuclears, Ltd., and the building, which was designed by W. S. Atkins and Partners, was constructed by W. E. Chivers and Sons. The whole project was financed by a grant from the Department of Scientific and Industrial Research (now the Science Research Council). The reactor has been constructed and is being operated by Imperial College on behalf of the University at the College Field Station at Silwood Park, near Ascot.

The University has also provided funds for a residential block of nine study-bedrooms for the use of those carrying out experiments at the reactor site.

The purpose of the reactor is to provide experimental facilities for research and training in nuclear engineering and applied nuclear science for the University and other educational institutions in the London area. *Consort* has therefore been designed to act as a source of neutrons for experimental purposes. The essential design feature of a neutron-source reactor is that the experimental facilities

should approach as closely as possible the reactor core so that the largest possible external fluxes are available for a given power.

Fig. 1 is a drawing of the general arrangement of the reactor, biological shield and experimental facilities. The reactor core consists of up to 24 fuel elements, each comprising twelve slightly curved plates of uranium-aluminium alloy clad in high-purity aluminium. The core is approximately 15 in. square by 24 in. high and is situated in a tank of water about 22 ft. deep and 3 ft. 9 in. in diameter; at the level of the core the tank is narrowed in one direction and has two flat faces where the distance between the core and tank is only 3.75 in. This water gap is provided to ensure nuclear decoupling of the core from any external assembly.

On one of the flat faces is constructed a hollow graphite thermal column to provide horizontal and vertical 'feeds' of thermal neutrons into large external assemblies and to provide spaces for the neutron irradiation of samples. On the other side is constructed the 'bare face' facility; this consists of a large space cut away in the fixed concrete of the biological shield with a short graphite section to lead the neutrons into it and to provide mechanical protection for the tank wall. In this space assemblies up to 4 ft. 6 in. wide and 9 ft. high can be placed.

In addition to these large irradiation facilities, twelve beam tubes are provided in which fluxes of between  $10^9$  and  $3 \times 10^{11}$  n/cm<sup>2</sup>/sec are available, and a small (1 in. diam.) tube is provided in the centre of the core in which a flux of  $2 \times 10^{13}$  n/cm<sup>2</sup>/sec can be obtained. If required, additional in-core irradiation space can be provided in one of the fuel-element positions.

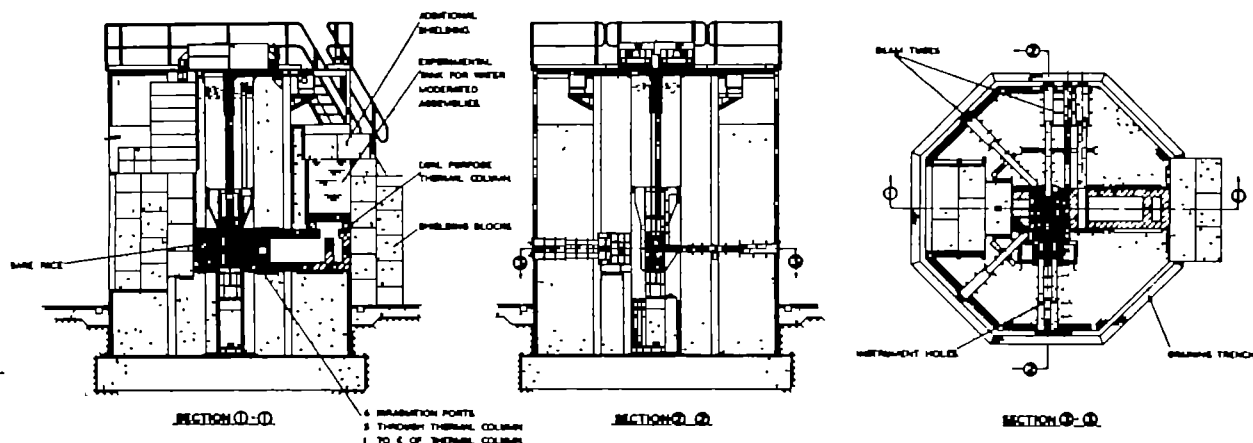


Fig. 1. General arrangement of *Consort* reactor with dual-purpose thermal column. (By courtesy of General Electric Co., Ltd., England)

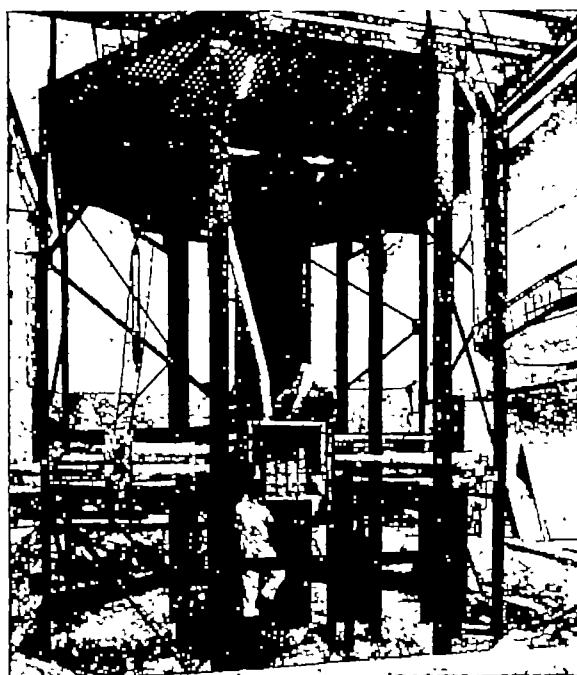


Fig. 2. Consort reactor under construction. (By courtesy of General Electric Co., Ltd., England)

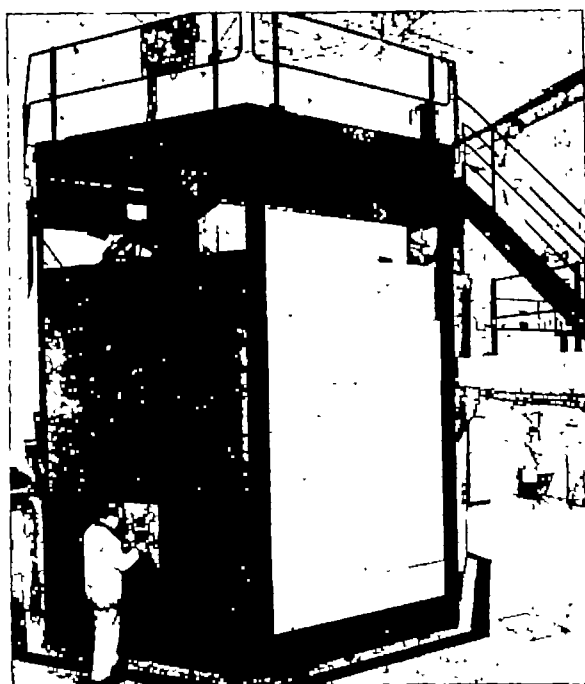


Fig. 3. Consort reactor, general view on completion. (By courtesy of General Electric Co., Ltd., England)

Figs. 2 and 3 show general views of the reactor from the 'bare face' side during construction and after completion. Fig. 2 indicates that good use has been made of the possibilities for extraction of beams of neutrons.

Heat from the reactor core is removed by natural circulation of water within the tank. At 100 kW power the temperature rise of the water passing through the core is about 10° C. In order to maintain a steady temperature, up to 2,000 gallons per hour of water are extracted from the tank, passed through an air-cooled radiator in an adjoining plant room and returned to the bottom of

the tank. 10 per cent of the water flow is by-passed through an ion-exchange column to maintain a high water purity.

The reactor is controlled by four control rods. Three of these, two 'coarse' and one 'safety', are of cadmium clad in stainless steel and each has a reactivity worth of about 1.5 per cent; the fourth, the 'fine' rod, is of stainless steel and has a worth of 0.5 per cent. All the rods move vertically in aluminium shrouds occupying spaces between fuel elements.

The instrumentation for the control and safety of the reactor is conventional. It comprises a low-power pulse channel using a fission counter and logarithmic d.c., linear d.c. and two shut-down channels, all operating from ion chambers in the instrument thermal column. The circuits are arranged so that at least three nucleonic safety channels are in operation at any time; initiation of a trip sequence by any one of these shuts down the reactor. In addition to the nucleonic trips, the reactor is shut down by failure of any control rod mechanism to operate correctly and by any significant rise in the water temperature above its normal value. Besides the trip conditions, warnings are automatically given to the control room of nucleonic or physical failures which do not prejudice the safety of the reactor but which might prevent its proper operation.

The main reactor shield is of monolithic ordinary concrete except where it is cut away for the bare face and thermal column facilities. These are shielded by means of concrete blocks. In the vertical direction the core is shielded by about 11 ft. 6 in. of water, which is sufficient protection against gamma radiation from the core in the shut-down condition, and by two movable concrete slabs which are wheeled over the top of the reactor when it operates. Interlocks ensure that the shield doors are in position before the reactor can be made critical.

Normally, fuel need never be removed from the reactor tank. Fuel storage positions are provided at the bottom of the tank for fuel elements which are not required for a particular loading or if the core has to be dismantled so that access may be obtained to bare face facility without the necessity for working in the gamma-ray back-

Table 1. Consort Reactor Core Main Parameters

Power	100 kW
Fuel element	U.K.A.B.A. standard M.T.B. type
Plate material	U-Al alloy
Enrichment	80% <sup>235</sup> U
No. of plates per element	12
Wt. of <sup>235</sup> U per element (nominal)	138 g
Critical mass	3.045 kg (22.25 elements)
Core	
Operating mass	3.114 kg (22.75 elements)
Excess reactivity	0.54%
Moderator, coolant and reflector	Light water
Control rods and reactivity inserted	
Fine control rod	0.5%
Coarse control rods (2)	1.5% each
Safety rod (1)	1.5%

Table 2

Facility	Dimensions	Thermal neutron flux
'Bare face'	Flux available over area 3 ft. square. Space for erection of assemblies: 4 ft. 6 in. wide x 9 ft. high	$5 \times 10^{12}$
Dual-purpose thermal column	Horizontal and vertical 'feeds' of neutrons over areas 3 ft. x 3 ft.	$10^9$
Thermal column irradiation holes	Internal irradiation cavity: 6 ft. x 1 ft. 9 in. x 1 ft. 9 in. Five holes 3 in. square. One hole 7 in. square. All 3 ft. long	$5 \times 10^9$
Through tube	Passes through fixed graphite near tank	$5.5 \times 10^{12}$
Beam tube terminating in cave in shield	Three in. diameter hole in graphite near tank leads to cave 2 ft. 6 in. cube	$3.5 \times 10^{12}$ at input end
Beam tubes (9)	All 6 in. internal diameter at narrowest point. Increasing to 8 in. diameter at step half-way along length	$3 \times 10^{11}$ (3 tubes), $3 \times 10^9$ (6 tubes)
Vertical tube in centre of core	One in. internal diameter	$2 \times 10^{12}$
Vertical tube terminating in graphite near tank	5.8 in. internal diameter	$3.5 \times 10^{12}$



ground from the shut-down core. Fuel handling is carried out by means of a simple hand-operated tool under direct visual control.

Table 1 lists the main reactor parameters and Table 2 summarizes the fluxes available in the experimental facilities.

It is expected that about 75 per cent of the reactor operating time will be allotted to research and the remainder to postgraduate and undergraduate teaching. The initial demand for experimental time for research has been heavier than expected. About fifteen proposals have

already been authorized by the Reactor Safety Committee and nearly half of these are large-scale experiments requiring prolonged use of the reactor. Two short experiments were completed during the commissioning period. So far, the majority of the experiments have been put forward by Imperial College staff, who have been in closer contact with reactor progress than other potential users, but general demand is expected to grow rapidly now that routine operation has begun. It is clear that the reactor is meeting a real need, and we look forward to a long and useful operating life.

## OBITUARIES

### Sir John Gaddum, F.R.S.

THE death, on June 30, 1965, of Sir John Gaddum has deprived physiology and pharmacology of one of their leading personalities. He died after a long illness, uncomplainingly borne, at his home in Cambridge at the age of sixty-five. Born in Bowdon, Cheshire, he was educated at Rugby and at Trinity College, Cambridge. There he read mathematics and, in his last two years, physiology as part of the Natural Sciences Tripos. It was later a source of merriment to him and of solace to his fellow sufferers that he only gained a 'second class' in physiology. From Cambridge he went to University College Hospital, London, where he obtained his medical qualifications in 1925. For the next two years he worked with J. W. Trevan at the Welloome Physiological Research Laboratories in Beckenham.

Gaddum's first paper there on the antagonism of adrenaline and ergotamine on the uterus of the rabbit was an indicator of things to come: his interest in drug antagonism, his mathematical treatment of biological data, the use of smooth muscle as a convenient quantitative measure of drug action, were indicative of much of his later work. The years 1927-34 were spent at the National Institute for Medical Research, Hampstead, under the directorship of H. H. (now Sir Henry) Dale. Many of the papers of these years are exercises in quantitative pharmacology and have helped to lay the foundation of accurate measurement of drug effects, and of estimation of errors due to sampling and other sources. He introduced the 'normal equivalent deviation' in the evaluation of quantal assays. He devised or improved the biological assay of substances which occurred in tissues in such small quantities that their chemical determination was impossible. There was also the discovery, with U. S. v. Euler, of a biologically active polypeptide in tissues ('substance P'), and with H. Schild, of a fluorimetric test for adrenaline. However, the investigation he most enjoyed during this period was one which led to the discovery, with W. Feldberg, that acetylcholine was the chemical transmitter at preganglionic autonomic synapses.

During his stay at Hampstead, Gaddum married a former fellow student, Dr. Iris Mary Harmer, with whom he shared many interests during their long married life, and who now survives him. They had three daughters, all of whom are married.

During 1934-58 Gaddum held chairs in pharmacology in the University of Cairo, University College, London, the College of the Pharmaceutical Society of Great Britain, and Edinburgh. Here he succeeded A. J. Clark in 1942 after several war years spent in work for the Ministry of Supply, to which he remained an adviser for many years to come. The release of histamine, the nature of the transmitter at adrenergic nerve endings, the role of 5-hydroxytryptamine in brain activity, and the assay of bradykinin were among his interests in the Edinburgh years. In two papers he drew attention to the fact that many biological phenomena exhibit a lognormal

distribution. He greatly improved the specificity of biological assays. This was done either by the use of specific antagonists, if necessary by desensitizing the tissue with a large dose of the substance the presence of which was suspected; or unambiguous clues to the identity of the unknown material were obtained by measuring its effect on many organs in parallel and estimating its potencies in relation to known reference substances. To the end of his life, Gaddum worked on active substances extractable from tissues, but his interest shifted increasingly to the search for new transmitters in the central nervous system. His last paper, started before he became ill and completed during the years of illness, deals with the chemical and structural requisites of central nervous activity. It is now in the press. In 1958, Gaddum accepted an offer from the Agricultural Research Council to succeed I. de Burgh Daly as director of the Council's Institute of Animal Physiology at Babraham, Cambridge. Always ready for new experience, he took up the challenge of learning about the needs of the physiology of farm animals and travelled all over the world to find out what was done in this field elsewhere. The growth of the Institute during his directorship is a telling tribute to his success.

Gaddum's singular achievements were due to a combination of mathematical ability, technical inventiveness (he constructed many useful research tools, the last one, the 'push-pull cannula', in 1961), with an unflinching interest in all biological, in fact all natural, phenomena, of which physiology and pharmacology were but one section. He had the mind of a naturalist, looking for plants and birds on his travels, and possessed an intellectual curiosity which did not fail him to the end. The capacity to take interest in new disciplines stood him in good stead when he gave up his chair to become director of the Babraham Institute.

During the years that Gaddum held professorships he had pupils from many lands. These will remember not only his brilliant mind but also his complete lack of prejudice, his willingness to hear all views, his integrity and sense of duty, his kindness and modesty, and the way in which a busy man gave freely of his time to their many needs. Gaddum considered it a scientist's duty to encourage international co-operation, and he certainly made an outstanding ambassador of good will.

Among the honours conferred on him was the fellowship of the Royal Society in 1945, an honorary LL.D. from the University of Edinburgh in 1964, and a knighthood in the same year.

MARTHE VOGT

### Dr. H. J. Gough, C.B., M.B.E., F.R.S.

AFTER a working life devoted to research in engineering at the National Physical Laboratory, the War Office, the Ministry of Supply and Unilever, Ltd., Dr. H. J. Gough for the past ten years had been living in retirement at Rottingdean; but he had maintained his interest in his old pursuits and during his retirement had continued to

serve on several boards and committees. He died on June 1.

Gough will chiefly be remembered for his pioneer investigations into the mechanism of the fatigue of metals. On joining the staff of the Engineering Department at the National Physical Laboratory in 1913, Gough began his investigation of fatigue under the tutelage of Stanton and Barstow. He soon made the subject his own, and after service with the Royal Engineers throughout the First World War (in which he gained promotion from the ranks in the field and was twice mentioned in dispatches) he returned to the National Physical Laboratory to continue his research. His book on *The Fatigue of Metals* was published in 1924, and this book, with the book by Moore and Kommers published about the same time, remain the basic references on this subject. In the 1920's, Gough, with Hanson of the Metallurgy Department at the National Physical Laboratory, began to investigate the process of fatigue in single crystals of metals. In crystals of aluminium he demonstrated that under all stress conditions, including alternating torsional stresses, plastic deformation occurred by slip on the slip planes and in the slip directions subjected to maximum ranges of shear stress, and he showed that fatigue cracks developed at the points of maximum resolved shear stress and in regions of heavy bands of massed slip. Afterwards, by tests on single crystals of zinc, silver, antimony, bismuth and iron, they demonstrated the importance of slip as a precursor of fatigue cracking in all the truly ductile metals, and also made observations on deformation twinning and cleavage. By comparative tests on pieces containing one or two large crystals, Gough demonstrated that the intercrystalline boundary played no essential part in the fatigue process: the fatigue crack still developed within the crystal grains, and in extending it neither avoided nor sought out the crystal boundaries.

In 1930 Gough succeeded Sir Thomas Stanton as superintendent of the Engineering Division, and the consequent increase in his administrative duties compelled him to delegate much of the further prosecution of his researches to his junior colleagues; but he continued to play an active part in investigations into corrosion fatigue, fatigue under combined stresses, fretting corrosion and study by X-rays of the nature of fatigue damage.

In 1938 Gough was called to the War Office as director of scientific research; and in 1942 he was awarded the C.B. and became director-general of research and development, Ministry of Supply. Of the many contributions Gough made to the war effort, most were not, of course brought to public notice, but the system developed under his guidance for rendering innocuous unexploded bombs earned the gratitude of us all.

After the War, Gough became engineer-in-chief to Unilever, Ltd., and was responsible for building up the Engineering Department of the Company's advisory technical division in London and for co-ordinating engineers' activities through the organization at home and abroad. The work he did in these fields established the importance of good engineering and did much to improve the status of the engineer.

Gough was elected to fellowship of the Royal Society in 1933 and was president of the Institution of Mechanical Engineers in 1949. During the period 1945-50 he played a large part in the establishment of the Mechanical Engineering Research Laboratory and in the transfer of research in engineering from the National Physical Laboratory to that establishment (now the National Engineering Laboratory). In the formative years of the National Engineering Laboratory, Gough acted as chairman of the Committee which supervised research there into all matters relating to strength of materials, and he took an active part in the organization of the conference on fatigue of metals held at the Institution of Mechanical Engineers in co-operation with the American Society of Mechanical Engineers in 1956.

H. L. COX

Prof. D. S. Hendrie

PROF. D. S. HENDRIE's death at the early age of fifty-six when he was at the height of his powers and when assuredly a great future lay ahead of him was a severe blow to Scottish agriculture. One could feel the acute sense of depression that settled over his College when the news came through. Although a man of immense ability, he had a most becoming modesty and the rare gift of making even the most junior lecturer feel important. His door was ever open to all callers and he was a ready listener to new ideas. He was at his happiest when mixing socially with his staff and students and he was a great champion of students' rights and privileges.

In 1954 Donald Hendrie was appointed principal of the West of Scotland Agricultural College and first professor of agriculture in the University of Glasgow. He brought with him a freshness of approach stemming, no doubt, from the five years he spent in New Zealand, where he was agricultural adviser to the High Commissioner for the United Kingdom. He was, in a real sense, coming home, for he had been a distinguished scholar at both of the institutions where he was now to play a leading part. He graduated B.Sc. with first-class honours in agricultural economics at Glasgow, winning many prizes and attaining the highest aggregate mark in the examinations for the National Diploma of Agriculture. He also held a B.Sc. in estate management of the University of London and a diploma in agricultural science of the University of Cambridge. As recipient of a Ministry of Agriculture scholarship, he spent a year at the Ontario Agricultural College, Canada. From 1932 until he went to New Zealand in 1949 he held a succession of posts, first as lecturer in the West College and then in Yorkshire with the Ministry of Agriculture.

It is a tribute to Prof. Hendrie's remarkable versatility and adaptability that he was as well thought of by the farmers of South-West Scotland as he was by the highest academics of the universities. He was as much at home in the fields of his native Ayrshire as he was in the classrooms and laboratories, but it was as a committee man and as an administrator that he was supreme.

The memoranda which flowed regularly from his pen were models of clarity, and they produced results. The complex interrelationships between university and college were a challenge to his abilities; they were soon sorted into satisfactory orderliness. It was also no easy matter to administer a College that existed in two parts separated by thirty miles, but under Prof. Hendrie's guidance the fusion was complete.

His talents were early recognized and he was asked to advise on the setting up of Faculties of Agriculture in two universities in Africa. He was a Governor of the Scottish Horticultural Research Institute, and of the Grassland Research Institute, Hurley. The many committees on which he served are too numerous to mention, but among the more important were the Scottish Milk Records Association, the Blackface Sheep Breeders Association, the Agricultural Research Council Standing Committee on Research Affecting Plants and Soils, the Livestock Records Bureau, and the West of Scotland Conservancy of the Forestry Commission. He was a member of the Government committee of enquiry into grassland farming of the Secretary of State's Scottish Agricultural Improvement Council, and chairman of its grassland committee.

He forged close links with industry, recognizing the important part that chemical firms have played and are playing in the development of agriculture.

In fine, we could ill afford to lose such a man, but we have cause to remember with gratitude his wisdom in developing agriculture in this area and his influence in wider spheres. He was a strong family man. In all his work he was aided and encouraged by his wife Jenny, and he leaves a son who is at present studying agriculture.

W. FLETCHER

## Prof. Jacques Bourcart

THE death of Jacques Bourcart on June 24 at the age of seventy-three removed from the scene of marine science in France a man whose richly varied life embraced very much more than great and successful activity applied in his own field of marine geology.

Bourcart was a patriot of patriots and it was doubtless the extraordinarily robust life which he led in both World Wars, inclusive of remarkable service in the Resistance, that made of him the man who became established as 'Patron' in the affections of his numerous students at the Sorbonne. It was in the field that Bourcart particularly excelled, and his detailed acquaintance with the geology of Morocco and Albania gave him that wide sweep of knowledge which drew so many admirable young people into his line of work. It was as an 'animator' of students that Jacques Bourcart won special fame. Conversation with him was not only rewarding and enjoyable; it was exhilarating—so buoyant and enthusiastic was he when engaged on some topic such as his well-known theory of continental flexure! This theory, which held that shorewards of a certain submerged zone elevation had taken place whereas seawards subsidence had occurred, was first announced in 1926 as a result of his detailed studies of the Moroccan littoral regions. It was indeed a good thing that Joubin did when he drew Bourcart into oceanography. The theory of continental flexure was applied to elucidate many puzzling changes of sea-level in the geological past.

Bourcart's thesis for his doctorate in science (1922) dealt mainly with the geology and geography of Albania, but it was in character that it strayed a good deal into discussions of the Albanian people and their language. In 1925 he was director of research in dynamical geology at the Sorbonne, and, with the passage of the years, he turned increasingly to littoral and submarine geology with particular attention to deep-sea sedimentation and bottom topography. His knowledge of Arabic led to service in Lebanon and Syria in the early days of the Second World War, but a later stage in that conflict saw him applying his vast knowledge of coastal bathymetry and morphology to the benefit of the allied forces. After remarkable military services which gained him great honour, Bourcart was appointed full professor at the Sorbonne in 1950. Five years later came the crowning of his university career when he became professor of physical geography and dynamical geology in succession to Prof. Lutaud. In this post he remained until he reached the age limit in 1961.

Bourcart was the first in France to have recognized the scientific importance of, and to have foreseen a great future for studies of, sea-bed geology and especially of abyssal sediments. He was a prolific writer and to him is due a fine set of bathymetric charts of the Mediterranean

which delineate those remarkable canyons which he had studied in detail, but the nature of which had been kept veiled in secrecy during the Second World War.

It was typical of Bourcart that, when a fund was raised to present him with the usual sword on his election to the Academy, he chose that the subscriptions should endow an academic prize able to pay the travelling expenses of a meritorious student wishing to pursue some study in physical oceanography.

In his later years Bourcart found it possible to extend the range of his busy life enough to include bathyscape descents and trips in the 'Diving Saucer'. The death of such a man will be felt far beyond the confines of his own country which he served so devotedly alike in the field of science and on the field of battle. J. N. CARBUTHERS

## B. M. Kupletsky

BORIS MIKHAILOVICH KUPLETSKY, a prominent Soviet petrologist, died on January 14 at the age of seventy-one. Kupletsky is best known as the pioneer investigator of the Kola Peninsula igneous alkaline complexes. He began his strenuous field work as a member of A. E. Fersman's 'heroic team' in 1920. From 1920 until 1930 he was an assistant of F. Y. Loewinson-Lessing in the Leningrad Polytechnical Institute. His most important works on the rocks of the Kola Peninsula are: "Petrography of the Khibina Tundra" (1926) and "Petrography of the Kola Peninsula" (1932). Besides descriptive petrology, Kupletsky published very important works dealing with the classification and genesis of various igneous rocks. These are: "Nepheline Rocks" (with T. M. Oknova, 1934); "Genesis of Alkaline Rocks" (1936); "Nepheline Syenites in the U.S.S.R." (1937); "Classification of Granitoids" (1939); "Classification of Lamprophyres" (1944).

S. I. TOMKHEFF

## A. A. Saukov

ALEXANDER ALEXANDROVICH SAUKOV, a prominent Soviet geochemist, died in Moscow on October 23, 1964. He was born on August 15, 1902, and studied under F. Y. Loewinson-Lessing and D. S. Belyankin in the Leningrad Polytechnical Institute, from which he graduated in 1929. He began his research on the geochemistry of mercury under the guidance of A. E. Fersman. Besides numerous papers on various geochemical topics, Saukov published an excellent text-book entitled *Geochemistry* (first edition, 1950; second edition, 1952), which gained a German translation (*Geochemie*, 1953). His second book bore the title *Geochemical Methods of Prospecting for Mineral Deposits* (1963). In 1961 Saukov came to Britain at the invitation of the Royal Society of London and lectured at a number of universities.

S. I. TOMKHEFF

## NEWS and VIEWS

## C.S.I.R.O. Division of Biochemistry and General Nutrition: Dr. A. T. Dick, O.B.E.

DR. A. T. DICK has been appointed chief of the Division of Biochemistry and General Nutrition of the Commonwealth Scientific and Industrial Research Organization of Australia. Dr. Dick succeeds Dr. H. R. Marston, who relinquishes his post at the end of August (*Nature*, 207, 465; 1965). He joined the Division of Animal Health as a research officer in 1933 and took up duties with Dr. A. W. Turner at Townsville, Queensland. There he applied his knowledge of chemistry and microbiology to some of the animal health problems of the region. In 1936 he joined Dr. Turner at the Parkville regional laboratory of the Division of Animal Health and Nutrition,

where he continued his microbiological studies of the causal organism of bovine contagious pleuropneumonia. Later in 1936 he took up work on chronic copper poisoning in sheep and in 1938 this was extended in a co-operative investigation of so-called toxæmic jaundice of sheep, which proved to be a pyrrolizidine alkaloidosis due to the consumption of *Heliotropium europæum*. The disease frequently became manifest as a hepatogenous chronic copper poisoning. This led to studies of copper metabolism in the sheep, and he demonstrated for the first time the interactions between molybdenum, inorganic sulphate and copper. He has continued experimental work on the toxic action of the pyrrolizidine alkaloids and on copper metabolism. He graduated B.Sc. with honours in 1932, M.Sc. in 1938, and D.Sc. in 1954 from

the University of Melbourne. He shared the David Syme Research Prize of the University of Melbourne in 1954, was elected a Fellow of the Royal Australian Chemical Institute in 1952 and a Fellow of the Australian Academy of Science in 1964. He was appointed an Officer of the Order of the British Empire in 1965 and in the same year he was awarded a senior foreign scientist fellowship by the National Science Foundation to work at the University of Missouri which he will take up in 1966. He has held various offices with the Royal Australian Chemical Institute, the Institute of Medical Laboratory Technology and the Victorian Society of Pathology and Experimental Medicine.

#### Statistics in the University of Edinburgh:

Prof. D. J. Finney, F.R.S.

PROF. D. J. FINNEY has been appointed to the newly instituted chair of statistics in the University of Edinburgh as from September 1966. Prof. Finney, who is well known for his work in statistics, is at present professor of statistics in the University of Aberdeen. He was born in Lancashire and educated at Lymm and Manchester Grammar Schools before obtaining an Open Scholarship at Clare College, Cambridge, where he graduated in mathematics in 1937. He took his M.A. in 1941, and a D.Sc. at Cambridge in 1947. After two years' research at Cambridge and London, he was from 1939 until 1945 assistant statistician at Rothamsted Experimental Station and, from 1945 until 1954, lecturer in the design and analysis of scientific equipment at the University of Oxford. In 1954 he moved to Aberdeen as reader in statistics, and director of the Agricultural Research Council Unit of Statistics. He was appointed to the chair of statistics in Aberdeen in 1963. In addition he has acted as consultant statistician to numerous institutions; he was visiting professor at the University of North Carolina in 1949, and at Harvard in 1962-63. Since 1961 he has been honorary director of the Research Group in Biometric Medicine of the Scottish Hospitals Endowments Research Trust, and since 1963 a member of the Adverse Reactions Sub-Committee of the Committee on the Safety of Drugs. He is a vice-president and past-president of the Biometric Society, a former vice-president of the Royal Statistical Society, and a member of numerous other societies in the fields of statistics and genetics. Although originally a mathematician, Prof. Finney is primarily interested in the application of statistical methods to biology, including agriculture and medicine. His research has been particularly concerned with statistical estimation, biological assay, experimental design, human genetics, screening and selection, and forest sampling.

#### Bland-Sutton Institute of Pathology:

Prof. R. W. Scarff

PROF. R. W. SCARFF, who retires this month, has for the past seventeen years been director of the Bland-Sutton Institute of Pathology at the Middlesex Hospital Medical School and responsible for all the teaching, research and routine hospital pathological investigations carried out there. Himself a student of the Middlesex, he joined the staff of the Bland-Sutton Institute in 1924 shortly after qualifying. The title of professor of morbid anatomy and histology was conferred on him in 1946, and two years later he succeeded Prof. J. McIntosh as director and professor of pathology. The Bland-Sutton has always had a reputation for cancer research, and Prof. Scarff has done much to enhance this. He has for long been closely connected with the British Empire Cancer Campaign for Research, having been appointed secretary of its Scientific Advisory Committee in 1935, a post he held until 1954 when he became honorary secretary. Nearly twenty years ago, sensing the need

for a new publication to deal with the rapidly increasing number of papers on cancer research, he started, and became editor of, the *British Journal of Cancer*, under the auspices of the British Empire Cancer Campaign for Research. This new *Journal* he developed along the same lines as the *British Journal of Experimental Pathology*, which he was already editing at that time. A highlight in his career was the 1958 International Cancer Congress held in London, for the great success of which he was, as secretary-general, largely responsible. Prof. Scarff's personal research work has covered a variety of subjects through the years, but has mainly been connected with malignant disease, and in 1958 he was appointed head of a World Health Organization International Reference Centre for the study of mammary tumours. Since 1960 he has been consultant adviser in pathology to the Ministry of Health, while among his increasing international activities has been his appointment as secretary and treasurer of the International Council of Societies of Pathology, in the formation of which body he played a leading part.

Prof. G. Dick

PROF. G. DICK has been appointed to succeed Prof. R. W. Scarff as from April 1, 1966. Prof. Dick will direct the Bland-Sutton Institute and head the Department of Medical Microbiology. After graduating M.B., Ch.B. at the University of Edinburgh in 1938 and B.Sc. with first-class honours in pathology in 1939, he served as pathologist in the Royal Army Medical Corps in Africa during the Second World War. In 1946 he joined the Colonial Medical Research Service and worked in Uganda on the natural history of yellow fever and other arboviruses. He was awarded a Rockefeller Foundation fellowship in 1948 and worked in New York and Baltimore on the relationship of Mengo virus to encephalomyocarditis virus and on methods of preparing inactivated poliovirus vaccines. In 1949 he graduated M.P.H. at Johns Hopkins University. In 1951 he became a member of the scientific staff of the Medical Research Council and worked at Mill Hill on the aetiology of mouse hepatitis. Since 1955 he has held the chair of microbiology at the Queen's University, Belfast. During this period he has worked on the development and evaluation of poliovirus vaccines. He has served on several Medical Research Council and Ministry of Health committees concerned with problems of immunization against virus diseases. Prof. Dick graduated M.D. in 1949 and D.Sc. in 1958. He was awarded the Singapore Gold Medal on both occasions. He was admitted M.R.C.P.(Edin.) in 1953 and elected Fellow in 1955, and was admitted M.R.C.P.(Lond.) in 1960 and F.C.Path. in 1964.

#### Zoology and Comparative Physiology in the University of Birmingham:

Prof. L. H. Finlayson

DR. L. H. FINLAYSON, senior lecturer in the Department of Zoology and Comparative Physiology in the University of Birmingham, has been appointed to the second chair in the Department of Zoology and Comparative Physiology in that University. Dr. Finlayson was educated at Kilmarnock Academy and the University of Glasgow, where he obtained a first-class honours degree in zoology in 1945. Having served for a year in the Pest Infestation Laboratory at Slough, he was appointed to the staff of the Department of Zoology at Birmingham, where he became senior lecturer in 1960. In 1952-53 he held a Rockefeller Foundation fellowship at Harvard, and in 1964-65 he was temporarily seconded to the Medical School of the University College of Southern Rhodesia and Nyasaland to fill the place of reader in biological sciences. Dr. Finlayson has worked in various fields of experimental entomology and is internationally known for his work on the fate of certain insect muscles during metamorphosis

and on stretch receptors associated with the insect body-musculature. During his stay in Africa, Dr. Finlayson opened up new aspects of research into the metamorphosis of the tsetse fly. In the field of higher education he took an active part in the modernization of biological teaching both in the grammar school and in the university. The new biology building at Birmingham owes much to his untiring participation in its planning and equipping.

#### Royal Armament Research and Development Establishment: Dr. R. H. Barker

DR. RONALD BARKER has been appointed the second deputy director of the Royal Armament Research and Development Establishment. Born in Dublin in 1915, Dr. Barker was educated at University College, Hull, where he obtained the London B.Sc. honours degree in physics in 1938. He was awarded a Ph.D. in 1945 for research on digital servo systems. Dr. Barker started his career with the Standard Telephone and Cable Co., at Woolwich, with whom he spent three years. In 1941 he entered the Civil Service, and joined the Signals Research and Development Establishment of the Ministry of Supply, where he was promoted to senior principal scientific officer in 1954. His interests there included the digital transmission of fire-control data and telemetry for guided weapons. In 1954 he attended the thirteenth course at the Joint Services Staff College and, on completion of this course, he was appointed assistant director electronics research and development (air) at the Ministry of Supply headquarters, with special responsibility for airborne radar, air communications and navigation aids. In 1957 he returned to the Signals Research and Development Establishment as superintendent of research. He left the Civil Service in 1959 to become deputy director of the Central Electricity Generating Board's laboratory at Leatherhead, where he remained for two years. In 1961 he joined the board of R. B. Pullin and Co., Ltd., which later became part of the Rank Organization, and it was from this firm that he rejoined the Scientific Civil Service to take up his present post. In his new appointment, Dr. Barker has direct responsibility for two Divisions of the Establishment dealing respectively with basic techniques and the applications of explosives.

#### Physics in the University of Manchester:

Prof. P. G. Murphy

DR. P. G. MURPHY, who has been appointed to the newly established additional chair of physics at the University of Manchester, is at present a senior member of the research staff of the Rutherford High Energy Laboratory at Chilton, Berkshire. A Cambridge graduate, Dr. Murphy gained his Ph.D. at the University of Chicago, where he studied problems in atomic and nuclear physics under Prof. S. K. Allison. He was for several years an I.C.I. Fellow in the University of Liverpool, working with Prof. J. M. Cassels in high-energy physics research at the 156-in. synchrocyclotron. In 1960 he took a fixed-term research appointment at the Rutherford Laboratory, and spent the next eighteen months on detached duty at the University of California at Berkeley, where he carried out several experiments at the 6-GeV Bevatron accelerator as a member of the group led by Drs. E. J. Lofgren, B. Cork and W. A. Wenzel. This group developed the first spark chamber to be used in a high-energy physics experiment, and studied  $K$ -meson scattering, and polarization effects in  $\pi$ -meson scattering and in  $\Lambda$  and  $\Sigma^+$  hyperon decays. Since the autumn of 1961 Dr. Murphy has worked in the *Nimrod* research programme, as joint leader of the resident counter group. He and his colleagues have completed experiments at *Nimrod* on the scattering of  $\pi^+$  and  $\pi^-$  mesons by liquid hydrogen and by a polarized proton target which they and Dr. H. H. Atkinson developed. He gave a course of lectures at the University of Reading on symmetry

properties in elementary particle physics. He was promoted to senior principal scientist in 1964.

#### Physical Chemistry in the University College of Swansea: Prof. J. H. Purnell

DR. J. H. PURNELL, at present lecturer in physical chemistry in the University of Cambridge, and a Fellow of Trinity Hall, has been appointed to the newly established chair of physical chemistry in the University College of Swansea. He will therefore be returning to South Wales, of which he is a native, having taken his first degree at the University College of Cardiff in 1946. Following this he was successively assistant lecturer and lecturer at Cardiff, during which period he was awarded the Ph.D. degree of the University of Wales. In 1952 he migrated to Cambridge in order to work with Prof. R. G. W. Norrish; there he obtained a second Ph.D. in 1955. In the same year he was appointed demonstrator in physical chemistry at Cambridge, becoming lecturer in 1960; he became a Fellow of Trinity Hall in 1958. His work has been mainly concerned with the kinetics of pyrolysis of hydrocarbons and metal alkyls, and related processes such as inhibition. It has been distinguished by the application of gas chromatography to the analysis of products, which has made possible a more detailed and deeper insight into the mechanisms of reactions than was possible previously. Dr. Purnell's interest in gas chromatography has resulted in his becoming a pioneer not only in its application but also in the understanding of the basic physical chemistry of the technique. It has led him into thermodynamic considerations and the extension of the general techniques to solution and adsorption studies, liquid surface properties and the measurement of molecular weights. He is the author of a distinguished monograph on the subject of gas chromatography. At present he has an active school of research at Cambridge with interests in photolysis at low temperatures, the pyrolysis of olefines, and the simulation of complex chemical reactions by computer techniques. He may be expected to pursue these lines of work, among others, at Swansea.

#### British Electrical Power Convention

THE seventeenth British Electrical Power Convention was held at the Dome, Brighton, during June 21-24, 1965, and records of the proceedings have recently been made available by the press officer of the Electrical Development Association in London. The presidential address on "Electricity and Economic Policy" was delivered by Sir Ronald Edwards, chairman of the Electricity Council; his theme was the planning of the electricity supply industry to meet a maximum demand of 54,000 MW in 1970 (compared with 30,000 MW in 1964) and the economic and technical factors in such planning; he had much to say on competition in the field of fuel and power, particularly the future of the gas industry which "... will, as soon as it can, turn its back on coal and rebuild its future on oil and natural gas". The Citrine Lecture was presented by Lord Hinton of Bankside, former chairman of the Central Electricity Generating Board, and entitled "The Future of Power Technology"; he considered that future trends of technology in the energy industries depend on a combination of economic, social and commercial factors, remarking that "... the future of the coal industry depends on electricity and the future of gas is part of the future of the oil industry". On the subject of nuclear power, Lord Hinton said that it "... was certainly emerging from its expensive adolescence and should be able to compete commercially with conventional power in favourable conditions before 1975". "The Future of Gas" was the subject of a paper by Sir Henry Jones, chairman of the Gas Council; commenting on the comparative efficiency of gas and electricity production, he said that an engineer may still "question a system

which involves the loss at the power station in flue gases and cooling water of two-thirds of the heat in the fuel. The gas industry uses production processes in which roughly a tenth of the heat in the feedstock is used. Up to 92 per cent of the heat value in the raw material is available in the gas produced". Mr. John Davies, vice-chairman of Shell-Mex and B.P., Ltd., read a paper on "The Future of Oil" in which he prophesied that "Thirty years from now the U.K. will be using 200 million tons of petroleum compared with the 61 million tons of today". This was a paper of most exciting forecasts for the oil industry in the years ahead. The picture of the coal industry, as now phased and evolving, was presented by Lord Robens, chairman of the National Coal Board, in his paper on "The Future of Coal"; he said that there was great potential for increasing productivity in the coal industry; that the task of rationalization should be completed by 1970; and that a completely new approach to mining techniques, elimination of uneconomic capacity and increased output from profitable pits were among the modern developments to be reckoned with. "The goal of cheap and abundant supplies of electricity for British homes . . . to be achieved much sooner than most people thought" was the subject of a joint paper "Electricity's Future in the Home—The Complete and Competitive Service" by E. G. Plucknett (Tube Investments, Ltd.) and S. F. Steward (British Electrical and Allied Manufacturers' Association).

### Engineering Manpower In Profile

AN intensive investigation of contemporary American engineering manpower, not without interest and significance in comparable disciplines in the United Kingdom, has resulted in a report from the National Engineers Register, based on responses to a detailed questionnaire sent to a cross-section of engineers in the United States by the Engineers Joint Council, made possible through a contract with the National Science Foundation, entitled *Engineering Manpower in Profile* (Pp. 30. New York: Engineers Joint Council, 345 East 47th Street, 1965. 1 dollar). More than 57,000 individuals representative of the membership of 41 leading engineering societies returned the required information, revealed by an exhaustive questionnaire (reproduced in this report). "The nation's engineer is a man, median age 41, who works in industry or is self-employed, and, though he leans towards electronics and work management, may be employed in any of 115 areas of technology. In addition, he and his fellow engineers tend to concentrate in New York and California." Among the pertinent findings of this survey, it is established that: about 71 per cent are employed in industry or self-employed; another 8 per cent work for educational and non-profit institutions; 15 per cent are employed by government; more than 1 out of every 5 engineers possess a doctorate or master's degree; only one-half of one per cent of responding engineers are women; about 42 per cent of the respondents are receiving some degree of support or sponsorship from Federal programmes; and the profession is characterized by its great variety of specialities. The survey has further demonstrated that there is a growing realization that engineering is "... the vital element in translating far-ranging scientific discoveries into practical use". This report provides the factual information latterly demanded by industries, educational institutions and Congressional groups in the United States and it is certain to provide a strong basis for guiding and executing many plans and projects now in contemplation in the engineering field in that country.

### German Aid to Developing Countries

THE Overseas Development Institute has published a survey of the sources, policy and structure of German aid to developing countries, aimed at promoting economic

development in those countries (*German Aid: a Survey of the Sources, Policy and Structure of German Aid*. By John White. Pp. 221. London: The Overseas Development Institute, Ltd., 1965. 20s.). Successive chapters of the survey describe the growth and volume of the aid programme, its administrative structure, sources of funds and general policy. Other chapters deal with classification and terminology, bilateral financial assistance, the relations between Germany and other donor countries, problems of private investment, trade, technical assistance in developing countries, and technical and academic programmes in Germany. There are two chapters dealing with technical assistance by non-Government organizations, and with public opinion in Germany. Of 920 German experts overseas on December 31, 1963, 81 were in Europe, 298 in Africa, 439 in Asia and 102 in Latin America. Of the 6,228 technical trainees financed from Federal or L nder funds up to December 31, 1963, 507 were from Europe, 3,277 from Africa north of the Sahara, 1,029 from Africa south of the Sahara, 289 from the Middle East, 414 from Southern Asia, 307 from the Far East, 113 from Latin America and 229 from South America. Of 5,649 Government-sponsored technical trainees up to September 30, 1963, 529 were senior staff, 3,505 engineers, 409 foremen and 1,206 skilled workmen. At the end of the summer term, 1963, there were 27,000 foreign students in Germany, including 18,000 from developing countries, out of a total student body of 370,000.

### Institute of Biology Examinations

THE Council of the Institute of Biology has approved proposals concerning the examination for membership (*Institute of Biology Journal*, 2, No. 4; 1964). Courses are designed to last for three years of part-time study and assume a Higher National Certificate in applied biology as the entry requirement. At the end of one year's study a Part 1 examination will be taken in the principal subject, a cognate subject and principles of biology. The remaining two years of the course will be devoted to the special subject, and lead to the Part 2 examination. The special subjects so far approved are biochemistry, entomology, microbiology, pharmacology and plant pathology. Courses are expected to commence in September 1965. It is also reported that, in the summer of 1964, examinations for the Ordinary Endorsed Certificate in Biology were held in 21 centres in England and Wales and Northern Ireland. There were 210 candidates, of whom 156 passed, 36 failed and 18 were referred in one subject. Ten students who had been referred in the Higher Endorsed Certificate in 1963 succeeded in passing the examination in 1964. The first examinations for the Higher National Certificate in Applied Biology were held at 12 colleges in England and Wales. There were 106 candidates, of whom 86 passed, 6 with distinction. One student was referred and 19 failed. In Scotland, courses for the Ordinary National Certificate were offered by five colleges: 83 candidates produced 58 passes, 19 failures and 6 referrals. Six students received distinction or special mention. Two colleges presented candidates for the Higher National Certificate. Of the 25 entrants, 17 passed, 3 failed and 5 were referred. Three students attempted to add a supplementary subject to their Higher National Certificate, but only one succeeded.

### Bee-keeping by John Evelyn

OVER many years John Evelyn (1620–1706) worked at a book treating every aspect of gardens and gardening, with the title *Elysium Britannicum*. At the time of his death this work was neither finished nor published and the manuscript has since lain in Christ Church, Oxford. One section of this manuscript, probably written in the 1680's, deals with bees and apiaries, and has now been reproduced in full, together with facsimiles of some of the



rough illustrations, in the journal of the Bee Research Association: *Bee World* (46, No. 2, 48; 1965). While this compilation does not add to our present knowledge of the honey-bee, it does contain much that is of interest, such as his clearly illustrated account of several types of sectional hives and how to use them, and a description of the "Transparent Hives" in the apiary of the "most ingenious Mr. Mewes". The wasteful methods of the housewife who kills the bees to get the honey are roundly condemned. An interpolation by another hand on "The Method to govern Bees" contains advice on which colonies to keep over the winter and which to destroy. This includes the sound physiological observation that "a poor or small swarm will require more feeding and tendance than a great swarm; the reason is, that a hive full of Bees keep a heat within which is nourishing; for the stomach requires least meat in hot weather". The dancing of the scout bees and the guiding of the colony to their new quarters are well described. A scout brings the tidings and gives notice "by a certain touch which he imparts to the out guards that by a kind of shivering motion communicate it to the whole swarm and centre bees in a moment"—which then disperse and fly immediately to the new home.

### Countryside In Wales

THE twentieth annual report of the Caernarvonshire Branch of the Council for the Protection of Rural Wales covers the year April 1964–March 1965 (Pp. 28. Bangor: H. W. Owen, Honorary Secretary, 202 High Street, 1965). While affirming the Council's belief that rightly sited and designed development is possible with acceptable loss to the landscape, the Council holds that a heavier toll than ever before has been taken during the year in Caernarvonshire, mainly through the 400-kV line across south Caernarvonshire. The Council is unimpressed by the purely economic argument of the Central Electricity Generating Board, and points out that on the Northern route of the 400-kV line the Board could still meet the wishes of the Council and of the County Planning Authority by taking a lower-sited line from Bwlch-y-Deufen and a shorter crossing of the Conway Valley, with certain existing cables placed underground. The Council shares the concern of the National Parks Commission as to the intrusion of mass recreational facilities and in all that is left of "Wilder Wales" urges that the emphasis should be on low-cost maintenance conservation. It also welcomes the "Operation Neptune" of the National Trust, and questions the Minister's decision to approve a new chalet town adjoining the sandhills at Morfa Bychan. It asks for priority in designating the Coed Helen Estate and the adjoining coastal area to Llandwrog as an 'Area of Outstanding Natural Beauty'.

### International Commission on Zoological Nomenclature

NOTICE is hereby given of the possible use by the International Commission on Zoological Nomenclature of its plenary powers in connexion with the following cases, full details of which will be found in the *Bulletin of Zoological Nomenclature* (22, Part 2; May 18, 1965): (1) Designation of a type-species for *Limacia* Müller, 1781 (Gastropoda). *Z.N.(S.)* 1665. (2) Designation of a type-species for *Leuctra* Stephens, 1835 (Insecta, Pleocoptera). *Z.N.(S.)* 1671. (3) Designation of a type-species for *Nupedia* Karl, 1930 (Insecta, Diptera). *Z.N.(S.)* 1691. (4) Designation of a type-species for *Heteroptrypa* Nicholson, 1879 (Bryozoa, Trepostomata). *Z.N.(S.)* 1693. (5) Designation of a type-species for *Peronopora* Nicholson, 1881 (Bryozoa, Trepostomata). *Z.N.(S.)* 1693. (6) Designation of a type-species for *Bastis* [Leach, 1815] (Insecta, Ephemeroptera). *Z.N.(S.)* 1620. (7) Suppression of the specific name *Ephemera bioculata* Linnaeus, 1758 (Insecta, Ephemeroptera). *Z.N.(S.)* 1620. Any zoologist who wishes to comment on any of the foregoing cases should

do so in writing to the Secretary, International Commission on Zoological Nomenclature, c/o British Museum (Natural History), Cromwell Road, London, S.W.7, before November 18, 1965.

### Mycobacterial Skin Ulcers

In the past two and a half years, 28 cases of mycobacterial skin ulcers have been seen and treated at the Medical Research Centre, Bulawayo. Drs. P. J. Barnard and L. Jacobson suggest (*Cent. Afr. J. Med.*, 11, No. 5; 1965) that this entity begins as a phlegmon in the subcutaneous tissues with peripheral spread causing pressure necrosis of overlying skin, thus forming an ulcer. The ulcer is associated with wide undermining of skin edges and induration of the surrounding skin. Diagnosis has been made by history and clinical appearance, by taking a smear from underneath the edges of the ulcer, and staining for acid-fast bacilli, and by biopsy of the ulcer edges, especially the gelatinous material. The only effective treatment has been wide excision of the involved tissue followed later by split-thickness skin grafts. Much remains to be done to determine the epidemiology of this disease. Some workers suggest that *Mycobacterium ulcerans* is a soil saprophyte; others feel that certain fish may act as a reservoir of infection.

### Venereal Infections

THE dramatic decline in the reported incidence of venereal syphilis and gonorrhoea in many countries between the late nineteen-forties and the early nineteen-fifties, following the introduction of penicillin and other antibiotics, was a landmark in the history of public health. Unfortunately, in both these diseases, a persistently rising incidence has been noted in many countries after an all-time low in and around the mid-nineteen-fifties. The reasons for this disturbing trend are analysed in a comprehensive report on the World Health Organization's programme in the endemic treponematoses and venereal diseases, originally submitted to the thirty-fourth session of the WHO Executive Board, in May 1964 (*International Work in Endemic Treponematoses and Venereal Infections, 1948–1963*. Pp. 47. Geneva: WHO; London: H.M.S.O., 1965. 2 Sw. francs; 3s. 6d.). It is stressed that the public health methods available for the control of venereal syphilis are as effective to-day as they were before the recrudescence of the disease became apparent in many countries a few years ago, but that there is an urgent need for a more determined application of present-day knowledge in public health programmes in this field. In gonorrhoea, on the other hand, there is increasing evidence of resistance to one or more of the antibiotics at present in use, though this is a localized rather than a general phenomenon. The chief difficulty is that of bringing cases and contacts to treatment quickly enough to overtake the rapid spread of the infection in the community, and it seems unlikely that the disease can ultimately be controlled except by mass treatment or some form of immunoprophylaxis. In the endemic treponematoses of childhood, striking progress has been made—thanks in large part to the work of the World Health Organization in this field. In most countries where mass campaigns against these diseases have been carried out, the prevalence among rural populations has rapidly regressed to a fraction of 1 per cent. Some 50–60 million people, however, still live in areas of medium or low prevalence where no intensive effort has been made to control these diseases. It is also hoped that it will now be possible to accelerate their elimination in these areas by means of selective, multi-purpose, or integrated campaigns. In the light of this report, the WHO executive board urged member States "to exert a determined effort to maintain adequate and effective measures to reduce incidence of the endemic treponematoses, particularly those of childhood, and the venereal



diseases and, where indicated, to increase their efforts to combat, at the national level, the recrudescence of these infections".

### Psychiatry In General Practice

A PRICE has to be paid for every advance in medicine. With mental disease, for example, an increasing burden is laid on the family doctor by the policy of the 'open door'. The wisdom or charity of this new concept of retaining the mentally disturbed patient in the community is generally accepted, although the family doctor may wonder whether the enthusiastic progenitors of the concept have ever realized what it means to him. He is willing to cope with the situation to the best of his ability, and the aim of a symposium in *The Practitioner* (194, No. 1163; 1965) is to help him in his onerous task. In the available space only a few aspects of the subject are considered. Those included have been selected either because they represent conditions commonly encountered, such as depressive illness which, as Dr. J. Pollitt points out, is the commonest psychiatric condition in general practice, or deal with new developments in this field such as therapeutic communities. The symposium concludes with an article on "The Neurotic in General Practice", which epitomizes one of the most worrying problems facing the family doctor at the present moment. Dr. N. Kessel notes that "Until the theoretical understanding of how to treat neurosis is achieved, until there is considerably more money to spend within the N.H.S. [National Health Service], the problem of how to manage the mass of neurotic patients known to their doctors cannot be satisfactorily solved". This gloomy outlook is relieved by the evidence that "there is a high proportion of spontaneous remissions". In other words, "most neurotic patients get better".

### The International Hydrological Decade

In November 1961, on the authority of the U.S. Department of State, a resolution was tendered to the Executive Board of Unesco "... calling attention to the importance of scientific hydrology and recommending that a meeting of experts should be convened, to examine the proposition to institute an international programme for hydrology, extending over a ten-year period". The General Conference of Unesco, in November 1964, approved the plans prepared by meetings of these experts for an International Hydrological Decade, to start in January 1965. The events leading up to this decision have been recorded by R. L. Nace in a paper entitled "The International Hydrological Decade" (*Trans. Amer. Geophys. Union*, 45, 413; 1964); a later and succinct account of the whole project is contained in an article similarly titled by E. S. Hills (*Austral. J. Sci.*, 27, 10; April 1965). This sets forth the main objectives of the scheme, which may be summarized as follows: (a) appraisal of the state of knowledge of world hydrology and identification of the principal gaps in that knowledge; (b) standardization of instruments, observations, techniques and terminologies used for compilation and reporting of data; (c) establishment of basic networks and improvements of existing networks to provide fundamental data on hydrological systems covering small watersheds to the world as a whole; (d) research on hydrological systems in selected environments, called 'representative basins'; (e) research on specific hydrological problems the urgency and special nature of which demand action at international level; (f) theoretical and practical training in hydrology and related subjects; (g) systematic exchange of information. E. S. Hills is chairman of the Australia-Unesco Committee for the International Hydrological Decade and in his article he gives a brief account of Australian participation in this laudable and vital scheme. This is followed in the same issue of the *Journal* by an important paper entitled "Water—Essential Factor of Economic Develop-

ment", by R. L. Nace (U.S. Geological Survey), reproduced from *Impact* (14; 1964); much of the background, both theoretical and technical, of the International Hydrological Decade is disclosed in this essay. The German version of this project is described in an article "Die Internationale Hydrologische Dekade (IHD)" by U. de Haar, Bad Godesberg (*Umschau in Wissenschaft und Technik*, Frankfurt/M, 14; July 1965); this includes a novel diagram in colour depicting the incidence and passage of rainfall in its circulation on and below the surface of the Earth, what is lost, what is used, and how the inevitable water-cycle is completed.

### History under the Sea

THE Smithsonian Institution has provided a very useful compendium of the rapidly growing work of underwater exploration in a book, *History under the Sea*, by Mendel Peterson (Pp. xiii+108+56 plates, publication 4538. Washington, D.C.: Smithsonian Institution, 1965). The subject is treated from all aspects and even discusses modern methods of spotting underwater wrecks by magnetometers and induction detectors. A useful section is devoted to the preservation of materials recovered from the water, both as regards first-aid work in the field and subsequent treatment in the laboratory. A selected bibliography and illustrations of typical finds complete this eminently worth-while publication.

### Photography and the Graphic Arts

THERE are many publications catering for the interests of photographers, both amateur and professional, but one entitled *Visual* is perhaps not so widely known as it deserves to be. *Visual* is the house organ of Ilford Ltd. (Ferguson House, 15-17 Marylebone Road, London, N.W.1), and appears three times annually. Two recent copies (2, No. 3; 1964, and 3, No. 1; 1965) are notable both for contents and certainly for the excellence of the black-and-white and colour illustrations generously distributed throughout the texts, as one would naturally expect from this particular firm. Among the titles in the former issue is "Assignment Tokyo", depicting briefly, in colour, contemporary life in this modern Japanese city; an account of the activities of the Institute of Incorporated Photographers; papers on "Photographic Paper Base", "Medical Photography", and "The Production of Photolitho Printing Surfaces for Offset Printing". In the more recent issue there are articles on "The Polytechnic Contribution to the Development of Photography"; "Printing and Publishing—15 Years Hence?"; "Photographic Processing Chemicals". The journal also includes an article entitled "Profile: Douglas F. Lawson", dealing with this internationally recognized authority on photomicrography; the colour illustrations to this are quite outstanding, ranging in subject from aspirin crystals, marine-floor foraminifera, natural onyx stone, section of a cat's tongue showing the surface hairs, and the eggs of a moth, to a section of fused phloridzin, and a reproduction of a slide of *Amphioxus*, part colour, which demonstrates the value of annotated drawings to accompany photomicrographs. Incidentally, as mentioned in this article, D. F. Lawson is the author of the well-known *Technique of Photomicrography*; his work "... has been reproduced in hundreds of publications in many parts of the world, and used in educational television programmes". *Visual* is yet another example of a high-class house magazine wherein advertisement is relegated to a modest background (a brief section entitled "Ilfordia") and the major appeal to the reader made by the quality of its contents, by its inviting format, and especially by its beautiful illustrations.

### Creep and Shrinkage of Concrete

ARTICLES, papers and books dealing with the subject of creep and shrinkage of concrete, held by the library of

the Cement and Concrete Association, are the basis of a recent publication entitled *Annotated Bibliography on the Creep and Shrinkage of Concrete* (Pp. 88. London: Cement and Concrete Association, 1965. 20s.). This derives from an extensive literature search on the factors influencing creep and shrinkage and the effect of these functions on concrete as a structural material. The results of this search are presented in three parts: an annotated bibliography of 68 publications; an Appendix (1) comprising a bibliography of more than 800 references to the subject; and an author index (Appendix 2), which greatly facilitates reference to the previous items where their names are known. In the annotated section, the years covered are from 1964 to 1967 (in that order); similarly in Appendix 1, from 1964 to 1968. The scope of this *Bibliography* is, of course, international, and for architects, civil engineers, cement and concrete technologists, and research students concerned with these problems, its possession will prove not only an invaluable guide to a widely scattered literature, but also a certain time-saver when it comes to reference work.

#### Anglo-Icelandic Field Research Group

THE Anglo-Icelandic Field Research Group was formed in 1961 to aid and co-ordinate field research in Iceland by specialists, by amateurs and by expeditions. A committee of specialists in geology, botany, ornithology, glaciology and so on deals with requests for information, bibliographies, suggestions for projects, or criticism of plans made. Informal contacts are maintained with Icelandic scientists, and otherwise elusive Icelandic scientific periodicals are subscribed to, thus allowing the most up-to-date information to be given at all times. The Group publishes a *Bulletin*, of which the third issue, just published, contains news of field work planned for the 1965 and 1966 seasons, brief notes on the progress of work undertaken in 1964, and a comprehensive review of recent scientific literature concerning Icelandic field research topics. Further items in the current issue include a comprehensive general bibliography, notes on information sources and a suggested itinerary for geologists visiting the important volcanic district of Myvatn in north-east Iceland. Further information can be obtained from the secretary of the Group, Mr. P. W. Sowan, 161 Piccadilly, London, W.1.

#### University News:

##### Bristol

DR. L. E. HAWKES has been appointed to the chair of mycology. Dr. I. M. Ward has been appointed senior lecturer in the physics of materials in the Department of Physics. The following lecturers have also been appointed: Dr. W. H. H. Banks (mathematics); Dr. B. M. H. Bush (comparative physiology in the Department of Zoology); P. W. Fitt (mechanical engineering); Dr. H. A. Osmaston (geography).

##### Southampton

DR. M. H. QUENOUILLER has been appointed to the chair of statistics in the Department of Mathematics. Dr. L. E. Tavener has been appointed reader in geography. The following lecturers have also been appointed: P. W. Fortescue (aeronautics and astronautics); P. C. Ryall (civil engineering); C. B. Chapman (econometrics); Dr. A. G. Bailey and Dr. J. A. Coekin (electronics); J. H. Dunmore (mechanical engineering); Dr. P. J. Williams (oceanography); Dr. N. Maclean and Dr. A. E. Wild (zoology).

##### Swansea

DR. J. H. PURNELL has been appointed professor of physical chemistry. Dr. T. O. Jeffries has been appointed to the newly established chair of industrial engineering. The following appointments have also been made: *Senior Lectureships*, Dr. E. G. Brown and G. T. Goodman

(botany); Dr. J. D. Davies and H. W. Goaling (engineering); Dr. H. E. Hallam (chemistry); Dr. C. N. Linden (pure mathematics); T. S. Walters (applied mathematics); *Lectureships*, T. H. Walton (pure mathematics); Dr. J. T. Davies and Dr. R. Phythian (physics); Dr. J. H. Williams (chemistry); Dr. D. T. Herbert (geography); Dr. J. S. Ryland (marine biology in the Department of Zoology); Dr. R. A. E. Tilney-Bassett (genetics in the Department of Zoology).

#### Announcements

Dr. J. W. Cornforth and Dr. G. J. Popjak, of the Milstead Laboratory of Chemical Enzymology, Shell Research Ltd., have been jointly awarded the first OIBA Medal of the Biochemical Society for their fundamental work on the biosynthesis of cholesterol and the stereospecificity of enzymatic reactions. The Medal will be awarded annually in recognition of outstanding research.

THE next awards of the Ethel Behrens Fund of the Chemical Society are to be made in connexion with their anniversary meetings to be held in Oxford in March 1966. This fund provides grants towards travelling and maintenance expenses to help Fellows of the Society studying for a degree at a British university or technical college to attend the anniversary meetings of the society. Application forms and further information can be obtained from the General Secretary, the Chemical Society, Burlington House, London, W.1.

THE autumn meeting of the Chemical Society will be held in the University of Nottingham during September 21-22. Further information can be obtained from the General Secretary, the Chemical Society, Burlington House, London, W.1.

A SYMPOSIUM on "Reservoir Yield", organized by the Water Research Association, will be held at the University of Oxford during September 21-23. Further information can be obtained from the Water Research Association, Medmenham, Marlow, Bucks.

A CONFERENCE on "The Fundamentals, Production and Applications of Hard Magnetic Materials" will be held in Vienna during September 21-24. Further information can be obtained from Verein Deutscher Eisenhüttenleute, 4 Düsseldorf, Breite Strasse 27.

A SYMPOSIUM on "Food Science Research in the United Kingdom" will be held at the School of Pharmacy, University of London, on September 22. Further information can be obtained from the Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London, S.W.1.

A SYMPOSIUM on "Modern Trends in Space Physics", organized by the British Interplanetary Society, will be held at University College, London, on September 23. Further information can be obtained from the Executive Secretary, the British Interplanetary Society, 12 Beesborough Gardens, London, S.W.1.

A TWO-WEEK summer school on "Methods of Numerical Approximation", organized by the University of Oxford Computing Laboratory and the Delegacy for Extra-mural Studies, will be held in Oxford during September 20-October 1. Further information can be obtained from the Secretary, Delegacy for Extra-mural Studies, Rewley House, Wellington Square, Oxford.

THE fifth international Aerosol Congress and the third International Aerosol Exhibition, organized by the Italian Aerosol Association on behalf of the Federation of European Aerosol Associations, will be held in Milan during September 21-26. Further information can be obtained from the British Aerosol Manufacturers' Association, Cecil Chambers, 86 Strand, London, W.C.2.

## FEDERATION OF EUROPEAN BIOCHEMICAL SOCIETIES

By DR. J. R. VILLANUEVA

Instituto de Biología Celular, C.S.I.C., Madrid

THE Federation of European Biochemical Societies was founded in 1964 to promote contacts and the interchange of information between European biochemists. It now comprises twenty-one Societies. Much of the work of the Federation is concentrated on a comparatively few clear-out projects including organization of meetings and courses in advanced research techniques. Meetings are held every year at some centre of research where symposia are held and a number of papers dealing with the most recent research topics are delivered. Two meetings have been organized; the first in London in 1964 and the second in Vienna in 1965. The third Federation meeting will be held in Warsaw in April 1966.

Organized by the Société Belge de Biochimie, the first summer course of the Federation of European Biochemical Societies was held at the Catholic University of Louvain during June 8-18; the subject was "Centrifugal Fractionation of Animal Cells: Theoretical Basis and Practical Procedures". The course, which was organized in detail by the Department of Physiological Chemistry, Institute of Physiology, gave a very wide view of the problem through lectures and practical demonstrations. It was sponsored by the Federation of European Biochemical Societies and the International Cell Research Organization of Unesco, which provided grants covering half the expenses of all the participants, who numbered about 20 and represented more than 15 nationalities; the places had been selected from a large number of applications. English was the official language of the course.

To comment on the importance of selecting the topic of separation of macromolecules and particles, it should be remembered that it is significant that some of the most remarkable recent advances in biochemistry have depended on new and improved separation procedures. Findings on the behaviour of a component in a separation process dependent, for example, on sedimentation velocity, provide a convenient basis for preliminary quantitative characterization. If such work is done systematically in correlation with investigations of biochemical function and with electron microscopy studies it may provide very interesting information which would have been difficult to obtain by other means.

Prof. C. de Duve (Department of Physiological Chemistry, University of Louvain, and Rockefeller Institute, New York) was the chairman of the course. Specialists will not need to be told that the science of centrifugation has made considerable progress during the past decade and that the Louvain group has made important contributions in this field.

Prof. de Duve described the general principles of centrifugal fractionation, theory of sedimentation, analysis of sub-cellular fractions, differential centrifugation in homogeneous medium, density gradient, isopycnic centrifugation, and the calculation, presentation and interpretation of results. Prof. J. Berthet (Department of Physiological Chemistry, Louvain), who was responsible for the internal and scientific organization of the course, together with Drs. H. Beaufay and P. Jacques, gave lectures on homogenization, centrifugation equipment, density gradient centrifugation, practical procedures, density gradient differential centrifugation and density gradient centrifugation for analysis of macromolecules. Prof. Drochmans (Department of Experimental Cytology, Institut Bordet, University of Brussels) gave a lecture and practical demonstration of the preparation of particulate glycogen; Dr. D. B. Roodyn (Department of Biochemistry, University College, London) described methods of preparing nuclei, and Prof. E. Schram (Department of Animal

Morphology, University of Brussels) gave a lecture on density gradient differential centrifugation and analysis of ribosomes and polysomes, followed by a very fascinating demonstration.

Practical experiments and demonstrations were also supervised by Profs. Berthet and H. Beaufay, Drs. P. Jacques, A. Trouet, G. Vacs, M. Robb and Thines-Sempoux (Department of Physiological Chemistry of Louvain), Drs. A. Burny, G. Huez and G. Marbaix (Department of Biological Chemistry, University of Brussels) and Prof. R. Wattiaux (Department of Physiological Chemistry, Namur), and took place in the laboratories of the Department of Physiological Chemistry of the Institute of Physiology. Fundamentally, the practical work comprised the preparation of gradients and a general but very detailed survey of the techniques of centrifugal fractionation, in which cells were disrupted in a homogenizer and then spun in a centrifuge at successively higher speeds to yield a number of fractions containing organelles of different types. The organelles were then explored by means of biochemical methods determining the association of a number of enzymes with the cellular structures. The importance of this systematized technique has been dramatically emphasized in recent years by the isolation of a new cell organelle, the lysosome, which has already been shown to have fundamental importance for cell biology.

The organization of the course was well carried out. Equipment was of a high order and very specialized, and a team of expert assistants helped so that participants were able to obtain the maximum advantage. This report would be incomplete without recording the appreciation of the participants for the excellent arrangements made by Prof. H. G. Hers, who assumed the administrative responsibility of the course with meticulous attention to detail, and for the hospitality extended to them.

Mention must be made of the enthusiasm of Prof. C. Liébecq, secretary of the Société Belge de Biochimie in persuading Prof. de Duve to start a new line for the Federation of European Biochemical Societies with this course and in particular for the Society's ready willingness at all times to co-operate with the scientific staff and participants in providing the required facilities.

To sum up the course, Prof. C. de Duve's group attempted in the lectures and practical work to draw an overall and consistent picture of the succession of processes constituting a mechanism of separation of sub-cellular particles. The participants of the course were given every facility to appreciate the methods developed by these skilled specialists, and the progress which can result from detailed analysis of the enzymatic properties of sub-cellular particles separated by differential and density gradient centrifugation and from the characterization of the various particles. The course, which was much appreciated by those present, ended with a general discussion of the practical results.

This first summer school of the Federation brought together biochemists of many different specialities. Throughout the course an atmosphere of the greatest cordiality and friendship prevailed. Many problems of common interest were discussed, and evening meals at the Faculty Club of the University, a beautiful and modern building, kept members gathered together after the laboratory work. The course in fact performed two interesting functions: one European and the other scientific. An official dinner was held on June 11, and presided over by Prof. C. de Duve. A succession of national toasts was offered by the participants to the staff of the Depart-

ment and the organizers, one representative of each country thanking in his own language the staff and all the people collaborating in the success of the course. In reply, Prof. de Duve thanked all the participants for their co-operation and expressed his satisfaction with the result of this first summer school; he expressed the conviction that this would be the starting point for a series of similar courses for which interest was amply evident.

The Federation of European Biochemical Societies is to be congratulated on organizing courses of such quality.

The general nature of this kind of course will ensure its future success, and there is clearly no shortage of subjects. The importance of ensuring conditions that will facilitate free international co-operation and interchange in any field of scientific endeavour is not small, and the organization of this course is an important new landmark in the development of relations among scientists of different countries. We are sure that in the very near future similar meetings will be held in other biochemical institutes.

## BIOCHEMISTRY IN MAGDEBURG

WE have recently returned from a short visit to East Germany, where we were invited, as guests of the G.D.R. Government, to participate in the second annual conference of the Biochemical Section of the Society for Experimental Medicine of the G.D.R. held at Magdeburg during June 24-26.

The conference was held at the Medical Academy in Magdeburg under the presidency of Prof. K. Lohmann. It was attended by about 300 biochemists, including a notable group from the Federal Republic of Germany and representatives of many countries of Eastern and Western Europe. Prof. Eberhard Hofmann, of Magdeburg, in his organization of the conference, followed the best traditions of international scientific meetings.

The meeting centred about three symposia: (a) "Functional Biochemistry of Cell Membranes and Mitochondria"; (b) "Problems of the Regulation of Glycolysis and Fermentation"; (c) "Aspects of the Training of Biochemists". There were also sessions where short communications were read. In all, about 100 papers were heard.

By far the most interesting event was the symposium on glycolysis and fermentation. This session, conducted admirably by Prof. H. Frunder, of Jena, was introduced by Prof. Benno Hess, Dortmund. In his exposition, Hess centred his arguments on mathematical models of glycolysis and on the oscillatory behaviour of metabolites under transitional conditions in living yeast and in yeast press juice. The importance of phosphofructokinase in regulatory mechanisms was emphasized in many contributions. Thus Hommes (Nijmegen) described a number of phosphofructokinases in different yeasts which were all inhibited by ATP. The phosphofructokinases from yeasts that showed an aperiodic change in pyridine nucleotide levels after anaerobiosis were activated by fructose-6-phosphate and not by AMP, while the enzymes from 'asymptotic' yeasts were activated by neither of these compounds. 'Oscillatory' yeasts had phosphofructokinases which were inhibited by ATP and activated by fructose-6-phosphate and AMP. Freyer and Kopperschlager (Magdeburg)

suggested that the high aerobic glycolysis of ascites tumour cells could be explained by the insensitivity to ATP of the phosphofructokinase, together with the low pyruvate utilization and the limited power of the mitochondria to oxidize cytoplasmic NADH. The inhibition of glycolysis in ascites tumour cells by alkylating cytostatic agents appears to be due to an activation of NAD-splitting enzymes (Grunicke, Richter and Holzer, Freiburg). Lamprecht (Munich) discussed the regulation of glycolysis in human heart muscle. Rapoport (Berlin) considered the regulatory significance in erythrocytes of an NADP-dependent lactate dehydrogenase and, in ascites tumour cells, of a lactate oxidase not requiring a pyridine nucleotide.

In the symposium on the training of biochemists, we learned from Prof. Hofmann's introductory remarks that courses for biochemists in the Faculty of Science existed in only two German universities: Tübingen in the West and the Humboldt University in East Berlin. Hofmann emphasized the need for additional courses of this kind. We had the opportunity of describing the existing courses in London and some of the proposed developments in Britain's new universities. Prof. Bücher (Munich) expressed enthusiasm for the relative brevity of degree courses in Britain and was joined by Prof. Rapoport (Humboldt University, Berlin) in a plea for the elimination of the time-consuming medical course as a prerequisite for a biochemical career in Germany. It was evident that these matters were of vital interest to the younger members of the conference.

We were impressed by the high quality of the scientific work as it was described in the communications and by the evident desire of our East German colleagues to improve their contacts with the countries of the West. It is deeply disturbing that the absurdities of politics make it difficult for us to contribute to this improvement and to make a full return for the hospitality which we received.

D. F. CHERRMAN  
J. R. LAGNADO

## INDUSTRIAL FEEDING AND CANTEN MANAGEMENT

THE report on the joint symposium on "Industrial Feeding and Canteen Management in Europe", held in Rome during September 2-7, 1963, has now been published under the auspices of the Food and Agriculture Organization, the International Labour Organization, and the World Health Organization\*. After explaining the reasons for the interest of these organizations in the subject, the report summarizes the situation to-day in various European countries and suggests some steps to

be taken if these specialized agencies are to assist member countries to solve their more urgent problems.

In most European countries industrial feeding is regarded as an integral part of the facilities of the factory, even though the provision of food to workers is frequently not required by law. The determining factor in provision of a canteen and in the type of service offered is usually the demand and need of the workers. The use of canteen services varies in different countries and is often disappointingly small. The effect of nutrition on occupational health and on working efficiency and such matters as food supply, levels of food consumption and food habits, as well as the problems encountered in pro-

\* Food and Agriculture Organization of the United Nations. FAO Nutrition Meetings Report Series, No. 36: *Report of the Joint Symposium on Industrial Feeding and Canteen Management in Europe, Rome, Italy, 2-7 September, 1963*. Pp. 44. (Rome: Food and Agriculture Organization of the United Nations. London: H.M.S.O., 1964.) 2s. 6d.; 50 cents.

moting acceptance of improved diets, are dealt with in successive chapters.

The symposium agreed that the newer knowledge derived from research in the behavioural sciences must be taken into account when planning for the educational aspects of canteen operation. It was suggested that much more attention should be directed to scientific research on food habits and food preferences among specific groups of workers in particular areas, on rates and channels of change in nutritional and diet practices, the effectiveness of different types of activities, and the results of educational methods and materials used. It was considered that carefully planned training in educational methods of techniques, as well as in relation to the psycho-social and cultural determinants of human behaviour about nutrition, should be included in all professional courses for people who would be responsible at a relatively high level for the management of industrial canteens.

Participants at the symposium urged the need for more exchange of information and better distribution of current literature. A suggestion was strongly supported that an international organization, possibly the Food and Agriculture Organization, should establish a clearing house for current literature, research and information on the nutrition of workers. It was also recommended that a

joint study group of specialists in industrial feeding and canteen management should be sponsored by the three Specialized Agencies. Such a study group, meeting at regular intervals, could co-ordinate and, where necessary, sponsor investigations and research in industrial feeding and suggest suitable planning and methods of research into the various aspects of industrial feeding. These might include food consumption and nutritional status of workers; times and amounts of food required by workers in relation to the type of work and the work environment; the influence of time-intervals between meals, length of meal periods and amount of food consumed on workers' efficiency; the psycho-social, socio-economic and cultural patterns that lead to the use or disuse of industrial feeding units; new foods and food processing methods; kitchen planning and lay-out in relation to work efficiency, quality of food and economic production; kitchen equipment, its use and upkeep; training and education of managers, supervisors and workers; education in nutrition for industrial workers. It was also suggested that the group could study with specialists, the Governments concerned, and organizations of employers and workers any additional problems related to industrial feeding and encourage the development of a uniform system of terminology to facilitate exchange of information in this field.

## NATIONAL COAL BOARD MINING RESEARCH ESTABLISHMENT

THE Mining Research Establishment held open days during July 19-23, of which three days were reserved for overseas delegates to the fourth International Mining Congress and two days for visitors from Great Britain. The demonstrations illustrated the wide application of the research results to mining problems.

Much effort has been put into supporting the National Coal Board's drive to offset by instrumentation the reductions in manpower expected in the future. On the coal face, automatic steering of the getting machine to keep it in the coal poses problems analogous to those met in the guided-missile field. Information as to the position of the machine is supplied by a nucleonic device that measures the thickness of coal left above or below the machine in terms of the back-scatter of gamma rays directed into the floor or roof from a radioactive source. The vertical orientation of the machine is given by a tilt transducer, and the two signals are fed into an electronic control unit operating the steering jacks. The actual system required depends on the delays inherent in the input and output sides and on the geometry of the machine, characteristics which vary with each type of machine. A certain amount of information has to be obtained from full-scale field observation, but the amount of field work is kept within practical bounds by simulating transducer outputs and machine performance on an analogue computer.

Remote-control techniques have already been successfully applied experimentally on the coal face to control both getting machine and roof supports, and the Mining Research Establishment is heavily involved in the extension at Bevercotes Colliery of analogous techniques to give remote control of other sectors of the mine and to communicate production information to central points underground and on the surface as an aid to management.

Almost all underground instrumentation, whether for remote control, monitoring or research, must be intrinsically safe, that is, incapable under any conditions of operation or failure of producing a spark of energy sufficient to ignite a methane-air mixture. Research at the Mining Research Establishment on inductive break-sparks has shown that ignition can be brought about at an energy-level one-fifth of that hitherto accepted as the minimum and of the same order as that previously asso-

ciated only with capacitative circuits. This lends support to the minimum energy concept of ignition which can be applied to the design of intrinsically safe circuits. Two new protective devices, the Zener diode and the surge arrester, have been introduced into this field.

Experiments on the mode of breakage of rock and coal under simple forces or when attacked by tools have led to a theory of breakage based on brittle fracture. The deductions have led to a rationalization of the design of mining tools and machinery to give faster production for less power with the added benefits of a larger product and less dispersion of respirable dust and firedamp. Systematic study of the forces and movements around mine workings is suggesting improvements in the design and use of supports. Comparison of results obtained with those reported elsewhere indicates that many of the basic phenomena follow the same laws in all coal-fields of the world.

A considerable amount of research is directed to increasing the safety of the atmosphere in the mine by reducing the dangers from firedamp accumulations and from the respirable (1-5  $\mu$ ) dust which causes pneumoconiosis. Airflow near the coal face is studied using scale models in which the flow patterns are made visible by illuminating tracer particles suspended in water. Results have been verified underground by ventilation surveys and by the use of tracer gases and smoke. Tracer gas is also one of the means used for studying the dispersion of firedamp from the coal seam. The Mining Research Establishment has pioneered the development of instruments for sampling respirable dust. The 'long-running thermal precipitator' samples the respirable fraction of the dust in the air during a full shift. The sample is usually evaluated by counting, but an optical densitometer has been designed which gives a rapid evaluation either in number of particles or mass. A gravimetric dust sampler collects a sample, again during a whole shift, on a fibreglass filter. Accuracy of evaluation is considerably higher than that with the 'long-running thermal precipitator' and mass is probably a more important parameter clinically than particle count. Research on dust dispersion and suppression has shown the overriding importance of good design, maintenance and use of machinery so as to minimize dust formation and

dispersion. The most effective ways of using water for dust suppression have been demonstrated and the use of exhaust ventilation with filtration is being studied.

Each year the National Coal Board spends something like £100 million on metal products: working out proper specifications of the materials involved is an important part of the work at the Mining Research Establishment. Of equal importance are investigations to ensure that advances in metallurgical science are applied to modern mining techniques. Corrosion can cause serious losses and mine waters vary widely in pH and salinity. Research into the resistance of different steels to the various contaminations and the protection afforded by metallic and other coatings is aimed at minimizing corrosion losses. Oil-in-water and invert emulsions are being introduced underground as fire-resistant substitutes for hydraulic oils. The stability and corrosion-inhibiting properties of these fluids are therefore being studied in addition to the relationship between their physical properties and performance. Aqueous glycols and phosphate esters are also being examined in this context.

A requirement peculiar to steels for mine supports is that they shall withstand repeated slow plastic bending; safety demands that neither initially nor after such treatment shall they be liable to brittle fracture. The value in these conditions of heat-treated carbon manganese steels has been established and means are being sought of further increasing strength without embrittlement in a continual effort to reduce the size and weight of mining equipment. The advantages and possible dangers of the use of high-strength steels, including spring steels, are being studied with the same objective. The fatigue properties of alloy

steels for suspension gear have been determined with and without stress raisers. From this work the degree of advantage of alloy steels over manganese steel as used at present has been determined.

Cemented carbides and other hard facings are being studied to establish the best compromises for different mining tools between abrasion- and chipping-resistance. An electron microscope is being used to further this work. Methods of brazing the carbide tips on to the tools are included in the study.

Nearly 8 million yards of conveyor belting is used in British collieries and specification tests have been evolved for fire-resistant belting. The tests include not only fire resistance and performance but also surface electrical resistance in order to ensure that static charges do not build up on the surface of the plastic (polyvinyl-chloride), which has replaced flammable rubber in underground belting. The same problems of fire-resistant and anti-static properties arise with all plastics used underground and there is a steady stream of articles arriving at the Mining Research Establishment for examination as underground applications are found for these new materials.

Conveyor belting has improved enormously in strength over recent years; it has been necessary to improve methods of jointing. A new type of fastener has been devised at the Mining Research Establishment which gives a performance several times better than fasteners at present in use, and work is continuing further to improve jointing methods. Simultaneously the fastener-holding properties of belts are being examined and the effect of varying the fabric in the carcass is being determined.

C. S. MAKOWER

## DEFORMATION OF SOLIDS BY THE IMPACT OF LIQUIDS, AND ITS RELATION TO RAIN DAMAGE IN AIRCRAFT AND MISSILES, TO BLADE EROSION IN STEAM TURBINES, AND TO CAVITATION EROSION

By DR. J. H. BRUNTON, DR. J. E. FIELD and G. P. THOMAS

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A DISCUSSION on "The Deformation of Solids by the Impact of Liquids" was held by the Royal Society on May 27. In his opening remarks, Dr. F. P. Bowden, who had organized the discussion, stated that the meeting would begin by considering the physics of liquid impact and the nature, magnitude and duration of the stresses which are produced when a solid is struck by a drop of liquid. Even with moderate impact velocities the pressures developed in the solid are considerable, and at high velocities the shock pressures are very great indeed and are sufficient to produce deformation of the strongest solids. These observations have a bearing on three technical problems which are of considerable importance at the present time. The first is the damage caused to a high-speed aircraft or missile when it strikes a raindrop. The second is the erosion of turbine blades rotating at high speed in wet steam. The third is the damage produced by 'cavitation' in liquids. There is now evidence that the stress due to liquid impact is a common factor in all three of these processes. One interesting development of this work is that liquid impact is being used as a practical method for 'cutting' or fracturing solids.

In the section on the physics of impact, papers by Dr. J. H. Brunton and Dr. J. E. Field (Cavendish Laboratory, Cambridge) described investigations of the high-speed impact of cylindrical and spherical drops on hard surfaces. At velocities in the region of 1,000 m/sec, they found that the impact pressures reached values of about 370 kg/mm<sup>2</sup>, a figure appreciably greater than the strength of many

common structural materials. They found that the behaviour of the drop on impact was adequately described by the 'water-hammer' equation. The main characteristics of this behaviour were an initial short duration (1-2 μsec) water-hammer pressure and a high-speed flow from under the impacting drop. The large magnitude and short duration pressure peak gave the drop impact an 'explosive' character and the high-flow velocities led to erosion at surface discontinuities in the target specimen. In brittle materials the impact characteristics gave rise to ring fracture patterns, and in metals to smooth depressions with eroded edges. In thin-plate specimens 'scabbing' (spalling) occurred near surfaces where the compression wave was reflected. The influence of surface flaws on failure in brittle materials was described.

An interesting account of the high-speed jets which arise when concave liquid surfaces collapse under pressure from behind was given by Dr. F. P. Bowden (Cavendish Laboratory, Cambridge). Examples were shown of liquid Munroe jets, and of jets formed in collapsing cavities and between coalescing drops. The implications of jet formation in cavitation were discussed in a later paper by Dr. T. Brooke Benjamin and Prof. A. T. Ellis.

Sir Geoffrey Taylor (Cavendish Laboratory, Cambridge) described an investigation of the problem of the oblique impact of a liquid jet on a plane surface. The transformation of the jet into a thin sheet was examined and compared with experiment. The maximum steady-state pressure was always the stagnation pressure, but it



seemed that much higher pressures were possible if the jet was moved sideways during impact.

A useful method for investigating the impact force produced by a liquid drop using a dislocation etch-pit technique was described by Dr. K. H. Jolliffe (Central Electricity Research Laboratories, Leatherhead). The method depended on comparing the size of the etch-pit 'rosettes' produced by a steel ball, for which the stresses can be calculated, with the size of the rosettes produced by the impact of a liquid drop.

Multiple impact experiments were described in papers by Mr. G. P. Thomas (Cavendish Laboratory, Cambridge), and by Dr. J. B. Marriott and Dr. G. Rowden (English Electric Co., Ltd., Whetstone). In Mr. Thomas's experiments the initial stages of deformation were examined for very low impact velocities, and at stresses well below the yield strength of the metals used. It was observed that the metal deformed initially only in small 'weak' areas (approximately 1  $\mu$  in diameter) forming depressions. Some depressions grew and eventually metal was eroded either by ductile or brittle failure in these larger depressions. In the paper by Drs. Marriott and Rowden the specimens were cobalt-based stellites eroded at high-impact velocities. They found general deformation by slip in the softer matrix at an early stage and observed flaring and pitting at phase boundaries. Erosion occurred as a result of cracking at the carbide/matrix boundaries.

When the discussion turned to the rain erosion of aircraft the problem was reviewed by Mr. A. A. Fyall (Royal Aircraft Establishment, Farnborough). Factors including component design, velocity of flight and rain intensity were discussed, together with their significance in practice. One point which emerged was the possibility of rain at quite high altitudes (> 30,000 ft.). However, commenting on this, Mr. R. F. Jones (Meteorological Office) said that the probability, although not negligible, was very small. Droplets would only form in association with vigorous convection currents and over limited horizontal distances; when they did occur they should be detectable by radar.

The possibility of rain droplets being disintegrated by the shock waves travelling ahead of fast-moving bodies was considered in a paper by Mr. D. C. Jenkins (Royal Aircraft Establishment, Farnborough). It was shown that although the droplets do start to break up in the shock the process takes a finite time. A combination of experiment and theory has enabled Mr. Jenkins to evolve an equation relating the time of break-up to the drop diameter and the velocity of the airstream.

Work in progress in Germany was reported by Dr. H. Busch (Dornier-System G.m.b.H., Friedrichshafen, Germany). A considerable achievement in their experimental programme has been the successful design of a rotating arm apparatus capable of reaching velocities of Mach 1.5. By operating the device in an atmosphere of simulated rain, a wide range of materials had been tested for their erosion resistance. High-speed photographs showed that during impact the water droplets disintegrated into a fine spray which quickly evaporated; this 'mechanical vaporization', under some conditions, caused cooling of the solid surface, a result of some relevance in the problem of the icing-up of aircraft.

In the next part of the meeting Mr. J. Caldwell (English Electric Co., Ltd., Rugby) described the damage found on steam turbine blading, and discussed the effect it had on the design and efficiency of turbines. Attempts at removing the water, shielding parts of the blade with more resistant material and increasing the spacing between rotating and stationary blades had achieved partial success.

Dr. T. Broom (Central Electricity Research Laboratories, Leatherhead) presented a paper by Dr. D. G. Christie and Mr. G. W. Haywood (Central Electricity Generating Board) on the formation of the water droplets which cause erosion in steam turbine. A film of the conditions inside a turbine was presented which showed how

water collected in large quantities on the stationary blades and was then afterwards swept off by the steam into the path of the rotating blades. Steam tunnel experiments were also described, and films showed the size, velocities and behaviour of drops in the steam flow.

In a paper by Mr. D. W. O. Baker (Central Electricity Generating Board, South-east Region), Dr. K. H. Jolliffe and Mr. D. Pearson (Central Electricity Generating Board, Marchwood), the form and microstructural characteristics of erosion damage in turbine blade materials were described. The progress of the damage was illustrated and a relation for the erosion resistance of a material was given. This relation was interpreted in terms of a fatigue mechanism of damage.

The session on cavitation was opened by Dr. T. Brooke Benjamin (Department of Applied Mathematics, Cambridge), who presented a paper by himself and Prof. A. T. Ellis (California Institute of Technology, Pasadena), and also one by Prof. M. S. Plesset (California Institute of Technology, Pasadena), who could not attend. The papers were concerned with the collapse of cavitation bubbles and the pressures produced. A particularly interesting point which emerged was that there is now considerable theoretical and experimental evidence to show that a cavity collapsing near a solid boundary may develop a jet of liquid by involution of its far side. This high-speed jet then passes through the cavity and may strike the solid boundary. Although the contribution of this phenomenon to cavitation damage has yet to be established, it seems likely that it will prove important.

Prof. F. G. Hammit (University of Michigan, Ann Arbor) was primarily concerned with the damage in materials caused by cavitating flow. The observed pitting was a mixture of randomly located symmetrical pits, presumably the result of single events, and irregular fatigue-type failures. There were interesting similarities between some of the pits illustrated by Prof. Hammit and those shown earlier for droplet erosion damage. The closeness with which cavitation and liquid impact erosion are linked was emphasized in the contribution of Dr. J. M. Hobbs (National Engineering Laboratory, East Kilbride) on the practical aspects of cavitation; one of the accelerated tests used by Dr. Hobbs for evaluation of cavitation erosion resistance was, in fact, a drop impact apparatus. Dr. Hobbs found that in this test the velocity of collision and the jet diameter had significant effects. These observations agree well with the present picture of the physics of the impact process, since the magnitude of the 'water-hammer' pressure depends on the velocity of impact, and its duration on the jet diameter.

The final paper, by Dr. S. J. Leach and Dr. G. L. Walker (Safety in Mines Research Establishment, Sheffield), considered the application of high-speed liquid jets to cutting. Fine jets had been produced at velocities up to 1,000 m/sec; the maximum velocity being produced by pressures of 5,000 atm. A detailed study of nozzle design, and of the pressures produced by the impacting liquid, had been made. Optical and X-ray photography demonstrated that the jet was by no means completely atomized at the nozzle, but had a central coherent core. Although the full potentialities of this method have yet to be evaluated, it is hoped that this form of cutting will prove useful in mining where frictional heating from more conventional cutting appliances can cause ignition of methane gas.

Prof. K. K. Shalnev (Institute of Mechanics, Moscow), who had prepared a paper on scale effects in cavitation, was unable to be present, but his contribution, together with the other papers and discussion, will be published in the *Proceedings of the Royal Society* dealing with the meeting. The meeting was attended by some 150 physicists and engineers from different countries.

In his closing remarks the president, Lord Florey, emphasized the importance which the Society attached to this interplay between science and technology.



# GEOMAGNETIC DEPTH-SOUNDING AND CORRELATION WITH OTHER GEOPHYSICAL DATA IN WESTERN NORTH AMERICA

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THE geomagnetic depth-sounding method is based on the comparison of variations in the natural geomagnetic field recorded at different locations. The vertical component in particular reflects the distribution of electrical conductivity at the depths where the bulk of the induced currents flow. For fluctuations of intermediate period (500–1,000 sec) the penetration depth is sufficiently large to permit use of the method for upper mantle research. The method is as yet insufficiently developed to permit direct quantitative interpretation; but it can provide a very powerful tool if used in conjunction with other geophysical information. A possible correlation between the geomagnetic 'coast effect' and discontinuities in the seismic low  $S$ -velocity layer has been suggested<sup>1,2</sup>. The apparent coincidence between seismic low  $P$ -velocity zones and some inland anomalies<sup>3,4</sup> has also been pointed out<sup>5</sup>.

The present communication presents the application of such a combined geophysical approach to the particular case of western North America. The available geomagnetic data<sup>6-7</sup> are summarized in Fig. 1. The ratio  $I = |\Delta Z|/[(\Delta D)^2 + (\Delta H)^2]^{1/2}$  (ratio of vertical to horizontal component amplitudes) is derived from peak-to-peak amplitudes of fluctuations in the range of periods 10–120 min. It is a somewhat crude parameter, and cannot be readily interpreted in the immediate vicinity of lateral discontinuities. However, it is very useful for comparisons between 'normal' stations (that is, over horizontally stratified distributions in conductivity) at roughly similar geomagnetic latitudes. Consistently lower  $I$ -ratios at any group of stations indicate that the external vertical component is being compensated more effectively by induced internal contributions, that is, that the highly conducting layers lie nearer to the surface than at other stations with higher  $I$ -ratios. In this particular case the contrasts in  $I$ -ratios are sufficiently large (changes by factors of 2–3 over distances of 100–200 km) to permit valid comparison of the results without more sophisticated analysis. At anomalous stations (shown circled) the recorded fluctuations differ significantly from those recorded at stations on either side; they can be assumed to be within 50–100 km of major lateral discontinuities. As Schmucker<sup>8</sup> pointed out, only these stations can be considered truly 'anomalous'; all others (whatever their  $I$ -ratios) are 'normal' for the particular horizontally stratified conductivity structure of the region. The anomalous stations shown near the coast represent the geomagnetic 'coast effect'<sup>9,10</sup> and are not directly relevant to the present investigation.

Some of the available seismic results<sup>11-14</sup> have also been summarized on Fig. 1. Those in the south-western United States are sufficiently dense to permit  $P_n$  velocity contours to be drawn<sup>5</sup>; the results in the north-west are as yet too sparse for reliable contouring, but tentative results indicate that the structure turns sharply to the north-west from about lat. 48° N. (ref. 15). In both cases the edge of the seismic low  $P_n$  velocity zone is roughly correlated with the edge of the geomagnetic low- $I$  zone, in location as well as strike. Exact spatial correlation cannot be expected, since seismic  $P_n$  data represent

average values over fairly long distances; also the relevant 'edge' would be a transition band between about 7.0 and 8.1 km/sec, rather than the 8 km/sec contour.

The foregoing comparison demonstrates the possible value of geomagnetic depth-sounding as a complementary method to seismic refraction work. Large areas can be economically surveyed by geomagnetic depth-sounding to find the location (and possibly the strike) of lateral discontinuities. These can then be investigated quantitatively by parallel seismic profiles on either side. Apart

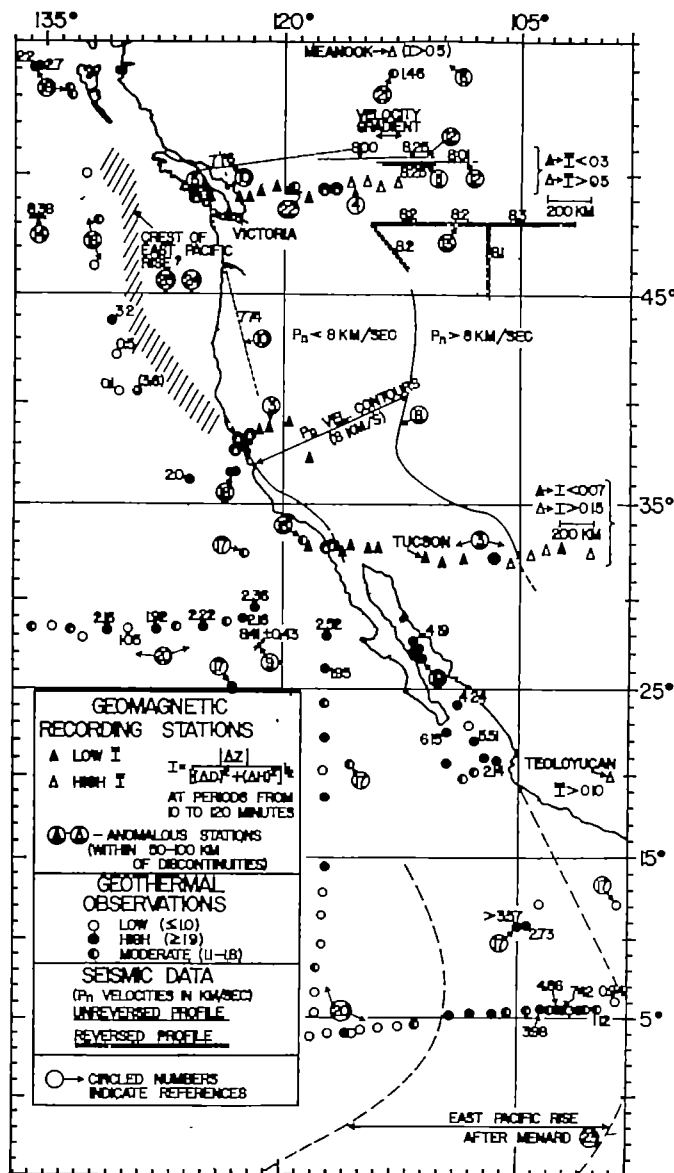


Fig. 1. Geomagnetic and other geophysical data, western North America.

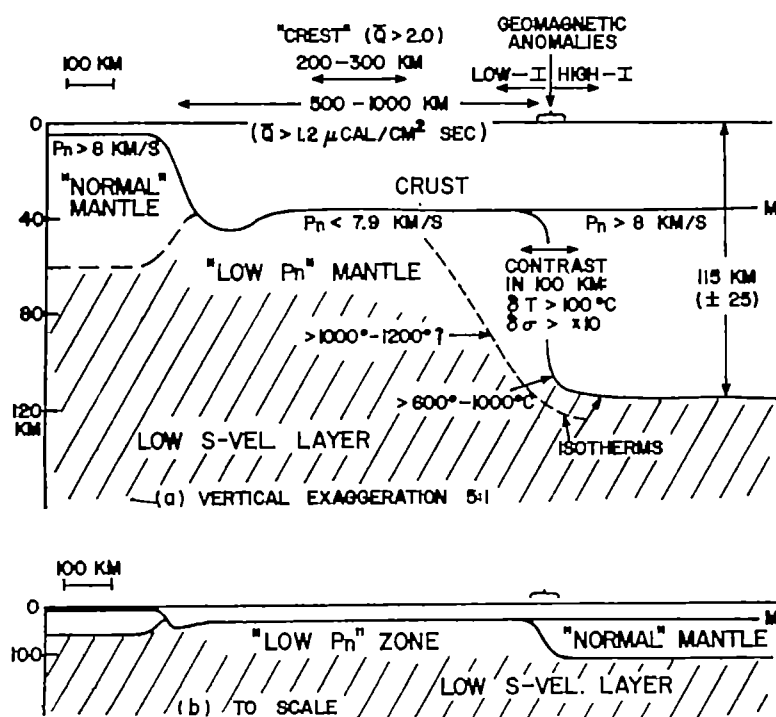


Fig. 2. Generalized upper mantle model, western North America

from this practical aspect of geographical delineation, the correlation between seismic low-velocity and geomagnetic low- $I$  zones carries implications about the nature of these zones. Since the electrical conductivity at upper mantle depths is primarily a function of temperature, the correlation lends strong support to the hypothesis that the low  $P$  velocities are caused primarily by increased temperatures rather than by changes in composition. The low  $P_n$  velocity zone in California has generally been interpreted on the basis of composition changes. In at least one case, however<sup>16</sup>, it was suggested that increased temperatures (by about 200° C at depth 30 km) could improve the consistency between gravity data and seismic  $P$  delays. This suggestion is strongly supported by the existence of major geomagnetic anomalies associated with the low  $P$ -velocity zones.

Indirect support for this hypothesis of increased upper mantle temperatures under western North America comes from the pattern of heat flow observations<sup>17-23</sup> shown in Fig. 1, and from the suggested continuation of the East Pacific Oceanic rise under the continent<sup>24</sup>. The East Pacific rise is characterized by a broad belt (about 200-1,000 km wide) of moderately high (more than 1.2 microcal/cm² sec) heat-flow, with a narrower band (about 100-200 km wide) of very high (more than 3 microcal/cm² sec) heat flow at the crest. Low seismic  $P_n$  velocities have been observed associated with the crest<sup>9</sup>. Menard<sup>25</sup> suggests that this crest enters the continent through the Gulf of California; this is strongly supported by the extremely high heat flows observed in the Gulf<sup>18</sup>. The continental continuation is not clearly delineated, but it is generally accepted<sup>22,24</sup> that the crest leaves the continent at about latitude 40° N. and continues as shown in Fig. 1. This assumed continuation is based primarily on the distribution of shallow earthquakes, without definite confirmation by high heat flow observations. Recent work on earthquakes off western Canada<sup>26</sup> indicates that the distribution of maximum strain-energy release (rather than the frequency of occurrence) runs closer to Vancouver Island. It is therefore possible that the 'crest' is located further east—perhaps even as far as the arc of recent and Pleistocene volcanoes which parallels the west coast about 150 km inland<sup>26</sup>. This suggestion is based on the admittedly tenuous assumptions that: (a) the continental

continuation of the East Pacific rise is delineated by the geomagnetic low- $I$  and seismic low  $P_n$ -velocity zone; (b) the 'crest' (here defined as the narrow band of very high heat flow) would be located near the centre of the rise. Due to the scarcity of continental heat flow data, this extrapolation of oceanic rise properties to its assumed continental continuation remains speculative. However, the proposed model shown in Fig. 2 is at least compatible with all the available geophysical information. The model is shown in detail with an expanded vertical scale, and also to scale in order to demonstrate the geometrical plausibility of the proposed upwelling. A discussion of the mechanisms responsible for the proposed isotherm upwelling is beyond the scope of this article, but the model is fully compatible with suggested mantle convection theories<sup>20,22,27</sup>.

Whatever the validity of some of the speculations presented in this article, it is clear that in some cases geomagnetic depth-sounding can serve as a powerful tool for upper mantle research, provided that its limitations are recognized and that it is used in conjunction with other geophysical methods. Even though it cannot provide a unique interpretation, it can impose useful restraints on choice of

models as well as geographical delineation of anomalous zones. Surveys in the different branches (seismology, geomagnetism, magneto-tellurics, heat-flow) will be carried out in western Canada over the next few years by various Government and university research groups. Although there is as yet little active co-ordination between the different disciplines, the sum total of the data which will become available should permit verification of our hypothesis in so far as western Canada is concerned. Some geomagnetic depth-sounding will also be carried out in New Mexico by the Department of Geophysics of the University of British Columbia, to investigate a suggestion made by Slawson (private communication) that the geomagnetic anomalies of Schmucker<sup>3</sup> may correlate with a pattern of lead isotope abundances<sup>28</sup>. Since very good seismic control is available for this region, the proposed correlation should be easy to verify.

A major uncertainty is presented by the region between latitude 36° and 46° N.; at present the low  $P_n$  velocities are the only support for our assumption of a continuous structure (rather than just two localized upwellings, in the south-western United States and in western Canada). Further work in this region will be required to verify this assumption.

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## LAYERING IN THE GALWAY GRANITE

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THE Galway Granite, western Ireland, is about 400 square miles in extent and is intruded into acid and basic migmatites and Dalradian metasediments. According to Giletti, Moorbatch and Lambert<sup>1</sup> the last major metamorphism of the Dalradian was 470 m.y. while the age of the Galway Granite is given as 365 m.y.; thus it is about Middle Devonian.

The present investigation in the Slieve Moidaun area between Invermore and Carna was started during the summer of 1963 and several mappable granite types were recognized, but no contacts between the different granites were found. The commonest granite consists of a rather variable porphyritic adamellite which is similar to the Errisbeg Townland Granite of Wager<sup>2</sup>. From the coast, this extends about 2 miles northwards towards the contact with the migmatites, but between a quarter and half a mile from the contact the phenocrysts diminish in size and eventually disappear. This non-porphyritic granite resembles, both in chemical composition and texture, the Murvey Granite also described by Wager<sup>2</sup>, though it may contain a little more biotite. When traced westwards near the contact this granite merges into a lighter, biotite-poor granite which is similar to the Garnetiferous Murvey Granite of Murvey, except that it is slightly coarser and does not contain garnets. The transition zone is about 100 ft. wide.

During the summer of 1964 a portable scintillation rate-meter (Type 1418A) was used to survey the gamma-radiation of the area. This has a 1½-in.-diameter 1-in.-thick thallium-activated sodium iodide crystal coupled to an E.M.I. photomultiplier tube. The meter is of the direct-reading type and is calibrated against natural radium. There are four scales to read from; the one most used when working on granites gives a full-scale deflexion of 0.060 mr./h. The instrument is fully transistorized and runs off three 1.5-V batteries and weighs about 9.25 lb. It is easily carried in a rucksack.

When using the instrument, a base station is set up and a routine check, which includes background, is carried out twice daily, the first reading in the morning and the last reading at night. The values are averaged and the field readings adjusted accordingly. During June–September the base station readings varied between 0.010 and 0.012 mr./h. Some field positions were checked three or more times on different days; the greatest variation was 0.001 mr./h. In Fig. 1, the values quoted included cosmic radiation as well. This is 0.003 mr./h when it is measured over deep open water. When taking field measurements the scintillation counter was placed on an open, lichen-free, rain-washed rock surface, which was as flat as possible. No readings were taken in hollows or gullies, or on narrow ridges, as anomalous values would have been obtained due to the solid-angle effect. The instrument is very sensitive and will differentiate between gamma-radiation from localities as close as 3–4 ft. When taking readings at each station there was a random fluctuation which, at its worst, was  $\pm 0.005$  mr./h. If fluctuations were great, three or more readings were taken at the one station and the values

averaged. During this radiometric survey, over continuous exposures a 100-yd. grid was maintained where possible, except when contacts were present and closer readings were taken.

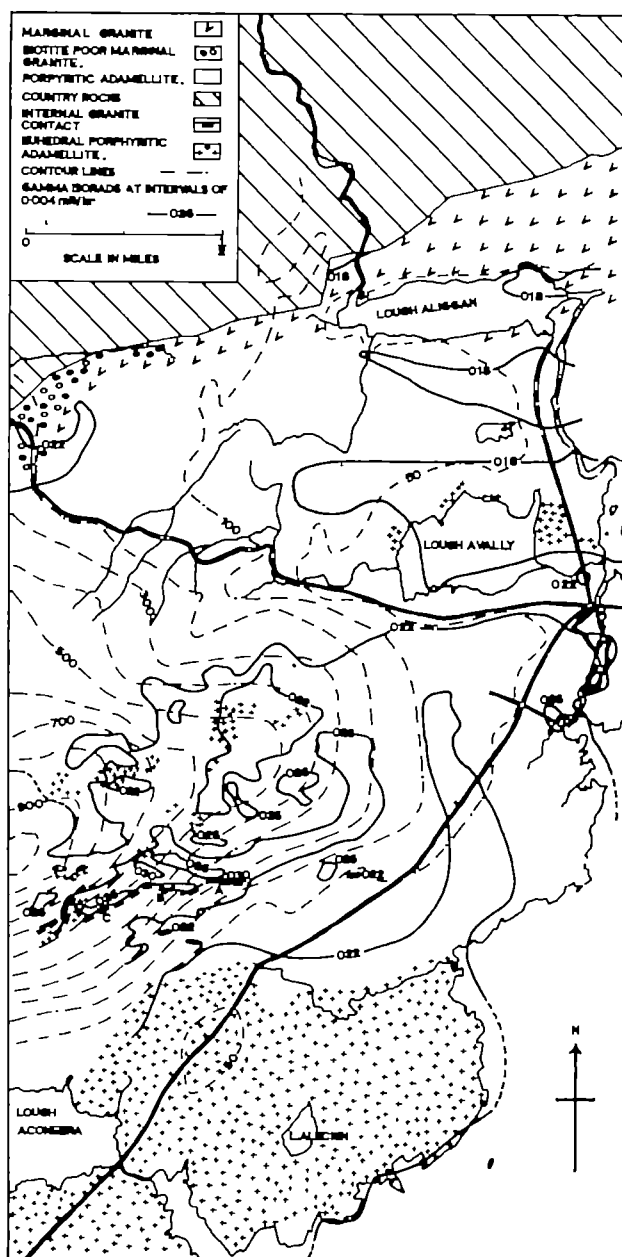


Fig. 1. Geological sketch map, showing the relationship between geology, activity, and topography

A gamma-isorad map is shown in Fig. 1. The contours are drawn ignoring the faults, as it is difficult to estimate displacements within a granite. It can be seen that values range from 0.014 to 0.032 mr./h in the granite.

Slieve Moidaun rises to just more than 1,000 ft. and extends parallel to the coast in an east-north-easterly direction. To the north and south of Slieve Moidaun the isorads are wide apart, while to the east they sweep around the hill. The steeper face shows the isorads to be set very close together, and generally trending east-north-east to west-south-west and consisting of a series of highs and lows. To explain the geometry of the isorad map, coupled with field evidence, it is proposed that the granite is disposed in layers which dip to the north or north-west. The north to south isorad anomaly west of Lough Alligean near the contact coincides with the transition between the two types of Murvey-like granite.

The granite exposed on Slieve Moidaun and on the coastal flat to the south is usually a porphyritic adamellite varying from a coarse-grained good porphyritic adamellite with euhedral potash feldspar phenocrysts to a poorly porphyritic adamellite with subhedral phenocrysts. Sometimes the adamellite is coarse grained and non-porphyritic. The layered disposition of the granite is shown by the wider, more uniform nature of the rock when exposed on the coastal flat south of Slieve Moidaun and on the low dipping north side. The steep south face of Slieve Moidaun shows the widest variety of granite types as exposed in 'strike' section. There is a distinct zone varying between 10 and 60 ft. thick where the porphyritic adamellite diminishes in grain size, upwards, becoming granitic and often aplitic and showing a sharp contact with normal coarse-grained adamellite above it. The contact is considered to be unchilled and can be traced for more than 0.25 mile. Biotite layers may occur along this contact and when seen they strike north-east to south-west and dip north-west. Biotite layers are common in the adamellite and also strike north-east to south-west with a dip which varies from 10° to 50° north-west. Some of the biotite layers show crystal settling with sharp bases grading upwards into more felsic material. The biotite layers may also cut one another similar to the mafic layering in the Alangorssuaq area in Greenland, as described by Harry and Emelius<sup>4</sup>. Basic xenoliths occur throughout the area and their long axes trend north-east to south-west or east-west dipping either vertically or northwards towards the contact. The contact between the finer granite and the overlying coarse adamellite dips north-west between 5° and 25°, and is always sharp. It is usually straight, but in some places small irregular lobes protrude upwards into the coarse adamellite. This contact is consistent along the length so far examined, with the granite above always consisting of a coarse-grained inequigranular hypidiomorphic adamellite with subhedral

to euhedral potash feldspar phenocrysts. In one or two localities the potassium feldspar phenocrysts are orientated parallel to the contact just below. A slight foliation may also be developed in the adamellite above the contact.

Below the contact, the granite is fine-grained, sometimes granophyric, increasing in grain-size downwards until the normal porphyritic adamellite is reached. This zone, in which the transition occurs between the porphyritic adamellite and the contact, is variable in thickness. At point A it is 70 ft., at point B it is 20 ft., while at point C the only evidence of the transition and contact is a 3-in. band of fine-grained granite in the porphyritic adamellite. The top of the zone is always sharp with the porphyritic adamellite above, but the granite below can also consist of a layered zone up to 20 ft. thick, consisting of fine- and coarse-grained granite intermingling. Because of the variability of the zone below the contact as regards the thickness of the finer-grained granite, the layers are thought to be lensoidal. This is supported to a certain extent by the radiometric evidence as the gamma-isorads close as they are traced along the strike. Above the contact differently textured porphyritic adamellites strike east-north-east and several zones of porphyritic adamellite with good euhedral potash feldspar phenocrysts merge laterally into a porphyritic adamellite with anhedral phenocrysts.

The radiometric evidence and the field evidence suggest that the porphyritic adamellite is layered and is not a uniform granite as it has been considered. Possible minor differentiating cycles in the sequence could be indicated by the increase of gamma-radiation from 0.024 mr./h in the adamellite to 0.032 mr./h in the more acid granite just below the contact with the overlying adamellite. This adamellite gives a much lower reading of 0.026–0.028 mr./h.

The layered nature of the intrusion is indicated by the work of Wright<sup>5</sup>, who mapped five distinct granite types in the Carna area, just to the west of the present investigation. He indicated that the various granite types were intruded as sub-horizontal lacoliths which also dip northwards towards the contact with the country rocks. Further geochemical work is being undertaken, the radiometric survey is being extended westwards and it is hoped to present more information in due course.

I thank Mr. S. H. U. Bowie and the Atomic Energy Division of the United Kingdom Geological Survey for suggesting the radiometric survey and the loan of the rate-meter, Dr. B. E. Loake and Mr. I. H. Ford for advice, and the Department of Scientific and Industrial Research (now the Science Research Council) for a research grant.

<sup>1</sup> Gillett, B. J., Moorhath, S., and Lambert, R. St. J., *Quart. J. Geol. Soc.*, 117, 253 (1961).

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<sup>3</sup> Harry, W. T., and Emelius, O. H., *Internat. Geol. Cong.*, Pt. 14, 172 (1960).

<sup>4</sup> Wright, P. O., *Proc. Roy. Irish Acad.*, 63, B, 261 (1964).

## EVOLUTION OF THE OCEANS AND THE ORIGIN OF FINE-GRAINED DOLOMITES

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CONTROVERSY has arisen concerning the origin of certain fine-grained, so-called 'primary' dolomites which are widespread in nature<sup>1</sup>. One of the major questions involved is whether the dolomite precipitated directly from sea-water or instead replaced sediments composed of calcium carbonate. Much geological evidence suggests direct chemical precipitation in marine evaporite basins, yet no one has been able to demonstrate experi-

mentally such a process, and modern sediments comparable to the extensive strata of high-purity dolomite have not been observed. If, on the other hand, the dolomite is a diagenetic replacement of pre-existing calcite, the cause and the mechanism of the alteration process remain to be explained.

Recently, some very important work of Epstein *et al.*<sup>2</sup> has opened up a new approach to the problem. Hydro-

thermal experiments by those authors, and isotopic analyses of high-temperature, natural quartz-calcite and quartz-dolomite pairs by Clayton and Epstein<sup>8</sup>, indicate that isotopic equilibration of calcite and dolomite at Earth surface temperatures should result in oxygen-18 enrichment in dolomite (with respect to the coexisting calcite) of 5–8 per mil<sup>4</sup>. Even under hydrothermal conditions, dolomite is difficult to crystallize, and Epstein *et al.* concluded that in some experiments calcite formed first. This mineral was then replaced by dolomite by some mechanism in which magnesium was incorporated in the solid phase without alteration of the  $^{18}\text{O}/^{16}\text{O}$  ratio of the carbonate ion of the original calcite. The bearing on the dolomite problem is immediately obvious; if the explanation of the experimental data is valid, and if natural 'dolostones' were formed by diagenetic replacement of pre-existing calcite, then examination of sedimentary rocks containing coexisting calcite and dolomite should show no fractionation of oxygen isotopes between these two minerals. Thus a good case for secondary dolomite might be established.

However, if dolomite were found to have a higher  $^{18}\text{O}/^{16}\text{O}$  ratio than coexisting calcite, could this observation be used as proof for primary chemical precipitation of the dolomite? Unfortunately, the answer is negative since such results cannot be interpreted uniquely. Possible explanations are: (1) the dolomite is primary and both calcite and dolomite were precipitated in isotopic equilibrium with sea-water; (2) the dolomite is a late diagenetic replacement product, but during dolomitization, isotopic equilibration occurred; (3) the dolomite formed authigenically very soon after deposition of the calcite, before lithification, and the slow authigenic alteration of calcite permitted isotopic equilibration (that is, replacement may have involved a solution-recrystallization mechanism rather than one of solid-state diffusion); (4) dolomite replaced pre-existing calcite without any change in the  $^{18}\text{O}/^{16}\text{O}$  ratio, but subsequent contact with intrastratal fluids and formation waters (usually enriched with oxygen-18) involved exchange of oxygen isotopes with calcite but not with the less-soluble and less-reactive dolomite. Each of these explanations could account for oxygen-18 enrichment of dolomite with respect to calcite in ancient sedimentary rocks. Before reviewing data available at the present time concerning  $\delta^{18}\text{O}_{\text{calc}}$  and  $\delta^{18}\text{O}_{\text{dol}}$  and before evaluating each of these hypotheses, a further complication requires some discussion: has the  $^{18}\text{O}/^{16}\text{O}$  ratio for sea-water changed with time?

The precipitation of both silica and calcium carbonate from sea-water involves fractionation of the oxygen isotopes so that the solid phase is enriched in oxygen-18. Silverman<sup>9</sup> noted that there is a net accumulation of these deposits with time, and thus as oxygen-18 is preferentially extracted from sea-water in calcareous sediments and chert, the  $^{18}\text{O}/^{16}\text{O}$  ratio of sea-water must have decreased with time, so that the  $^{18}\text{O}/^{16}\text{O}$  ratio of the present oceans is lower than that of Cambrian sea-water. Degens<sup>6</sup> and others have expanded this argument.

I have criticized<sup>7</sup> this hypothesis on the basis of evidence to be discussed later, and have proposed the argument that the  $^{18}\text{O}/^{16}\text{O}$  ratio of sea-water has increased with time. If the oceans have evolved by degassing of the Earth throughout geological history<sup>8</sup>, the addition of juvenile water to the oceans may have influenced the isotopic composition of sea-water. Juvenile or magmatic water is extremely elusive, and both the rate of escape from the Earth's interior and the isotopic composition are difficult to determine. Using the most reliable estimates<sup>7</sup> it is possible to construct a mass balance equation whereby the oxygen-18 content of the present oceans must equal that of the ancient oceans, less the oxygen-18 extracted by carbonate and non-detrital siliceous sediments, plus the oxygen-18 added by juvenile water. Imprecision of the estimates involved does not permit calculation of the  $^{18}\text{O}/^{16}\text{O}$  ratio of Cambrian sea-water, but it is found

that the estimated oxygen-18 contribution of juvenile water is of sufficient magnitude to influence the  $^{18}\text{O}/^{16}\text{O}$  ratio of sea-water.

There are, therefore, two opposing hypotheses concerning the oxygen isotopic composition of ancient sea-water. It will be shown that  $\delta^{18}\text{O}$  analyses of coexisting calcite-dolomite pairs in sedimentary rocks may be used to investigate further this problem.

(1) *Calcareous limestones.* It has been known for some time that the older carbonate rocks are relatively enriched in oxygen-18. Variation of the  $^{18}\text{O}/^{16}\text{O}_{\text{calc}}$  ratio with time was suggested by the early analyses<sup>8</sup>, but the trend was not well defined until a large number of rocks had been analysed. Keith and Weber<sup>10</sup> reported  $\delta^{18}\text{O}$  analyses for more than 500 specimens ranging in age from Cambrian to the present. These samples varied widely in geographic locality, and since the depositional environment exerts some influence on the  $^{18}\text{O}/^{16}\text{O}$  ratio of the carbonate, only limestones containing marine fossils were included. Mean values for each age category are: (Precambrian, -11.39); Cambrian, -9.72; Lower Palaeozoic, -8.65; Upper Palaeozoic, -4.95; Jurassic, -4.98; Cretaceous, -4.18; Tertiary, -2.33; Quaternary, -1.18, relative to the PDB standard  $\text{CO}_2$  (expressed as the oxygen-18 difference, in parts per mil, between the sample and the PDB standard carbon dioxide by the relation:

$$\delta^{18}\text{O} = \left( \frac{^{18}\text{O}/^{16}\text{O}_{\text{sample}} - ^{18}\text{O}/^{16}\text{O}_{\text{std}}}{^{18}\text{O}/^{16}\text{O}_{\text{std}}} \right) 1,000$$

Three explanations might be advanced to account for these data: (i) Since the fractionation factor,  $\alpha = [^{18}\text{O}/^{16}\text{O}_{\text{H}_2\text{O}}]/[^{18}\text{O}/^{16}\text{O}_{\text{calc}}]$ , is temperature dependent, a change in the mean annual temperature of sea-water may be responsible. The inadequacy of this explanation is immediately evident since temperatures calculated from the  $^{18}\text{O}/^{16}\text{O}$  ratio of Cambrian carbonates are much above the maximum temperature tolerated by living organisms. (ii) Sea-water has become enriched in oxygen-18 with time by the addition of juvenile water. (iii) Ancient calcites have partially equilibrated with formation waters, and the degree to which oxygen isotopes have reached a state of equilibrium is more or less a function of the age of the limestone.

(2) *Dolomitic limestones.* Degens and Epstein<sup>11</sup> examined a "wide range of carbonate specimens representing a variety of genetic types", and "care was taken that the selection included representative dolomites of recent and ancient, continental and marine, and synsedimentary and late diagenic origin". Their results for ancient rocks indicate that dolomite is enriched with oxygen-18 with respect to coexisting calcite, but the data are too few to demonstrate that  $\delta^{18}\text{O}_{\text{dol}}$  is proportional to the age of the sample, or that  $\delta^{18}\text{O}_{\text{dol}} - \delta^{18}\text{O}_{\text{calc}}$  varies with time. On the basis of the hydrothermal experiments of Epstein *et al.*, and the fact that Degens and Epstein observed no fractionation of oxygen isotopes between calcite and dolomite from relatively recent sediments in the Bahamas, Florida and the Coorong of Australia, Degens and Epstein concluded that: (a) all dolomites are derived by metasomatic replacement of calcite or aragonite; (b) ancient calcite is depleted in oxygen-18 with respect to associated dolomite because of oxygen isotope equilibration of the calcite with sub-surface waters.

The argument that observed differences in the  $^{18}\text{O}/^{16}\text{O}$  ratio of ancient coexisting calcite and dolomite are evidence of primary precipitation of the dolomite can probably be dismissed on the grounds that this hypothesis contradicts an enormous amount of experimental work directed toward precipitating dolomite from solution under conditions encompassing those in Nature. The alternatives are: (1) equilibration of dolomite, calcite and fluid occurred during dolomitization, or (2) dolomitization occurred without alteration of the  $^{18}\text{O}/^{16}\text{O}$  ratio, but the calcite later exchanged oxygen isotopes with sub-surface water.

In the first case, the  $\delta^{18}\text{O}_{\text{ct}}$  and  $\delta^{18}\text{O}_{\text{dol}}$  would be equilibrium values; in the latter they may not.

I suggest that the dolomite and calcite may have differed in isotopic composition before lithification, at least so far as the fine-grained, so-called "syngenetic dolomitic limestones" are concerned, and that subsequent equilibration with formation waters is not a major factor. One approach to this problem is the examination of fine-grained dolomitic limestones of freshwater origin. If the interpretation of Degens and Epstein is correct, then dolomite replacing freshwater calcite should have an isotopic composition similar to modern or Tertiary freshwater calcites. Associated calcite would be relatively enriched in oxygen-18 if equilibration with sub-surface waters took place. If, on the other hand, such dolomites show  $\delta^{18}\text{O}/\delta^{16}\text{O}$  ratios significantly higher than modern freshwater calcites, early equilibration of calcite and dolomite might be indicated. Two such freshwater deposits have been studied: the Palaeocene-Eocene Flagstaff formation<sup>11</sup> (central Utah) where  $\delta^{18}\text{O}_{\text{ct}} = -9$  to  $-10$  per mil, and  $\delta^{18}\text{O}_{\text{dol}} = -3$  to  $-4$  per mil, and the Upper Carboniferous Freeport formation<sup>12</sup> (central Pennsylvania), where  $\delta^{18}\text{O}_{\text{ct}} = -7.87$  per mil, and  $\delta^{18}\text{O}_{\text{dol}} = -3.04$  per mil. These values may be compared with  $\delta^{18}\text{O}$  analyses of eleven freshwater calcium carbonates of Quaternary age<sup>10</sup> (mean  $-8.15$  per mil, standard deviation  $2.08$ ) and  $63$  of Tertiary age (mean  $-9.65$ , *S.D.*  $4.48$ ). The data are difficult to acquire and are therefore few in number at the present time. There is insufficient information to characterize variation or 'noise', and the influence of possible variation of the  $^{18}\text{O}/^{16}\text{O}$  ratio of sea-water with time must be taken into account since the oxygen-18 content of freshwater is to some extent related in the oxygen-18 content of the oceans. The preliminary data appear encouraging and further investigation is warranted.

The fundamental questions are: (1) Has the  $^{18}\text{O}/^{16}\text{O}$  ratio of sea-water changed with time or not? (2) Is oxygen-isotope equilibration of dolomite and calcite in "syngenetic limestones" common or not? (3) Is post-depositional oxygen-isotope equilibration of calcite and sub-surface water a common phenomenon or not? With sufficient data some of these questions may be answered. Models for the three major cases may be constructed (see Figs. 1-3). In Fig. 1, the  $^{18}\text{O}/^{16}\text{O}$  ratio of sea-water is assumed to have increased with time, according to the hypothesis of Weber<sup>1</sup>. Two extreme sub-cases involve 'early syngenetic' dolomitization and late diagenetic dolomitization; there appear to be petrographical and lithological criteria to distinguish these varieties in many cases. For each sub-case, there are two further possibilities: either  $^{18}\text{O}/^{16}\text{O}$

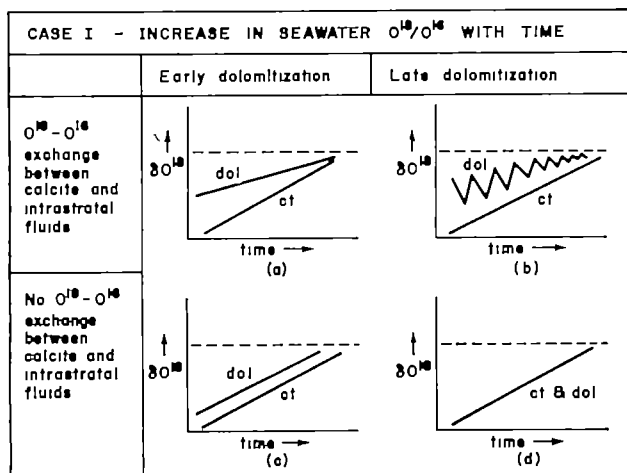


Fig. 1. Generalized models predicting the oxygen isotopic composition for coexisting calcite and dolomite in marine carbonates if the  $^{18}\text{O}/^{16}\text{O}$  of sea-water has increased with time. Dashed line in each model represents the  $\delta^{18}\text{O}$  value of modern marine calcites. The extremes on the time-scale represent Cambrian at the left and Recent on the right.

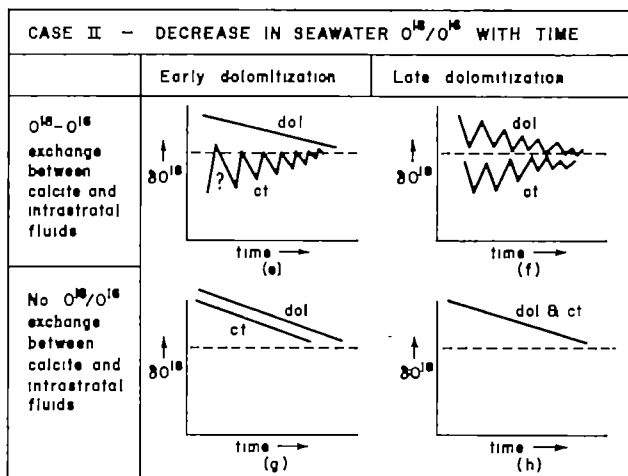


Fig. 2. Generalized models, similar to Fig. 1, where the  $^{18}\text{O}/^{16}\text{O}$  ratio of sea-water has been assumed to decrease with time.

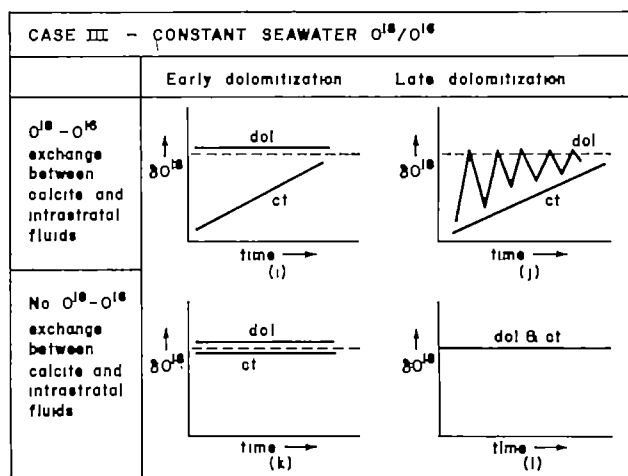


Fig. 3. Generalized models, similar to Fig. 1, where the  $^{18}\text{O}/^{16}\text{O}$  ratio of sea-water is assumed essentially constant from the Cambrian to the present.

exchange between calcite and formation waters was substantial, or it was not.

Fig. 2 represents analogous categories in Case II, where the  $^{18}\text{O}/^{16}\text{O}$  ratio of sea-water is assumed to have decreased with time, according to the hypothesis of Silverman<sup>8</sup>, Degens<sup>9</sup> and others. Case III, shown in Fig. 3, is included for completeness; the  $^{18}\text{O}/^{16}\text{O}$  ratio of sea-water is assumed to have remained constant. It is possible, of course, that major changes in the isotopic composition of the oceans may be cyclic or even random, but no evidence of this has appeared and no hypotheses concerning such phenomena have been advanced. In each model, the dashed line represents the isotopic composition of modern marine calcites.

In model *a* (Fig. 1), calcite has been precipitated from sea-water becoming increasingly enriched in oxygen-18. Syngenetic dolomite is heavier than the associated calcite and its isotopic composition is represented by a line located above that for calcite. Originally the two lines would have been parallel. Oxygen isotope exchange between sub-surface water and calcite, however, will shift the slope of the line so that  $\delta^{18}\text{O}_{\text{dol}} - \delta^{18}\text{O}_{\text{ct}}$  is greater for more ancient carbonates, assuming that the extent of equilibration is proportional to the time available for the process.

Model *c* is equivalent to model *a* except that equilibration of calcite and formation waters has not been significant.

In model *e*, ancient calcite was enriched with oxygen-18 with respect to later calcites. Dolomite was enriched with oxygen-18 with respect to the associated calcite. Equili-

bration of calcite with sub-surface water would shift the line representing the  $\delta^{18}\text{O}$  of calcite to a position somewhere within the zigzag area. Model *g* is identical with *e* except that the calcite isotopic composition has remained unaltered since deposition.

If sea-water  $^{18}\text{O}/^{16}\text{O}$  has remained constant (Fig. 3),  $\delta^{18}\text{O}_{\text{dol}}$  should be greater than  $\delta^{18}\text{O}_{\text{ct}}$ , but neither should vary with time if equilibration of fluids and calcite is insignificant (model *k*). With oxygen-18 - oxygen-16 exchange between sub-surface water and calcite,  $\delta^{18}\text{O}$  for ancient calcites should be less than that for more recent specimens, but  $\delta^{18}\text{O}_{\text{dol}}$  would remain unchanged (model *i*).

Similar arguments can be applied to situations where dolomitization occurred without alteration of the  $^{18}\text{O}/^{16}\text{O}$  ratio of the calcite, as proposed by Degens and Epstein. In Case I, where the  $^{18}\text{O}/^{16}\text{O}$  ratio of sea-water has increased with time, the line representing the  $\delta^{18}\text{O}$  of calcite slopes to the left. With no post-depositional oxygen isotope exchange between calcite and sub-surface water (model *d*)  $\delta^{18}\text{O}_{\text{ct}} - \delta^{18}\text{O}_{\text{dol}} = \text{zero}$  for coexisting pairs. With equilibration (model *b*), the isotopic composition of the dolomite will vary much more than that of the calcite, since the value of  $\delta^{18}\text{O}_{\text{dol}}$  depends not only on the age of the calcite but also on the length of the time-interval between precipitation of the calcite and dolomitization. For example, if dolomitization of a Cambrian limestone occurred soon after lithification,  $\delta^{18}\text{O}_{\text{dol}}$  would be similar to the isotopic composition of calcites in Cambrian time. If dolomitization of the same limestone occurred long after lithification, for example, in Mesozoic time, the  $^{18}\text{O}/^{16}\text{O}$  ratio of the dolomite would be much lower than that of the calcite in Cambrian time because in the time-interval separating the Cambrian from the Mesozoic, the calcite would have been enriched in oxygen-16 as a result of equilibration with sub-surface waters. With sufficient data, perhaps many hundreds of samples, variation about the line representing  $\delta^{18}\text{O}_{\text{dol}}$  should be significantly greater than variation about the line for  $\delta^{18}\text{O}_{\text{ct}}$ . This is repre-

sented by a zigzag curve in model *b*. With this explanation, models *f*, *h*, *j* and *l* require no further elucidation.

With present information, which models appear plausible? The trend of  $\delta^{18}\text{O}_{\text{ct}}$  with time is fairly well known from the analysis of more than 500 specimens of carefully selected limestones<sup>10</sup>. On this basis, models *g*, *h*, *k* and *l* are eliminated.

The isotopic data for coexisting calcite and dolomite from Degens and Epstein<sup>11</sup> indicate that for ancient dolomitic limestones,  $\delta^{18}\text{O}_{\text{dol}}$  is greater than  $\delta^{18}\text{O}_{\text{ct}}$ , and  $\delta^{18}\text{O}_{\text{dol}}$  is less than the  $\delta^{18}\text{O}_{\text{ct}}$  of modern marine calcites (dashed line, Figs. 1-3). Models *d*, *e*, *f*, *g*, *h*, *i*, *k* and *l* are therefore unlikely.

Remaining are models *a*, *b*, *c* and *j*. The available data are insufficient to evaluate further these models since background noise arising from other variables is high enough to camouflage any  $\delta^{18}\text{O}_{\text{dol}}$ -time trend.

Further investigation of 'syngenetic' dolomitic limestones may provide a basis for choosing one of the remaining models. If this is possible, the rewards are enormous; the use of oxygen isotope ratios for palaeotemperature determination and for environmental analysis may be extended far past the Mesozoic.

<sup>1</sup> For reviews of the dolomite problem, see Sonnenfeld, P., *Bull. Canad. Petrol. Geol.*, 12, 101 (1964), Fairbridge, R. W., in *Regional Aspects of Carbonate Deposition*, Soc. Econ. Pale. Mineral. Spec. Pub. 5, 125 (1967), and Ingerson, E., *Geochim. Cosmochim. Acta*, 26, 817 (1962).

<sup>2</sup> Epstein, S., Graf, D. L., and Degens, B. T., in *Isotopic and Cosmic Chemistry*, 169 (North Holland Publishing Co., Amsterdam, 1963).

<sup>3</sup> Clayton, R. N., and Epstein, S., *J. Geology*, 66, 351 (1958).

<sup>4</sup> Because low temperature isotopic equilibrium has not been demonstrated experimentally at this time,  $^{18}\text{O}$  enrichment in dolomite is contended, see Friedman, I., and Hall, W. B., *J. Geology*, 71, 238 (1963).

<sup>5</sup> Silverman, S. R., *Geochim. Cosmochim. Acta*, 2, 25 (1961).

<sup>6</sup> Degens, B. T., *Neues Jahrb. Geol. Palaeontol., Monatsb.*, No. 4, 180 (1959).

<sup>7</sup> Weber, J. N., *Ann. Meet. Geol. Soc. Amer., Preprints (Abstr.)*, 218 (1964), *Geochimica*, No. 6, 674 (1965).

<sup>8</sup> See Riney, W. W., in *The Origin and Evolution of Atmospheres and Oceans*, 1 (John Wiley and Sons, New York, 1964).

<sup>9</sup> Degens, B. T., and Epstein, S., *Bull. Amer. Assoc. Petrol. Geol.*, 46, 534 (1962).

<sup>10</sup> Keith, M. L., and Weber, J. N., *Geochim. Cosmochim. Acta*, 28, 1787 (1964).

<sup>11</sup> Degens, B. T., and Epstein, S., *Geochim. Cosmochim. Acta*, 28, 23 (1964).

<sup>12</sup> Weber, J. N., *Science*, 145, 1303 (1964).

<sup>13</sup> Weber, J. N., p. 972 of this issue of *Nature*.

## NICKEL CONTENT OF PACIFIC OCEAN CORES

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HIGH nickel content in central Pacific sediment cores, relative to continental rocks and sediments, was reported by Pettersson and Rotschi<sup>1</sup>, who advanced the hypothesis that meteoritic dust settling on the Earth's surface is the origin of a nickel surplus. Their examination of three long cores indicated significant variation of nickel concentration with depth, but an abundance everywhere greater than the estimated average crustal value of 0.008 per cent<sup>2,3</sup>. Smales and Wiseman<sup>4</sup> concluded that meteoritic material contributes little to the content of deep-sea sediments, on the basis of comparisons of nickel-cobalt, nickel-copper and copper-cobalt ratios in Pacific red clays and in meteorites. Their interpretation of these ratios has been challenged by Öpik (as reported by Pettersson<sup>5</sup>). Extensive neutron activation investigations of terrestrial and oceanic rocks, marine sediments and meteorites<sup>6</sup> were taken to provide general apparent support to the findings of Smales and Wiseman but without elimination of possible local meteoritic influence. It was shown in 1958 that spherules magnetically separated from deep-sea sediments are similar in composition to iron meteorites<sup>7</sup>, although it had previously been reported that the content of magnetically separable spherules in a core of high nickel content

appeared too low to contribute significantly to the observed bulk content of that element<sup>8</sup>. Pettersson<sup>9,10</sup>, nevertheless, estimated a value for the rate of extra-terrestrial nickel accretion, on the basis of particles captured by atmospheric filtration at high altitude, which is within an order of magnitude of his previous estimate based on an assumed extra-terrestrial origin of nickel excess in deep-sea cores. Turekian<sup>11</sup> estimated an accretion rate similar to Pettersson's, by combining carbon-14 sedimentation rate data and nickel analyses for an Atlantic equatorial core. He does not consider this a corroboration of the extra-terrestrial origin hypothesis, however, on the ground that accumulation rates of other elements such as copper and lead were high in the particular core examined.

While strong evidence of cosmic origin for the magnetic spherules has accrued<sup>7,12,13</sup>, the origin of the apparent high-bulk nickel content of Pacific sediments seems to remain unresolved. There can be no doubt that a portion at least of this nickel is of terrestrial origin; but one could still assume an increment of nickel resulting from a steady influx and settling of fine cosmic dust, generally similar to meteorites in composition and either non-magnetic or, if magnetic, too small in particle size for efficient separation in the usual magnetic device. If such a dust does, in fact,

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contribute significantly to the content of pelagic sediments, broad correlations in nickel content between separate cores should be observable, although local meteoritic events would of course still be expected to cause local fluctuations. In the work recorded here profiles of nickel content of two Pacific cores have been obtained and compared.

Samples of the two deep-sea cores V18-306 (07° 20' S.; 133° 03' W.; depth 4,491 m) and V18-307 (08° 32' S.; 131° 57' W.; depth 4,711 m) were obtained from the Lamont Geological Observatory of Columbia University. The region involved is far removed from major land masses; we assume the rate of sedimentation to be low, probably of the order of millimetres per thousand years, as reported for another core taken at similar latitude<sup>13</sup>. The two cores were collected on successive days of the 1962 voyage of the *Verna* thus providing material from separate localities of the same general region. In addition to the core samples, sediment material dredged from the south Pacific ocean bottom has been made available to us.

Gross samples weighing 1–2 g were taken from regions centred about the positions of core depth chosen for analysis. These were leached to remove all water-soluble material. In order to achieve high sensitivity over a wide range of nickel concentration in small sizes of sample, the method of neutron activation analysis was used. The procedure applied was similar to that of Smiles *et al.*<sup>14</sup> in its wet chemical aspects, but included additional chromatographic and electrochemical stages. The usual sample size was 100 mg of dry sediment; each such sample, along with a 10-mg standard of spectroscopically pure nickel, was irradiated for 1 h at a flux of about  $10^{13}$  neutrons  $\text{cm}^{-2} \text{sec}^{-1}$  in the Brookhaven graphite reactor. After dissolving the sample and addition of inactive nickel and appropriate holdback carriers, radiochemical purification was achieved by successive precipitations of nickel dimethylglyoxime and manganese dioxide, passage in 9N hydrochloric acid through a 'Dowex LX-4' anion exchange column, and final preparation for counting by electro-deposition of the nickel on one side of a copper disk. Chemical yield was determined by the weight of nickel deposited. An aliquot of the irradiated nickel standard was simultaneously deposited on a separate copper disk, with carrier added so that standard and sample deposits were of approximately equal thickness. The quantities of  $^{60}\text{Ni}$   $\beta$  activity in standard and sample were compared by proportional counting, continued over several half-lives of the nuclide. In view of the close coincidence in the half-lives of  $^{60}\text{Ni}$  (2.56 h) and the possible contaminant nuclide

$^{54}\text{Mn}$  (2.58 h),  $\gamma$ -ray spectrometry was used to monitor the efficacy of manganese decontamination. Samples taken from regions of very high manganese concentration were found to require one or more additional  $\text{MnO}_2$  precipitations. Confidence in the analytical method was gained by its application to National Bureau of Standards basic open-hearth steel samples. Analyses were performed on whole sediment, with only water-soluble constituents removed. Inorganic carbonate content was determined separately. Assuming all carbonate present to be  $\text{CaCO}_3$ , the data have been corrected to a  $\text{CaCO}_3$ -free basis. Carbonate content was generally significant only in the uppermost layers of these cores.

The nickel concentration profiles obtained for the two cores are shown graphically in Fig. 1. Results of replicate analyses are shown in Fig. 1 for material at several depths. Although our experience with standard steels indicated a reproducibility to within about 2 per cent, there are several instances of greater dispersion in the results of replicate sediment sample analyses, as shown. Seven measurements on dredged sediment material gave a mean value of 270 p.p.m., with minimum and maximum values of 218 and 302 p.p.m. respectively. We believe that heterogeneity of the sediment materials, with respect to nickel, is strongly indicated.

Within the limitation already described here, we note the following features of the two profiles:

- (1) The observed nickel concentration is everywhere greater than the average crustal rock value<sup>3</sup> of 0.003 per cent, although at some points not significantly so; it is also nearly everywhere greater than the basalt average of 150 p.p.m. given by Taylor<sup>15</sup> from the data of Turekian<sup>14</sup>.
- (2) The values are in the same general range as those reported by Pettersson and Rotschi<sup>1</sup>.
- (3) Both cores exhibit broad maxima in the upper regions, that in 307 being more pronounced and somewhat displaced toward increased depth, with respect to 306.
- (4) In both cores the observed broad maxima are followed by broad minima again more pronounced in the case of 307.

The broad features described here may be considered consistent with the hypothesis of extra-terrestrial origin if one assumes a roughly constant influx of meteoritic nickel during the total period of sedimentation involved and a rate of total sedimentation that fluctuates with climatic conditions. Thus the higher nickel concentration of recent deposit, observed in both cores, may be attributable to a lowered rate of arrival of sediment of continental origin in this region during recent glaciation. This is at variance with the view expressed by Turekian<sup>11</sup> that terrestrial control of supply is indicated by climatic variation in the rate of accumulation of nickel. The sedimentation rates in the Atlantic equatorial core he investigated are very large, however, and the average nickel contents exceedingly low. Under these conditions the measurements may well have lacked sensitivity to any possible increment above the undisputed terrestrial supply. Accounts of single 'average' values over extended intervals of core material may be misleading in the search for an incremental concentration, particularly when the values are as low (18 and 17 p.p.m.) as those reported<sup>11</sup>. Finally, given the high gross sedimentation rates involved, we do not believe Turekian's observation of high rates of accumulation of copper and lead to be necessarily meaningful to the question at issue.

Assuming the top 200 cm of the cores investigated here to represent a total time span of  $10^4$  years, and an average of 200 p.p.m. of total nickel to be in excess of normal terrestrial supply, we estimate an annual accumulation of extra-terrestrial nickel for the Earth as a whole of  $4 \times 10^{11}$  g. This value, based on a generous allowance for nickel of terrestrial origin, is in agreement with the estimate of Pettersson<sup>1</sup> of  $3.5 \times 10^{11}$  g based on atmospheric particle collection. Our evidence of heterogeneity in nickel concentration supports the idea that at least some of the high

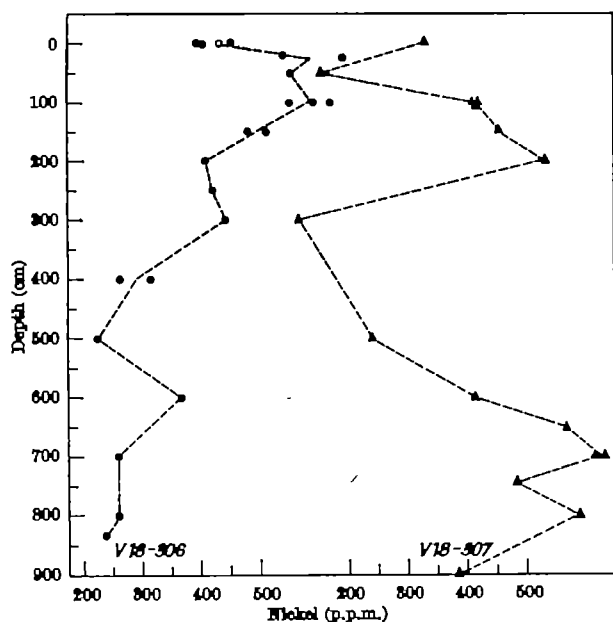


Fig. 1

nickel content found in these cores is present in particulate form.

Our observations do not constitute a 'proof' in any sense of the hypothesis of extra-terrestrial origin of a nickel surplus in deep-sea cores. We do maintain that the observed broad consonance in nickel concentration profiles in two cores, separate in space within a region far removed from major land masses in which the rate of sedimentation has been low, is consistent with that hypothesis, and that the question cannot be regarded as settled. Correlations between cores, as in the present investigation, co-ordinated with measurements of rates of sedimentation should assist materially in further pursuit of the issue.

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Laboratory for providing reactor irradiation services; Dr. D. R. Christman for carbonate analyses; and Dr. O. A. Schaeffer for his advice.

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## DISTRIBUTION OF NEWLY FORMED SOLUBLE SULPHATE DURING THE FIRING OF A RADIOACTIVE-LABELLED BRICK

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IT has recently been shown that during the firing of a high-sulphate building brick, considerable redistribution of sulphate occurs<sup>1</sup>. The brick was made artificially high in sulphate by incorporating  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  labelled with sulphur-35 in the unfired clay, which was a Weald clay containing some organic matter and sulphide. It was remarked that the redistribution was surprising in view of the fact that calcium sulphate is relatively insoluble and thermally stable; hence one would not expect sulphate migration to occur during the drying of the moist clay, nor any appreciable thermal breakdown to occur at the firing temperatures used. The results showed that there was a loss of activity to the atmosphere during firing, presumably from the formation of gaseous sulphur oxides. Also, the redistribution of labelled sulphate occurred in such a way that the centre of the brick was almost devoid of sulphate, whereas just below the surface the peak concentration exceeded the original mean concentration of sulphate put into the clay. This was taken as evidence for a zone of re-concentration of sulphate just below the surface, for such a distribution cannot be obtained simply by a loss of active gaseous sulphur, in whatever chemical form it may exist.

The assays of water-soluble sulphate were both made radiometrically and gravimetrically, and it was noted that there was some discrepancy near the peak of the distribution curve. This has now been shown to be due to oxidation of sulphide in the unfired clay, with the consequent formation of new soluble sulphate. This newly formed soluble sulphate does not affect the radiometric assay, but obviously increases the gravimetric assay, and hence caused the puzzling drop in specific activity which had already been noted.

The results shown in Fig. 1 were obtained from samples taken from the brick 31 days after manufacture. The samples were taken from drillings normal to the  $8\frac{1}{2}$  in.  $\times$   $2\frac{1}{4}$  in. face and passing through the central plane  $2\frac{1}{4}$  in. from the surface. The upper curve shows the variation in total water-soluble sulphate assayed gravimetrically by barium sulphate precipitation. The peak in this curve occurs slightly more than 0.5 in. below the surface, and its level is far in excess of the mean concentration of labelled sulphate added to the unfired clay. The intermediate curve shows the variation in water-soluble radioactive

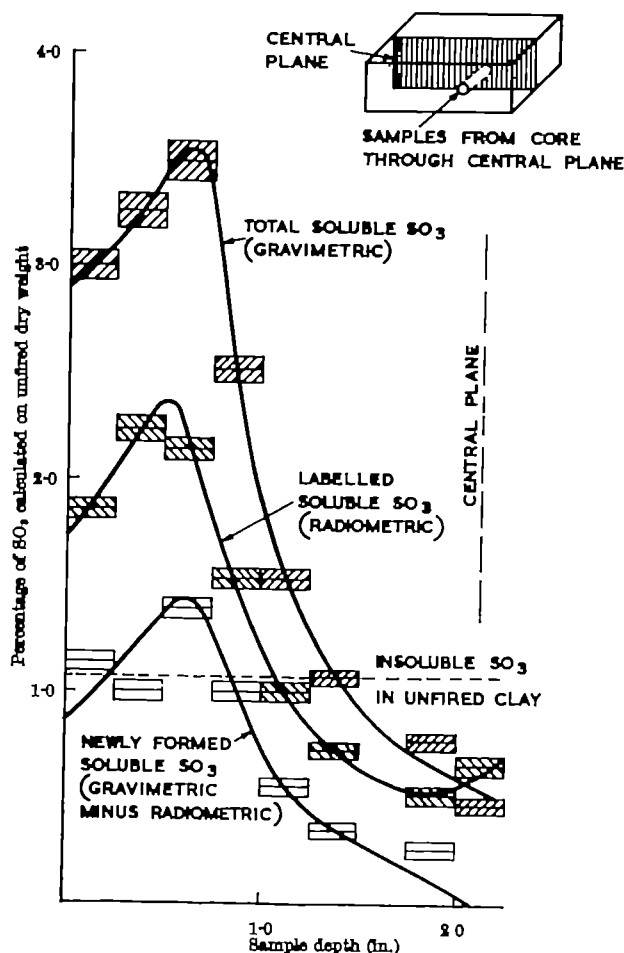


Fig. 1. The variation of soluble sulphate concentration in a fired building brick. Labelled  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  was added to the unfired clay, which was otherwise free from soluble  $\text{SO}_3$ . The insoluble  $\text{SO}_3$  content (presumably as  $\text{FeS}_2$ ) was about 1 per cent before firing, but negligible afterwards. The newly formed soluble  $\text{SO}_3$  has a distribution as shown by the lowest curve, and an integrated mean level of about 1 per cent

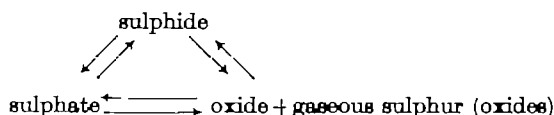
sulphate assayed radiometrically by a liquid scintillation counting technique<sup>2</sup>. The peak is slightly nearer the surface (0.5 in.) and is approximately at the same level as the incorporated radioactive sulphate. (The initial sampling immediately after firing the brick showed that this peak had exceeded the level of added radioactive sulphate.) The two upper curves are widely divergent near their peaks but agree reasonably well near the central plane, which indicates that the divergence is not due to systematic errors in analysis. The lower curve is obtained by taking the difference between the two upper curves, and must represent newly formed sulphate which has appeared during the firing of the brick. This also shows a peak about 0.5 in. below the surface, and is practically zero at the centre of the brick. This non-radioactive sulphate was not present in the original unfired clay, which had a water-soluble sulphate content of 0.07 per cent as  $\text{SO}_3$ .

It was presumed that the source of the newly formed sulphate was insoluble sulphide present in the unfired clay, and subsequent analysis has proved this to be correct. The unfired clay was assayed in both our laboratories for total sulphur by four different methods; peroxide fusion, carbonate nitrate fusion,  $\text{Br}/\text{CCl}_4/\text{HNO}_3$  oxidation and  $\text{Br}/\text{H}_2\text{O}/\text{HCl}$  oxidation. The mean total sulphur content was 1.06 per cent as  $\text{SO}_3$ . In the fired brick, most of the sulphur was present as soluble sulphate, the residual insoluble sulphur content being only  $0.11 \pm 0.04$  per cent as  $\text{SO}_3$ , of which  $40 \pm 11$  per cent was radioactive. Hence the total sulphur available for oxidation was  $1.06 - 0.07 = 0.99$  per cent as  $\text{SO}_3$ . The mean level of newly formed sulphate, obtained by numerical integration throughout the volume of the brick, was 1.06 per cent as  $\text{SO}_3$ , which is in reasonable agreement. This has been confirmed by analysis of a further series of drillings from the radioactive brick and by measurements on non-radioactive bricks constructed in an identical manner.

It is reasonably certain that the sulphur in the unfired clay exists as some form of the very insoluble sulphide  $\text{FeS}_2$ , for the acid volatile sulphide content is very low. If all the iron sulphide had uniformly oxidized to sulphate the constant level throughout the brick would have been 1.06 per cent as  $\text{SO}_3$ , whereas in fact there is a peak of newly formed sulphate which exceeds this by about 30 per cent. These results show that the newly formed sulphate

behaves in a similar manner to the incorporated sulphate, and supports the former conclusion that there is a zone of sulphate reconcentration just below the surface of the brick.

The mechanism whereby sulphate is broken down and formed during the firing of the brick is still a matter for conjecture. The brick, for example, can be regarded as an inert porous matrix in which the following series of solid-state reactions are possible:



The sulphate  $\rightarrow$  sulphide part of the reaction if it exists is very small, because of the low residual sulphide content. The sulphate  $\rightarrow$  oxide part of the reaction and the oxide  $\rightarrow$  sulphate part do not necessarily occur at the same site; the latter reaction could well be responsible for the zone of reconcentration just below the surface.

It is still not clear why, in the centre of the brick, both sulphide and sulphate disappear, for the first requires oxidizing conditions while the second is known to require reducing conditions. (Gypsum heated in a reducing atmosphere with silica and alumina is a commercial source of  $\text{SO}_2$ .) It is possible, of course, that the two reactions are not independent. For example, the dissociation temperature of sulphate is lowered by the presence of  $\text{SiO}_2$  or  $\text{Fe}_2\text{O}_3$  (ref. 3) and presumably the dissociation temperature of  $\text{CaSO}_4$  could be lowered by the presence of  $\text{FeSO}_4$ . Another possibility is that the heated sulphide eliminates sulphur in the elemental form, which afterwards oxidizes after diffusion nearer to the surface. A further possibility is the formation of hydrogen sulphide by reaction of the heated sulphide with water from the clay mineral; there is an unmistakable smell of hydrogen sulphide from the centre of the freshly opened brick. However, apart from the mechanisms of the reaction, it is now apparent that newly formed sulphate distributes itself in the same way as added sulphate.

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## ELECTRICAL POTENTIAL AT AN OIL/WATER INTERFACE

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MANY reports are available<sup>1</sup> about the electrical potential of the oil membrane, which consists of an 'oil' (pentanol, nitrobenzene, etc.) between two water layers; very little, however, is known about the nature of the potential. The importance long attached to the oil/water interface as a model system of biological interest is especially obvious when the oil contains, or is in contact with, biologically important materials such as phospholipids<sup>2</sup>, proteins and others.

The extensive work on the oil/water potentials culminated in the controversy between Beutner and Baur. Beutner<sup>3</sup> maintained that the electrical potential is generated by a partition equilibrium of the ions between oil and water; Baur<sup>4</sup> held that the observed potentials are produced by the selective adsorption of the ions into a double layer at the oil/water interface. Notwithstanding Baur's admission that Beutner was right and the assurance given by Adam that really both parties were right in the

circumstances<sup>5</sup>, the conceptual differences between the two points of view remain so powerful as to prompt re-investigation of the problem.

In the accepted approach<sup>3</sup>, the electrical potential of the oil cell was a function of the diffusion of the ions (small<sup>6</sup> and large<sup>1</sup>) under a concentration gradient and was described variously as a diffusion<sup>6</sup>, concentration or distribution potential by the Nernst and Henderson equations, which are still being used<sup>1,6</sup>. On the other hand, the simplified  $\Delta V$ -log  $c$  plot of the Gouy equation<sup>7,8</sup> for charged monolayers carries the limitations of the Gouy potential, which is restricted to the ideal (monovalent  $\text{NaCl}$ ,  $\text{KCl}$ ) dilute electrolyte and ignores the specific interactions between ions (including sodium and potassium) and charged interface. These interactions, as described by the Freundlich and Langmuir isotherms<sup>9,10</sup>, must be as fundamental to the structure and function of biological colloids as they are to mineral colloids<sup>11</sup>. The fixed-charge theory<sup>12</sup> had not been applied to the oil membrane; interestingly, however, the Donnan term was

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shown to be identical with the Gouy potential<sup>11</sup> and obviously could not account for specific interactions (ref. 8, p. 176).

With cogent arguments, which were neither rebuffed to satisfaction<sup>12</sup> nor explored further, Ehrensvard and Sillen<sup>13</sup> provided momentum and clarity to the Beutner-Baur controversy. They<sup>13</sup> pointed out the inconsistencies and inadequacy of the distribution and Henderson potentials and proposed an equation containing the Langmuir isotherm in two terms, one of which is a modified Gouy potential. The new equation, vindicating Baur, brings forth the concept of the adsorption; however, neither theory nor experiment with this<sup>13,14</sup> and the other equations explained the role of the fixed head charges in the origin of the potential. The practical  $\Delta V$ -log  $c$  plot of all these equations has the form of the Nernst, concentration, diffusion, distribution and Henderson potentials, all of which look alike, put the emphasis on the electrolyte and tend to make one lose sight of the charged components of the membrane. In the present article I shift the emphasis from the mobile ions to the charged interface, and develop the concept that the membrane potentials are caused by the appearance and disappearance of the fixed head charges of the amphipathic molecules of phospholipids and other surfactants orientated at the oil/water interface.

The experiments were performed at 25° C in a modification of the 'hanging drop' cell described by Dean<sup>15</sup> and similar to the cells used by Beutner<sup>3</sup> and Baur<sup>4</sup>. A layer of water-saturated pentanol separated two electrolyte solutions at the lower (A) and upper (B) interfaces (Fig. 1). Two saturated calomel electrodes connected the lower and upper electrolytes respectively to the positive and negative poles of a high impedance electrometer, Keithley model 610A. The electrolyte solutions were prepared by dissolving the salts in pentanol-saturated water, which was glass-distilled and had a specific resistance of a megohm cm before shaking with the alcohol. Diameter of the circular interface was 2.5 cm.

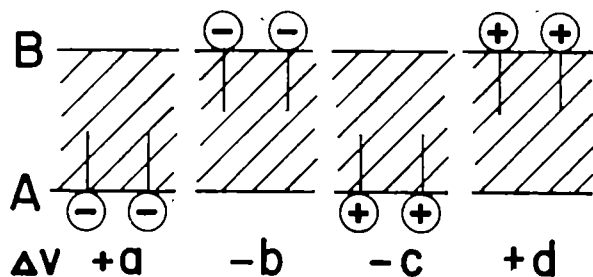


Fig. 1. Relation of sign of  $\Delta V$  to sign of head charge of surfactant

The change of potential at a given interface was studied by injecting given quantities of surfactant at that interface without disturbing the other interface. It is expected<sup>4</sup> that the amphipathic molecules at the interface orientate themselves with the head charges in the water and the non-polar chains in the oil. The potential was measured immediately before and after the injection, and the difference  $\Delta V$  was calculated. A sudden change of potential ensued on injection, and the new potential decayed at a rate depending largely on the surfactant's solubility and disappearance from the interface.

In brief (Fig. 1), a negative head charge at the lower interface A or a positive head charge at the upper interface B produced a positive change of potential (+a, +d); conversely, a positive head charge at the lower interface A or a negative head charge at the upper interface B produced a negative change of potential (-b, -c). Conclusively, the sign of the potential was determined by the sign of the fixed head charges of the amphipathic molecules<sup>4</sup> as they appeared at the oil/water interface. Baur<sup>4</sup> claimed that the sign of the charged interface determines the sign of

the potential; but no evidence was given for it before these experiments.

The size of the potential changes varied with the concentration of the electrolyte<sup>4</sup> in line with the Gouy equation<sup>11</sup> and with the equation and data of Ehrensvard and Sillen<sup>13,14</sup>. Typically (Table 1), the change of potential was smallest in 1 M potassium chloride and increased with decreasing electrolyte concentration. As expected, the absence of electrolyte causes expansion of the double layer, which then shows a conspicuous head charge and a large potential; conversely, a large salt concentration compresses the double layer with consequent masking of the head charges and decrease of the potential. Similarly, the presence of polyvalent ions (calcium, magnesium, sulphate) in the water phase caused changes of potential consistent with the adsorption of the counter-ions and masking of the head charges.

Table 1. EFFECT OF ELECTROLYTE CONCENTRATION ON THE OIL/WATER POTENTIALS OF TYPICAL ANIONIC (SODIUM DEOYL SULPHATE, SDS) AND CATIONIC (OCTYLTRIMETHYLAMONIUM BROMIDE, CTAB) SURFACTANTS

Surfactant Electrolyte (M)	Potentials in mV $\pm 2$				
	SDS KCl	SDS CaCl <sub>2</sub>	CTAB KCl	CTAB K <sub>2</sub> SO <sub>4</sub>	Cephalin CaCl <sub>2</sub>
1.0	39	22	-40	—	0
0.1	65	48	-62	-8	3
0.01	110	70	-84	-18	63
0.001	140	109	-150	-80	105
Water	158	—	-220	—	180

A solution of 0.001 M KCl was kept at interface B, while 1 mmole SDS or CTAB in 0.1 ml. water or animal cephalin in 0.3 ml. pentanol was applied with a micropipette at interface A on the electrolyte solutions of the given concentration. Membrane thickness: 10 mm.

As predicted by Fig. 1, the largest potentials were obtained when charges of opposite sign lined the opposing interfaces (+a +d; and -b -c), where no difference in electrolyte concentration was required. These potentials, obviously, increased with decreasing electrolyte concentrations and became largest on water. On injection of SDS and CTAB on water at A and B respectively,  $\Delta V$  reached the value of 370 mV. Wilbrandt<sup>16</sup> obtained these large 'asymmetry' potentials with collodion membranes and used them as the strongest argument against the assumption that they were diffusion potentials.

By comparison with the anionic SDS and the cationic CTAB (Table 1), a preparation of animal cephalin<sup>4</sup> as well as certain preparations of synthetic phosphatidylethanolamine and phosphatidylcholine (Nutritional Biochemical Corp.) behaved like anionic surfactants. Thin-layer chromatography of these preparations showed the presence of large quantities of acidic components, which would account for the appreciable size of the anionic-type potential on water; under the same conditions, chromatographically homogeneous egg lecithin produced a zero change of potential.

In similar experiments (Table 2), a solution of surfactant in pentanol separated a solution of 1 M potassium chloride at interface B and solutions of variable electrolyte concentration at interface A.

Table 2. EFFECT OF ELECTROLYTE CONCENTRATION ON THE ELECTROCAL POTENTIAL OF ANIONIC AND CATIONIC OIL MEMBRANES

At A KCl (M)	Potential in mV $\pm 2$			
	SDS	OTAB	Myristoylcholine chloride	Acetylcholine bromide
0.1	40	-48	-51	-15
0.01	92	-98	-98	-30
0.001	125	-142	-144	-49

The anionic membrane of sodium decyl sulphate reflects the combination of events +a and -b of Fig. 1. The positive  $\Delta V$  is the sum of a large positive potential of the anionic head charge in dilute potassium chloride at interface A and a much smaller negative potential of the same head charge in concentrated potassium chloride at interface B. Conversely, the negative  $\Delta V$  of the cationic membranes of CTAB, myristoylcholine chloride and acetylcholine bromide is the combination of a large negative potential (-c) of a cationic surfactant in dilute potassium chloride at A and a smaller positive (+d)

potential of the same cationic in concentrated potassium chloride at B. As expected, the shorter the chain of the amphipathic molecule (acetylcholine versus CTAB) the smaller the potential, or the longer the chain the more stable the interfacial film. Accordingly, injection of acetylcholine bromide on 0.01 M potassium chloride at interface A caused a potential change of -15 mV, which vanished in 2-3 sec; whereas CTAB produced -82 mV, which decayed only 5 mV in the first minute.

The potentials in Tables 1 and 2 were independent of the type of electrode pair used: that is, saturated calomel, Ag/AgCl in 4 M potassium chloride, copper or platinum in 4 M potassium chloride. These electrodes are not suitable to measure concentration potentials of either the small (potassium chloride) electrolyte or the surfactant in bulk; and, experimentally, the electrodes had no contact with the surfactant. With that in mind, the observed oil/water potentials cannot possibly be concentration or distribution potentials, both of which are electrode potentials, are irrelevant and (Baur<sup>4</sup>) "do not exist" as membrane potentials.

The electrical model of the oil membrane is not yet available. The foregoing data, however, indicate that this type of membrane has two properties (sign and electrolyte dependency of the potential) which distinguish it sharply from the ordinary ion exchange membrane. When I used cationic and anionic 'Amphion C-60 and A-60' (American Machinery and Foundry Company) solid membranes in place of the oil membrane in Fig. 1, the resulting potentials had signs opposite to those in Tables 1 and 2 and increased with increasing electrolyte (potassium chloride) concentrations. Similarly, the electrical potential of the anionic clay membrane<sup>17</sup> as calculated from the fixed charge theory<sup>11</sup> and as measured in the laboratory<sup>17</sup>

increased with the electrolyte concentration, whereas the potential of the oil membrane decreased with increasing concentration of potassium chloride. Henceforth, the electrolyte does not play a direct part in the origin of the potential of the oil membrane except for masking and unmasking the head charges at the interface. Polyvalent ions (calcium, magnesium, sulphate) and all kinds of long-chain ions, which are known to be preferably adsorbed on charged colloids, were particularly effective in depressing the oil/water potentials, which in that respect resemble the electrokinetic or zeta potentials<sup>8</sup>. Once the interfacial charge is destroyed the membrane potential vanishes; and that is true also for ordinary ion exchange membranes. The cited differences and similarities between the two types of membranes and biological membranes remain to be investigated.

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## NEW PYRETHRIN-LIKE ESTERS WITH HIGH INSECTICIDAL ACTIVITY

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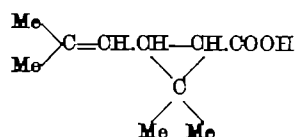
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NEW esters of chrysanthemic acid have been prepared with highly specific insecticidal action. Two of the new compounds are more toxic to some insect species than the natural pyrethrins or allethrin, and are more stable than the natural pyrethrins.

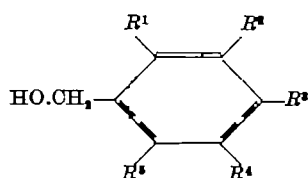
Several non-ketonic esters of chrysanthemic acid (I), such as dimethrin (from 2,4-dimethylbenzyl alcohol, IIa) and barthrin (from 6-chloropiperonyl alcohol), have useful insecticidal activity<sup>1</sup> but are less toxic to many insect species than are esters from pyrethrolone (IIIa), cinerolone (IIIb), and allethrolone (IIIc). In 2,4-dimethylbenzyl alcohol (IIa), the 4-methyl group is held by the benzene ring in a steric relation to the H—O bond of the alcohol similar to that between the side-chain CH<sub>2</sub>X and the HO link in the cyclopentenolones (III). Unsaturated side-chains CH<sub>2</sub>X in the chrysanthemates of the ketols (III) are important for insecticidal activity<sup>2,3</sup>, so 4-alkenylbenzyl chrysanthemates were examined for their activity.

To adult female *Musca domestica* L. (house-flies), treated topically with measured drops of solutions in acetone<sup>4</sup>, 4-allylbenzyl (+)-*trans*-chrysanthemate (from IIb) was approximately four times, and the (±)-*ois-trans*-chrysanthemate twice, as toxic as the mixture of esters of the natural pyrethrins. By this method the mixed pyrethrins, allethrin (from IIIe), and pure pyrethrin I (ref. 5) were equitoxic and 4-allylbenzyl (±)-*ois-trans*-chrysanthemate (ABC) was seven times more toxic than

dimethrin. ((±)-*Ois-trans* esters were prepared from the commercially available ethyl ester, which contained approximately 30 per cent *ois*-isomers; (+)-*trans*-chrysanthemic acid was naturally derived.) But to adult *Phaedon cochleariae* Fab (mustard beetles), treated similarly, allethrin was twice as toxic as dimethrin and six times more toxic than ABC. (Relative toxicities are expressed as inverse ratios of median lethal doses, LD<sub>50</sub>.) Methyl groups on the benzene ring of ABC increased toxicity to mustard beetles considerably. Thus, 4-allyl-2,6-dimethylbenzyl (±)-*ois-trans*-chrysanthemate (DMABO) (from IIc) was twice as toxic as allethrin and half as toxic as the natural pyrethrins mixture to mustard beetles and just significantly more toxic than ABC to house-flies. (New compounds are protected by British Patent applications 10701/63, 10702/63 and 42715/63, and corresponding foreign applications assigned to the National Research Development Corporation.) 4-Allyl-2,6-dimethylbenzyl and 4-allylbenzyl (+)-*trans*-chrysanthemates were more toxic to house-flies than was any other pyrethrin-like ester, natural or synthetic; also with other insect species (for example, *Tribolium castaneum* (Herbst.), *Periplaneta americana* (L.), *Aphis fabae*, Scop., and the red spider mite *Tetranychus telarius* (L.)), both in laboratory tests and conditions nearer to practical use, the 2,6-dimethyl compound (DMABO) was as toxic or more so than allethrin. The insecticidal activity of a film of DMABO, irradiated with ultra-violet light, persisted longer than that of



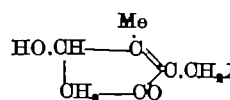
(I)



(II)

Table 1

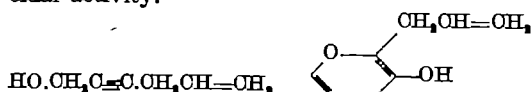
	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	R <sup>5</sup>
a	Me	H	Me	H	H
b	H	H	CH <sub>2</sub> CH=CH <sub>2</sub>	H	H
c	Me	H	CH <sub>2</sub> CH=CH <sub>2</sub>	H	Me
d	H	H	CH=CH <sub>2</sub>	H	H
e	H	H	CH=CH <sub>2</sub>	H	H
f	Me	Me	CH=CH <sub>2</sub>	Me	Me
g	H	H	CH <sub>2</sub> CH=CH <sub>2</sub>	H	H
h	H	H	CH <sub>2</sub> CH=CH <sub>2</sub>	H	H
i	H	H	CH <sub>2</sub> CH=CH <sub>2</sub>	H	H
j	Me	H	CH <sub>2</sub> CH=CH <sub>2</sub>	H	Me
k	Me	Me	CH <sub>2</sub> CH=CH <sub>2</sub>	Me	Me
l	H	H	CH <sub>2</sub> CH=CH <sub>2</sub>	H	H

a X = CH=CH<sub>2</sub>b X = CH=CH<sub>2</sub>c X = CH=CH<sub>2</sub>

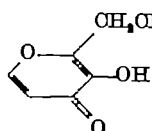
(III)

comparable deposits of allethrin or the natural pyrethrins.

The relationship between toxicity to house-flies and mustard beetles and chemical structure in esters related to the allyl compounds already mentioned was examined in detail. To both species, 4-allylbenzyl esters were more toxic than 4-*n*-propyl compounds; similarly, allethrin was more active than its *n*-propyl analogue<sup>4</sup>. 4-Allylbenzyl esters were about ten times more active than 3-allyl compounds and 2-allylbenzyl esters gave no kill at the largest doses. This showed that it was important to maintain the appropriate steric relation between the unsaturated side-chain of the alcohol and the linkage to the acid portion of the molecule. Esters in which the side-chain double bond was conjugated with the benzene ring (from 4-vinyl and 4-propenylbenzyl alcohols, II*d* and II*e*, respectively) did not kill mustard beetles and were only slightly toxic to house-flies. The ester from the enyne alcohol (IV) (ref. 6) in which an acetylenic link maintained a steric relation between the hydroxyl and allyl groups similar to that in 4-allylbenzyl alcohol was not toxic either to mustard beetles or house-flies, so this steric relation on its own was insufficient to give insecticidal activity.



(IV)



(V)

Most of the alcohols formed more toxic chrysanthemates when the hydrogen atoms *ortho* to the CH<sub>2</sub>OH group (II, R<sup>1</sup> and R<sup>2</sup>) were replaced with methyl groups. The 4-allyl-2,6-dimethyl ester was the most toxic compound to mustard beetles, but to both species the ester from 4-allyltetramethylbenzyl alcohol (II*f*) was less toxic than the 2,6-dimethyl compound. The 4-*trans*-crotylbenzyl alcohol (II*g*) gave esters less toxic to both species than esters of 4-allylbenzyl alcohol (II*b*) but, to mustard beetles only, the ester from 4-(2'-methallyl) benzyl alcohol (II*h*) was better than that from 4-allylbenzyl alcohol.

The ester from the readily accessible 4-*trans*-sorbitylbenzyl alcohol (II*i*) was only one-twentieth as toxic as the 4-allyl compound to house-flies and one-tenth as toxic to mustard beetles.

2-, 3- or 4-Allyloxybenzyl chrysanthemates had little insecticidal activity, less than that of the corresponding methoxy compounds. Free hydroxyl groups suppressed toxicity; the ester from the alcohol (V), easily made from *O*-allyl kojic acid<sup>7</sup>, was not insecticidal. Methoxyl groups at any position on the ring decreased the toxicity of otherwise active compounds. Thus, Barthel *et al.*<sup>8</sup> made 3-allyl-4-methoxy- and 4-ethoxybenzyl chrysanthemates, and we made 3-allyl-4-methoxy- and 4-allyl-3-methoxybenzyl chrysanthemates; but all were much less effective than the related unmethoxylated allyl benzyl esters.

The high toxicity of the 4-alkenylbenzyl chrysanthemates led to an investigation of methylbenzyl compounds. The 2,4-, 3,4- and 2,6-dimethyl esters had already been tested<sup>1</sup>. 2,4,6-Trimethylbenzyl (±)-*cis-trans*-chrysanthemate (from II*j*) was approximately one-third as toxic as DMABO to house-flies and about twice as toxic as dimethrin to house-flies and mustard beetles. Penta-methylbenzyl (±)-*cis-trans*-chrysanthemate (from II*k*) was significantly more toxic than the 2,4,6-trimethyl ester to mustard beetles, but less active to house-flies. 2,4,6-Triethyl benzyl alcohol (II*l*) gave a non-toxic ester. Other dimethyl esters were less toxic than the 2,4- and 3,4-dimethyl compounds. 2,4- and 3,4-Dichlorobenzyl chrysanthemates were less toxic than the corresponding dimethyl compounds.

Thus, those benzyl alcohols the structures of which relate them most closely to pyrethrolone, cinerolone, etc. (III), give the most toxic chrysanthemates. 4-Allylphenyl and 4-allyl-2,6-dimethylphenyl chrysanthemates, which have some structural features in common with the benzyl esters but differ stereochemically, had no insecticidal activity; this indicated that there must be a specific and appropriate stereochemical relationship between the acidic and alcoholic parts of the esters. The 4-allyl-2,6-dimethylanilide of (±)-*cis-trans*-chrysanthemic acid differs little from DMABO stereochemically but obviously lacks the requisite chemical properties, for it was not toxic.

Molecular models show that, when the allyl groups in allethrin and in 4-allyl-2,6-dimethylbenzyl chrysanthemates are orientated similarly relative to the chrysanthemic acid parts of the molecules, one of the 2,6-dimethyl groups in the benzyl ester occupies a position equivalent to that of the methyl group on the cyclopentenolone ring; a possible explanation for the lack of toxicity of 2,4,6-triethylbenzyl chrysanthemate is that here there is no room for an ethyl group to be similarly accommodated. Additional evidence that the high insecticidal activity of the 4-allyl and 4-allyl-2,6-dimethylbenzyl esters is associated with their similarity in molecular shape to the natural esters and allethrin is that (+)-*trans*-chrysanthemic acid gives esters approximately twice as toxic to house-flies as those from the (±)-*cis-trans*-acid, and that, to house-flies and mustard beetles, the 2,4,6-trimethylbenzyl ester of (+)-*trans*-chrysanthemic acid is much more active than that from (-)-*trans*-chrysanthemic acid. Analogous results were obtained with cyclopentenolone esters of (+), (-), and (±)-*cis-trans* acids<sup>9,10</sup>. The results support an earlier suggestion<sup>8</sup> that the insecticidal activity of the pyrethrins and related compounds depends partly on the cyclopentenolone ring acting as a planar activating nucleus to hold the unsaturated side-chain in an appropriate stereochemical relationship to the alcoholic link and thence to the acid. In these benzyl esters the benzene ring fulfils this role.

Neither ABC nor DMABO was well synergized by piperonyl butoxide in activity against house-flies.

Details of the synthesis of the new compounds and of the biological data will be published.

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Mr. F. P. W. Winteringham for a helpful discussion at an early stage in this work and Dr. C. Potter for his help and advice.

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## STEREOSPECIFICITY OF CERTAIN SOLUBLE AND PARTICULATE PREPARATIONS OF MITOCHONDRIAL REDUCED NICOTINAMIDE-ADENINE DINUCLEOTIDE DEHYDROGENASE FROM BEEF HEART

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IN recent years several preparations of soluble and particulate NADH dehydrogenase have been described, and the question, whether any or all of these are derivatives of the mitochondrial, respiratory chain-linked NADH dehydrogenase, has been the subject of intense investigations and discussions<sup>1</sup>. Recently, it has been established that the respiratory chain-linked NADH dehydrogenase reaction involves the 4B hydrogen atom of NADH, as revealed by studies with submitochondrial particles from beef heart<sup>2,3</sup> and rat liver<sup>4</sup>. Simultaneously it was found that a number of other NADH-oxidizing enzymes, including pyridine nucleotide transhydrogenase<sup>5,6</sup>, DT diaphorase<sup>7</sup>, the microsomal NADH-cytochrome c reductase<sup>8,9</sup>, as well as a liver-mitochondrial NADH-cytochrome c reductase catalysing the oxidation of extramitochondrial NADH<sup>10</sup>, are 4A-specific with regard to NADH. From these results it appeared that determination of the stereospecificity with regard to NADH might be a useful tool in characterizing various purified preparations of NADH dehydrogenase. This article presents results regarding the stereospecificity of samples of soluble NADH dehydrogenase prepared from beef heart according to various current procedures. Results are also presented regarding the stereospecificity of NADH oxidation as catalysed by the Keilin-Hartree heart-muscle preparation<sup>7</sup>, as well as a purified preparation of Straub's diaphorase<sup>6</sup> ( $\alpha$ -lipoyl dehydrogenase<sup>11</sup>). As will be shown, the NADH dehydrogenase reaction catalysed by all these preparations involves the 4B hydrogen atom of NADH.

NADH dehydrogenase of King and Howard<sup>12</sup> was prepared as described by these authors<sup>13</sup>, except that fractions were isolated using narrower cuts. The preparation was free of FAD. Singer's<sup>14</sup> NADH dehydrogenase was prepared according to the improved procedure described by Cremona and Kearney<sup>15</sup>. The purification was pursued until the step before the centrifugation on a sucrose gradient. Mackler's<sup>16</sup> procedure for preparing NADH dehydrogenase was followed precisely as described.  $\alpha$ -Lipoyl dehydrogenase was purified essentially as described by Massey<sup>17</sup>. The preparation contained no FMN. Heart-muscle preparation was made by Keilin and

Hartree's<sup>7</sup> method as adapted by King<sup>18</sup>. The preparation was slightly deficient in cytochrome c. NADH-4A-<sup>3</sup>H and NADH-4B-<sup>3</sup>H were prepared from NAD<sup>+</sup>-4-<sup>3</sup>H by reduction with unlabelled UDPG+UDPG dehydrogenase and with unlabelled ethanol + alcohol dehydrogenase, respectively, as previously described<sup>19</sup>. NAD<sup>+</sup>-4-<sup>3</sup>H (specific activity 0.7 mo./mmole) was prepared by the method of Krakow *et al.*<sup>19</sup>.

All enzyme preparations were assayed in the presence of NADH-4A-<sup>3</sup>H or -4B-<sup>3</sup>H, under two sets of conditions: (1) with an excess of ferricyanide as the electron acceptor; (2) in the absence of electron acceptor. In the former case, the reaction was followed by measuring the reduction of ferricyanide at 420 m $\mu$  in a Beckman DK-2 recording spectrophotometer; the exact conditions and the reaction rates measured are stated in Table 1. When virtually all NADH was oxidized (after about 2-3 min), the samples were frozen in a dry ice-acetone mixture, and water was isolated by sublimation as previously described<sup>20</sup>. A portion of the isolated water was used for counting radioactivity with a Packard model 314 HX 'Tri-Carb' liquid scintillation counter. In the absence of added electron acceptor the incubation mixture was the same as when acceptor was present except that larger amounts of enzyme were used (compare Table 2) and the time of incubation was 5 min. The Keilin-Hartree heart-muscle preparation was also assayed with respect to NADH oxidase activity by following the aerobic oxidation of NADH at 340 m $\mu$ . Again the reaction was followed until virtually all NADH was oxidized. The tritium in water was determined as already described here.

The results are summarized in Tables 1 and 2. Both in the presence (Table 1) and in the absence (Table 2) of a terminal electron acceptor, there occurred a substantial

Table 1. STEREOSPECIFICITY OF NADH OXIDATION BY DIFFERENT ENZYME PREPARATIONS FROM BEEF HEART

Preparation	Terminal electron acceptor	Activity, $\mu$ moles NADH oxidised/min/mg protein	Per cent <sup>3</sup> H in H <sub>2</sub> O after oxidation of NADH-4A- <sup>3</sup> H	NADH-4B- <sup>3</sup> H
(1) (a) Keilin-Hartree prep.	Oxygen	0.15	0	100
(b) Keilin-Hartree prep.	Ferricyanide	4.7	1	53
(2) NADH dehydrogenase				
<i>see. to:</i>				
(a) King and Howard <sup>12</sup>	Ferricyanide	23.3		83
(b) Cremona and Kearney <sup>15</sup>	Ferricyanide	48.2	0	91
(c) Mackler <sup>16</sup>	Ferricyanide	30.6	0	69
(3) $\alpha$ -Lipoyl dehydrogenase	Ferricyanide	17.8	8	73

The incubation mixtures contained in a final volume of 2.5 ml.: (1a) 0.2 mM NADH, 5 mM *tris*-HCl, 0.1 M phosphate buffer, pH 7.5, and 0.37 mg enzyme protein; (1b) 0.2 mM NADH, 1 mM ferricyanide, 5 mM *tris*-HCl, 0.1 M phosphate buffer, pH 7.5, and 0.072 mg enzyme protein; (2a) 0.2 mM NADH, 1 mM ferricyanide, 5 mM *tris*-HCl, 0.1 M phosphate buffer, pH 7.5, and 0.023 mg enzyme protein; (2b) 0.2 mM NADH, 1 mM ferricyanide, 45 mM *tris*-HCl, pH 7.5, and 0.023 mg enzyme protein; (2c) 0.2 mM NADH, 1 mM ferricyanide, 5 mM *tris*-HCl, 0.1 M phosphate buffer, pH 7.5, and 0.080 mg enzyme protein; (3) 0.2 mM NADH, 1 mM ferricyanide, 5 mM *tris*-HCl, 0.167 M acetate buffer, pH 4.8, and 0.017 mg enzyme protein. The temperature was 30° C. The samples were incubated for 1.5 min before the addition of the enzyme.

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Table 2. STEREO-SPECIFICITY OF EXCHANGE OF HYDROGEN ATOMS BETWEEN NADH AND WATER, CATALYSED BY DIFFERENT ENZYME PREPARATIONS FROM BEEF HEART

Preparation	Per cent <sup>3</sup> H in H <sub>2</sub> O	
	NADH-4A- <sup>3</sup> H	NADH-4B- <sup>3</sup> H
(1) Keilm-Hartree prep. (+ 4 μM rotenone)	4	96
(2) NADH dehydrogenase acc. to:		
(a) King and Howard <sup>10</sup>	11	75
(b) Cremona and Kearney <sup>12</sup>	4	94
(c) Mackler <sup>14</sup>	10	90
(3) α-lipoil dehydrogenase	4	92

The incubation mixtures were similar to those in Table 1, except that no electron acceptor was added, and the amounts of enzyme (mg protein) were as follows: (1) 0.37; (2a) 0.14; (2b) 0.22; (2c) 0.18; (3) 0.064. The samples were incubated at 30° C for 5 min.

detrition of NADH-4B-<sup>3</sup>H, and essentially no detrition of NADH-4A-<sup>3</sup>H, with all the enzyme preparations tested. Thus, all preparations contained almost exclusively 4B-specific NADH dehydrogenase, and catalysed also an exchange of hydrogen atoms between NADH and water. The NADH oxidase activity of the Keilm-Hartree preparation was likewise 4B-specific (Table 1), as expected on the basis of earlier results<sup>1</sup>. The finding that the detrition was not complete in the samples containing ferricyanide (Table 1), in spite of virtually complete oxidation of NADH (as assessed spectrophotometrically), is apparently due to an isotope effect, indicating that the actual transfer of hydrogen from NADH to the enzymes was rate-limiting. Complete detrition occurred, in fact, in the NADH oxidase reaction of the Keilm-Hartree preparation, the rate of which was much lower than that of the corresponding ferricyanide reduction, that is, the rate-limiting step apparently was on the acceptor side of the NADH dehydrogenase. Rotenone, which blocked the NADH oxidase reaction of the Keilm-Hartree preparation but not the reduction of ferricyanide with any of the preparations tested, had no effect on the rate of detrition. This finding, in accord with previous results<sup>2,3</sup>, indicates that rotenone does not block the transfer of hydrogen from NADH to NADH dehydrogenase with any of the preparations studied.

Table 3 is a summary of previous and present conclusions regarding the stereochemical properties of various enzymes catalysing the oxidation of reduced pyridine nucleotides. The respiratory chain-linked NADH dehydrogenase and the α-lipoil dehydrogenase are specific for the 4B hydrogen atom of NADH. The soluble NADH

Table 3. STEREOCHEMICAL PROPERTIES OF SOME ENZYMES CATALYSING THE OXIDATION OF REDUCED PYRIDINE NUCLEOTIDES

Enzyme	Stereo-specificity	Exchange with water	Ref.
NADH dehydrogenase (mitochondrial, resp chain-linked)	B	+	2, 3
α-lipoil dehydrogenase	B	+	This paper
NADH dehydrogenase (microsomal)	A	-	4, 6
NADH dehydrogenase (liver-mitochondrial, 'external')	A	-	4
NADH-NADPH transhydrogenase (mitochondrial, energy-linked and non-energy-linked)	A	B	2, 3, 5
DT diaphorase	A	A	2

dehydrogenase of King and Howard<sup>10</sup>, Singer<sup>13</sup>, and Mackler<sup>14</sup> are 4B-specific. Since these preparations were free of FAD and/or of α-lipoil dehydrogenase activity, they most probably represent derivatives of the NADH dehydrogenase of the respiratory chain. A similar conclusion may hold for Mahler's<sup>17</sup> enzyme, the stereo-specificity of which has been determined by Drysdale and Cohn<sup>18</sup>. In contrast to these enzymes are the microsomal NADH-cytochrome c<sup>4</sup> or -cytochrome b<sub>5</sub> reductase<sup>8</sup>, the 'external' NADH-cytochrome c reductase of liver mitochondria<sup>9</sup>, DT diaphorase<sup>2</sup>, and the pyridine nucleotide transhydrogenase<sup>2,3,5</sup>, which are specific for the 4A hydrogen atom of NADH; interestingly, the transhydrogenase is 4B-specific with regard to NADPH, whereas DT diaphorase is 4A-specific. Another interesting feature is that the two 4B-specific NADH-oxidizing enzymes, NADH dehydrogenase and α-lipoil dehydrogenase, catalyse a ready exchange of hydrogen atoms between NADH and water, whereas the 4A-specific NADH-oxidizing enzymes seem to be devoid of this capacity. These and further aspects of the stereochemistry of enzymes catalysing the oxidation of reduced pyridine nucleotides may be of value for the study of their reaction mechanism and metabolic function.

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## AMINO-ACIDS AND PEPTIDES IN HUMAN GASTRIC JUICE WITH PARTICULAR REFERENCE TO PERNICIOUS ANAEMIA: A REVIEW

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NUMEROUS investigations have been carried out, in the past, on the gastric juice of both normal subjects and pernicious anaemia patients. In the main, these researches have been concerned with the protein fraction of gastric juice, often in an endeavour to isolate the 'intrinsic factor' first proposed by Castle<sup>1</sup>. This factor, which has never been isolated, has been assumed to be protein in nature since the work of Ternberg and Eakin<sup>2</sup> and of Glass *et al.*<sup>3</sup>. Much of this research has been well described in reviews<sup>4-10</sup>, and a good deal of knowledge of

the proteins of gastric juice has accrued from the search for this elusive factor. Less information seems to be available concerning the non-protein nitrogenous fraction of human gastric juice, and the present review is an attempt to summarize our existing knowledge of this fraction.

The earlier investigations<sup>11,12</sup> on protein-free gastric juice were inconclusive and it was not until 1931 that Martin<sup>13</sup> carried out the first meaningful examination. He precipitated the protein by means of sodium tungstate and,

after filtration, analysed the filtrates for nitrogen by the method of Folin and Wu<sup>14</sup>. The non-protein nitrogen in normal samples was found to vary over the range of 20–48 mg/100 ml. gastric juice. In simple achlorhydria, the range was 30–90 mg per cent, while in pernicious anaemia, it was 60–150 mg per cent. Martin also observed that the volume of gastric juice produced by the fasting pernicious anaemia patient was very small when compared with that produced by a fasting normal subject.

In a further investigation<sup>15</sup>, carried out two years later, Martin showed that both the amino-acid-nitrogen and the non-protein-nitrogen were twice as great as the normal values in cases of benign achlorhydria and three times as great in pernicious anaemia. Martin's investigations were carried out before the advent of paper chromatography, and he obtained no details of the individual amino-acids which were present. In the next section, we shall examine results which have been obtained in more recent studies on normal gastric juice.

**Amino-acids in Normal Gastric Juice.** Cagianut, Zehnder and Nager<sup>16</sup> precipitated the proteins from normal gastric juice by the addition of excess alcohol and examined the protein-free solution, after centrifugation, by means of paper chromatography. They found small quantities of alanine, leucine, histamine and glucosamine.

Two years later, Gilligan, Moor and Warren<sup>17</sup> compared the results obtained by alcoholic precipitation and dialysis. On examination of the protein-free solutions these authors found alanine, aspartic acid, glycine, valine and the leucines (not separated) to be present in all cases. In addition  $\gamma$ -amino-butyric acid, glutamic acid, glutamine and tyrosine were often present; other amino-acids occurred on rare occasions. The dialysates and alcoholic filtrates proved to be almost identical in amino-acid composition, and the leucines, valine and alanine accounted for two-thirds of the total concentration of the free amino-acids. The individual amino-acids were estimated by visual comparison of the colour produced by ninhydrin with that produced by standard amounts of each acid.

Merlevede, Pottiez and Verhelle<sup>18</sup>, in 1955, precipitated the proteins of gastric juice with trichloroacetic acid (20 per cent), removed them by filtration, and then demineralized the filtrate by passing it through a column of resin ('Amberlite IR-120'). The amino-acids were eluted by means of ammonia, and the eluate was concentrated *in vacuo* at room temperature. The samples were chromatographed as bands, and each band was then resolved into its component amino-acids by paper electrophoresis. In the gastric juice from ten normal subjects, lysine, histidine, leucine, phenylalanine, glycine, alanine, serine, tyrosine, glutamic acid, glutamine, valine and citrulline were found in every case. In a few samples ornithine, threonine, aspartic acid, proline and methionine were also found. The reported presence of ornithine and citrulline was rather surprising.

Chiba and Ishikawa<sup>19</sup> deproteinized their samples of normal gastric juice by heating to 80° with perchloric acid (8.6 per cent) and then filtering. Paper chromatography showed the presence of aspartic acid, cystine, alanine, histidine, valine and/or methionine, leucine and/or isoleucine. Some of these amino-acids may possibly have been produced by partial hydrolysis of peptide links during the preparation of the protein-free filtrate using hot perchloric acid.

Shimizu<sup>20</sup> examined seven samples of gastric juice from patients with non-gastric disease. He deproteinized the juice by alcoholic precipitation and then examined the filtrates by chromatography. Valine, leucine, alanine, serine and tyrosine were present in all cases. This was in agreement with the results obtained by Merlevede *et al.* already mentioned.

After removal of the protein in their samples by dialysis, Bouda and Veselý<sup>21</sup> used paper electrophoresis and paper chromatography to separate the amino-acids in the filtrates. In six samples of normal fasting juice which they

examined, histidine, arginine, serine, tyrosine, phenylalanine, aspartic acid and glutamic acid were found. Bouda and Veselý<sup>21</sup> reported that Hais and Macek<sup>22</sup> found the same amino-acids as Gilligan *et al.*<sup>17</sup>, and in addition they found serine and threonine to be present.

The technique of adsorption on to a column of 'Dowex-50' resin was used by Borkowski<sup>23</sup> to examine a series of filtrates of normal fasting gastric juice, after the protein had been removed by means of alcohol. The amino-acids were then eluted with dilute ammonia and identified by paper chromatography in the usual way. Borkowski found altogether some 17 amino-acids of which ten were always present. These were leucine, valine, glycine, alanine, glutamic acid, serine, lysine, threonine, aspartic acid and tyrosine. A quantitative estimation was possible for eight of these (Table 1). He found no histidine or citrulline, in contrast to Merlevede *et al.*<sup>18</sup>, but claimed the additional presence of glutamine, arginine,  $\beta$ -amino-isobutyric acid, phenylalanine, proline, ornithine and hydroxyproline in this decreasing order of occurrence.

Table 1. THE CONCENTRATION OF AMINO-ACIDS IN NORMAL GASTRIC JUICE (BORKOWSKI, 1960)

Amino-acid	Concentration (mg/100 ml.)
Aspartic acid	0.64
Glycine	0.53
Glutamic acid	0.47
Lysine	0.46
Tyrosine	0.35
Alanine	0.34
Threonine	0.27
Serine	0.26

Fucik and Bolková<sup>24</sup> investigated normal gastric juice for amino-acids and peptides. The juice was first treated with a mixture of alcohol and acetone, and the protein-free filtrate was examined by paper chromatography and electrophoresis.

They found, in addition to the amino-acids reported by Borkowski, methionine, valine, phenylalanine, leucine, isoleucine, histidine, arginine and  $\gamma$ -aminobutyric acid to be present in nearly all the subjects examined.

**Comparative Investigations of Normal and Pernicious Anaemia Juice.** Hiller and Bischof<sup>25</sup>, in 1953, compared the free amino-acids in the dialysates of the gastric juice of five normal subjects and several patients suffering from diseases of the stomach. Two of the patients with gastritis had atrophy of the gastric mucosa which may well have been incipient pernicious anaemia. The samples of dialysate were concentrated over phosphorus pentoxide and then examined by paper chromatography.

Qualitatively, they found little difference between the amino-acids of normal gastric juice and those of the patients. In all cases they found serine phosphoric acid, aspartic acid, serine, glutamic acid, glycine, lysine, threonine, alanine, arginine, valine, and leucine. There was an estimated increase in the concentration of amino-acids in the two cases of gastric atrophy; but, unfortunately, it was not possible to determine quantitatively the small amount of amino-acids present in normal juice. The values which these authors quoted for the concentrations of the amino-acids in the cases of gastric atrophy were about one-fiftieth of the concentrations reported by Washington<sup>26</sup> for the amino-acids present in pernicious anaemia (see following).

In a comparative investigation of the protein-free filtrates of normal gastric juice and those from pernicious anaemia patients, Norpoth, Surmann, and Clöges<sup>27</sup> used alcohol for the precipitation of the protein. They found, using paper chromatography, that valine, leucine, alanine, glutamic acid, aspartic acid and phenylalanine were frequently present, while histamine, tryptophan, lysine, threonine and  $\gamma$ -amino-butyric acid were less frequent. The gastric juice of pernicious anaemia (and of gastric cancer) contained greater numbers of amino-acids than normal gastric juice. The amino-acids were also present in increased concentration.

In 1960, Bouda and Veselý<sup>21</sup>, using the same techniques as before<sup>21</sup>, carried out a direct comparison between the

gastric juice of normal subjects and of patients suffering from gastric atrophy. In normal juice, they found most of the amino-acids reported by Norpoth *et al.* and in addition methionine, tyrosine, serine, glycine and arginine. In diseases associated with gastric atrophy, such as cancer and pernicious anaemia, both the number and concentration of free amino-acids were increased.

Washington<sup>17</sup> has recently examined the non-protein fraction of two samples of pooled normal human gastric juice and compared his results with those obtained from a few (individual) samples of juice from pernicious anaemia patients. All samples were of fasting juice and, except in the case of one sample of pernicious anaemia, were taken without any artificial stimulation such as histamine or alcohol. Washington deproteinized the gastric juice from normal subjects by three different methods, namely, dialysis, precipitation with trichloroacetic acid, and precipitation with ethanol. On paper chromatography and staining with ninhydrin the protein-free filtrates, however prepared, produced a prolonged streak which was interspersed with regions of increased intensity. The streaking was eventually shown to be due to the presence of a peptide which was causing the individual amino-acids to bond together and to be transported, in part, to atypical positions along the line of the chromatogram. The regions of increased intensity represented the true main positions of the amino-acids. The problem of separation of the individual components was resolved by developing a band of the filtrate in one solvent and eluting the main component bands thus produced. The elution was carried out with water and, after concentration, the eluates were chromatographed again as individual bands in a second solvent system. The new bands (sub-fractions) from these second chromatograms were eluted and concentrated as before, and chromatographed again a third time, either in the first solvent or in a different one. At this third stage discrete bands were obtained, on staining. The bands which corresponded to the same individual amino-acids in each sub-fraction were treated together for colorimetric determination.

The gastric juice from patients with pernicious anaemia proved to be more easily analysed owing to the absence of peptide and, in this case, it was possible to carry out two-dimensional chromatography directly on the eluates of the bands derived from the first chromatographic separation.

The main findings are summarized briefly in Table 2, from which it can be seen that the sample of pernicious anaemia gastric juice has an overall concentration of amino-acids which was almost five times as great as that of the average sample of normal gastric juice. Individual samples of juice, of either kind, contained other amino-acids such as methionine or phenylalanine, but Washington found no trace of ornithine or of citrulline.

Alcohol-stimulation of the production of gastric juice in one pernicious anaemia patient was found to increase the concentration of the amino-acids in the non-protein filtrate above the value obtained without such stimulation.

Only a few samples were examined by Washington, but the quantitative recovery of the amounts of amino-acids which are present, in non-stimulated normal gastric juice, was good. Furthermore, the values which he obtained, for the overall concentration, were within the ranges quoted by earlier workers in the field (Table 3). There is every reason to believe that the results which were obtained in the pernicious anaemia juice were, if anything, even more reliable, owing to the larger concentrations of amino-acids present and the relative simplicity of the analysis.

**Peptides in Gastric Juice.** On hydrolysis of the non-protein filtrates of normal gastric juice, Gilligan *et al.*<sup>18</sup> observed that the concentration of the amino-acids increased ten-fold and this seemed to indicate that many of the amino-acids were present combined in peptide chains. In their examination of the alcoholic filtrates and dialysates of normal juice, they claimed that a peptide was often present which migrated faster than leucine in chromato-

Table 2. FREE AMINO-ACIDS IN FASTING GASTRIC JUICE (WASHINGTON, 1964)

Amino-acid	Amino-acid concentration in gastric juice (mg/100 ml.)	Pernicious anaemia (PA)*	Normal†	Ratio of PA/normal
Alanine	9.3	2.6	3.6	3.6
Arginine	4.7	1.3	3.6	3.6
Aspartic acid	4.1	0.4	10.2	10.2
$\gamma$ -Aminobutyric acid	4.5	1.0	4.5	4.5
Cysteine	2.7	0.2	13.5	13.5
Glutamic acid	3.5	0.5	7.0	7.0
Glutamine	2.9	2.0	1.4	1.4
Glycine	4.3	1.4	3.1	3.1
Leucine plus isoleucine	6.4	1.0	6.4	6.4
Proline	1.8	0.3	4.8	4.8
Serine	4.1	1.2	3.4	3.4
Threonine	1.9	0.5	3.8	3.8
Tyrosine	6.4	1.2	5.8	5.8
Valine	3.9	0.7	5.6	5.6
Total	60.0	14.3	4.2	4.2

\* Average value derived from three individual samples, except where marked  $\alpha$ .

† Average value derived from two pooled samples, except where marked  $\alpha$ .

Table 3. CONCENTRATION OF  $\alpha$ -AMINO-NITROGEN IN NORMAL GASTRIC JUICE

Author	$\alpha$ -Amino-nitrogen (mg/100 ml. of juice)
Borkowski (1960)	0.4
Martin (1931)	0.6*
Gilligan, Moor and Warren (1963)	0.9
Martin (1931)	7.2
Ohba and Ishikawa (1965)	17.5
Washington (1964)	1.5

\* Obtained from the figure quoted for the amino-acids by assuming a value of 120 for the average mol. wt.

graphic solvents, and which they called the 'high streak' peptide.

Norpoth, Surmann and Clöages<sup>19</sup> also reported the incidence of a similar 'high streak' peptide.

Bouda and Vesely<sup>20</sup>, in 1960, described the presence of "peptide streaks" in some experiments with normal gastric juice, but these were not investigated further. These workers also found that the juice of patients suffering from gastric atrophy (either pernicious anaemia or cancer) rarely contained peptides.

In 1960 also, Brummer and Kulonen<sup>21</sup>, after precipitation of the proteins of gastric juice, found a spot to be present on paper chromatograms of all alcoholic filtrates where the pH of the gastric juice was below 3.0. This spot, which they called spot "A", migrated faster than leucine, but could not be characterized adequately. In the next year, Brummer, Seppala and Kulonen<sup>22</sup>, by means of column chromatography, separated spot "A" from other components and then examined it by means of paper chromatography. They found "A" to consist of two components—a weakly-staining, slow-moving spot and a much more strongly staining fast-moving one. After acid hydrolysis of the 'fast' spot, the following amino-acids were identified: aspartic acid, glutamic acid, serine, glycine, alanine, valine, leucine and isoleucine. The leucines were present in the highest concentration.

An acidic gastric juice from which spot "A" was absent was adjusted to pH 2.0 with dilute hydrochloric acid. After this mixture had been allowed to stand at room temperature for 16 min, spot "A" appeared as a trace on staining. The spot achieved maximum staining intensity within 1 h. Brummer *et al.* suggested that spot "A" was connected with the conversion of pepsinogen to pepsin and this they confirmed with pure hog pepsinogen.

Brummer, Seppala and Kulonen<sup>23</sup> also found a strong spot, which they again called "A", in six out of eight patients suffering from pernicious anaemia, after acidification of their gastric juices with dilute hydrochloric acid. They recognized, however, that the stomach in pernicious anaemia would be very unlikely to contain more pepsinogen than either the normal subject or the patients with simple achlorhydria. They could only suggest, therefore, that the spot obtained after the acidification of pernicious anaemia gastric juice, although chromatographically identical with spot "A", was due to another polypeptide.

The enigma of the existence of spot "A" in pernicious anaemia gastric juice was re-examined by Brummer and Seppala in 1963<sup>24</sup>, using eight patients with pernicious

anaemia, and seven patients with histamine-fast achlorhydria. Each sample of gastric juice was dialysed and the non-dialysable fraction was subjected to paper electrophoresis. On staining, bands were found which corresponded to four main protein fractions which were designated: albumin,  $M_1$ ,  $M_2$ ,  $M_3$ , and in addition there were some vague bands due to peptides. After acidification of these anacid juices, followed by electrophoresis, they found that the fraction  $M_3$  had disappeared and the peptide bands had increased in intensity. They concluded that the spot "A", which they had noticed previously in pernicious anaemia gastric juice, was probably due to polypeptides which had been freed from the albumin and mucous substances when the gastric juice had been acidified.

Heathcote and Washington<sup>22</sup> have reported recently the isolation of a peptide, from two pooled samples of normal human gastric juice, which was completely absent from the individual samples of pernicious anaemia juice which they examined. The peptide had a low  $R_F$  value in the usual chromatographic solvent systems for amino-acids and there was no evidence for the existence of a 'high streak' peptide such as was reported by Brummer and Seppala. The presence of the peptide in normal gastric juice causes marked streaking on paper chromatograms of the protein-free filtrates or dialysates. It gave a single peak in the analytical ultracentrifuge which corresponded with a molecular weight of about 1,000. Preliminary analyses indicated an amino-acid composition which was similar to, though not identical with, that of the faster moving component of Brummer *et al.*<sup>20</sup>. Washington has since reported the presence of this peptide in a third sample of normal gastric juice.

### Discussion

The results which have been obtained for the concentrations of the amino-acids in gastric juice can now be summarized. We must keep in mind, however, that in addition to the usual biological variation there are two other variables. These are, first, that the concentration may alter according to the method of production of the juice, that is, whether stimulated artificially or not, and secondly, that the method of deproteinization may affect, appreciably, the results of the analysis<sup>27,28</sup>.

Qualitatively, it would seem that almost all the common naturally occurring amino-acids which one might expect have been reported to exist in normal human gastric juice, although not always in the same sample. Taurine, citrulline and ornithine have also been reported to occur on occasions. The total amount of amino-acid nitrogen which is present in normal gastric juice has been reported to vary between 0.4 and 17.6 mg per 100 ml.

With regard to the gastric juice of pernicious anaemia patients, there are only eight reports extant and most of these refer to only a few patients. The four qualitative studies all agree on the increase in number of the amino-acids when compared with normal juice. Of the four quantitative studies, two<sup>18,19</sup> indicate a three-fold increase in the value of total amino-acids over the normal value; the third, by Hiller and Bischof<sup>26</sup>, gave individual concentrations which were about one hundred times lower than the values reported for normal gastric juice by other workers and must, therefore, be presumed to be erroneous; finally, the most recent investigation<sup>27</sup> indicates an increase of between four and five times the concentration of amino-acids in pernicious anaemia gastric juice over the normal value.

Normal gastric juice, when administered together with cyanocobalamin, orally to pernicious anaemia patients, caused remission of the symptoms<sup>24</sup> and the question is still open as to how this effect could have arisen. Could it be due, for example, to the presence of certain amino-acids in normal gastric juice, which are absent from, or present only in small amounts in, pernicious anaemia gastric juice? Simple mixtures of glutamic acid and cobalamin have

indeed been shown to be more effective than cobalamin alone<sup>25-27</sup>. At first sight, this would not seem to be a likely explanation for the improved activity because the concentration of amino-acids is greater in the pernicious anaemia juice. However, according to Ibre<sup>25</sup>, the volume of fasting juice in the normal subject may be fifteen times the volume in the pernicious anaemia patient and, accordingly, the amounts of many amino-acids, including glutamic acid, are quite likely to be present in much greater overall amount in normal gastric juice.

A further possible explanation for the improved therapeutic effect of a mixture of normal juice and cyanocobalamin could be the presence of some low molecular weight peptide. Heathcote and Mooney<sup>29,30-31</sup>, and Withey, Jones and Kilpatrick<sup>32</sup>, have reported therapeutic success in pernicious anaemia with low molecular weight peptides derived from fermentation sources. The recent discovery by Washington of a peptide in normal human gastric juice, which was found to be absent from the gastric juice of the pernicious anaemia patients examined, would seem to be of possible significance in this connexion. It would seem quite possible that such a peptide, with its strong ability to bind amino-acids, could be involved in the increase in therapeutic value of oral vitamin B<sub>12</sub> when administered with normal gastric juice. However, irrespective of the significance of these recent results in relation to the fraction of gastric juice which may be responsible for the enhanced therapeutic activity of oral vitamin B<sub>12</sub>, it is hoped that this review will be of interest to all concerned with the secretions, and pathology, of the stomach.

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AN IMPROVED METHOD FOR THE CHARACTERIZATION OF HUMAN  
HAEMOGLOBIN MUTANTS: IDENTIFICATION OF  $\alpha_2\beta_2^{95\text{GLU}}$ ,  
HAEMOGLOBIN N (BALTIMORE)

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ONE of the problems during investigations of human haemoglobin mutants has been the difficulty of separating the  $\alpha$  and  $\beta$  chains in a pure form from small quantities of starting material. While chain separation is not a pre-requisite for all chemical investigations, such work can be carried out more easily on the isolated  $\alpha$  and  $\beta$  chains. The method for the separation of the peptide chains of human haemoglobin described in this communication is relatively easy to use and has an advantage over those previously reported in that it will not only give separation of the  $\alpha$  and  $\beta$  chains, but is also capable of resolving a charged mutant chain from the normal, thus enabling the globin of a heterozygous charged mutant to be fractionated into all its component chains.

A second difficulty usually encountered is the familiar one of incomplete digestion of proteins by proteolytic enzymes. Digestion of human  $\alpha$  and  $\beta$  haemoglobin chains with trypsin normally leaves a trypsin-resistant 'core' with the result that a region consisting of about 30 per cent of the molecule cannot easily be examined by fingerprinting. However, conversion of the cysteine residues of the  $\alpha$  and  $\beta$  chains to aminoethyl-cysteine by reaction with ethylenemine<sup>1</sup> has been shown by Jones<sup>2</sup> to give derivatives which no longer contain the trypsin-resistant region. Thus, fingerprints of aminoethylated  $\alpha$  and  $\beta$  chains show all the expected trypsin peptides plus additional ones due to splits at aminoethyl-cysteine.

The combination of a high-resolution chromatographic separation of the  $\alpha$  and  $\beta$  chains and the conversion of the separated chains into aminoethyl derivatives susceptible to trypsin<sup>3</sup> affords a procedure considerably more rapid and more sensitive than any previously available for studying haemoglobin mutants. With the technique described here, recoveries of the separated  $\alpha$  and  $\beta$  chains are quantitative and sufficient material for amino-ethylation and subsequent fingerprinting can be obtained from as little as 10 mg of starting globin. In addition, quantitative amino-acid analyses of peptides eluted from fingerprints of 2 mg of the digested chains have been routinely achieved.

*Isolation and characterization of the  $\beta$  chain of haemoglobin N (Baltimore).* An 8-month-old Negro female was found to have two haemoglobins by routine electrophoretic

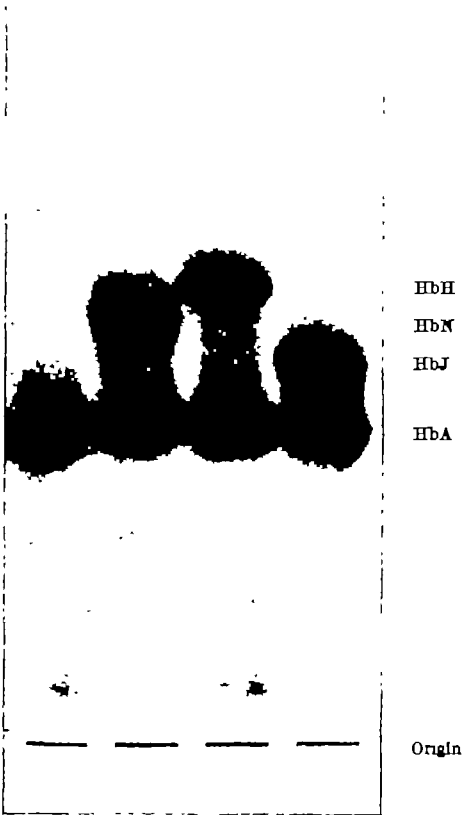


Fig. 1. Starch-gel electrophoresis (*tris*-EDTA-borate, pH 8.6) of haemolysates containing (left to right), haemoglobin A, haemoglobins A and N, haemoglobins A and H, and haemoglobins A and J.

screening. Apart from a mild iron deficiency there were no significant haematological abnormalities either in the child or in the mother, who also carried the abnormal variant. The relevant haematological values and the proportions of normal and abnormal haemoglobins are summarized in Table 1. On starch-gel electrophoresis at pH 8.6 (Fig. 1) the abnormal haemoglobin migrated

Table 1. HAEMATOLOGICAL AND ELECTROPHORETIC DATA

Family member	Age	Haemoglobin (g/100 ml.)	Red cell count (millions/mm <sup>3</sup> )	Reticulo-cytes (%)	MCV ( $\mu^2$ )	MCH ( $\mu$ /g)	MCHC (%)	Haemoglobin constitution	Haemoglobin fractionation			Alkali-resistant haemoglobin (%)
									HbA (%)	HbN (%)	HbA <sub>2</sub> (%)	
Propositus	8 months	10.2	4.06	5.1	81	28	31	A + N	46.3	44.3	1.0	8.4
Mother	24 years	13.8	4.13	1.1	102	33	33	A + N	48.6	49.6	1.4	0.4

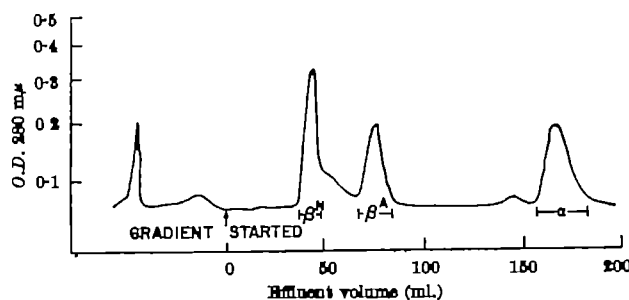


Fig. 2. Gradient elution chromatography on carboxymethyl-cellulose of globin from haemoglobin N heterozygote

faster than haemoglobin J (Baltimore)<sup>8</sup> but slower than haemoglobin H. These electrophoretic characteristics are similar to those previously described for haemoglobin N<sup>4</sup> and the variant has therefore been designated as haemoglobin N (Baltimore). Comparative gel-electrophoresis experiments with other known haemoglobin mutants suggested that at pH 8.6 haemoglobin N (Baltimore) possesses four more negative charges per 68,000 molecular weight than does haemoglobin A. Hybridization with canine haemoglobin indicated that the abnormality in haemoglobin N (Baltimore) was in the  $\beta$  chain. Fingerprints of tryptic digests of whole haemoglobin N (Baltimore) or of isolated  $\beta^N$  (Baltimore) chain showed no detectable differences from those of haemoglobin A or normal  $\beta$  chain.

20 mg of the child's globin, prepared by 2 per cent acid-acetone precipitation of a whole red-cell lysate, was dissolved in 2 ml. of a buffer consisting of 8 M urea, 0.05 M 2-mercaptoethanol, and 0.005 M  $\text{Na}_2\text{HPO}_4$ , adjusted to pH 6.7 with phosphoric acid. The solution was dialysed at room temperature against three changes of a 50-fold excess of the same buffer for a total of 2.5 h and then applied to a 1 cm  $\times$  10 cm column of carboxymethyl-cellulose (0.7 m.equiv. g) equilibrated against the same buffer. After the column had been washed to remove any unretarded material the peptide chains were eluted at a flow rate of 1 ml./min by means of a linear  $\text{Na}^+$ -ion gradient made by mixing 100 ml. of starting buffer with 100 ml. of a buffer consisting of 8 M urea, 0.05 M 2-mercaptoethanol, and 0.03 M  $\text{Na}_2\text{HPO}_4$ , adjusted to pH 6.7 with phosphoric acid. The column effluent was monitored continuously at 280 m $\mu$  and the resulting chromatogram is shown in Fig. 2. Of the three peaks obtained, two were found at the expected elution volumes of normal  $\alpha$  and  $\beta$  chains, while the third peak emerged much earlier than normal  $\beta$  chain, indicating the presence of a more acidic  $\beta$  chain. This confirmed the findings of the hybridization experiments.

The fractions corresponding to the two  $\beta$  chains were collected and solid  $\text{KSCN}$  was added to each solution to give a concentration of 1 M. After the pH of the solutions had been adjusted to 9.2 with concentrated HCl, ethylenimine was added to a final concentration of 0.5 M (that is, a 10-fold molar excess over the 2-mercaptoethanol). The aminoethylation reactions were then allowed to proceed at room temperature until no free sulphhydryl groups were detectable by the nitroprusside test<sup>9</sup> (usually after about 2.5 h). The pH was then adjusted to 3 with concentrated HCl and the solutions passed through a 'Sephadex G-25' column equilibrated with 0.5 per cent formic acid, in order to remove urea, salts, etc. Finally, the recovered protein fractions were freeze dried.

Tryptic digests of amounts of protein greater than 10 mg were carried out at pH 9.0 for 2 h at room temperature in a radiometer pH-stat. Digestion was terminated by adjusting the pH to 4.7 and freeze-drying the solution. It was preferable to digest smaller amounts of protein in 1 per cent  $\text{NH}_4\text{HCO}_3$ , pH 8.2 (the amounts of salt introduced by the automatic procedure being sufficient to cause smearing of peptide spots when it was necessary to apply all the digest to a single fingerprint). After digestion was complete, the  $\text{NH}_4\text{HCO}_3$  was removed by repeated freeze-drying. For all the digestions the trypsin/protein weight ratio was 1/100. The dried digests were dissolved in pH 4.7 buffer (1.25 per cent pyridine, 1.25 per cent acetic acid) and aliquots corresponding to 2 mg of the original proteins were subjected to electrophoresis in this buffer on 108 cm  $\times$  57 cm sheets of Whatman No. 3 MM paper for 3.25 h at 33 V/cm in a 'Varsol'-cooled tank. After drying, the papers were chromatographed overnight in *n*-butanol, acetic acid, water, pyridine (15:3:12:10) (ref. 6). The resulting fingerprints were stained by dipping in 0.02 per cent ninhydrin-acetone and developing at 60°. Photographs of the two fingerprints are shown in Fig. 3 and the significant differences between the mutant and the normal patterns are indicated by arrows. The other slight differences are due to the varying amounts of partially split peptides.

The fingerprint of the mutant  $\beta$  chain is characterized by the absence of peptides  $\beta\text{Tp}10$  and  $\beta\text{Tp}11$  and by the appearance of a new peptide which has a distinctive yellow colour. Since  $\beta\text{Tp}10$  also gives a yellow colour with ninhydrin, these observations suggest that the mutant peptide is a combination of  $\beta\text{Tp}10$  and  $\beta\text{Tp}11$ . (No tryptic cleavage at the aminoethyl-cysteine residue in  $\beta\text{Tp}10$  has been found, presumably because aspartic acid is the adjacent amino-acid, the sequence in this region being AECys (aminoethyl-cysteine)<sup>12</sup>-Asp<sup>14</sup>-Lys<sup>15</sup> (ref. 7). This absence of a split at AECys<sup>12</sup> was previously

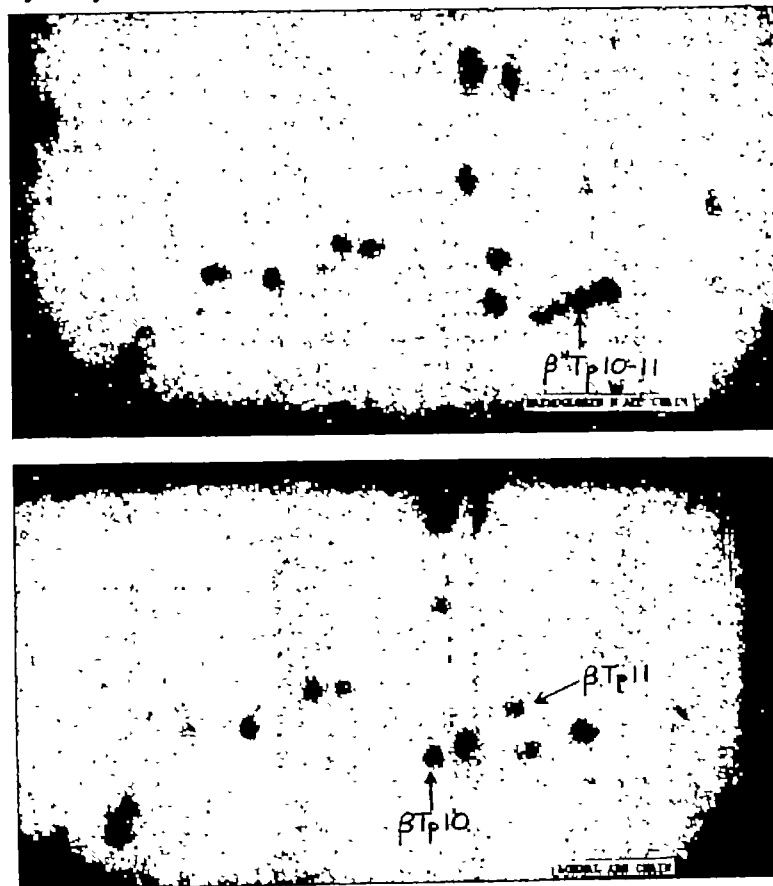


Fig. 3. Fingerprints of tryptic digests of AEM(Baltimore) (above) and normal AEM chains (below)

reported by Jones<sup>1</sup>.) The peptides to be analysed were cut from the fingerprints and eluted with 6 N HCl into 100  $\mu$ l. capillary disposable pipettes by the method described by Sanger and Tuppy<sup>2</sup>. Collection of 30–40  $\mu$ l. was sufficient to ensure complete removal of the peptide from the paper. The tubes were then sealed, and heated at 105° for 18 h. After hydrolysis, the contents of the tubes were washed into 0.5 ml. of distilled water and applied directly to a 'Technicon' 5-column amino-acid analyser. This made it unnecessary to dry down the hydrolysates, a procedure which has been shown to reduce the yield of amino-acids, possibly because of further reaction with ninhydrin<sup>3</sup>. Table 2 gives the amino-acid analysis and composition of the mutant peptide. From this it is apparent that the lysine residue (95) of  $\beta$ Tp10 has been replaced by glutamic acid (or glutamine), with the result that a conjugate peptide,  $\beta^N$ Tp10–11, is formed by tryptic digestion of the mutant  $\beta^N$ (Baltimore) chain. The evidence for a lysine to glutamic acid (rather than glutamine) change at position 95 in the haemoglobin N (Baltimore)  $\beta$  chain is provided by the starch-gel electrophoresis findings of a difference of four negative charges between haemoglobin N (Baltimore) and haemoglobin A. It explains why the mutant peptide  $\beta^N$ Tp10–11, occurring as it does in the 'core' region of the  $\beta$  chain, was not detected on the original fingerprints of tryptic digests of haemoglobin N (Baltimore) or of the isolated  $\beta^N$ (Baltimore) chain which had not been reacted with ethyleneimine.

Table 2. AMINO-ACID ANALYSIS AND COMPOSITION OF Amino-ACID CHAIN PEPTIDES

Amino-acid	Amino-acid (Baltimore)			Amino-acid (ref. 7)		
	$\beta^N$ (Baltimore)	Tp 10–11		$\beta^N$ Tp10	$\beta^N$ Tp11	$\beta^N$ Tp10 + $\beta^N$ Tp11
	Found (amoles)	Residues	Nearest whole residue	Expected	Expected	Expected
Lys	—	—	—	1	—	1
His	0.043	1.6	2	1	1	2
Arg	0.028	1.0	1	—	1	1
Asp	0.078	2.8	3	1	2	3
Thr	0.061	1.8	2	2	—	2
Ser	0.033	1.2	1	1	—	1
Glu	0.065	2.0	2	1	1	2
Pro	0.026	0.9	1	—	1	1
Gly	0.026	0.9	1	1	—	1
Ala	0.030	1.1	1	1	—	1
Val	0.028	1.0	1	—	1	1
AB-Cys	0.032	1.1	1	1	—	1
Met	—	—	—	—	—	—
Ileu	—	—	—	—	—	—
Leu	0.076	2.7	3	2	1	3
Tyr	—	—	—	—	—	—
Phe	0.047	1.7	2	1	1	2
Tryp	—	—	—	—	—	—

Overall recovery of Amino-acid (Baltimore) Tp10–11 was 24 per cent of amount originally applied to the fingerprint.

In accordance with the recommended nomenclature<sup>10</sup>, haemoglobin N (Baltimore) is designated as  $\alpha_2\beta_1^{N95}$ .

The procedure outlined in this article has the following advantages over those previously described:

(1) The chromatographic system enables globin from a heterozygous charged mutant to be resolved quantitatively into all its component chains, thus simplifying subsequent chemical investigations. In addition, the high resolution achieved during chromatography eliminates the need for hybridization experiments to determine in which chain an amino-acid substitution is located. (It should be mentioned that the pH of the chromatographic system is not critical and can be varied from at least pH 6.5 to 7.2, the actual choice depending largely on the nature of the mutant globin to be fractionated and the degree of resolution required. As an example of this flexibility, Fig. 4 shows a separation of 11 mg of sickle-trait globin into  $\beta^A$ ,  $\beta^S$  and  $\alpha$  components at pH 7.2.)

(2) All the tryptic peptides of the amino-ethylated  $\alpha$  and  $\beta$  chains can be resolved by fingerprinting.

(3) Quantitative amino-acid analysis of these peptides can be determined on samples obtained by elution from a ninhydrin-stained fingerprint of as little as 2 mg of digested chain.

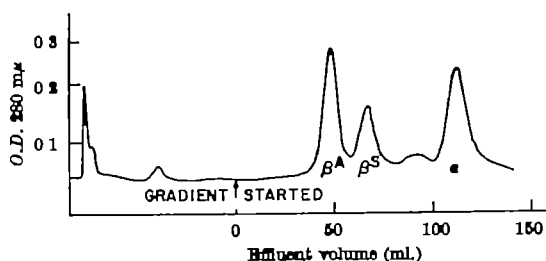


Fig. 4. Chromatography at pH 7.2 of globin from haemoglobin S heterozygote

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## A MODIFIED 'ASEPTIC ADDITION' ASSAY PROCEDURE FOR THE MEASUREMENT OF SERUM 'FOLIC-ACID' ACTIVITY

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THE 'folic-acid' activity of serum is now recognized as a reliable index of the folate state of an individual and is measured by a micro-biological assay technique with the organism, *Leuobacillus casei*. A reliable and accurate method, which is not time-consuming, is necessary because the investigation is now no longer restricted to the elucidation of the aetiology of a megaloblastic

anaemia and is being applied to patients suffering from a variety of diseases.

The 'aseptic addition method' of Herbert and Zalusky<sup>1</sup> fulfils the foregoing criteria. In this method, serum extraction is not used and the serum is added directly to the prepared assay tubes immediately before inoculation with the organism. However, further simplification of this technique is possible without detracting from the value of the test.

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The purpose of this article is to describe a modified 'aseptic addition' assay procedure for measuring the 'folio-acid' activity of serum.

**Method.** The assay medium is the double-strength assay medium of Waters and Mollin<sup>1</sup> with additional L-tryptophan (25 mg/100 ml.).

The stock solution (0.4 mg/ml.) of pteroyl-glutamic (folio) acid in 20 per cent ethanol is stored in an amber bottle in the refrigerator. It is used to prepare the standard curve.

Serum, without added ascorbic acid and in which no haemolysis is evident, is stored at  $-20^{\circ}\text{C}$ .

Ascorbic acid (2 mg/ml.) is dissolved in water just before use, to provide 'ascorbate water'.

De-ionized water from an 'Elgastat' de-ionizer is used in the preparation of all solutions.

*Lactobacillus casei* (ATCC 7469, NCIB 8010) is maintained as a stab culture in micro-culture agar (Difco) and sub-cultured weekly. The inoculum is prepared according to the method of Chanarin and Berry<sup>2</sup>, but sterile physiological saline is used instead of single-strength assay medium to wash the bacterial deposit.

The stock pteroyl-glutamic acid solution is diluted in water to a working solution of 0.4  $\mu\text{g}/\text{ml}$ . immediately before use. A duplicate set of tubes (2 ml. in each) containing 0.0, 0.1, 0.2, 0.4 and 0.8  $\mu\text{g}$  of folio acid is prepared from the working solution; 2 ml. of double-strength assay medium is added to each tube and the tubes are closed with aluminium caps. The final folio acid concentrations are 0.0, 0.025, 0.05, 0.1 and 0.2  $\mu\text{g}/\text{ml}$ . The tubes are autoclaved at 10 lb./in.<sup>2</sup> for 10 min.

For each serum to be assayed, 1.96 ml. of ascorbate water is added to each of two tubes containing 2 ml. of double-strength assay medium. After closing the tubes with aluminium caps, they are autoclaved at 10 lb./in.<sup>2</sup> for 10 min. On cooling, 0.04 ml. of serum is added to each of the two tubes (the serum dilution is 1:100). All the tubes, including those of the standard curve, are inoculated with one drop of the *L. casei* inoculum and then incubated at  $37^{\circ}\text{C}$  for 18 h.

The turbidities are measured in a photo-electric colorimeter ( $\lambda = 510\text{--}590$ ). A mixture of the duplicate 0.0 tubes of the standard curve is used to 'zero' the instrument. (An uninoculated serum blank tube is not necessary.)

The turbidities of the folio acid standard curve are plotted on arithmetic graph paper against the concentration of folio acid in  $\mu\text{g}/\text{ml}$ . The 'folio-acid' activity of the serum is calculated from the resultant curve, taking into consideration the serum dilution. For greater accuracy, sera with 'folio-acid' activities below 2.5  $\mu\text{g}/\text{ml}$ . and above 20  $\mu\text{g}/\text{ml}$ . are re-assayed at lower and higher serum dilutions, respectively.

**Effect of ascorbic acid on the growth of *L. casei*.** Two sets of the standard curve were set up in duplicate; one set was prepared in water and the other set in ascorbate water (2 mg/ml.). After being autoclaved, the tubes were inoculated with the *L. casei* inoculum and then incubated at  $37^{\circ}\text{C}$ . The turbidities were measured using a 'medium blank', an uninoculated single-strength assay medium, to 'zero' the instrument.

Table 1. EFFECT OF ASCORBIC ACID ON GROWTH OF *L. casei*

	Folio acid in $\mu\text{g}/\text{tube}$				
Standard curve	0.0	0.1	0.2	0.4	0.8
Turbidities in water	45	60	70	85	95
Turbidities in ascorbate water	44	58	73	85.5	99

Instrument set with 'medium blank'.

The results are indicated in Table 1. They show that, at the concentration used, the ascorbic acid has no effect, either stimulant or depressant, on the growth of *L. casei*.

**Effect of phosphate buffer on the growth of *L. casei*.** Water and phosphate buffer (0.1 M; pH 6.1) were first autoclaved at 10 lb./in.<sup>2</sup> for 10 min. (A) Three sets of

Table 2. EFFECT OF PHOSPHATE BUFFER ON THE GROWTH OF *L. casei*

A. Tubes containing no folio acid			
Water	2.0 ml.	1.6 ml.	0.0 ml.
Phosphate buffer	0.0	0.4	2.0
Assay medium	2.0	2.0	2.0
Turbidities	45	50	65

Instrument set with 'medium blank'.

B. The standard curve				
Standard curve	0.0	Folio acid in $\mu\text{g}/\text{tube}$		
		0.1	0.2	0.4
Turbidities in water	45	60	70	85
		15	25	40
Turbidities in buffer	65	78	86	103
		18	21	38

Instrument set with 'medium blank'.

five tubes were set up. 2 ml. water was added to each tube of the first set; 1.6 ml. water and 0.4 ml. buffer was added to each tube of the second set; 2 ml. buffer was added to each tube of the third set. 2 ml. double-strength assay medium was then pipetted into all the tubes. (B) Two sets of the standard curve were prepared in duplicate; water was used for one set and the buffer for the second set. All the tubes of 'A' and 'B' were capped, autoclaved, inoculated and incubated at  $37^{\circ}\text{C}$ . The turbidities were measured after 18 h, using a 'medium blank', an uninoculated single-strength assay medium, to 'zero' the instrument.

The results are given in Table 2 'A' and 'B'. They demonstrate that there is increased growth of *L. casei* in the presence of phosphate buffer and that the inclusion of an inoculated 'buffer blank' tube is necessary.

**The *L. casei* activity of stored serum.** The *L. casei* activity of four sera, without and with ascorbic acid (5 mg/ml.), which had been in storage at  $-20^{\circ}\text{C}$  for more than 3 months, was studied. The modified 'aseptic addition' method was used with and without the ascorbate water.

The results are given in Table 3. They show that the *L. casei* activity of the 'stored serum without ascorbic acid' was similar to that of the 'stored serum with ascorbic acid' when ascorbate water was used, but was less when water replaced the ascorbate water in the assay procedure.

Table 3. *L. casei* ACTIVITY OF STORED SERUM

Mod. 'As. Ad.' method using	Serum With ascorbic acid		Without ascorbic acid	
	Water	Ascorbate water	Water	Ascorbate water
1	48	47	28	51
2	107	106	85	104
3	97	94	70	98
4	75	76	60	72

Instrument set with 'medium blank'.

**Comparison of the *L. casei* activity of serum measured by the method of Waters and Mollin<sup>1</sup> and the modified 'aseptic addition' method.** Twenty paired sera, that is, sera with and without ascorbic acid, were used. The assay procedure of Waters and Mollin<sup>1</sup> was used on the serum containing ascorbic acid (5 mg/ml.); an inoculated 'buffer blank' tube, containing an equivalent amount of autoclaved ascorbate phosphate buffer, was included for the purpose of setting the instrument when the turbidities of these tubes were measured. The modified 'aseptic addition' method was used on the sera not containing ascorbic acid; an inoculated 'water blank' tube, single-strength assay medium in water, was included with these tubes to set the instrument for measuring the turbidities.

The resultant turbidities of the corresponding sera are given in Table 4. They show that the *L. casei* activity of the corresponding sera measured by the two assay procedures is essentially similar.

Table 4. COMPARISON OF SERUM *L. casei* ACTIVITY MEASURED BY THE MODIFIED 'ASEPTIC ADDITION' METHOD AND THE METHOD OF WATERS AND MOLLIN

Mod. 'As. Ad.' method	123	49	45	112	75	74	59	160	80	55
Waters and Mollin method	127	53	42	109	70	77	50	145	35	52
Mod. 'As. Ad.' method	59	81	88	118	77	58	94	40	83	60
Waters and Mollin method	65	92	85	109	81	62	90	45	39	55

**Discussion.** The modified 'aseptic addition' method described in this article differs in certain respects from

that of Herbert and Zalusky<sup>1</sup>. The assay medium of Waters and Mollin<sup>2</sup> with additional L-tryptophan is used instead of the assay medium of Herbert<sup>4</sup>; the final volume in each assay tube is reduced from 10 ml. to 4 ml.; ascorbate water (2 mg/ml.) replaces ascorbate phosphate buffer (10 mg/ml.; pH 6.1).

A number of factors influenced the decision to use a final volume of 4 ml. in each assay tube. The most important was that with this volume it was possible to measure the 'folio/acid' activity on a very small sample of serum (0.04 ml.) without loss of accuracy which would have been the case with the high dilution necessary with a final volume of 10 ml. The use of a small volume of serum is particularly of great value in paediatric cases and in those adults in whom venepuncture is difficult.

Herbert<sup>4</sup> found that ascorbic acid and phosphate buffer stimulated the growth of *L. casei* and consequently used ascorbate phosphate buffer in the preparation of both the serum tubes and the standard curve tubes. Waters and Mollin<sup>2</sup> demonstrated that ascorbate phosphate buffer had no effect on the growth density of the organism. Ball and Giles<sup>5</sup>, using the method of Waters and Mollin for measuring serum *L. casei* activity, added ascorbic acid (1 mg/ml.) as a routine to the double-strength assay medium in order to stimulate the growth of the organism and to stabilize the relationship between the serum tubes and the standard curve tubes. The investigations reported here showed that, whereas the growth of *L. casei* was increased in the presence of phosphate buffer, ascorbic acid in the concentration used had no effect.

The phosphate buffer solution was eliminated from the modified 'aseptic addition' method because the buffering capacity of the assay medium was considered adequate and consequently its inclusion in the serum tubes and the standard curve tubes was regarded as superfluous.

In view of the finding that ascorbic acid, in the concentration used, has no effect on the growth of the organism, the ascorbate water is not used in the preparation of the standard curve. Its use in the serum tubes is essential because ascorbic acid is not added to serum either before or after storage. However, those who prefer to add ascorbic acid (5 mg/ml.) to the serum prior to storage may do so without affecting the eventual result, for the amount of ascorbic acid in the assay tube is still insufficient to destroy folate. Using the method as described, the tedium of weighing our ascorbic acid for each serum sample received, and the storage of the samples as paired sera, were avoided. The concentration of ascorbic acid was reduced to 2 mg/ml., for even at this concentration sufficient remains after autoclaving to restore any *L. casei* activity 'lost' in the stored serum.

The lability of the *L. casei* activity of serum is an established fact, as is the value of ascorbic acid in respectively preventing and protecting its decline and destruction. Herbert<sup>4</sup>, however, considered that the activity was stable in the frozen state. On the other hand, the results of an investigation by Waters and Mollin<sup>2</sup>, which were substantiated by Chanarin and Berry<sup>3</sup>, clearly demon-

strated a decline in the activity in serum stored without added ascorbic acid at -20° C; they also showed that the 'lost' activity could be restored by the addition of ascorbic acid (5-15 mg/ml.) to the serum after storage. The results of the investigation reported in this article showed that with the modified 'aseptic addition' method there was no difference in the activity of serum stored with or without ascorbic acid if ascorbate water was used in the serum assay tubes, but that there was a difference if the ascorbate water was replaced by water. An 'aseptic addition' method, using ascorbic acid in the serum assay tubes, thus masks any decline of *L. casei* activity which may have occurred in the serum during storage at -20° C, by restoring it to its original level during the assay itself.

Herbert<sup>4</sup> emphasized that stringent aseptic techniques were not essential when handling the serum. He did not regard bacterial contamination as a problem because of the rapid growth of *L. casei* and the quantity of lactic acid produced during the assay. His experience was confirmed in this laboratory by sub-culturing on to blood agar plates a number of the serum assay tubes before the measurement of their turbidities; these sub-cultures were incubated at 37° C and examined after 18 h; they revealed no contamination with other organisms.

Before adopting, it was essential to evaluate the modified 'aseptic addition' method, especially with the view of deciding on the necessity of determining the normal range of serum 'folio/acid' activity for the method. Comparison of *L. casei* growth densities obtained by the modified 'aseptic addition' method and the method of Waters and Mollin on paired sera showed that the results were comparable; Herbert<sup>4</sup> observed a similarity between his results and those of Waters and Mollin. Because of these observations and findings, the figures reported by Herbert and Zalusky<sup>1</sup> are accepted for the modified 'aseptic addition' assay procedure. Levels of serum 'folio/acid' activity below 3 µg/ml. are regarded as diagnostic of folate deficiency, while levels between 3 and 4.9 µg/ml. are strongly suggestive; levels between 5 and 7 µg/ml. are considered to be equivocal and the normal range is given as 7-16 µg/ml.

The modified 'aseptic addition' assay procedure is less time-consuming than other methods; a small volume of serum only is required and this enables the technique to be applied to paediatric cases, without the necessity of resorting to venepuncture. *Lactotacillus casei* is an organism which is sensitive to many antibiotics *in vitro*, including the anti-tuberculous drugs, and consequently it is advisable not to carry out the assay on patients receiving antibiotics.

I thank Dr. K. V. Lodge for her advice and help.

<sup>1</sup> Herbert, V., and Zalusky, R., *J. Clin. Invest.*, **41**, 1253 (1962).

<sup>2</sup> Waters, A. H., and Mollin, D. L., *J. Clin. Path.*, **14**, 335 (1961).

<sup>3</sup> Chanarin, I., and Berry, V., *J. Clin. Path.*, **17**, 111 (1964).

<sup>4</sup> Herbert, V., *J. Clin. Invest.*, **40**, 81 (1961).

<sup>5</sup> Ball, M. W., and Giles, C., *J. Clin. Path.*, **17**, 165 (1964).

<sup>6</sup> Herbert, V., *Proc. Roy. Soc. Med.*, **57**, 877 (1964).

## MECHANISM OF INTESTINAL ABSORPTION IN MAN

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ABOUT six years ago, a new kind of inhibitor of intestinal absorption was discovered, namely, cetrinide<sup>1</sup>. This substance blocked the absorption of a variety of nutrients, but it also caused reversal of flow of glucose, from the blood to the intestinal lumen. In the

rabbit and guinea-pig, this reversal occurred at a concentration of the drug which left the absorption of methionine and butyrate undiminished, thus indicating direct interference with the active transport of glucose. The examination of the mode of action of this and other

compounds resulted in a new theory of the mechanism of intestinal absorption, the main features of which were summarized recently<sup>1</sup>. In the course of these investigations, striking species and strain variations came to light, and, hence, it appeared particularly important to find out what actions the various intestinal inhibitors possessed in man, and whether the new concepts regarding the mechanism of intestinal absorption applied to our species with parallel validity. Experiments were planned along lines indicated by these previous studies in animals, and a preliminary account of the results is given in this article.

The experiments were carried out with complete safety on volunteers undergoing abdominal operations for a variety of reasons. After washing through a length of jejunum, beginning at a point 15 cm distal to the duodeno-jejunal flexure, with 0.9 per cent (w/v) saline at 37° C, four segments, 12 cm in length each, were firmly but gently clamped off, forming four completely isolated sacs. Into these sacs were injected 50 ml. of saline-nutrient solutions, containing 0.9 per cent sodium chloride and 0.2 per cent (w/v) of D-glucose, sodium butyrate and DL-methionine, and the effect of different concentrations of the drugs ( $0.5 \times 10^{-4}$  –  $8 \times 10^{-4}$ ) was investigated. In most experiments, the four sacs contained cetrinide, phloridzin, cetrinide + phloridzin and a control solution without drug. The order of sacs in relation to the drug used was varied, so as to isolate the effect of distance from the duodeno-jejunal flexure on absorption by an

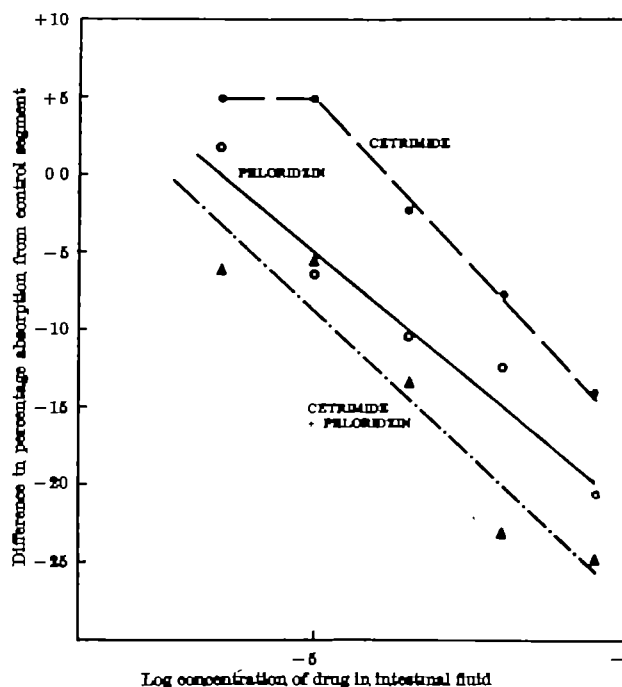


Fig. 1

Table 1. MEAN PERCENTAGE ABSORPTION OF GLUCOSE IN MAN FROM 12-CM JEJUNAL SEGMENTS CONTAINING DIFFERENT CONCENTRATIONS OF CETRINIDE, PHLORIDZIN, AND CETRINIDE+PHLORIDZIN

Values represent mean  $\pm$  S.E. of four experiments, except where number is indicated in parentheses

Concentration of drug	Cetrinide	Phloridzin	Cetrinide + phloridzin
0.0 (control)	52.46 $\pm$ 2.27 (36)		
$0.5 \times 10^{-4}$	52.2 $\pm$ 8.9	49.1 $\pm$ 7.8	41.2 $\pm$ 5.2
$1 \times 10^{-4}$	60.1 $\pm$ 4.1 (19)	33.5 $\pm$ 3.7 (7)	40.2 $\pm$ 4.6 (5)
$2 \times 10^{-4}$	44.2 $\pm$ 7.5	36.1 $\pm$ 5.4	33.1 $\pm$ 6.3
$4 \times 10^{-4}$	48.3 $\pm$ 3.4	43.6 $\pm$ 4.5	33.1 $\pm$ 7.7
$8 \times 10^{-4}$	31.1 $\pm$ 4.0	27.1 $\pm$ 4.2	23.9 $\pm$ 8.1
Mean of group for all doses	47.2	37.9	34.1

Table 2. DIFFERENCE IN PERCENTAGE ABSORPTION OF GLUCOSE IN MAN FOR 12-CM JEJUNAL SEGMENTS CONTAINING DIFFERENT CONCENTRATIONS OF CETRINIDE, PHLORIDZIN, AND CETRINIDE+PHLORIDZIN FROM VALUES IN CORRESPONDING CONTROL JEJUNAL SEGMENT

Values represent mean of four experiments, except where number is indicated in parentheses. In the last column, the figures in parentheses represent the corresponding theoretically calculated values from the dose-response curves for cetrinide and phloridzin respectively, on the basis of logarithmic synergism alone

Concentration of drug	Cetrinide	Phloridzin	Cetrinide + phloridzin
$0.5 \times 10^{-4}$	-4.976	-1.600	6.060 (-4.90)
$1 \times 10^{-4}$	-4.996 (19)	6.443 (7)	5.400 (0.99)
$2 \times 10^{-4}$	2.300	10.425	13.450 (11.15)
$4 \times 10^{-4}$	7.825	12.525	23.060 (20.60)
$8 \times 10^{-4}$	14.250	20.725	24.960 (28.16)

Table 3. ANALYSIS OF VARIANCE, DOSE-RESPONSE LINES, DIFFERENCES BETWEEN SLOPES, AND POTENCY RATIOS FOR CETRINIDE, PHLORIDZIN, AND CETRINIDE+PHLORIDZIN IN 5-MIN ABSORPTION EXPERIMENTS IN MAN, FROM 12-CM JEJUNAL SEGMENTS IN MAN

	Cetrinide (a)	Phloridzin (b)	Cetrinide + phloridzin (c)
Analysis of variance			
Source of variation			
Linear regression	$P < 0.001$	$P < 0.001$	$P < 0.01$
Deviation from regression	$P > 0.2$	$P > 0.2$	$P > 0.2$
Dose-response formula ( $s = \log \text{conc.} + 6$ )	$y = 21.28s - 26.27$	$y = 16.63s - 11.74$	$y = 18.70s - 9.92$
Combined analysis of variance			
Between group (a) and (b)			
Source of variation			
Between drugs	$P < 0.001$	$P < 0.001$	$P < 0.1 > 0.05$
Between intestinal segments	$P > 0.2$	$P > 0.2$	$P > 0.2$
Between doses	$P < 0.001$	$P < 0.001$	$P < 0.01$
Difference between slopes	4.75 $P < 0.5 > 0.4$	2.68 $P < 0.8 > 0.7$	2.07 $P < 0.8 > 0.7$
Potency ratio	2.87	4.47	1.73

analysis of variance. The solutions were withdrawn after an absorption interval of 5 min, the volume measured, and estimations carried out on glucose, butyrate and methionine as previously<sup>1</sup>. The percentage absorption of the nutrients was calculated from the amount of nutrient recovered out of the total 100 mg put in. During the experiment, the segments were kept as close to normal body temperature as possible. The duration of the whole experiment averaged 11 min. The injection tracks were sealed with fibrin foam at the end. We shall confine ourselves to an analysis of the effects of the drugs on glucose absorption and some comments on the absorption of butyrate and methionine. A more detailed account will follow elsewhere.

The mean percentage absorption,  $\pm$  S.E., for glucose, butyrate and methionine in 36 control sacs was  $52.46 \pm 2.27$ ,  $44.27 \pm 2.37$ , and  $40.17 \pm 1.92$  respectively. The design of the experiment, and the inclusion of a control sac in each subject, was fully justified by the results. As anticipated, the variation between experiments on different individuals was such as to preclude the establishment of any particular effect at a satisfactory significance level, from a small number of experiments. This is evident from Table 1, where the mean percentage absorption values of glucose are shown for different concentrations of the drugs. There is probable stimulation of absorption at  $10^{-4}$  cetrinide, and inhibition at larger doses. From the bottom line, which gives the mean percentage absorption for each column, one may conclude greater inhibitory effect for phloridzin than for cetrinide, and synergistic inhibition when the drugs were combined. It is clear that no satisfactory significance for regression of dose on response would emerge from these data, nor for any of the other usual analytic comparisons. On presenting the results as the difference in the percentage absorption for each sac from its control value on the same subject, however, we obtain a strikingly different picture (Table 2 and Fig. 1). We are now able to demonstrate linear regression and draw other conclusions with a high order of certainty. The importance of this experimental design in our study is thus established with little doubt.

The results of an analysis of variance for the three dose-response lines of cetrinide, phloridzin, and cetrinide + phloridzin are given in Table 3, together with the dose-response formulae. Deviation from regression was

not significant for any of the lines, nor was the difference between slopes. The potency ratios showed the inhibitory effect of phloridzin to be 2.87 times that of oestrime, though this difference must be exaggerated by the stimulating effect of oestrime at low concentrations. The effect of the two drugs together was greater by a factor of 4.47 as compared with the effect of oestrime alone, and by 1.73 as compared with phloridzin. All the differences mentioned showed a high order of significance except the latter, that is, the difference in potency between phloridzin and oestrime + phloridzin, for which  $P$  was only just smaller than 0.1. However, the parallel greater activity of the mixture, which was also obtained with regard to butyrate and methionine absorption, lends greater significance to this result. Further, Table 3 shows no significance for order of segment on intestinal absorption.

Increased absorption of glucose was obtained at the lower concentrations of oestrime,  $0.5 \times 10^{-3}$  and  $10^{-4}$ . This is in agreement with the results obtained previously in animals<sup>1,2</sup>. Since regression evidently stops at  $10^{-4}$  oestrime, the lower concentration of  $0.5 \times 10^{-4}$  was not included in the calculation of the dose-response formula, nor in the analysis of variance. A 't' test carried out for the mean difference of these two groups (23 expts.) from their control established stimulation at the lower doses of oestrime with a  $P \approx 0.001$ . A similar increase of butyrate absorption at these doses occurred with a mean of 4.8 (= 11 per cent), which gave a 't' test with  $P < 0.05$ , but the difference was smaller for methionine absorption with a mean of 2.2 (= 5.5 per cent), for which no statistical significance was obtained.

The present investigation was restricted to concentrations of  $8 \times 10^{-4}$  or less, but in spite of this limitation, significant linear regression with dose was obtained for all three sets of experiments. The results in man reinforce in several aspects those obtained in animals<sup>1,2</sup>, but there are nevertheless some interesting differences. The stimulation of glucose and butyrate absorption with low doses of oestrime was parallel to the stimulation previously demonstrated with the same substance in the mouse and rat, and may be concluded to have the same significance, namely, that it is the result of the combination of oestrime with mobile intracellular proteins acting as carriers for the different nutrients. This conclusion follows from the analysis of the stimulant effect of inhibitors at low concentrations, previously shown<sup>1,2</sup> to require several mobile protein carriers, which can only be accommodated in the intracellular plasma. Presuming that, as in the mouse and rat, phloridzin acts primarily on a first receptor in relation to a portal of entry through the cell membrane, the site of action of oestrime would constitute the second receptor in this two-stage mechanism of intestinal absorption. Within the present dose range, no stimulation of glucose absorption was obtained with low concentrations of phloridzin, though this may be demonstrated at still lower concentrations, if phloridzin exhibits

affinity for the second receptor as in the mouse. Stimulation of the absorption of butyrate of about 10 per cent, and of methionine of about 15 per cent, was, however, obtained with phloridzin, so that combination with the protein carriers of butyrate and methionine may be presumed.

As in the mouse and rat, and contrary to its effect in the rabbit and guinea-pig, phloridzin showed greater potency in man than did oestrime. On the other hand, the synergistic effect exhibited when the two drugs were combined was of a much smaller order than that obtained in the mouse and rat. In these two species, synergism was shown to require conservation of phloridzin from silent receptors through displacement by oestrime, in addition to logarithmic synergism through action on two distinct receptors in series. The weaker synergism obtained in man suggested at first that only logarithmic synergism may be involved, due to the combined action of phloridzin at the first receptor and of oestrime at the second receptor. A theoretical curve based on a hypothesis of logarithmic synergism can be constructed from the two dose-response formulae for phloridzin and oestrime. The results give reasonable agreement with experiment for the three highest doses of the combined drugs, but show striking deviation at the lowest doses, where logarithmic synergism gives stimulation of absorption (Table 3). Furthermore, no stimulant effect was obtained when the two drugs were used together at the lower concentrations of any of the three nutrients. This suggests strongly that conservation of phloridzin from silent receptors must still be a factor to be reckoned with, in addition to the expected logarithmic synergism, though this conservation must be of considerably smaller value than in the mouse, and assumes importance only at lower concentrations of the drugs.

In summary, the present investigation of the effect of oestrime and phloridzin on intestinal absorption in man suggests that these compounds have activities similar to those already demonstrated in the mouse and rat. Both the stimulation at lower doses of oestrime and the potentiation between oestrime and phloridzin suggest that the theory previously advanced for the mechanism of absorption in the lower species also holds in man. Besides a portal of entry, which in the case of glucose and butyrate is blocked by phloridzin, the function of active transport against a gradient is vested with a spectrum of intracellular proteins, which have overlapping but distinctive affinities. Hence, detailed study of these proteins, involving their affinities to nutrients and drugs and their fractionation and identification, constitutes the logical next step in the further elucidation of the mechanism of absorption.

<sup>1</sup> Nisum, J. A., *Nature*, **185**, 232 (1960); **187**, 308 (1960); **204**, 148 (1964); *Brit. J. Pharmacol.*, **24**, 205 (1965); also observations to be published elsewhere.

<sup>2</sup> Hart, B. L., and Nisum, J. A., *J. Physiol.*, **169**, 119P (1963); *Nature*, **204**, 51 (1964); also observations to be published elsewhere.

## NOREPINEPHRINE METABOLISM IN HYPERTROPHIED RAT HEARTS

By DR. JOSEF E. FISCHER, W. DALE HORST and IRWIN J. KOPIN

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CIRCULATING norepinephrine is initially inactivated largely by being taken up and bound in sympathetic nerve endings<sup>1</sup>. In certain pathological conditions such as hyperthyroidism, an increased sensitivity to norepinephrine has been suggested on clinical grounds. It has afterwards been shown that, in hyperthyroid rats, because of blood flow changes a greater portion of intravenously administered catecholamine reaches the enlarged

hearts of hyperthyroid animals, but the hypertrophied myocardium is not able to bind more administered norepinephrine than normal hearts<sup>2</sup>. There probably is no increase in sympathetic nerve supply as the heart hypertrophies. It was therefore suggested<sup>3</sup> that the net decrease in density of these important catecholamine-inactivating structures was the reason that less norepinephrine is bound per gram of heart muscle in hyperthyroidism<sup>3,4</sup> and

Table 1.  $^{45}\text{K}$  and  $^3\text{H}$ -NOREPINEPHRINE UPTAKE BY THE HYPERTROPHIED RAT HEART

Heart weight group (g)	Mean heart weight (g/100 g of rat)	$^{45}\text{K}$ (20 sec)		$^3\text{H}$ -Norepinephrine o.p.m. $\times 10^3$ (2 min)	
		per heart	per g heart	per heart	per g heart
<1.0 (27)	0.28	1.65 $\pm$ 0.16	1.71 $\pm$ 0.94	222.2 $\pm$ 12.1 (7)	222.2 $\pm$ 8.6
1.0-1.25 (21)	0.33	1.68 $\pm$ 0.40	1.46 $\pm$ 0.39	301.7 $\pm$ 20.8 (7)	257.3 $\pm$ 24.4
1.25-1.50 (19)	0.38	1.87 $\pm$ 0.61	1.36 $\pm$ 0.44	291.8 $\pm$ 12.8 (6)	213.0 $\pm$ 10.3
1.50+ (5)	0.51	2.60* $\pm$ 0.13	1.26 $\pm$ 0.6	181.2* $\pm$ 28.5 (5)	102.0* $\pm$ 6.0

\* Differs from control  $P < 0.01$ .Rats received  $^{45}\text{K}$  and  $^3\text{H}$ -norepinephrine, 20 sec or 2 min, respectively, before being killed. Hearts were assayed as described in the text. The results are the means  $\pm$  S.E.M. for the number of animals shown in parentheses.Table 2. DISAPPEARANCE OF  $^3\text{H}$ -NOREPINEPHRINE FROM THE HYPERTROPHIED RAT HEART

Heart weight (g/100 g rat)	o.p.m. $\times 10^3$ per heart per 2 min	Percentage of initial content			
		2 min	1 h	6 h	24 h
0.28	222.2 $\pm$ 12.1	100 (7)	86.8 $\pm$ 3.5 (6)	55.0 $\pm$ 4.0 (6)	18.2 $\pm$ 2.9 (8)
0.33	301.7 $\pm$ 20.8	100 (7)	66.5 $\pm$ 5.6 (4)	49.8 $\pm$ 0.65 (5)	19.0 $\pm$ 2.6 (5)
0.38	291.8 $\pm$ 12.8	100 (5)	72.0 $\pm$ 5.8 (4)	33.5* $\pm$ 3.9 (5)	11.9* $\pm$ 1.4 (5)

\* Differs from control,  $P < 0.01$ . $^3\text{H}$ -Norepinephrine was administered intravenously and the animals killed 2 min, 1 h, 6 h, or 24 h after administration of the labelled amine. The results are expressed as the percentage of the initial (2 min)  $^3\text{H}$ -norepinephrine contents and are the means  $\pm$  S.E.M. for the number of animals shown in parentheses. † Hearts were assayed as described in the text.

that this contributes to the supersensitivity to catecholamines.

Cardiac hypertrophy accompanies a variety of clinical entities. The sympathetic nervous system is thought to play an important part in cardiovascular physiology but presumably is anatomically unaltered in cardiac hypertrophy. It was therefore desirable to study the effects of cardiac hypertrophy on the disposition of norepinephrine, in the absence of the hypermetabolic state of hyperthyroidism.

Male Sprague-Dawley rats, weighing 140-160 g, were anaesthetized with ether, and the abdominal aortae constricted above the superior mesenteric artery with a single No. 40 cotton ligature. The ligature was tightened while a 20-gauge hypodermic needle was held adjacent to the aorta, to obtain consistency in the degree of constriction.

Two months later, a similar procedure was performed on litter mates of the first group. Operative mortality was 5-10 per cent. In another group of rats the ligature was tightened over a 23-gauge needle, but only a small number of rats survived this severe degree of aortic constriction.

Sham-operated animals of the same age and weight served as controls. Animals were weighed periodically, and any animals with evidence of infection (poor weight gain) were discarded.

In the following experiments, endogenous and administered labelled norepinephrine in the myocardium, as well as the fraction of the cardiac output perfusing the heart, were estimated in each animal. Blood flow was estimated using the  $^{45}\text{K}$  technique of Sapirstein<sup>4</sup>.

Two or four months after aortic constriction, the experimental animals and their sham-operated controls each received 90  $\mu\text{C}$  d,l-7-norepinephrine- $^3\text{H}$  (20 mc/mg, New England Nuclear Corp.) intravenously and were killed by decapitation 2 min, or 1, 6, or 24 h later. Twenty seconds before decapitation, 1  $\mu\text{C}$   $^{45}\text{K}$  (as KCl, adjusted to 4 mequiv./ml.) was injected intravenously. Hearts were rapidly removed, cooled on ice, weighed, and assayed for  $^{45}\text{K}$  in a gamma ( $\gamma$ ) well counter. A  $^{45}\text{K}$

standard representing 1 per cent of the injected dose was assayed periodically to correct for  $^{45}\text{K}$  decay. Hearts were then homogenized in 10 volumes of cold 0.4 N perchloric acid and assayed for tritiated<sup>5</sup> as well as endogenous<sup>6</sup> norepinephrine. The 5 animals which survived aortic constriction to the diameter of a 23-gauge needle constituted a single group killed 2 min after intravenous injection of  $^3\text{H}$ -norepinephrine.

Cardiac weights of the animals with aortic constriction to a 20-gauge needle ( $1,284 \pm 24$  mg) were significantly greater ( $P < 0.001$ ) than those of the sham-operated controls ( $934 \pm 9$  mg).

None of the hearts of the sham-operated animals exceeded 1 g. The hearts of animals with aortic constriction (over a 20-gauge needle) were divided into two groups on the basis of weight. Hearts weighing between 1 and 1.25 g were classified as mildly hypertrophied, and those weighing 1.25-1.50 g as moderately hypertrophied. Severe cardiac hypertrophy ( $> 1.50$  g) was seen only in rats with marked (23-gauge needle) aortic constriction.

The body weight of rats with aortic constriction ( $349 \pm 18$  g) was not greater than the sham-operated controls ( $358 \pm 15$  g). There was no evidence of congestive heart failure, pulmonary congestion, hepatomegaly, effusions, cyanosis, or oedema in any of the animals.

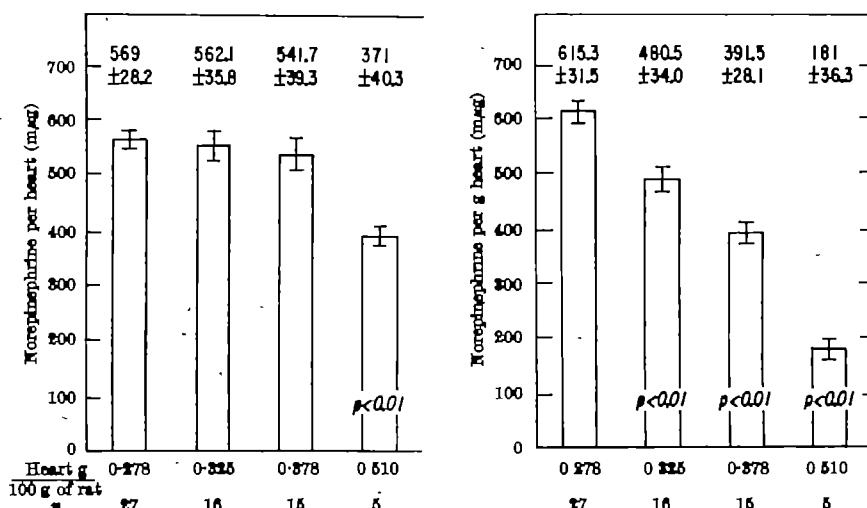


Fig. 1. Norepinephrine content of normal and hypertrophied rat hearts, expressed in terms of content (left histogram) and concentration (right histogram)

Myocardial concentration of norepinephrine was diminished in hypertrophied hearts, but with mild or moderate cardiac hypertrophy the total norepinephrine content was unaltered (Fig. 1). This is consistent with the view that norepinephrine is largely confined to the sympathetic nervous tissue and that this tissue is unaltered with myocardial hypertrophy. When the heart was markedly hypertrophied, the total norepinephrine content, as well as concentration, was diminished.

The total content of  $^3\text{H}$ -norepinephrine two minutes after administration of the labelled catecholamine was not altered in mildly or moderately hypertrophied hearts, but was decreased in markedly hypertrophied hearts (Table 1); the concentration was, however, reduced in all cases and was inversely related to the degree of hypertrophy.

When  $^{42}\text{K}$  is injected intravenously, the labelled ion reaches the various organs in proportion to the percentage of the cardiac output perfusing the organ. Although there is not complete extraction of  $^{42}\text{K}$  by the tissue, a large portion delivered to each organ remains there, presumably because of the large intracellular store of potassium and the rapid equilibrium of intracellular and circulating potassium. The percentage of injected  $^{42}\text{K}$  found in an organ has been used to estimate the proportion of cardiac output perfusing the organ<sup>4</sup>.

With myocardial hypertrophy subsequent to abdominal aortic stenosis the proportion of injected  $^{42}\text{K}$  found in the heart, and presumably the proportion of the cardiac output perfusing this organ, increases (Table 1) so that no significant difference was found in the proportion of cardiac output perfusing each gram of normal or hypertrophied heart. The proportion of administered norepinephrine- $^3\text{H}$  delivered to normal and hypertrophied hearts would be expected to parallel the uptake of  $^{42}\text{K}$ . Thus, although each gram of hypertrophied myocardium received about the same amount of injected norepinephrine- $^3\text{H}$  as did normal heart muscle, two minutes later there was a lower concentration of the tritiated catecholamine in the moderately and markedly hypertrophied hearts.

Initially the total amount of tritiated norepinephrine present in moderately hypertrophied hearts was the same as in hearts of normal animals, but less of the labelled catecholamine was present 6 or 24 h later (Table 2). These results suggest that there is a more rapid rate of turnover of norepinephrine in this group. The increased turnover rate in hypertrophied hearts may be the consequence of marked sympathetic overactivity or diminished ability to retain catecholamines. Since the norepinephrine concentration is unaltered in moderate hypertrophy (Fig.

1), it appears that replacement can keep pace with increased net loss of catecholamine and implies an increased rate of norepinephrine synthesis. With marked hypertrophy (Fig. 1) or cardiac failure<sup>5</sup>, the endogenous norepinephrine content of the left ventricle is diminished. The diminished norepinephrine content indicates that replacement no longer keeps pace with loss. This may be a consequence of further increase in loss of norepinephrine or a diminished rate of synthesis. Further increase in loss of norepinephrine could be a consequence of further increase in sympathetic nerve activity, or diminished rebinding of norepinephrine. The data suggest that the second mechanism may be operative, for in severely hypertrophied hearts there is a decrease in uptake of tritiated norepinephrine not related to blood flow (Table 1).

Only about 20 per cent of the norepinephrine content of the heart is derived from the circulation<sup>6</sup>. Thus, while decreased uptake could account for a small portion of the diminished catecholamine content, this could not account for the observed 35 per cent decrease in total norepinephrine content of markedly hypertrophied hearts.

When norepinephrine is released from sympathetic nerve endings, a large portion of the neurotransmitter is inactivated by being re-bound in the nerve ending. This conserves norepinephrine and diminishes the need for synthesis in maintenance of the catecholamine stores. If the mechanism for binding of norepinephrine were impaired, the net rate of norepinephrine loss would be increased, especially if increased sympathetic nerve activity occurred. The diminished uptake of exogenous norepinephrine suggests an impaired binding mechanism, and this may partially explain the increased norepinephrine loss.

With moderate hypertrophy an increased rate of synthesis might keep pace with the increased loss associated with the more rapid turnover rate that has been demonstrated. With severe hypertrophy or cardiac failure, however, metabolic derangements might prevent the maintenance of a balance between synthesis and loss, resulting in a diminished store of norepinephrine.

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## POST-NATAL ORIGIN OF MICRONEURONES IN THE RAT BRAIN

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**M**ITOTIC neurones are seldom, if ever, seen after birth in the brains of mammals, from which it is commonly concluded that neurogenesis is a pre-natal phenomenon. This conclusion is not often questioned, even though post-natal neurogenesis in some brain structures has been well established for some time. Thus it was recognized in the nineteenth century<sup>1-3</sup> that a class of small nerve cells, the granule cells, shows a high rate of proliferative activity in the cerebellar cortex in young animals. Likewise, good quantitative evidence was obtained some time ago<sup>4</sup> of an increase in the total number of nerve cells in the cerebral cortex of rats up to the 20th day after birth. In this article we shall describe recent autoradiographic evidence from our laboratory which shows clearly that a large proportion of the short-axoned neurones present in various brain structures are formed after birth in the rat.

In these investigations we injected rats systemically with tritiated thymidine. Thymidine is a specific precursor of chromosomal DNA and it is incorporated into cell nuclei only when new DNA is formed by cells preparing for multiplication<sup>5</sup>. When animals are injected with tritiated thymidine, the cells proliferating at the time of injection tend to incorporate the administered radiochemical and they thus become 'tagged'. The radioactively labelled cells are then easily identified with fine-resolution autoradiography. Moreover, by killing animals after varying periods of survival following the injection, a time-lapse record may be obtained of the fate of the originally labelled cells, such as the kinetics of their re-multiplication, and their migration and transformation.

In a pilot study<sup>6</sup> we made stereotaxic lesions in the lateral geniculate body in rats and then injected a small dose of tritiated thymidine into the lesion area. The

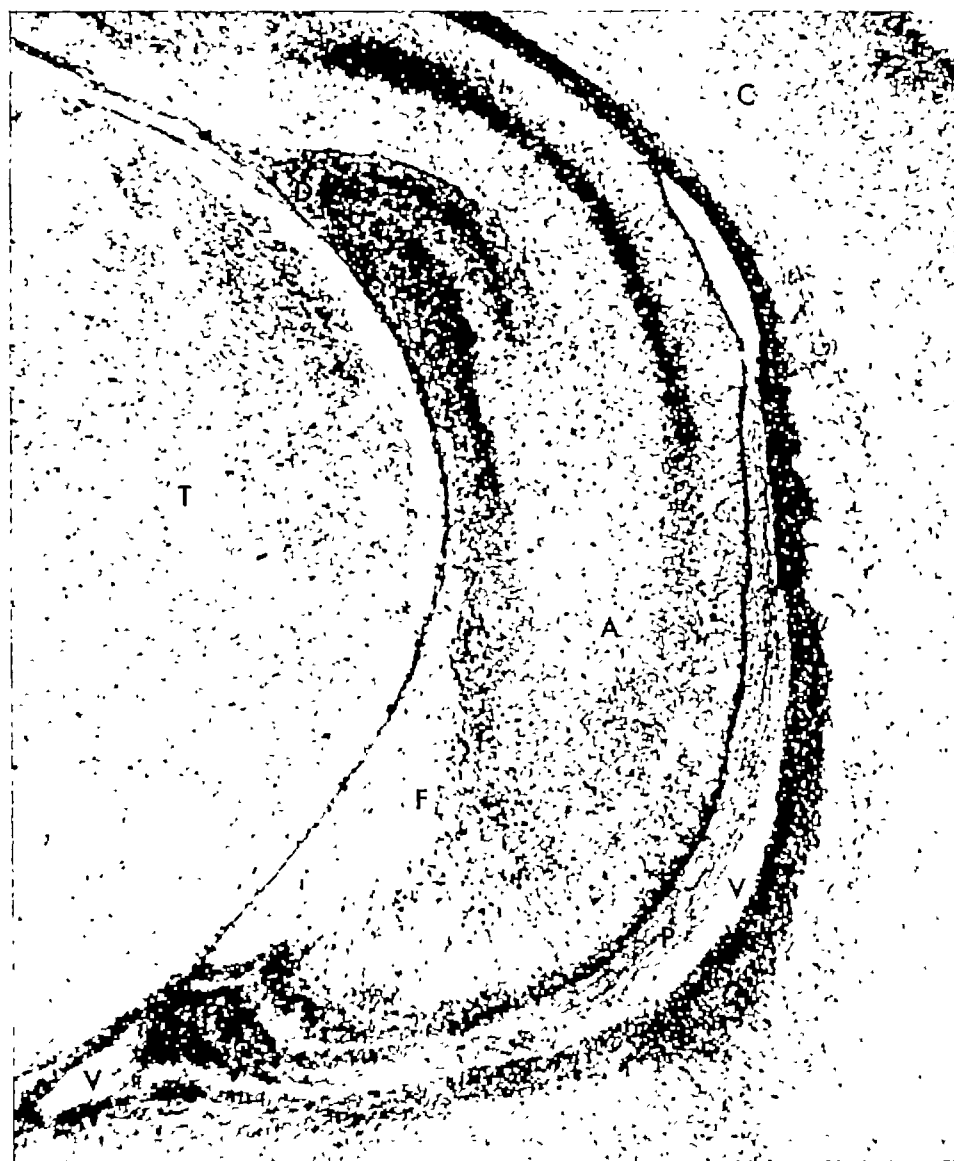


Fig. 1. Low-power photomicrograph of a galloxyanin-chromalum-stained autoradiogram of a brain section from a rat injected with tritiated thymidine at the age of 6 h and killed 24 h later. Black dots are the labelled cells. A, Ammon's horn; C, cortex; D, dentate gyrus; F, fimbria; H, hippocampus; P, choroid plexus; T, thalamus; V, lateral ventricle. ( $\times 68$ )

animals were allowed to survive for different periods after the injection and the utilization of the injected tritiated thymidine was examined by autoradiography. We found that numerous glia cells were labelled in brain regions structurally or functionally associated with the lesion site. This indicated induced proliferation of glia cells by the brain lesion. However, we found labelled glia nuclei in smaller numbers in areas which could not be related to the traumatized brain area, suggesting the possibility of glial multiplication as a normal phenomenon. In a subsequent study<sup>7</sup> we injected tritiated thymidine systemically into normal adult rats and intraventricularly into adult cats. The examination of this material showed that glia cells proliferate at a low but significant rate in virtually all parts of the normal, adult mammalian brain.

A more surprising finding was that not only the nuclei of glia cells but also the nuclei of occasional neurones were labelled in the brains of adult rats and cats<sup>8</sup>. We were particularly impressed by the labelling of granular nerve cells in the dentate gyrus of the hippocampus<sup>8</sup>. In all the animals (mature adults included) and all the sections examined a few labelled granule cells were always encountered. Since the granular layer of the dentate gyrus

is essentially devoid of the nuclei of glia cells, the labelling in this region could be attributed only to uptake of thymidine by the nuclei of the granule cells.

Accordingly, in our next investigation<sup>10</sup> we undertook to investigate in detail the nature and significance of DNA metabolism in the dentate gyrus of the hippocampus. Rats of varying ages (from neonates to adults) were injected systemically with tritiated thymidine and the animals were permitted to survive for selected periods after the injection. In addition, to obtain collateral material for histological evaluation the brains of a large series of non-injected rats of different ages were prepared for microscopy.

This study showed that the number of labelled cells in the dentate gyrus is very high in neonates and infants, and declines to a low but appreciable level in adults. Cell counting in collateral material showed that the number of differentiated granule cells in homologous sections increases six-fold from birth to 3 months of age, suggesting that the autoradiographic evidence may have reflected hippocampal neurogenesis. Counter-indicating this conclusion was the fact that mitotic cells were seldom encountered in the dentate gyrus. In further examination



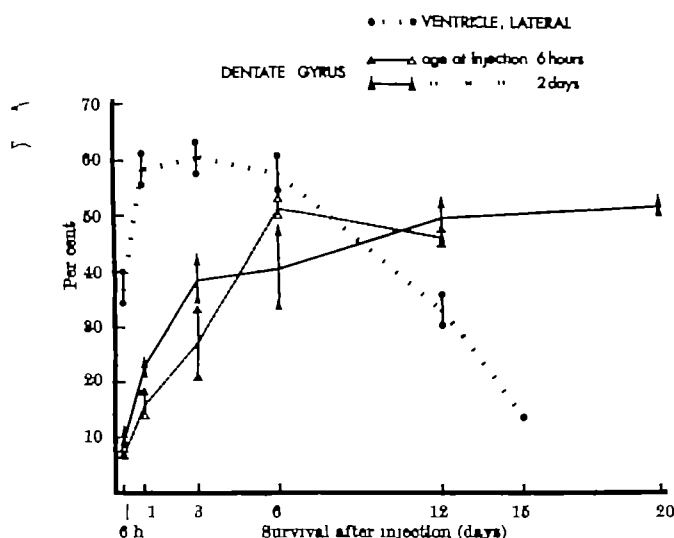


Fig. 2 Percentage of labelled cells in the germinal lateral ventricle and in the dentate gyrus as a function of survival time after injection

of this material we were impressed by the fact that in the brains of neonate and infant rats there is an extensive ependymal and sub-ependymal germinal matrix around the lateral ventricle, composed of cells with darkly staining nuclei and occasional mitotic ones. Such darkly staining cells were also seen in the fimbria, and they were particularly numerous in the dentate gyrus in the brains of the younger animals. Quantitative work showed that the number of cells and the areal extent of the lateral

ventricle declined rapidly after birth, with a transient rise at about 15 days. A delayed, rapid rise could also be seen in the number of small cells in the dentate gyrus, showing a decline also at 15 days of age. The decline in the latter was accompanied by a corresponding increase in the number of differentiated granule cells with a peak at about 3 months of age. On the basis of these observations we postulated that undifferentiated cells or neuroblasts multiply at the lateral ventricle, migrate from there by way of the fibrous tracts of the hippocampus to the dentate gyrus and become differentiated there in the course of development into granule cells.

The hypothesis of the previous investigation was confirmed in an extensive subsequent series of experiments in which groups of neonate, infant, adolescent and adult rats were injected systemically with tritiated thymidine (10  $\mu$ c./g body-wt.; specific activity 6.7 c./mmole; radiochemical dissolved 1 mc./ml. in isotonic saline). The animals were allowed to survive for 6 h, 1, 3, 6, 12, (15), 20, 30, 60, 120 and 180 days after the injection. In this article we shall deal merely with some of the results obtained in the rats injected as neonates, particularly at 2 days of age. (A detailed evaluation of the entire experimental material is in preparation.)

In neonates injected with tritiated thymidine (Fig. 2) 35–40 per cent of the cells in the ependymal and sub-ependymal layers of the lateral ventricle were labelled in the animals killed 6 h after the injection. The rate of cell labelling, with concomitant dilution of labelling within cells, rose to about 60 per cent 24 h after the injection. With prolonged survival after injection there was a considerable dilution of the label within the cell nuclei and, after 6 days of survival, a reduction in the total number of labelled cells. This suggested a continued

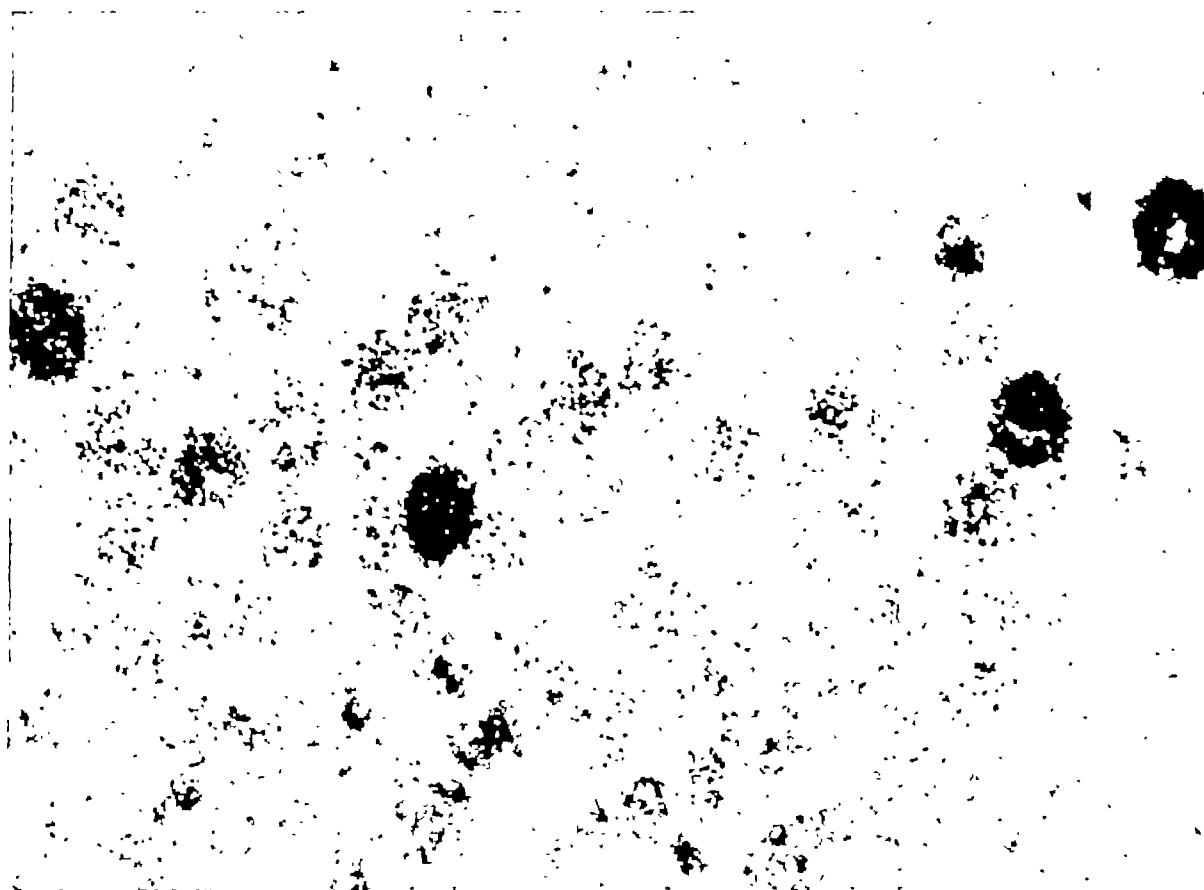


Fig. 3. High-power photomicrograph of a galloyanin-chromalum-stained autoradiogram of granule nerve cells in the granular layer of the dentate gyrus. The animal was injected with tritiated thymidine at 2 days of age and survived for 20 days after the injection ( $\times 880$ )

multiplication of the initially labelled cells and their migration to other brain regions. Cell labelling was initially low in the dentate gyrus (less than 10 per cent), and in the animals with short survival after injection these were typically small cells with darkly staining nuclei, situated in the plexiform layers surrounding the ill-developed granular layer. The number of labelled cells increased to about 45-50 per cent by the 12th day after injection, by which time the majority of labelled cells have migrated into the thickening and expanding granular layer, and many of the labelled cells had the appearance of differentiated granule cells.

The distribution and differentiation of labelled cells in the granular layer followed a regular pattern. In animals killed 20 days after the injection, the top row of cells in the granular layer (the cells in intimate contact with the superficial plexiform layer) were typically unlabelled. These we consider the 'oldest' or earliest-differentiating cells of the dentate gyrus; derived from cells which multiplied before the administered thymidine became available. Below this row of unlabelled cells were many intensely labelled cells. Absence of appreciable label dilution indicated that these cells ceased to multiply soon after the injection; that is, they represent the 'oldest' of the post-natally formed cells. Moving from the superficial to the basal rows of granule cells intensity of cell labelling decreased gradually, with many unlabelled cells to be found in the basal rows of cells; the latter represent the 'youngest' of the granule cells. Cell size and cellular differentiation showed the reverse pattern. The cells in the superficial row tended to differentiate first and were among the largest; the basal cells were smaller and often undifferentiated.

The post-natal genesis of granule cells is not an exclusive characteristic of the dentate gyrus of the hippocampus. Our results show clearly that the bulk of the granule cells in the granular, mitral and glomerular cell layers of the olfactory bulb are formed post-natally. In this region neuroblasts proliferate at a high rate around the wall of the olfactory ventricle (Fig. 4), then move out and invade gradually the layers of the olfactory bulb, where they presumably differentiate. A large proportion of the granule cells of the internal granular layer of the cerebellar cortex (and to a lesser extent the Golgi cells and basket cells) are formed post-natally. This occurs both by local multiplication and through the migration of cells multiplying in the external granular layer (which is a germinal matrix that disappears in the rat at about 20 days of age)

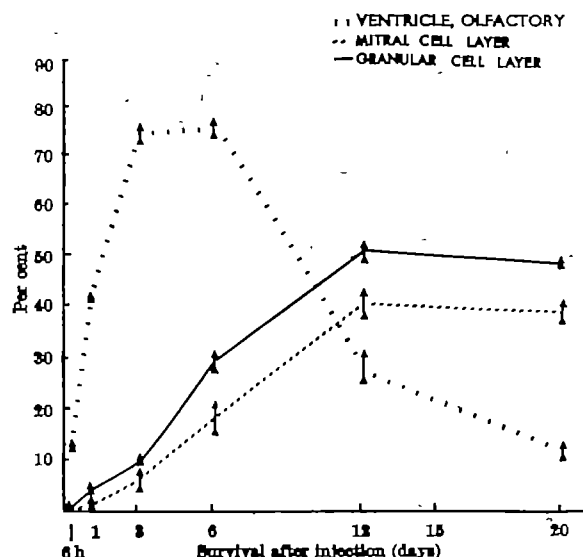


Fig. 4. Percentage of labelled cells in the germinal olfactory ventricle and in the mitral and granular cell layers of the olfactory bulb in rats injected with tritiated thymidine at 3 days of age.

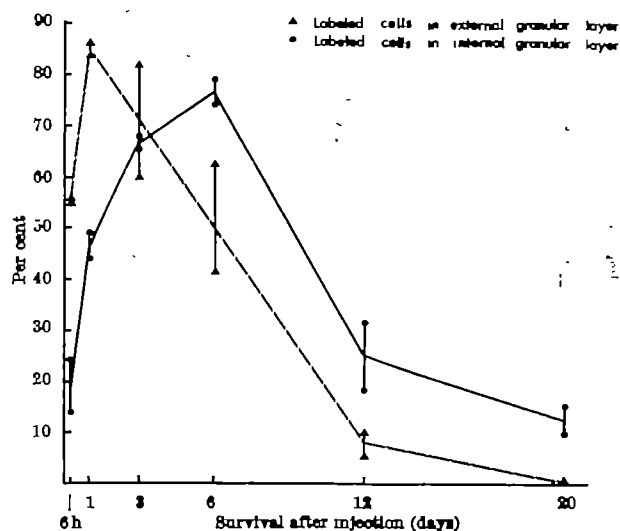


Fig. 5. Percentage of labelled cells in the external and internal granular layers of the cerebellum.

into the internal granular layer (Fig. 5). Similarly, the granule cells of the granular layer of the ventral cochlear nucleus are largely formed post-natally through migration of cells multiplying in the lateral recess of the fourth ventricle. It is very likely, though not conclusively proved, that many of the granular or stellate cells of the cerebral cortex also are of post-natal origin.

In the same structures in which a large proportion of the granule cells became labelled with tritiated thymidine, the nuclei of neighbouring large neurones remained typically unlabelled. The large mitral cells in the olfactory bulb were never seen with labelled nuclei, nor were the nuclei of the polymorph cells of the dentate gyrus, of the pyramidal cells of Ammon's horn, or the Purkinje cells of the cerebellum ever labelled. In general, large or long-axoned neurones (occasional ones in the cortex excepted) do not incorporate tritiated thymidine injected into neonates, whereas a considerable number of neuroblasts which afterwards differentiate into small, short-axoned neurones predictably utilize tritiated thymidine. From these findings we may conclude that there is a radical difference, from the developmental point of view, between the short-axoned neurones or microneurones<sup>11</sup> of the central nervous system and the long-axoned neurones or macroneurones. We shall not speculate here about the possible functional significance of the post-natal origin of the majority of microneurones. Elsewhere<sup>12</sup> we have made the suggestion that the pre-natally formed afferent, relay and efferent macroneurones represent the invariant or fixed components of the central nervous system, whereas the interposed microneurones, which develop post-natally as the animal comes to face and respond to its varied external environment, could be its modulatory and plastic elements.

This work was supported by the U.S. Atomic Energy Commission. We thank Elizabeth C. Altman and William J. Anderson for their assistance.

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## PROPRIOCEPTIVE INFLUENCE IN VOLITIONAL CONTROL OF INDIVIDUAL MOTOR UNITS

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HARRISON and Mortensen<sup>1,2</sup> recently showed that remarkably fine control over individual motor units was quickly established when auditory and visual cues to their activation were presented. Basmajian<sup>3</sup> confirmed these findings and also observed that such units could be recalled into activity without cues after training. He suggested that single anterior horn cells could be brought under conscious control by the training of descending pathways, and stated that the process by which such control is established may have a bearing on the general problem of learning.

These reports did not mention the possible role of the proprioceptive system, although it has long been recognized that such sensory information is essential for the co-ordination of motor performance<sup>4,5</sup>. It has also been shown<sup>6,7</sup> that the results of stimulation of the motor cortex of the cat can be influenced by changing the appropriate input from the responding limb.

In the course of our work with tungsten microelectrodes (ref. 8 and unpublished observations), we noted that proprioception was important in volitional activation of individual motor units. This consideration prompted us to re-examine the question of motor-unit control in the human in relation to knowledge of sensorimotor integration.

In the present investigation we used both our own type of microelectrodes and electrodes similar to those described by Basmajian. In 10 subjects, a number of different muscles were studied in minimal to moderate contraction, and the activity was recorded from one or two tungsten microelectrodes inserted into one muscle. A silver disk used as a reference electrode was placed on the skin over the tendon of the appropriate muscle. The electrodes were prepared from 10-mil straight tungsten wire by electrolytic etching until the tip was about 1  $\mu$  in diameter. The entire electrode except for the tip was insulated with either 'Formvar' or 'Epoxytite'. Before use, these electrodes, as well as the bipolar wire electrodes described below, were mounted in a special holder along with all accessory equipment and were sterilized in ethylene oxide.

The microelectrode was inserted into anaesthetized skin by placing it carefully either in a specially prepared inserter<sup>8</sup> or in a 22-gauge needle. After insertion in the muscle the carrier was gently withdrawn, leaving the electrode in place. Manipulation of the electrode was possible without bending or damage to the tip. The electrode was then connected to the recording apparatus by a fine wire lead soldered to a 1-cm length of 22-gauge

stainless steel tubing. With this type of electrode a unit was easily located when the muscle was minimally contracted.

The second type was a bipolar electrode made and inserted in a manner similar to that described by Basmajian<sup>3</sup>. Two 2-mil wires (either copper with 'Formvar' insulation or W. B. Driver Company's 'Evanohm' with nylon covering), the tips of which were bared of insulation for 1 mm, were inserted in a 22- or 24-gauge hypodermic needle so that their ends were bent back over the bevel of the needle about 4 mm from their tips. After insertion into the muscle (after sterilization) to an estimated appropriate depth, the needle was gently withdrawn, leaving the hooked wires in place. The separation of the tips of the wires was between 1.5 and 2.5 mm. The subject was rarely aware of any sensation attendant on the presence of these wires, even during fairly vigorous contraction of the muscle. This bipolar electrode was used to sample only minimal contractions in the abductor pollicis brevis, flexor pollicis brevis, and opponens pollicis in three experienced subjects (ourselves).

Both types of electrodes were connected to a remote cathode follower stage of a conventional *R-C* preamplifier. The output of the preamplifier was led into a cathode-ray oscilloscope and an audio system. The subject was comfortably seated facing the oscilloscope, so that he could both see and hear the activity. Even a naive subject quickly learned to identify single units and to distinguish one from others. We found, as did Harrison and Mortensen, that all subjects relied more on auditory than on visual cues.

In the course of the microelectrode experiments in the present investigation, it was found that factors other than auditory or visual cues played a part in the conscious activation of some individual motor units. Such units could often be followed with microelectrodes for as long as 15 min when the limb was held rigidly within a cast<sup>8</sup>. However, when no cast was worn individual units would often disappear when the subject changed his position slightly; there was generally no evidence in the traces that this could be due to damage to any unit caused by movement of the electrodes within the muscle. Occasionally, the same units would appear later if the electrode had not been moved. Little importance was attached to these observations until one subject pointed out that he could activate one specific gastrocnemius unit under study only if his ankle was maintained in plantar flexion. Fig. 1 is a recording from two such units in the gastrocnemius which showed a marked sensitivity to dorsiflexion of the ankle joint. The first part of both tracings shows the activation of these units under minimal contraction. However, when the foot was passively dorsiflexed only

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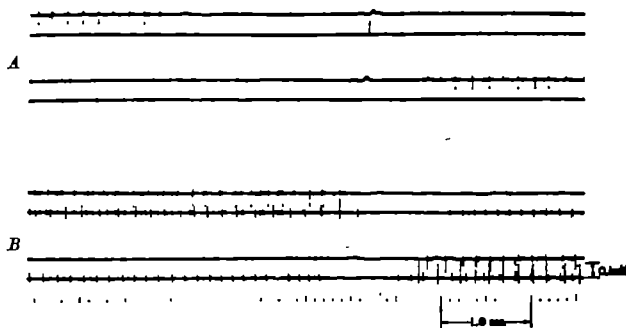


Fig. 1. Gastrocnemius unit activity recorded by means of two separate microelectrodes, each on a separate channel. In both A and B, the second pair of traces is continuous with the first pair. The indicated calibrations apply to both records. Slight baseline shifts are due to slight dorsiflexion and plantar flexion respectively. The unit shown in the top trace of A could be brought in by a minimal contraction. It could be activated at will, but only when the ankle joint was in plantar flexion. When the subject tried to reactivate the unit, he succeeded at his second attempt. The record illustrated in B was made about 5 min after that in A and with what the subject considered a slightly increased effort with the ankle joint in plantar flexion. Now at least two units are activated in the vicinity of the second electrode (bottom trace, B). One is activated along with the unit shown in the upper trace. The second is activated independently. The subject had been consciously attempting to bring in a unit during each of two different slight changes of position. When the foot was dorsiflexed  $3^\circ$  neither larger unit could be activated.

$3^\circ$  (as measured with a protractor) relative to the original position and held there and the gastrocnemius again contracted, these units were quiescent although the muscle was active. It was quickly established that in this and other subjects activation of these motor units depended on the resting length of the muscle, the position of the joint members in space, and the specific function that the muscle was called on to perform. Harrison and Mortensen made apparently somewhat similar observations, but did not point out the possible significance of these interactions since they only stated, in passing, that "slight changes in the position of the foot or leg were sometimes effective". From these experiments, which bring out the importance of the sensory system in the volitional activation of motor units, it was concluded that the neuromuscular system cannot be regarded as a simple hierarchy of motor units with thresholds of response graded according to the degree of contraction, as has often been suggested.

When these studies were extended to experiments with bipolar wire electrodes in the abductor pollicis brevis, it was found that because of the larger area of pick-up of the bipolar electrodes, a subject would invariably activate many units simultaneously when he contracted the muscle at the beginning of a session. In confirmation of Basmajian's report, it was found possible to gain control quickly over individual motor units singled out with the aid of auditory and visual cues. It was found even more clearly than in the experiments using microelectrodes that a unit that was singled out was highly dependent for its activation on the position of the thumb. In order to gain simultaneous control over more than one unit with the digit in the same position, an extended period of practice was essential. When such control was finally gained, the activation of each unit could be brought about without any detectable movement of the thumb, but the subject reported that the activation depended on recall of the original position and contraction effort necessary for activation.

Fig. 2 shows two units, each of which originally could be activated only when the thumb was in a markedly different position. After training, both units could be fired from one position; one unit could be stopped and the other activated discretely, within 100 msec; the necessary change in actual position of the thumb at this time was almost imperceptible, even to the subject.

The following additional observations were made in the course of these studies using minimal contractions. (1) When the thumb was immobilized by conscious effort with use of other muscles or by a cast, different units in the muscle under study could still be activated by alteration of contraction of the other muscles needed to stabilize the thumb. For example, different units in the abductor pollicis brevis could be activated by contraction of the opponens pollicis and/or the flexor digitorum longus. (2) The importance of peripheral input in establishing control over individual units was further emphasized by experiments in which the thumb and forearm were immobilized by a full forearm cast. The subject quickly learned to utilize the added sensation produced by pressure on the skin from the side of the cast as an aid in reproducing the state of muscle contraction under which the unit was originally singled out. (3) During any single training period, less and less effort from other muscles was necessary to activate specific units which were originally dependent in part on contraction of those other muscles. (4) As the ability of a subject to control motor units became progressively refined and accurate, he was able to activate certain higher-threshold units by using less tension than had originally been required to activate the lowest-threshold units.

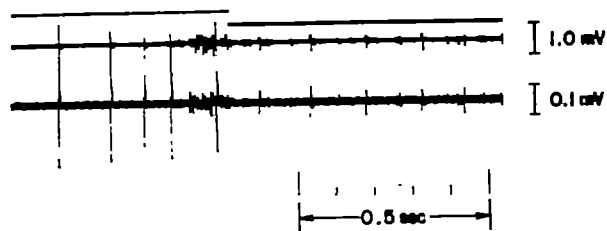


Fig. 2. Recordings made from the abductor pollicis brevis with percutaneous electrodes (middle trace) and a fine bipolar electrode (bottom trace). These recordings were made after the subject had trained himself to select a unit with an almost imperceptible change in position of the thumb. At the signal, which the subject activated with his other hand, an attempt was made to stop one motor unit. The units are clearly recorded with the bipolar electrode, although even the percutaneous electrode shows evidence of them (especially the smaller unit seen in the bipolar recording). The jitters in the tracings indicate transient activation of other units in the muscle during the attempt to bring in the second unit. This is probably a more accurate indication of the time the subject began his attempt to bring in this unit than is the signal marker, which he rarely synchronized exactly with such an attempt.

It appears from these observations that, without training, volitional activation of descending pathways does not result in invariable responses of specific motor cells. Otherwise, the response of particular motor units would not be dependent on maintenance of certain positions of the limb or reproduction of a certain pattern of muscle contraction. During minimal or moderate contractions, the action of the descending pathways is modified, and perhaps controlled, probably in the anterior horn of the spinal cord, by peripheral input—proprioception, muscle contraction, or cutaneous stimuli. Appropriate sensory information, therefore, must be present for volitional activation of specific motor units. However, after an intensive period of training, precise conscious control over motor units can be established, with a lessened influence of peripheral stimuli.

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# KINETICS OF ELIMINATION OF DRUGS POSSESSING HIGH AFFINITY FOR THE PLASMA PROTEINS

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THE classical method of determining the rate of elimination of a drug from the body is to examine the decline of the plasma concentration of the drug with respect to time. I have pointed out<sup>1</sup> that this method is only valid when throughout the period of investigation the plasma contains a constant proportion of the drug in the body and that this is not so when the drug is extensively bound to the plasma proteins. The application of the foregoing method to such drugs is therefore fundamentally unsound, and the values reported for the elimination rate or half-life of certain drugs, as determined from plasma data, may underestimate the rate of elimination. Certain factors operate which can cause the plot of log plasma drug concentration against time to be approximately linear and this has no doubt falsely encouraged belief in the validity of this method.

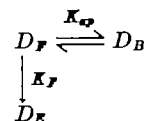
This article provides a theoretical consideration of the kinetics of elimination of a number of model drugs which have a high affinity for plasma proteins. A detailed analysis of the kinetics is complex, only a simple treatment is given here and the method of calculation cannot be regarded as mathematically exact.

In the previous work<sup>1</sup>, calculations were made relating to the distribution of a series of model drugs possessing different association constants ( $K_{sp}$ ) for a plasma protein, with the limitation that negligible binding of drug occurs in other tissues. Graphs were presented relating the amount of free drug in the body, and the amount of drug in plasma, to the total quantity of drug in the body. Using these data, the calculations are now extended to include the kinetics of elimination of these model drugs. For a given dose the amount of free drug in the body can be determined from the graph, and if an elimination rate constant applicable to the free drug is specified, the amount eliminated in 1 h can be calculated on the basis that the rate of elimination is proportional to the con-

centration of free drug. The amount eliminated is then subtracted from the original total to obtain the amount of total drug remaining and the amount of free drug again determined graphically; this process is repeated for each hour to construct the pattern of elimination over several hours. The corresponding amount of drug in the plasma at each stage of the elimination process is determined from the appropriate graph. Data relating to total drug decline, plasma drug decline and urinary excretion rate from two such calculations are plotted in Figs. 1a and b.

If  $D_f$  = concentration of free (non-bound) drug in body water,  $D_b$  = concentration of bound drug in plasma,  $V_p$  = plasma volume,  $V_t$  = volume of total body water, then: total drug in body ( $D$ ) =  $D_f V_t + D_b V_p$ ; free drug in body ( $D_f$ ) =  $D_f V_t$ ; bound drug in body ( $D_b$ ) =  $D_b V_p$ ; total drug in plasma ( $D_p$ ) = ( $D_f + D_b$ )  $V_p$ .

The model system may be represented as:



where  $D_e$  is the amount of drug eliminated.

In the process of drug elimination, the decline in  $D_f$  is the net effect of loss of free drug by elimination and of gain of free drug arising from the dissociation of  $D_b$ .

$$-\frac{dD_f}{dt} = -\frac{dD}{dt} + \frac{dD_b}{dt} \quad (1)$$

If the loss of drug by elimination is proportional to  $D_f$ , then:

$$-\frac{dD}{dt} = K_f D_f \quad (2)$$

where  $K_f$  is a first-order elimination rate constant applicable to the amount of free drug in the body.  $D_f$  is not a

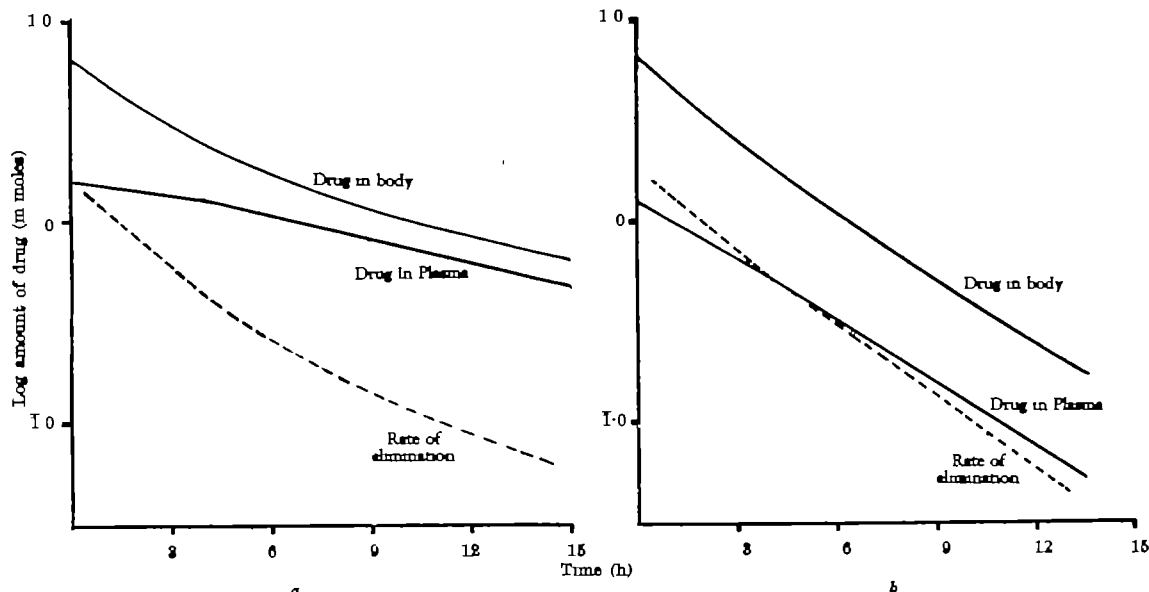


Fig. 1 Plots of the log drug in the body (m.moles), log drug in plasma (m.moles), and log rate of elimination (m.moles/h) against time for two model drugs of  $K_{sp} = 1 \times 10^3$  (Fig. 1a) and  $1 \times 10^4$  (Fig. 1b) undergoing elimination at a rate of 20 per cent/h of the amount of free (non-bound) drug. Initial drug present 6 m.moles. Data calculated for a 70-kg man on the basis previously described<sup>1</sup> ( $P = 5 \times 10^{-4}$  M,  $\alpha = 1$ ,  $V_p = 3$  l.,  $V_t = 42$  l.)

constant fraction of  $D$ ,  $(D_F/D)$  diminishes and elimination becomes relatively slower as it proceeds. Neither the decline of drug nor the decline in the rate of elimination is first order.

In respect of the decline of drug in plasma, the decrease in  $(D_F/D)$  as elimination proceeds is partly compensated by the increase in  $(D_F/D)$ , and the rate of decline of drug in plasma consequently underestimates the rate of drug elimination. This is particularly marked at high dosage. The variation of  $(D_F/D)$  and  $(D_P/D)$  for the model drug of  $K_{ep} = 1 \times 10^3$  is such that the plot of  $\log D_P$  against  $t$  is curved, but closely approximates to two linear sections of different slope, at first showing a gentle decline and then a steeper decline (Fig. 1a). The decline in  $\log$  plasma drug concentration not only underestimates the rate of drug decline at high levels but later also gives the false impression that the  $\log$  rate of elimination has increased, whereas elimination becomes relatively slower, rather than faster, at low drug concentrations.

These calculations relate to model drugs which exhibit no appreciable binding in other tissues. Several drugs will consequently show departures from this idealized behaviour, but probably in no instance can the rate of decline of high plasma drug concentrations be accepted as a valid indication of the rate of drug decline.

An increase in slope of the  $\log$  plasma drug concentration as elimination proceeds has been reported for procaine<sup>2</sup>, streptomycin<sup>3</sup>, dicoumarol<sup>4</sup>, ethyl bisacoumate<sup>5</sup> and phenylbutazone<sup>6</sup>, all of which are bound to the plasma proteins. This has been interpreted in terms of relatively slower elimination at higher doses. This may well be so—it is with salicylic acid<sup>7</sup>—but in the absence of additional evidence, the present considerations indicate that such a deduction is not justified and that the change in slope of the  $\log$  plasma drug decline can arise simply as a consequence of the interaction of the drug with plasma proteins.

The rate of decline of drug in the body can be assessed from urinary excretion data by constructing the pattern of decline of that fraction of the dose which is excreted unchanged, that is, by a plot of  $\log (D_{U\infty} - D_{Ut})$  against  $t$ , where  $D_{U\infty}$  is the amount of unchanged drug ultimately recovered in the urine, and  $D_{Ut}$  is the cumulative amount recovered to time  $t$ . The interpretation of data, which includes the excretion of drug metabolites, is complicated by the process of metabolite accrual<sup>8</sup>.

It is frequently asked, what is the effect of protein-binding on the rate of drug elimination? It has been suggested<sup>1</sup> that this depends not only on certain factors

which remain constant, but also on the amount of drug present in the body, and the magnitude of the effect will increase as elimination proceeds. There is therefore no simple answer, although the maximum potential effect can be stated. Alternatively, what would be the elimination rate constant of the drug if it exhibited no interaction with plasma protein? This theoretical concept has been introduced and the relevant rate constant ( $K_F$ ) is identified in equation (2). For certain drugs it may prove possible to estimate  $K_F$  directly from urinary excretion data only. If a drug has a high  $K_{ep}$  and its therapeutic dose is such that after one or more doses the plasma protein closely approaches saturation with drug, then limited elimination of drug can take place without appreciable change in  $D_B$ . If  $D_B \rightarrow$  constant,  $dD_B/dt \rightarrow 0$ , then from equations (1) and (2):

$$-\frac{dD_F}{dt} = -\frac{dD}{dt} = K_F D_F = K_F D_{Fe} - K_F t$$

hence:

$$\ln \left( -\frac{dD}{dt} \right) = \ln D_F K_F - K_F t \quad (3)$$

In terms of urinary excretion data<sup>9</sup>:

$$\ln \left( -\frac{dD_U}{dt} \right) = -K_F t + \text{constant} \quad (4)$$

A plot of the  $\log$  amount of unchanged drug excreted in a series of equal intervals of time ( $dD_U/dt$ ) is almost linear over this limited period and  $K_F$  can be assessed from its slope (Fig. 1a). This plot afterwards becomes distinctly curved and it is also at this time that the  $\log$  drug plasma plot exhibits a marked change in slope.

Two well-known methods for the treatment of urinary excretion data have been utilized in this work. In this instance they yield entirely different information,  $(D_{U\infty} - D_{Ut})$  is used to assess the decline of total drug, while  $(dD_U/dt)$  is proportional to the amount of free drug.

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## LIGHT-INDUCED STOMATAL OPENING AND THE POSTULATED ROLE OF GLYCOLLIC ACID

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THE history of investigation of the physiology of stomata has been one of successive hypotheses, usually based on one aspect only of the evidence, each attempting to explain in terms of a single relatively simple mechanism the way in which changes in environmental factors affect guard cell turgor and produce stomatal movement. Francis Darwin<sup>1</sup> himself accumulated enough experimental evidence of the complexity of stomatal behaviour in response to various stimuli to show that no very simple single explanation of the stomatal mechanism was to be expected, and of recent years this has been made even more apparent by the results of much experimentation under more rigidly controlled conditions. A new hypothesis, largely based

on observed effects of metabolic inhibitors on stomatal aperture, has now suggested that the photosynthetic production of glycollic acid plays a major part in the stomatal mechanism<sup>2-4</sup>.

In this article we discuss the complexity of the effect of light on stomatal movements, including some new findings concerning an effect of blue light which is apparently independent of carbon dioxide, and show, with some experimental evidence relating to the effects of metabolic inhibitors, the inadequate nature of the new hypothesis.

Metabolic inhibitors have proved most valuable in plant physiology when used in conjunction with measurements of the rate of a metabolic process, notably respiration, or photosynthesis as carried out by isolated chloroplasts or

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even unicellular algae. When the process measured is the resultant of a large number of simultaneous complex biochemical and physical processes, an exclusive reliance on metabolic inhibitors can sometimes result in "confusion worse confounded", for so-called specific inhibitors are seldom as specific as their users would like to think. The number of compounds which will, for example, inhibit growth of coleoptile sections, even when in low concentration, is legion. The finding that a 'specific' inhibitor for a particular enzyme will prevent or reduce growth does not necessarily show that the mechanism of growth is entirely, or even mainly, explicable in terms of that enzyme system.

A somewhat similar situation holds for stomatal movements. There is evidence, referred to here, that at least three light-operated mechanisms are involved in stomatal opening, one acting through the carbon dioxide concentration in stomatal guard cells, the second causing stomatal opening independently of carbon dioxide and the third controlling the phase of endogenous rhythms.

The first-mentioned mechanism is controlled partly by photosynthesis in the guard cells themselves<sup>6</sup> and partly by that in the mesophyll<sup>6,7</sup>. The photosynthetic production of osmotically active substances in the guard cells may be expected to assist opening but is of minor importance compared with the effect of photosynthesis in reducing the carbon dioxide concentration; this is shown by the facts that in light, increasing the ambient carbon dioxide concentration does not cause opening but partial closure<sup>8,9</sup> and that wide stomatal opening can be brought about in the dark by exposing leaves to air free of carbon dioxide<sup>6</sup>. The hydrolysis of guard cell starch is much affected by carbon dioxide concentration, but not by light *per se*<sup>6</sup>, with the possible exception of blue light.

Secondly, stomatal opening due to an effect of light on the guard cells operating independently of carbon dioxide concentration was postulated by Heath and Russell<sup>4</sup>, who found stomatal responses in wheat to changes in light intensity while the intercellular carbon dioxide concentration was kept between zero and 0.01 per cent; in that range, wheat stomata were found not to respond appreciably to changes in the carbon dioxide concentration<sup>4,5</sup>. The results of Mouravieff<sup>10</sup> later implied that if there was a carbon dioxide-independent effect of light on stomatal guard cells, this effect was probably due to short wave-lengths in the blue region. Recently we have tested stomatal responses to blue and red light, and have confirmed that in blue light stomata open further than in red light even when the intercellular space carbon dioxide concentration in blue light is higher than in red. Leaves of *Xanthium pennsylvanicum* Wall. were illuminated in a closed system and allowed to establish  $\Gamma$ , the minimum intercellular space carbon dioxide concentration<sup>11</sup>. When red or blue light (from broad-band sources, practically free of far- and infra-red) was applied so that the incident quantum supply was approximately the same in each wave band, namely, 3,400 ergs/cm<sup>2</sup>/sec of red and 4,550 ergs/cm<sup>2</sup>/sec of blue,  $\Gamma$  remained more or less constant at 127 p.p.m. of carbon dioxide (average for 8 leaves). However, in red light log stomatal resistances (measured in log Gregory and Pearse units)<sup>12</sup> were on average 1.22 whereas in blue light they were 1.34 for the same leaf area. In another experiment we have shown that with blue light at lower intensities than red, the former can still produce wider stomatal opening, in spite of a larger  $\Gamma$ -value, that is with a higher carbon dioxide concentration within and surrounding the leaf. In these experiments light-induced stomatal opening from darkness occurred with an output of carbon dioxide from the leaf into initially carbon dioxide-free air. Thus there could have been no net gain of photosynthate and the postulated production of new carbohydrate<sup>6</sup>, whether via glycolic acid or by any other path, could not have occurred. These findings will be reported more fully elsewhere.

Thirdly, the light effect controlling the phase of the rhythm which proceeds in darkness has been shown elsewhere<sup>13</sup> to be brought about by wave-lengths in the neighbourhood of 700 m $\mu$ . This light effect is distinguished from those more directly concerned with stomatal opening both by the action spectrum and the fact that the intensity required to elicit a phase shift ( $< 100$  ergs/cm<sup>2</sup>/sec) is well below that required for the production of detectable opening. The endogenous rhythm in darkness can considerably modify the re-opening in high-intensity light<sup>14</sup>, and consequently the light reaction which affects the phase of this rhythm may make a significant contribution to regulation of the opening response to light.

The foregoing complexities have been listed with reference to effects on guard cells alone, but it was first postulated by von Mohl<sup>15</sup> and demonstrated experimentally by Heath<sup>16</sup> that the degree of stomatal opening depends on the turgor difference between the guard cells and the neighbouring epidermal or subsidiary cells. A further physical complexity is added by the different types of thickening of the guard cell walls so extensively studied by Schwendener<sup>17</sup>.

With such a system it is naive to look for a single all-embracing explanation of stomatal movement, or even of stomatal responses to light. It is not surprising that a great many chemical agents damage one component or another sufficiently to cause stomatal closure or prevent opening. A great deal of early work showed that bathing isolated epidermis, portions of leaf or intact leaves with various buffer solutions at the same or different pH values, or various acids, alkalis and neutral salt solutions had complex effects on stomatal aperture and stomatal starch content<sup>18</sup>. More recently, Zelitch<sup>3</sup> listed eighteen compounds (mainly inhibitors, plant growth substances or chelating agents) which partially or completely prevented opening of the stomata on tobacco leaf disks floated on solutions of  $10^{-3}$  M or lower concentration (this list included glycolic acid—see below). In a later paper<sup>3</sup> he listed a further thirteen compounds which partially prevented opening in concentrations between  $5 \times 10^{-3}$  M and  $3 \times 10^{-2}$  M and truly remarked "the wide range of compounds that influence stomatal movements suggests that there are many sites at which the opening of stomata may be interfered with to cause closing". Since the relaxed position of the guard cells results in stomatal closure, substances causing opening are more unusual and likely to be of more potential interest, though here also there is a danger of misinterpretation as toxic effects may act differentially on the neighbouring epidermal cells which are in general more easily damaged than the guard cells; loss of turgor by the epidermal cells tends to cause stomatal opening. This danger has been avoided by Mouravieff<sup>10</sup>, who works mainly with isolated stomata.

Zelitch<sup>3</sup> has produced a scheme, based mainly on the results of studies with inhibitors, to account for both light-induced stomatal opening and carbon dioxide-induced stomatal closure. It is assumed that in the main light-induced stomatal opening is due to the photosynthetic formation in the guard cells of glycolic acid and its further metabolism to glyoxylic acid and thence to carbohydrate, resulting in an increase of osmotic pressure. The inhibitors used in the investigations leading to the formulation of this scheme were  $\alpha$ -hydroxysulphonates, and their action has been explained in terms of their effect on glycolic acid oxidase, so that it is not here the synthesis of glycolic acid but its further metabolism which is affected<sup>19</sup>. Glycolic acid is said to accumulate rapidly in the leaf if these inhibitors are present; within thirty minutes it may accumulate twenty-fold<sup>19</sup>. The closing effect of high carbon dioxide concentration, on the other hand, is supposedly due to inhibition of photosynthetic formation of glycolate<sup>3</sup>.

Zelitch has also speculated that stomatal opening may require adenosine triphosphate produced by photosynthetic phosphorylation and that a glycolic acid—



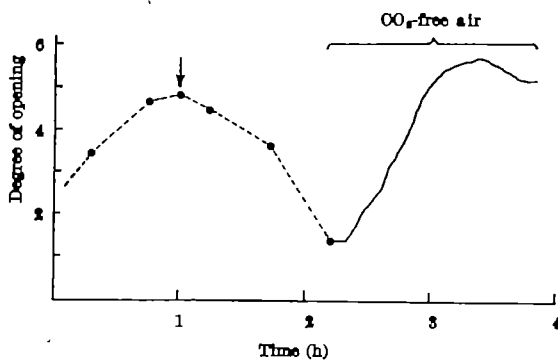


Fig. 1. Stomata on a detached leaf of *Xanthium pennsylvanicum*, in the course of opening in light of 8,000 lux, begin to close when  $10^{-3}$  M  $\alpha$ -hydroxy-2-pyridinemethanesulphonic acid is fed through the petiole (time of application indicated by arrow). Flushing the leaf with air free of carbon dioxide completely reverses the effect of the inhibitor, application of which was continued through the rest of the experiment. During the carbon dioxide-free air treatment a continuous record was obtained. Ordinate indicates degree of opening on the scale of the recording resistance porometer of Heath and Mansfield<sup>10</sup>.

glyoxylic acid shuttle may be involved. (We have discussed this elsewhere<sup>22</sup>, but it is not incorporated in his main scheme<sup>23</sup>.)

We have recently studied the effects of  $\alpha$ -hydroxy-sulphonate inhibitors on stomatal behaviour in *Xanthium pennsylvanicum*. Although our results fully confirm that these substances cause stomatal closure, we have also observed that the closing effects can be quickly and completely reversed by flushing the leaf with air free of carbon dioxide. A typical result from a series of experiments is shown in Fig. 1. This observation is open to two possible explanations, neither of which appears compatible with the glycollic hypothesis proposed<sup>1-4</sup>. One possibility is that it is the glycollic acid itself accumulating in the tissue (due to inhibition of its subsequent metabolism) that causes the closure. Flushing the leaf with air free of carbon dioxide would reduce photosynthetic production to a minimum and stop further accumulation of the acid. That already accumulated might then be removed if glycollic acid oxidase were not completely blocked. Zelitch's own observations (ref. 2, Table 2) have shown that glycollic acid at a concentration of  $10^{-3}$  M causes 26-50 per cent stomatal closure. However, results of recent experiments, to be published elsewhere, have shown that glycolate accumulation *per se* causes neither stomatal closure nor reduction in photosynthetic rate. The most likely possibility is that the inhibitor reduces photosynthetic rate, resulting in an increase in the intercellular space carbon dioxide concentration and thereby inducing stomatal closure. Asada and Kasai<sup>21</sup> have shown in barley, tobacco and *Chlorella* that  $10^{-3}$  M  $\alpha$ -hydroxysulphonate severely affects the rate of photosynthetic carbon dioxide uptake. Zelitch<sup>22</sup> considers that it is stomatal closure that causes reduced photosynthetic rate, but in view of the fact that photosynthesis in *Chlorella* was similarly affected this is most unlikely. The simplest explanation of our observation (Fig. 1) is that flushing the intercellular spaces with air free of carbon dioxide removes the respiratory carbon dioxide which is accumulating due to a reduction in photosynthetic rate. The normal sensitivity of the stomata to carbon dioxide does not appear to be affected by the inhibitor.

Zelitch<sup>2</sup> has proposed that stomatal closure in high carbon dioxide concentrations is caused by the inhibition of carbohydrate formation via the glycolate pathway. One would expect more carbohydrate to be formed at such concentrations, even if not by that pathway, but the observed effects of carbon dioxide on stomatal movement do not in any event support the hypothesis. Pritchard, Griffin and Whittingham<sup>23</sup> have shown for *Chlorella* that glycolate excretion into the medium is maximal with 0.1 per cent carbon dioxide in the gas phase and decreases both above and below this concentration.

At zero carbon dioxide concentration there can be little, if any, photosynthetic formation of glycolate, and on Zelitch's hypothesis illuminated stomata should therefore show closure in carbon dioxide-free air; they should open to a maximum value as the carbon dioxide concentration is increased and then close again at still higher concentrations. In fact stomatal aperture is maximal in carbon dioxide-free air, shows no detectable change with the first small increase of concentration (to about 0.01 per cent for wheat<sup>24</sup>) and then decreases to approach a steady value at a concentration which depends on the light intensity, but up to 800 ft.-candles is in the range of physiologically high concentrations from 0.05 to 0.08 per cent<sup>25</sup>. Concentrations of 1.8 or 0.5 per cent, as used by Zelitch, are far outside the physiological range. There thus appears no better evidence that reduction in the rate of glycolate synthesis accounts for the closing responses of stomata to carbon dioxide than there is that the further metabolism of glycollic acid to carbohydrate accounts for their opening response to light.

**Opening due to removal of carbon dioxide.** All wavelengths of light in which photosynthetic uptake of carbon dioxide occurs will produce opening. This effect can be simulated in darkness by flushing the leaf with air free of carbon dioxide<sup>24</sup> and it may, therefore, be said to be non-photosynthetic since it does not necessarily depend either on light or on carbon dioxide fixation; the role of photosynthesis is an indirect one.

**Opening component independent of carbon dioxide.** We have shown above that this is brought about by blue light. The opening occurred in a system in which there could be no net photosynthetic carbon dioxide uptake.

**Low-intensity light effect.** Light of very low intensity (most effective at 700 m $\mu$  wave-length) can alter the phase of a rhythm of 'opening ability' proceeding in darkness and so control the rate of light-induced opening in high light intensity.

**Mechanisms of light effects.** It seems most improbable that there is much in common between the unknown mechanisms by which the foregoing three light effects control guard cell turgor and hence stomatal opening.

**Role of glycollic acid.** Stomatal opening occurred in either red or blue light with no net carbon dioxide fixation and hence no production of osmotically active photosynthates via glycolate or by any other path. Appreciable formation of glycolate is not therefore essential for light-induced opening.

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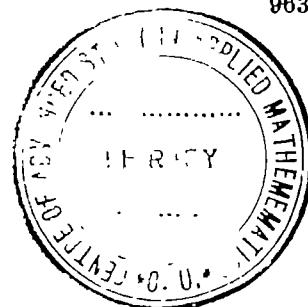
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## LETTERS TO THE EDITOR



## ASTROPHYSICS

## A Sensitive Test for the Presence of Atomic Hydrogen in Intergalactic Space

FIELD<sup>1</sup> has stressed the importance of the scattering of Lyman- $\alpha$  quanta on hydrogen atoms in determining the spin temperature of intergalactic hydrogen. The purpose of this communication is to point out that the scattering itself provides an extraordinarily sensitive indication of the presence of atomic hydrogen.

According to Field<sup>1</sup> (equation 11) radiation emitted at wave-lengths shorter than Lyman- $\alpha$  is scattered:

$$N = 2.4 \times 10^{14} n_H$$

times while it is red-shifted through the Lyman- $\alpha$  line. (Field assumed that Hubble's constant is 180 km/sec Mpc, but the order of magnitude of  $N$  should be correct in any case.) Thus, with a typical 'cosmological' density of  $n_H = 10^{-5}$  atoms  $\text{cm}^{-3}$ , a quantum emitted with a wave-length shorter than Lyman- $\alpha$  is scattered  $10^4$  times, and has a negligible chance of continuing in its initial direction. Even a density of  $10^{-6}$  atoms  $\text{cm}^{-3}$  would very effectively scatter such radiation. Thus, if any radiation is observed from a discrete extragalactic source in the range of wave-lengths between the local and the red-shifted Lyman- $\alpha$  wave-lengths, the density of hydrogen atoms in intergalactic space is  $< 10^{-6} \text{ cm}^{-3}$ .

Ultra-violet spectroscopy by rocket or satellite-borne telescopes cannot, as yet, reach the galaxies one would wish to examine. However, Schmidt<sup>2</sup> has shown that the optical object associated with the radio source 3C 9 has a red-shift  $z = 2.012$ , and its Lyman- $\alpha$  emission line is red-shifted into the visible spectrum. Schmidt's paper is concerned only with the two emission lines of 3C 9, and there is no explicit mention of a continuum. The Lyman- $\alpha$  line is some tens of Å units wide, and the fact that the centre of the line is not noticeably displaced might already be considered as evidence that there is no noticeable scattering within the first Mpc around 3C 9. However, it is quite possible that 3C 9 has a peculiar velocity of a few 100 km/sec, and it is also possible that intergalactic hydrogen in the vicinity of 3C 9 is exceptionally highly ionized. Thus a decision must await a new examination of Schmidt's spectrum.

If the scattering were appreciable, then the radiation emitted at a particular frequency would be scattered from a spherical shell around the source, the radius of which is proportional to the difference between the emitted wave-length and Lyman- $\alpha$ . Observation of such scattered light would, in principle, yield Hubble's 'constant' as a function of  $z$ , and hence provide a history of the expansion of space. Unfortunately, the detection of such faint radiation distributed over disks many minutes of arc in diameter is beyond the reach of observation.

If it turns out that 3C 9 has a continuum below 3660 Å, then either the mean density of hydrogen is exceedingly low, or its ionization is very complete. The conditions required to produce such complete ionization will be examined elsewhere. A third possibility, that the red-shift of 3C 9 is not cosmological<sup>3</sup>, cannot be ruled out at present.

*Note added in proof.* I understand from Dr. Schmidt that work on the lines suggested in the foregoing letter

has been carried out independently by Gunn and Peterson (*Astrophys. J.*, in the press).

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## RADIOPHYSICS

## Ordinary Mode Whistlers observed In Satellites

SMITH *et al.*<sup>1</sup> recently published dynamic spectra of a very interesting phenomenon observed in the satellites *Alouette I* and *Injun III*. One of these is reprinted here as Fig. 1A. This shows a whistler (rapidly falling part) produced by (extraordinary mode) dispersion of an atmospheric propagated upwards to the satellite. The whistler is followed by a rising trace which flattens out at a frequency which could correspond to the ion gyro-frequency.

Although such a sequence of events may suggest a 'triggering' process for the rising trace<sup>2</sup>, I would like to show that the spectral (frequency-time) shapes of both traces can be closely matched if both are interpreted as dispersed forms ('whistlers') of the original atmospheric. Thus the falling trace is an extraordinary mode whistler and the rising part an ordinary mode whistler.

The ordinary mode can propagate only below the ion (proton) gyrofrequency. For frequencies very much less than the electron gyrofrequency, the refractive indices (for longitudinal propagation) become<sup>3</sup>:

$$n^2 = a(g \pm f)^{-1}$$

where  $g$  is the proton gyrofrequency,  $f$  the wave frequency, and  $a$  the scale frequency (ratio of plasma frequency squared to the gyrofrequency, for either protons or electrons). The plus and minus signs correspond to the extraordinary and ordinary modes respectively ( $f < g$  for the latter). I have assumed a neutral plasma of electrons and protons only. The group refractive indices are then (signs as above):

$$n_g = a^{1/2}(g \pm \frac{1}{2}f)(g \pm f)^{-3/2}$$

The travel times of waves in the two modes can be calculated from this with an appropriate model. Fig. 1B shows the result of such a calculation using parameters ( $a, g$  and path length) to fit the observed dynamic spectrum in Fig. 1A.

The agreement for both modes is quite reasonable. Effectively only two independent parameters can be chosen to obtain a fit. These determine the time and frequency scale. The shape is mainly determined by the refractive index expression given here and only slightly by the model or form of the plasma density and magnetic field variation. The effects of other ions do not appear to be important.

Jacobs and Watanabe<sup>3</sup> have suggested that certain types of micropulsations which have dynamic spectral shapes like the rising part shown in Fig. 1A might be such ordinary mode whistlers (they used the term

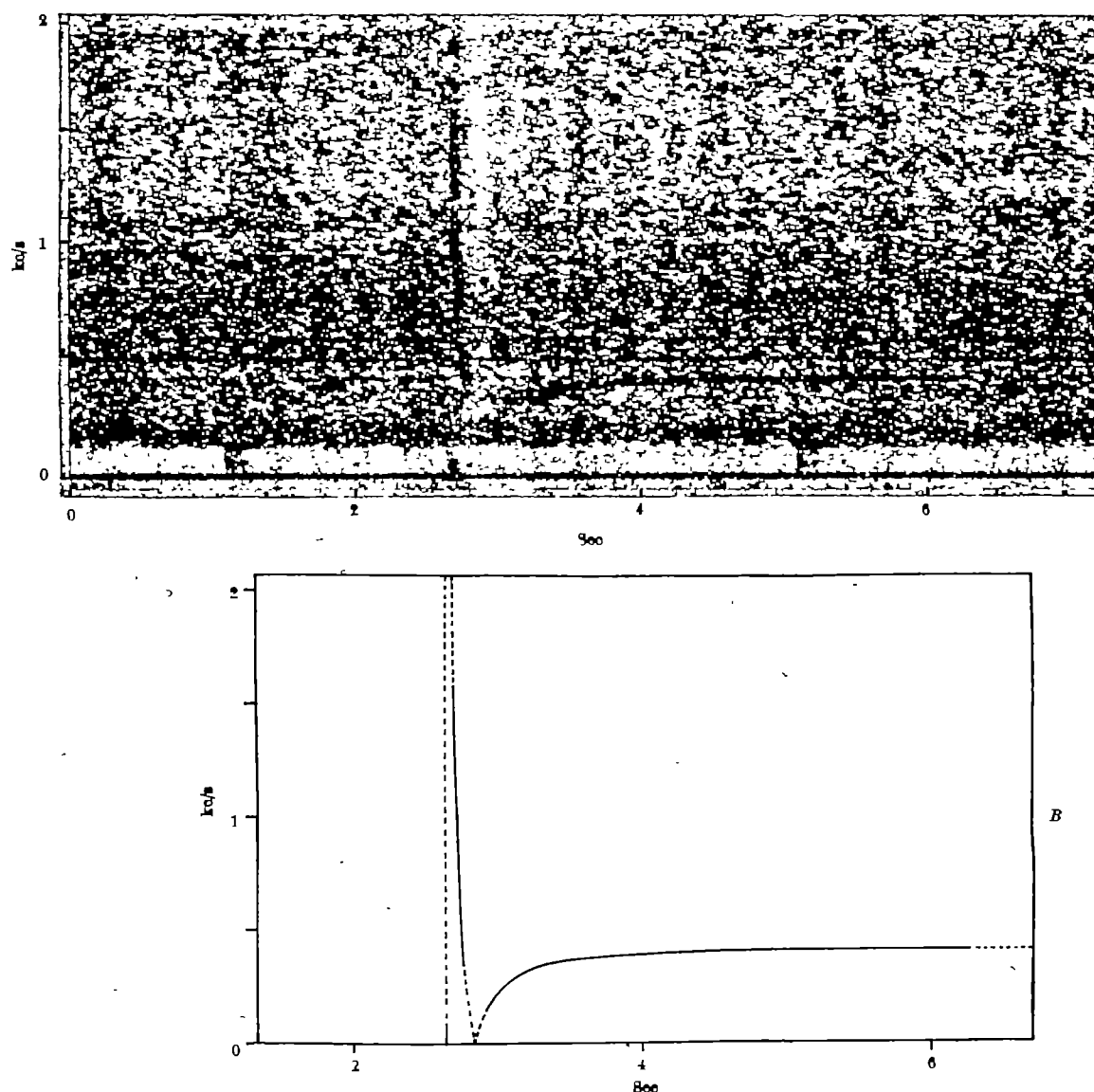


Fig. 1. A, Event observed by *Injun III* (after Smith *et al.*<sup>1</sup>). B, An attempt to match the event shown in A by the two-mode whistler hypothesis. The solid curves correspond to the portions actually observed. The time of the initiating atmosphere is shown by the vertical broken line.

'micropulsation whistlers'). This adds considerable weight to their theory and is perhaps the first direct evidence that these can be initiated by an atmospheric impulse.

*Note added in proof.* Since this letter was submitted the same idea has been presented by Gurnett *et al.*<sup>4</sup>

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### The Appleton Anomaly

In recent communications<sup>1,2</sup> and an earlier letter<sup>3</sup> various workers have shown that electrodynamic drift (that is, magnetohydrodynamic motion of the magnetic field lines) in the *F2* layer would give rise to a phenomenon

similar to the observed Appleton anomaly. However<sup>1,2</sup>, the required drift speeds are much smaller than the 20 m/sec or more predicted by dynamo theory<sup>4,5</sup>.

Meanwhile, work in progress elsewhere<sup>6-8</sup> has suggested that the topside *F2* layer is in diffusive or hydrostatic equilibrium. In fact, when the possible effects of electrodynamic drift are considered above the peak<sup>9,10</sup>, it is found that even drifts of the order of 10 m/sec should have very large effects indeed.

In view of this apparent discrepancy between what the drift should be, and what it actually is, we have investigated a possible explanation in terms of horizontal motion of the neutral air caused by ion drag. Earlier workers<sup>11-13</sup> have previously discussed the effects of such motion at moderate latitudes, and have shown that the apparent vertical electrodynamic drift is reduced by such motion, and the apparent horizontal drift is increased. In the case when the horizontal motion of the neutral air exactly matches the horizontal velocity of the ions we find<sup>14</sup> that in the absence of electrodynamic drift there is a deep equatorial trough in  $N_m$  of about the same depth as the trough in the observed Appleton anomaly. However, the latitudinal maxima of  $N_m$  occur at the approximate latitudes of 10°.

In the case of perfectly matched horizontal velocities, the apparent vertical drift is cancelled, and the horizontal drift is enhanced. It seems clear, therefore, that this horizontal drift, when included, would move the maxima outward to latitudes of  $15^\circ$ , as in the actual anomaly. Although we have not considered the dynamics of such horizontal circulation, other workers<sup>18</sup> have discussed the principal operative forces.

In view of this, it seems a reasonable conjecture that the complete description of the equatorial  $F_2$  layer may lie somewhere between these extremes. Account may have to be taken of production<sup>16</sup>, loss<sup>17</sup>, diffusion<sup>18,19</sup>, electrodynamic drift<sup>20</sup>, horizontal air motions<sup>21</sup> and temperature variations<sup>21</sup> (see also ref. 22) in assessing quantitatively the grosser features of the  $F_2$  layer at low latitudes.

Other possibilities include electric currents along the magnetic field lines between conjugate points of the dynamo region<sup>22</sup>, and electric field effects associated with the necessity for the electric field to be irrotational<sup>24</sup>. However, the possible importance of these further effects has not yet been investigated.

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## PHYSICS

### Lorenz Number of Plutonium Metal

RECENT work on the thermal conductivity ( $k$ ) and electrical conductivity ( $\sigma$ ) of pure  $\alpha$ -phase plutonium revives the question of the magnitude of the Lorenz number  $L (= k/\sigma T)$ .

The early work of Sandenaw and Gibney<sup>1</sup> gave  $L$  at  $20^\circ\text{C}$  between  $3.96$  and  $4.41 \times 10^{-8}\text{ V}^2\text{ deg}^{-2}$ , very much in excess of the theoretically expected value ( $2.45 \times 10^{-8}\text{ V}^2\text{ deg}^{-2}$  at a temperature above the Debye temperature, the latter being  $192^\circ\text{K}$  for plutonium). It is unlikely that the difference could be wholly accounted for by a lattice contribution to the thermal conductivity. However, their thermal conductivity value ( $0.020\text{ cal cm}^{-1}\text{ sec}^{-1}\text{ }^\circ\text{C}^{-1}$ ) was not confirmed by the later work of either Lee and Mardon<sup>2</sup> ( $k$  between  $0.008$  and  $0.010\text{ cal cm}^{-1}\text{ sec}^{-1}\text{ }^\circ\text{C}^{-1}$ ) or myself<sup>3</sup> ( $k = 0.0098\text{ cal cm}^{-1}\text{ sec}^{-1}\text{ }^\circ\text{C}^{-1}$ ). These lower values have now been confirmed by

a measurement of the room-temperature thermal diffusivity<sup>4</sup> as  $0.02\text{ cm sec}^{-2}$  which, using the specific heat data of Kay and Loesby<sup>5</sup> ( $0.034\text{ cal g}^{-1}\text{ }^\circ\text{C}^{-1}$ ) and a density of  $19.6\text{ g cm}^{-3}$ , gives a thermal conductivity of  $0.013\text{ cal cm}^{-1}\text{ sec}^{-1}\text{ }^\circ\text{C}^{-1}$ . The problem now arises that using these lower values of  $0.01$  for  $k$  and  $142 \times 10^{-8}\text{ ohm-cm}$  as the room-temperature electrical resistivity<sup>6</sup>,  $L$  is reduced to about  $2.0\text{ V}^2\text{ deg}^{-2}$ , nearly 20 per cent lower than the theoretical value and clearly not to be explained by the presence of any additional conduction mechanism.

Linde<sup>7</sup> seems to have been the first to point out the low values of  $L$  given by a number of pure metals and suggested that an additional term should be included by splitting the total thermal resistance of a metal into two components, one determined by impurities and lattice defects and hence independent of temperature, the other determined by lattice vibrations. This is an obvious thermal analogy to Matthiessen's rule for electrical resistivity. Linde's theory was extended by Bäcklund<sup>8</sup>, who removed Linde's restriction that the resistivity temperature curve had to be linear and suggested the amended form of the Weidemann-Franz law:

$$\frac{k(\rho + \bar{\rho}_0)}{T} = L$$

where  $\rho$  is the electrical resistivity and  $\bar{\rho}_0$  the intercept obtained by extrapolating back the linear low temperature part of the resistivity-temperature curve to zero temperature. Applying this to the foregoing values of  $k$  and the recent resistivity data of Meeden<sup>9</sup>, a value of  $2.48 \times 10^{-8}\text{ V}^2\text{ deg}^{-2}$  is obtained for  $L$ . This close agreement with the theoretical Lorenz number would appear to be a vindication of the Bäcklund theory. However, recent papers by King *et al.*<sup>10</sup> and Wigley<sup>11</sup> show that Matthiessen's rule fails completely for plutonium at low temperature due to the additional temperature-dependent resistivity caused by self-irradiation. It remains to be shown whether the thermal resistivity is similarly affected by self-irradiation. A theoretical justification for the applicability of an argument similar to Bäcklund's for the various electron scattering mechanisms awaits a fuller understanding of a peculiar behaviour of the electrical resistivity of plutonium<sup>11</sup>.

The value of  $\bar{\rho}_0$  for uranium from Meeden's results<sup>9</sup> amounts to only 3 per cent of the room temperature resistivity, a variation insignificant compared with the range of values of  $L$  consequent on the various available experimental values of  $k$ .

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### Infra-red Evidence of the Grinding Effect on Hydrargillite Single Crystals

SEVERAL investigations of the infra-red spectrum of hydrargillite powder have been reported<sup>1-4</sup>. However, no study of single crystals as yet seems to have been reported. In the present investigation, thin plates were cut from a single crystal of synthetic hydrargillite (product of Showa Denko Co.) about  $3\mu$  thick, some parallel to the (001) plane and some nearly perpendicular to the (001) plane. The absorption spectra were recorded in the region of  $4,000\sim 450\text{ cm}^{-1}$ . The spectra so obtained were

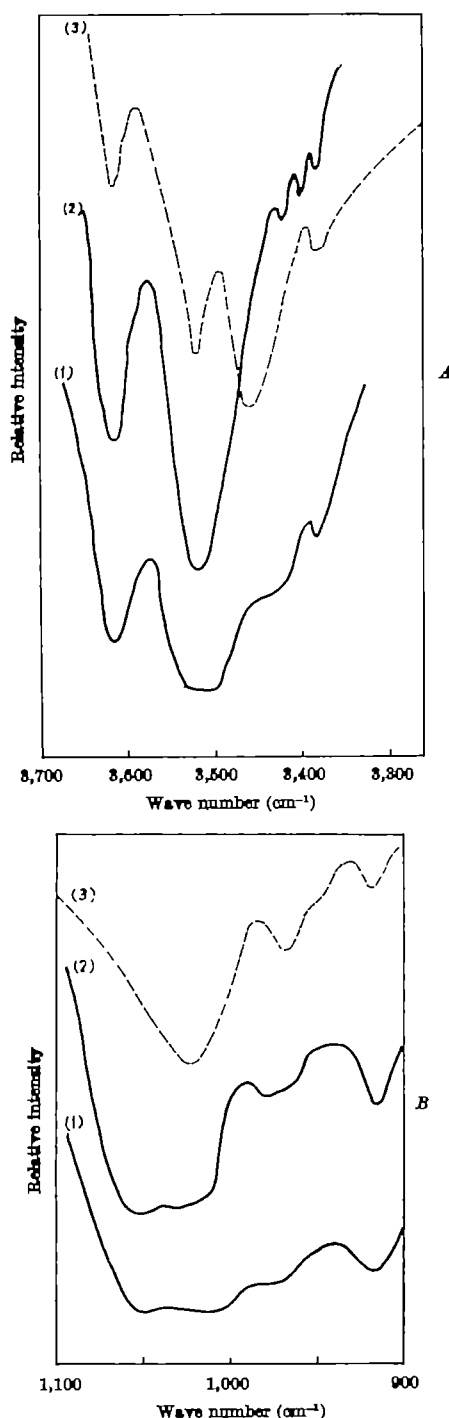


Fig. 1. Infra-red spectra of synthetic hydrargillite: A,  $\nu$ OH region; B,  $\delta$ OH region. Curve 1, single crystal, the incident beam is nearly parallel to (001) plane; curve 2, single crystal, the incident beam is perpendicular to (001) plane; curve 3, powder obtained by grinding the single crystal (nujol).

compared with the powder spectra and several points of difference were found.

According to the literature<sup>1-3</sup>, the spectrum of hydrargillite powder has characteristic OH bands at the following wave numbers: 3,617 (or 3,620), 3,520, 3,428 and 3,380 for  $\nu$ OH, and 1,060 (occasionally present)<sup>4</sup>, 1,020, 969, 914 and 560 for  $\delta$ OH (the 560  $\text{cm}^{-1}$  band can reasonably be assigned to  $\delta$ OH with the deuteration data by Kolesova<sup>5</sup>, though no report has appeared on the assignment).

The powdered sample in the present investigations was obtained by grinding the crystal, and gave characteristic OH absorption bands at the following wave numbers:

3,617, 3,520, 3,460, 3,420 and 3,380 for  $\nu$ OH (Fig. 1A, curve 3), and 1,020, 969, 914 and 560 for  $\delta$ OH (Fig. 1B, curve 3). The strong 3,460  $\text{cm}^{-1}$  band is believed to correspond to the 3,428  $\text{cm}^{-1}$  band reported in the literature due to the similarity of shape and intensity.

The OH absorption bands of the single crystal were found at the following wave numbers: 3,617, 3,520, 3,420, 3,400 and 3,380 for  $\nu$ OH (Fig. 1A, curves 1 and 2), and 1,060, 1,020, 969 and 914 for  $\delta$ OH (Fig. 1B, curves 1 and 2). Since the 3,420  $\text{cm}^{-1}$  band is very sharp but not so intense, it cannot be considered to correspond to the very strong 3,428  $\text{cm}^{-1}$  band reported in the literature<sup>1,2</sup>.

The main difference in the two spectra of the present samples occurs at three points: First, the single crystal shows no band at 3,460  $\text{cm}^{-1}$  while the powdered sample has a strong one. The 3,520  $\text{cm}^{-1}$  band of the crystal is very intense and somewhat broad, while the corresponding peak of the powder is sharper and lower in intensity. Secondly, the 1,060  $\text{cm}^{-1}$  band, which is very strong in the spectrum of the crystal, is considerably weakened or diminished in the spectrum of the powder. Finally, the 560  $\text{cm}^{-1}$  band is characteristic for the powder, but absent in the spectrum of the crystal.

The dependency of these bands on the period of grinding was examined. The intensity ratio of the 3,460  $\text{cm}^{-1}$  band to the 3,520  $\text{cm}^{-1}$  band was found to increase as the period of grinding increased, while the 1,060  $\text{cm}^{-1}$  band was extremely weakened. It is probable that these powdering effects are not due to any increase in the surface hydroxyl group, since this is estimated to be very small compared with that of inner lattice sites. It is more likely that these effects are due to crystal deformation by the mechanical stress during the grinding.

We thank A. Kato, of the Science Museum in Tokyo, for providing the samples.

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### Variance as a Measure of Line Broadening : Particle-size Determination

THE X-ray determination of the average particle size in a polycrystalline aggregate, using the variance as a measure of line breadth, gives two independent estimates of the average size<sup>1</sup>. The variance of the line (corrected for the background<sup>2</sup>) varies linearly with the range of scan in the region where the intensity decreases as the inverse square of the range. The slope of this linear function, after subtracting the contribution from the emission-spectrum variance, is inversely proportional to the apparent particle size, provided that the specimen is well annealed and free from 'mistakes'. The apparent size calculated from the slope is the same as that determined by the Fourier method<sup>3</sup>, and is the mean thickness of the crystallites measured perpendicular to the reflecting planes<sup>4</sup>. (The integral breadth, however, gives a slightly higher apparent size, this being the volume average of the thickness of the crystal, again in the direction normal to the reflecting planes<sup>5</sup>.) The 'true' size (the cube root of the volume) is the product of the apparent size and the appropriate Scherrer constant<sup>1,6</sup>.

The intercept of the linear variance-range function is inversely proportional to the square of what may be regarded as another apparent size. The ratio of the true size to this apparent value is  $\sqrt{L}$ , where  $L$  is the taper parameter<sup>1</sup>.  $L$  is a measure of the angularity, or rate of taper, of the crystallites, and has values in the range

0.3 for the regular shapes considered by Wilson<sup>1</sup>. The taper parameter displays a wider variation than the Scherrer constant for different  $hkl$  planes, and if the intercepts of the variance function are determined for several reflexions, information about the shape of the crystallites is obtained, as well as the average size.

The particle size in an annealed nickel powder has been measured using the variance method—the linear variance-range functions being obtained for the first eight reflexions. The intercepts and slopes of these functions were in each case corrected for the effect of truncation of the integrated intensity (ref. 8, equation 8). The apparent size, based on the slope, ranges from 77 Å to 115 Å. For each reflexion, the true size can be calculated on the assumption that the shape of the particles is spherical, cubic, tetrahedral or octahedral<sup>1</sup>. This estimate of the size, for a particular value of  $hkl$ , is to some extent dependent on the assumed shape, and in this case the Scherrer constant for tetrahedra gives the most consistent value of the true size ( $180 \pm 30$  Å). Since the 'slope size' averaged for all  $hkl$  is relatively independent of the assumed shape for crystallites of the face-centred cubic system, the value of 180 Å is probably close to the actual (average) particle size. (The standard deviation of 30 Å is partly a measure of the experimental error, but it also includes the scatter introduced by the assumption that all particles are of the same size and shape.)

It was found that the variance-range functions had a significant negative intercept for all  $hkl$ , and it was immediately apparent that the particles are far from spherical. The effective apparent size is in the range 83 Å–120 Å, and of the foregoing shapes, using the appropriate taper parameters, tetrahedra again give the most consistent estimate of the true size ( $140 \pm 20$  Å). Unlike the value determined from the slopes, the 'intercept size', averaged for all  $hkl$ , is strongly dependent on the shape, as is evident from Wilson's Table 2 (ref. 1). The low average value obtained from the intercepts in fact indicates that the crystallites taper more rapidly (that is, are more angular) than tetrahedra.

An average size of 220 Å was obtained by direct measurement from electron micrographs, which agrees well with the size determined by X-ray methods. The crystallites were also observed to be angular and irregular. However, the assumption of a regular shape for the purpose of calculating the average size gives a reasonable estimate of the actual value.

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## GEOPHYSICS

### Travel-time Tables for the Seismic Wave PKP

RECENTLY I published some contributions<sup>1,2</sup> on the problem of the structure of the Earth's core. In the course of this work a considerable amount of observational material on the seismic core phase PKP was exam-

ined and empirical seismological travel-time tables for two branches of the PKP curve were derived from the observations. A number of seismologists have suggested that it would be useful to them if such tables were published.

Revised PKP travel-times are required for two main purposes: in order to derive the structural detail within the core, and in routine computer location of hypocentres of earthquakes. In the latter problem, because of the relatively small gradient of the travel-time curve, PKP observations provide a valuable constraint on origin-time and focal-depth.

In this communication my present estimates of the average-travel-times of PKP are tabulated in Table 1. The general level of the curves is tied to the *P* travel-time tables of Jeffreys and Bullen<sup>3</sup>. This standardization is achieved for each earthquake by adjusting the observed PKP times by the mean residual  $\delta$  from the Jeffreys-Bullen tables for *P* observations at distances of  $40^\circ < \Delta < 105^\circ$ . This procedure minimizes errors in the estimation of focal-depth and relates Table 1 directly to the standard spherical Earth model with a continental crust of 33 km.

Table 1. TRAVEL-TIMES OF PKP

$\Delta$ deg	(Surface focus)		GH branch (m) (s)
	DEF branch (m) (s)		
110	D 18	35.0	
115		44.6	
120		54.1	
125	19	2.7	G (18 41.7)
130		13.3	18 55.5
135		23.6	19 9.2
140	(19	33.1)	19 23.9
145	(19	40.6)	19 36.4
150		48.8	(later than DEF)
155		56.9	
160	20	02.0	
165		06.9	
170		10.4	
175		13.4	
180	F 20	13.0	

The listed times are for the two branches of the PKP curve denoted by DEF and GH (Fig. 2, ref. 2). DEF gives the times for the longitudinal waves PKIKP which penetrate the terrestrial inner core. The branch GH has been interpreted<sup>1</sup> as arising from longitudinal waves PKHKP, which are refracted into an intermediate shell which separates the liquid outer core from the solid inner core.

Values are given at intervals of  $5^\circ$  allowing interpolation from the first differences only. Adjustments for focal depth are, of course, identical with those given in the Jeffreys-Bullen tables. The times in parentheses are not directly from observations in the corresponding distance range but are interpolations. At distances greater than  $150^\circ$ , PKHKP arrives later than the PKIKP phase; the material used to derive Table 1 did not yield usefully precise values for this portion of the GH curve.

The PKP curves were derived as follows:

**The branch DEF.** In the range  $110^\circ < \Delta < 140^\circ$ , the times in Table 1 are means of three independent sets of measurements. (1) Times of PKIKP to North American and European stations from 24 selected earthquakes in southern Asia and the Pacific provide the most numerous material<sup>4</sup>. A least-squares analysis of these data led to a linear relation with a gradient of 1.92 sec/deg in the entire distance range and a standard error for one observation,  $\sigma$ , equal to 2.4 sec. (2) A series of shallow-focus Aleutian earthquakes in 1957 provided a linear equation for the PKP times to African observatories<sup>5</sup> in the range  $126^\circ < \Delta < 137^\circ$ . The gradient was 1.80 sec/deg and  $\sigma = 2.0$  sec; the maximum time difference between this solution and the one for the Pacific earthquakes was 0.6 sec.

At distances between  $140^\circ$  and  $150^\circ$ , the branches AB (this branch is generated by the PKP<sub>1</sub> waves), DEF and

Table 2. PACIFIC NUCLEAR EXPLOSIONS YIELDING RECORDED PKP

Shot name	Date	Time		Co-ordinates	Height of explosion (ft.)	Shot position	Number of PKP times and source
		(h)	(m) (s)				
Mike	Oct. 31, 1962	19	14	59-4	20	Tower	4 ISS
Bravo	Feb. 23, 1964	18	45	00-0		Surface	4 BOIS
Romeo	Mar. 26, 1964	18	30	00-4		Barge	5 BOIS
Union	April 26, 1964	18	10	00-6		Barge	2 BOIS
Yankee	May 4, 1964	18	10	00-1		Barge	5 BOIS
Wigwam	May 14, 1965	20	00	00-0	-2,000	Under water	3 ISS
Zuni	May 27, 1965	17	56	00-3		Surface	1 BOIS
Navajo	July 10, 1965	17	56	00-3		Barge	4 BOIS
Tewa	July 20, 1965	17	45	00-0		Barge	2 BOIS
Wahoo	May 16, 1963	01	30	00-5	-500	Under water	1 BOIS
Oak	June 23, 1963	19	30	00-1		Barge	4 BOIS
Poplar	July 12, 1966	03	30	00-1		Barge	5 BOIS

Table 3. PKP RESIDUALS FROM NUCLEAR EXPLOSION DATA

Shot	Station	$\Delta$ (deg)	Onsets	Travel-time (s)		MHP correction (sec)	$\delta$ (sec)	Residuals (O-O) (sec)	
				(m)	(s)			J-B	Bolt
Wigwam	Quetta	120-1	e X	18	55	0-4	0-0	1-7	0-3 DFF
Mike	Lwiro	144-5	e X	19	41	0-8		1-9	0-3 DFF
	La Paz	131-0	e N	19	17-6	1-0	-1-8	4-5	3-2 DFF
	Pretoria	134-3	e X	19	16-6	0-8		-2-5	-3-9 DFF
	Kimberley	135-6	i X	19	10-6	0-7		-12-6	-1-8 GH
Wahoo	Tamanrasset	139-2	e X	19	18-6	0-9		-9-6	-1-2 GH
	Paris	117-5	e	18	56-0	0-1	-1-8	8-8	7-3 DFF
	Halley Bay	115-9	e	18	43-9	-0-6	-1-8	0-7	-0-9 DFF
	Paris	117-2	e	18	45-4	0-1		-0-2	-1-7 DFF
Poplar	Huancayo	123-9	i	19	2-9	1-1		3-3	2-0 DFF
	Tamanrasset	138-8	e	19	19-9	0-9		-7-2	1-2 GH
	Keara	114-0	e	18	37-9	0-4	-1-8	-1-3	-3-7 DFF
	Astoria	135-0	e	19	21-9	0-8		1-6	0-1 DFF
Zuni	Uvira	135-8	e	19	23-9	0-8		2-1	0-7 DFF
	Lwiro	135-8	e d	19	24-9	0-8		3-1	1-7 DFF
	Tamanrasset	140-4	e	19	25-9	0-9		-4-4	2-8 GH
	Tamanrasset	140-6	e	19	25-7	0-8	-1-8	-5-3	1-5 GH
Navajo	Tananarive	119-9	e	18	52-7	1-0	-1-8	1-2	-0-2 DFF
	La Paz	128-0	e	19	5-7	1-0		-1-7	-3-0 DFF
	Kimberley	139-5	e	19	18-7	0-7		-0-8	-1-7 GH
	Tamanrasset	140-5	e	19	28-7	0-9		-1-8	-3-4 DFF
Tewa	La Paz	128-0	e	19	10-0	1-0	-1-8	2-6	1-3 DFF
	Tamanrasset	140-5	e	19	20-0	0-9		-10-5	-3-4 GH
	Stuttgart	116-2	(4)	18	44-4 $\pm$ 0-6	0-1	-1-8	0-7	-0-8 DFF
	Tananarive	119-8	(2)	18	53-7 $\pm$ 0-6	1-0		2-2	0-8 DFF
Bikini 1964	Pretoria	137-3	(3)	19	15-4 $\pm$ 0-6	0-6		-9-2	0-9 GH
	Kimberley	139-5	(4)	19	23-0 $\pm$ 1-0	0-7		-5-5	1-5 GH
	Tamanrasset	140-4	(4)	19	25-0 $\pm$ 1-1	0-9		-4-3	2-0 GH

GH of the PKP curve intersect so that allocation of a wave onset to its proper branch is very difficult; resolution is further obscured by the caustic at the cusp at B. (3) Values of times of PKIKP for  $\Delta > 150^\circ$  were found from measurements<sup>8</sup> of the times of this phase from the deep-focus New Zealand earthquake of December 10, 1958. More than 70 travel-times were available in the ranges  $118^\circ < \Delta < 130^\circ$  and  $146^\circ < \Delta < 180^\circ$ . A cubic polynomial, fitted by least squares, yielded  $\sigma = 1.9$  sec. The cubic joins smoothly on to the linear curve from the 24 southern earthquakes at  $135^\circ$ . The close agreement between the three independent curves at distances of  $125^\circ < \Delta < 135^\circ$  provides the justification for combining them to obtain the single solution given in Table 1.

*The branch GH.* A reliable set of measurements<sup>3</sup> is available from readings of PKHKP at North American stations from the deep-focus Java Sea earthquake of April 16, 1957. Only times from stations with  $127^\circ < \Delta < 139^\circ$  were used although there is evidence that a faster wave path exists for distances as small as  $123^\circ$ . Some weight in the computation of the mean values for GH in Table 1 was also given to observations of the precursor to PKIKP summarized in the study of the 24 southern earthquakes<sup>4</sup>.

Travel-times for GH are not given in the Jeffreys-Bullen tables. The times for DFF in Table 1 are later by 1.8 to 0.8 sec than the corresponding times given by Jeffreys and Bullen. The latter times for  $\Delta < 145^\circ$  are based on an analysis of PKP readings from the deep-focus earthquake of January 9, 1932. A subsequent check of these times was made by Jeffreys<sup>8</sup> using observations from the deep-focus earthquake of June 29, 1934; of his two solutions, the preferred one gave a mean of

+1.2 sec for PKIKP residuals at  $\Delta < 140^\circ$  with a standard error of 0.25 sec. This adjustment, which would give close agreement with the values in Table 1, was not, however, incorporated into the Jeffreys-Bullen tables.

'Calibration' of the values at some distances in Table 1, all of which are derived from earthquake data, is possible from recorded PKP transmission times from surface Pacific nuclear explosions with known shot-times<sup>7</sup>. A search of the catalogues of the *International Seismological Summary* and the *Bureau Central International Seismologique* shows that only the shots listed in Table 2 provided PKP onsets which were measured routinely.

Although a number of stations recorded several PKP phases from a single shot, only the time of the first onset has been used here. The 16 PKP measurements from the 1954 series at Bikini have been discussed already by a number of authors, so that only the over-all mean at each recording station is listed in Table 3; the remaining 23 observations, not previously summarized, are tabulated in full.

To allow direct comparison with the earthquake data the observed PKP times for each shot must be adjusted to the standard reference Earth. The adjustment  $\delta$  was found in previous work<sup>1</sup> to be -1.8 sec for a surface source at Bikini atoll. In the absence of evidence to the contrary the same value is adopted for Eniwetok atoll.

The Wigwam shot occurred under water 4 km above the abyssal plains of the eastern Pacific. P readings at 12 stations with  $70^\circ < \Delta < 100^\circ$  have a mean  $\delta$  equal to 0.0 sec. The differences between this adjustment and that for the other explosions can be explained readily in terms of



the differing  $P$  velocity in water (1.4 km/sec) and the rock under the atolls (5.1 km/sec).

In Table 3 the observed  $PKP$  times, after adjustment for  $\delta$  and ellipticity, are compared with the Jeffreys-Bullen tables for the  $DEF$  branch and with Table 1. At all distances involved, the former tables, on the average, lead to larger residuals than do the new tables. The largest residual of 7 sec comes from an apparently late reading at Paris of  $PKP$  from the Wahoo shot. On rejection of this observation the 21 onsets identified as  $PKIP$  give a mean residual against Table 1 of  $-0.4 \pm 0.4$  sec; the 18 onsets taken to be  $PKHKP$  give  $0.7 \pm 0.5$  sec. Both mean residuals do not differ significantly from zero so that these nuclear explosion data do not suggest any further revision in the  $PKP$  tables.

Inter-comparison between the independent sets of observations used to derive Table 1 allows an assessment of the general accuracy. The greatest uncertainty in the mean values is probably in the values for  $GH$ . Wave energies for this branch are relatively weak and the phase is often missed. The 18 travel-times with  $136^\circ < \Delta < 141^\circ$  from the Pacific explosions yield a standard error of  $\sigma = 2.2$  sec. (The tables for  $GH$  may encourage publication of observations of this phase in station bulletins.) Average times for  $PKIP$  at  $\Delta < 140^\circ$  in the standard Earth model are now probably known within 1 sec; standard errors calculated from the independent populations considered for the times of  $DE$  are of order 2 sec. The standard error for one  $PKIP$  observation for  $\Delta > 150^\circ$  is about 2 sec. Many of the  $PKP$  observations which were made near the antipode from the 1958 New Zealand earthquake come from stations on the Iberian Peninsula, some of which were not well equipped at the time. However, most measurements are consistent between adjacent stations. For  $\Delta > 147^\circ$ , for example, only six of the 55 observations had residuals from the Jeffreys-Bullen tables exceeding 4 sec in magnitude. If these six are rejected the standard error for one observation falls to 1.5 sec and the corresponding travel-times in Table 1 are reduced by two-tenths of 1 sec.

In conclusion, it should be stressed that the revised  $PKP$  tables represent smoothed empirical times. They do not depend on any particular assumed velocity distribution within the core. However, the velocity distribution called  $T2$  which I have derived<sup>1</sup> predicts the times in Table 1 to about 1 sec.

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### Terrestrial Control in the Production of Aurora

AMPLE evidence exists for a physical connexion of some kind between certain solar events, typified by a flare, and such related phenomena as disturbances in the Earth's magnetic field and in the ionosphere, and the occurrence of aurora. A variety of model mechanisms has been proposed to describe the links in the chain of connexion. But whether they invoke a stream of particles emanating from the active solar area or an elongated envelope of solar magnetic field anchored on the area for the first stage in

the linkage from the Sun, few of the models continue the mechanism in detail nearer the Earth than the outer confines of the magnetosphere. There are defects in any model which tries to continue the particle stream directly down to auroral level in the Earth's atmosphere using the geomagnetic field as guide lines; and to introduce the outer radiation belt as an intermediate stage has incurred the difficulty, at least according to present notions, that the energy which can be stored there is inadequate to sustain great auroral displays.

As part of the difficulty in completing the chain of events is attributable to lack of observational evidence for supporting either a direct or indirect connexion between the Sun and the Earth's atmosphere, it may be useful to summarize a few aspects of auroral activity which are relevant to the problem. These were noted<sup>1</sup> at Fort Rae, north-west Canada, during the second polar year 1932-33.

The main evidence lies in the occurrence of clear-cut departures in the development of auroral displays on two or more successive nights from the pattern of development on a majority of nights. Ignoring colour effects the average pattern to which most displays tended to conform was broadly as follows: after a more-or-less diffuse homogeneous arc had persisted quietly for several hours from evening twilight it began to disintegrate into separate bands with vertical ray structure. The change to bands of rays was at first slow, but after a few minutes the whole scene became more active and filled the sky in a series of rapid transformations. The rays extended vertically, brightened, and moved about in bundles with progressively increasing speed and intensity of brightness, the movements being both to-and-fro in an east-west (magnetic) plane and in wave-like movements out and in from that plane. At this stage the rays extended towards the zenith forming a sheet of curtains or draperies traversed by rapid waves. This violent phase, which sometimes culminated in a corona lasting a few seconds, was short-lived: the whole display quickly degenerated. The rayed curtains became diffuse and increasingly confused and sluggish in movement; they fell back from the zenith to merge together in a ragged band or confused glow lower in the sky. For a time, of the order of a minute, the part of the upper sky where the activity had been concentrated was suffused with a tangled mass of luminescence which quickly became uniform and faded.

This cycle of high activity frequently repeated itself several times in quick succession within one night's display, but on the average such short-life outbursts did not affect the basic pattern: long and slow development, rapid culmination to violently active rayed structures, followed by a quick transition back to a protracted decay. The distribution of activity in time was such that any one display could span continuously the 16 h from dusk around 4 p.m. to dawn at 8 a.m. and the active phase occurred at any time in the 5-h period centred 0.5 h before midnight. The point to be inferred from the foregoing 'identikit' sketch is that although there were differences, sometimes considerable, from night to night in the intensity and time of the most violent phase, enough of a basic generic resemblance subsisted throughout the displays of the 1932-33 season at Fort Rae to allow departures from the average pattern to become immediately noticeable. It is these unusual features in the development of displays on two or more consecutive nights, and particularly the similarity of auroral behaviour at approximately the same time on these consecutive nights, which seem to provide evidence for a terrestrial control in the mechanism producing the aurora. The repetitions of detailed behaviour were the more striking in that they occurred at times when, according to the average pattern of development, quite other phases of the display should have been in progress. The following examples were noted:

(1) February 23 and 24, 1933. The climax was reached on both nights between 23 h 5 m and 23 h 30 m L.T., and

took almost identical form—particularly long-rayed curtains in violent motion. Similar concentrations of activity occurred on February 26 and 27 between 21 h 50 m and 22 h 15 m and again on March 2 and 3 between 23 h 35 m and 23 h 40 m.

(2) In contrast with the usual first appearance of aurora in the form of a diffuse quiet arc or low glow, the displays on August 18 and 19, 1932, started at about the same time with multiple arc systems overhead or slightly south of the magnetic prime vertical plane. Subsequent developments on both nights were also similar and different from normal.

(3) December 28 and 29, 1932. A homogeneous arc which had persisted quietly for many hours quickly disintegrated at precisely the same time on both nights (5 h 55 m L.T.). A similar feature occurred on December 31, 1932, and January 1, 1933.

(4) On the evenings both before and after January 8 and 9, 1933, aurora was noted as soon as twilight was weak enough, about 16 h L.T. But, though the sky was almost cloudless, aurora was not observed until 19 h 45 m on January 8 and 19 h 30 m on January 9. Instead of the normally continuous display after the start, a period without activity intervened at 21 h.

(5) August 8 and 9, 1932. On those nights features differing from the average pattern were repeated at about the same times. Both displays began between 20 h 45 m and 21 h 15 m L.T. with band forms in the south-south-east sky. These persisted until 22 h 15 m when, after a brief lull in activity on both nights, arcs appeared across the zenith: these quickly dissipated, to be followed by another interlude of weak aurora. From 22 h 45 m until 23 h 45 m the most active phase occurred, culminating in a corona on both evenings. A further lull intervened 15 min after midnight, followed by another outburst.

(6) Well-developed and clearly defined coronas occurred infrequently. Although aurora of one form or another was observed at Fort Rae for more than 1,500 h during the 1932–33 season, the total duration of all coronas probably did not exceed 10 min. If in those circumstances a corona appeared about the same time within a few minutes on consecutive evenings, there was reason for regarding the feature as unusual, more especially when, on other occasions, a corona did form as early as 21 h on one evening and as late as 3 a.m. in the next night's display. On September 9 and 10, 1932, coronas formed at 1 h 17 m, 1 h 50 m and 2 h 3 m. On other pairs of consecutive days of the same month they appeared at 22 h 45 m and 23 h 13 m. Variants of this kind of repetition occurred on other pairs of days separated by one day.

In the face of those examples it is difficult to accept any mechanism for the production of aurora which has not one constituent at least partially within the Earth's control. Despite present assessments that its stored energy is unable to account for prolonged displays the most likely intermediary is the outer radiation belt. Besides being amenable to explain the local time repetition of particular auroral features on consecutive days, it can assume again the role attributed to it a few years ago and since withdrawn, the role of serving as a local reservoir for auroral particles even in the Sun's least active times. That this is an important requirement of any mechanism may also be inferred from the Fort Rae data. Although 1932–33 was a period of very low solar activity, aurora was observed on 242 out of 270 nights in the one season, mid-August to mid-May, and the sky was obscured by cloud on the other 28 nights.

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### Comparison of the Diurnal Variation of the Horizontal Component of the Earth's Magnetic Field at Nairobi with that at Addis Ababa

THE small magnetic observatory at University College, Nairobi, was completed in time for the International Quiet Sun Year. The station is equipped with three component La Cour variometers and is standardized by means of two quartz horizontal force magnetometers and a magnetometric zero balance. These instruments were compared with standard instruments in March 1963 by Laursen and again in December 1964 by Wienert. Observations for the first year of operation are being analysed and the results for the diurnal variation in horizontal component  $H$  during the months of April and May 1964 are given here for comparison with similar data from Addis Ababa for the same period.

The geographical co-ordinates of the station are  $36^{\circ}82' \text{ E.}$ ,  $1^{\circ}32' \text{ S.}$ , the latitude being approximately  $10^{\circ}5' \text{ S.}$  of the magnetic dip equator, which in East Africa passes close to Addis Ababa. The measured dip of  $26^{\circ}9' \text{ S.}$  compares with the  $26^{\circ}7' \text{ S.}$  found by Whitham and Hoge<sup>1</sup> in 1959 for another site in Nairobi. The station is thus well outside the main influence of the electrojet<sup>2</sup> so that it is of interest to compare the diurnal variation in  $H$  with that observed close to the electrojet at Addis Ababa. Local time is similar at the two stations so that records may be directly compared (longitude Addis Ababa  $38^{\circ}46' \text{ E.}$  Both stations use Greenwich Mean Time plus 3 h). Fig. 1 shows plots of the mean hourly ranges for the months of April and May 1964 for 9 quiet days and 8 disturbed days at Nairobi and for the same days at Addis Ababa, together with the difference between the disturbed and quiet day values. We define the hourly ranges as the departures in  $H$  from the mean midnight value for the day (the mean midnight value being taken as the mean of the values for the hours of 01 and 24 for the same day). The diurnal range is the maximum departure from this midnight value.

It will be noted that the maximum ranges occur close to noon and that, although the Addis Ababa noon range is substantially greater than that for Nairobi on quiet days, the noon ranges at the two stations are similar for disturbed days. A greater difference exists between the disturbed and quiet day curves at Nairobi than is the case for Addis Ababa, and a pronounced maximum occurs in the records from both stations at approximately 03 h on disturbed days: Onwumechilli<sup>3</sup> notes that indentations occur simultaneously at stations of differing latitude near the equator. The Nairobi records throughout the year show similar features on disturbed days.

Whitham and Hoge<sup>1</sup> comment on the large diurnal range in East Africa, and this is in agreement with previous investigations<sup>4,5</sup>. For the period under consideration we find that the mean quiet day range is 57% compared with the 76% found at Addis Ababa. The ratio of the diurnal variation at Nairobi to that at Addis Ababa is about 0.75.

Unfortunately several different definitions of daily range are in use, and this makes it more difficult to compare our results with those obtained elsewhere than would otherwise be the case. Onwumechilli<sup>3</sup> has measured the total diurnal range in  $H$  (that is, the difference between the minimum and maximum values) as a function of latitude at a number of field stations in West Africa during the period November 1956–January 1957. His measurements extend only to about  $4^{\circ}$  on either side of the dip equator; he finds that the ratio of the total range at a station of about  $4^{\circ}$  dip latitude to that observed near the dip equator is approximately 0.55. The value of this ratio between Nairobi and Addis Ababa for quiet days in April and May 1964 is about 0.8 and is considerably higher than one would expect from Onwumechilli's observations. Onwumechilli<sup>3</sup> also estimates a total quiet-day

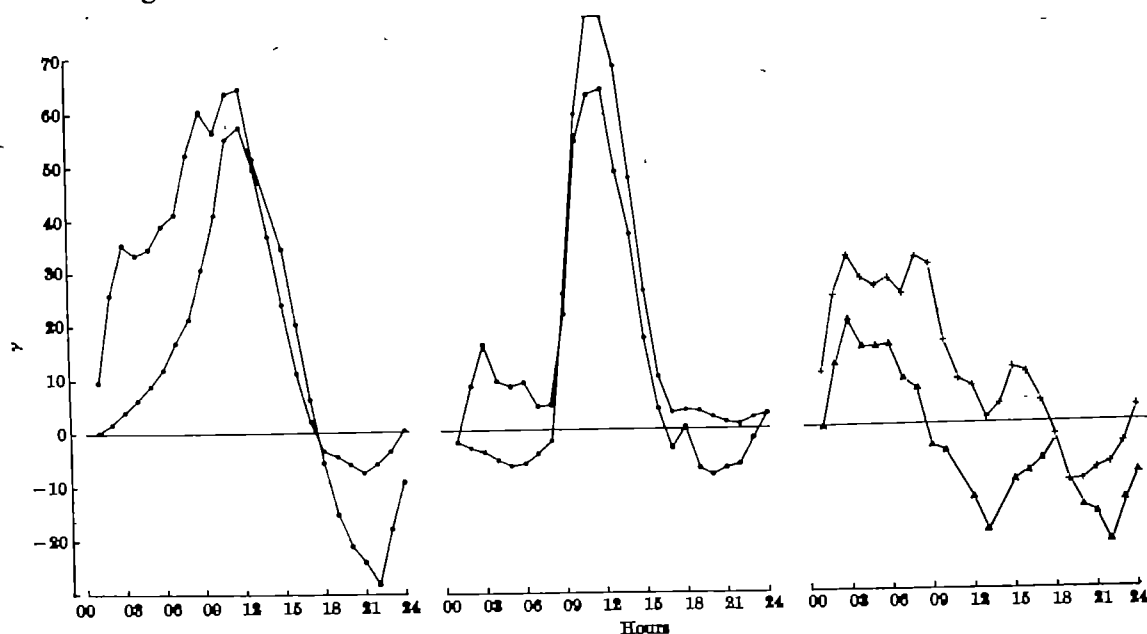


Fig. 1. a, Nairobi, ●, quiet; ○, disturbed; b, Addis Ababa, ●, quiet; ○, disturbed; c, disturbed less quiet values, +, Nairobi; Δ, Addis Ababa

diurnal range in  $H$  of  $64\gamma$  at the geographical equator by assuming a parabolic relation for the variation with latitude of the quiet day normal field (that is, the field not including the influence of the electrojet). The value found by us for the total quiet-day range at Nairobi for sunspot minimum is  $85\gamma$ . Data published for Addis Ababa<sup>5</sup> suggest that the daily range at sunspot minimum is about 60 per cent of that at sunspot maximum and thus lend support to the idea that the daily range is high at Nairobi.

Vestine *et al.*<sup>6</sup> quote 95–100 $\gamma$  for the total diurnal range on the geographical equator, averaged for the months of April and May over the period 1922–1933. Forbush and Casaverde<sup>7</sup> give curves which suggest a range of  $95\gamma$  for a station  $10^\circ$  from the dip equator and  $210\gamma$  for a station near the dip equator in Peru and at sunspot maximum; the value for the total range at Addis Ababa in 1958 is  $175\gamma$  (ref. 5). Whitham and Hoge<sup>1</sup> give curves for 5 quiet, 5 disturbed and 24 other days which show an average total diurnal range of  $120\gamma$  in March and April 1959 in Nairobi. The disturbed day total range at Nairobi for April and May 1964 is  $92\gamma$  and is substantially greater than the  $71\gamma$  observed at Addis Ababa.

The adoption of a standard definition of daily range would much simplify the comparison of data from different stations. Further comparisons of the data from Nairobi and Addis Ababa will be continued in order to obtain more reliable information and comparison of the seasonal effects.

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## MINERALOGY

### Stilpnomelane from the Dalradian

STILPNOMELANE has been found as a constituent of the schists of many low-grade areas throughout the world<sup>1–3</sup>. In the Dalradian it has been described from the South-west Highland epidiorites<sup>4–6</sup> but not from the greywackes and pelites of the Upper Dalradian, although it is mentioned as occurring in "the quartz-feldspathic green schists of the Southern Dalradian" by Voll<sup>7</sup>.

Two distinct members of the stilpnomelane group of minerals have been found in the Ben Ledi Grits of the Highland Border area. They occur in association with albite, microcline, quartz, muscovite and chlorite north-west of Aberfoyle in Perthshire.

On the basis of their pleochroism, the Dalradian stilpnomelanes may be divided into green varieties, rich in ferro-stilpnomelane, and brown varieties, rich in ferristilpnomelane. Pleochroism is strong with axial colours as follows. Ferro-stilpnomelane:  $\alpha$  = very pale yellow to pale yellow,  $\beta = \gamma$  = pale green to olive green; ferristilpnomelane:  $\alpha$  = pale golden yellow,  $\beta = \gamma$  = dark brown.

The stilpnomelane occurs either as radiating clusters lying in and across the schistosity planes or as thin scales wrapped around and embaying quartz and feldspar grains. Some microcline grains are completely replaced by finger-like growths of stilpnomelane which project into and grow within them. Stilpnomelane occurs in the grits both as the only ferromagnesian phase and as an accessory constituent in the more common assemblage, albite-microcline-quartz-muscovite-chlorite.

Table 1 lists the X-ray powder data for a brown stilpnomelane from a grit to the north of the slate belt. The measurements were obtained using a Debye-Scherrer camera, filtered cobalt radiation and a small amount of quartz as an internal standard. The data are tabulated alongside that of Gruner<sup>8</sup> for a brown stilpnomelane from Baern, Moravia. Unfiltered iron radiation was used, but in tabulating the data the lines due to  $K\beta$  have been ignored. The powder photograph also contains other lines, notably one at  $8.20 \text{ \AA}$ . The origin of these is not clear, but they may be spurious peaks as the X-rays were filtered but not otherwise monochromatized<sup>9</sup>. The basal spacing  $12.1 \text{ \AA}$  agrees with that of Gruner<sup>8,10</sup> and Hutton<sup>1</sup>.

Apart from the Aberfoyle area, stilpnomelane has been found as an abundant mineral in a grit from the Lemy

Table 1. X-RAY POWDER DATA FOR STILPNOHOMELANE

Line number	Present study	Gruner 1937
1	12.07	11.9
2	6.067	6.03
3	4.730	4.74
4		4.188
5	4.042	4.045
6	3.567	3.566
7	3.030	3.036
8	2.707	2.693
9	2.562	2.549
10		2.481
11		2.418
12	2.345	2.341
13	2.182	2.188
14	2.092	2.109
15	1.950	1.964
16	1.884	1.888
17	1.685	1.686
18	1.581	1.578
19	1.569	1.561
20	1.510	1.519
21	1.412	1.416
22	1.408	1.397
23	1.348	1.359
24		1.339
25	1.320	1.322
26	1.302	1.306
27	1.284	1.287
28	1.237	
29	1.151	1.151
30	1.093	
$d_{001}$ average	12.12	12.13

Grit Series south of the Birnam slate quarries near Dunkeld in Perthshire. Therefore it seems possible that stilpnomelane is widely distributed in the grits and greywackes of the Upper Dalradian and may be present throughout the Highland Border area.

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## GEOCHEMISTRY

### Oxygen Isotope Fractionation between Coexisting Calcite and Dolomite in the Freshwater Upper Carboniferous Freeport Formation

HYDROTHERMAL experiments of Epstein, Graf and Degens<sup>1</sup> have suggested that dolomite forms from pre-existing calcite by a replacement mechanism in which the  $^{18}\text{O}/^{16}\text{O}$  ratio of the original calcite remains unchanged. In an investigation of natural sedimentary dolomite-calcite rocks, Degens and Epstein<sup>2</sup> observed no fractionation of the oxygen isotopes between coexisting calcite and dolomite in relatively young deposits and concluded that all dolomite is secondary, that is a replacement product of pre-existing calcite. Older carbonate rocks examined by those authors, however, did contain dolomite which was enriched in oxygen-18 with respect to the associated calcite. This phenomenon was explained by possible isotopic equilibration of the calcite with intrastatal fluids (which are generally enriched in oxygen-16). Dolomite is believed to be more resistant to equilibration. Hence, according to Degens and Epstein, such calcite rocks are partly replaced by diagenetic dolomite without alteration of the isotopic composition of the carbonate. With time, oxygen isotope exchange between formation waters and the calcite, but to a negligible extent between the waters and the dolomite, results in a higher  $^{18}\text{O}/^{16}\text{O}$  ratio in the dolomite. Thus the difference in  $\delta^{18}\text{O}_{\text{dol}}$  and  $\delta^{18}\text{O}_{\text{cal}}$  ob-

served in ancient carbonate rocks does not represent original isotopic equilibrium between these two minerals.

Although the mechanisms postulated by Degens and Epstein are possible, there is some doubt as to whether 'all' dolomite deposits have been formed in this manner, and indeed, if even most of the fine-grained dolomites have such an origin. The question is an important one since problems concerning the evolution of the oceans and possible extension of the oxygen isotope palaeogeothermometer to older rocks are involved<sup>3</sup>. For this reason, a number of occurrences of very fine-grained, dense, compact carbonate rocks of freshwater origin are being examined on the basis of a working hypothesis that: (1) dolomite in such deposits originated soon after deposition of the calcite, before consolidation and lithification of the sediments, and (2) the slow growth of authigenic dolomite permitted oxygen isotope equilibrium between the two minerals. Observations consistent with this hypothesis would comprise: (1) oxygen-18 enrichment in dolomite with respect to calcite in the order of 5 to 8 per mil, (2)  $\delta^{18}\text{O}$  values for calcite in the range of present freshwater calcites, and (3)  $\delta^{18}\text{O}$  values for dolomite significantly greater (more positive) than those of present freshwater calcites. If the  $^{18}\text{O}/^{16}\text{O}$  ratios of a reasonable sampling of freshwater dolomitic limestones are not much different from modern freshwater calcites, then the conclusions of Degens and Epstein would seem valid.

The results of an investigation of the dolomite-calcite micrites of the Palaeocene-Eocene Flagstaff formation (central Utah) have already been reported<sup>4</sup>:  $\delta^{18}\text{O}_{\text{cal}} = -9$  to  $-10$  per mil, and  $\delta^{18}\text{O}_{\text{dol}} = -3$  to  $-4$  per mil relative to the PDB standard carbon dioxide. These values may be compared with the isotopic composition of eleven freshwater calcium carbonates of Quaternary age (mean  $-8.15$  per mil, *S.D.* 2.08) and 63 of Tertiary age<sup>5</sup> (mean  $-9.65$ , *S.D.* 4.48).

The fine-grained dolomitic limestones of the Freeport formation (Upper Carboniferous) outcropping over an area of about 100 square miles in central Pennsylvania constitute the second examination of this nature. The freshwater depositional environment is established by the presence of freshwater ostracods, *Spirorbis*, *Carbonicola* and other freshwater fossils, as well as by detailed palaeogeographic reconstruction. The fine-grained nature of the coexisting carbonates precludes physical separation of the calcite and the dolomite. The Degens and Epstein method of differential extraction of carbon dioxide based on the difference in the reactivity rate of these carbonates with phosphoric acid cannot be applied because of the presence of siderite in the Freeport samples. To obtain the values of  $\delta^{18}\text{O}_{\text{cal}}$  and  $\delta^{18}\text{O}_{\text{dol}}$ , a number of samples from each collecting site were analysed to obtain  $\delta^{18}\text{O}$  for the bulk carbonate, that is a mixture of calcite, dolomite and siderite. The mineral composition of the rock was obtained by quantitative X-ray diffraction (I thank Dr. R. E. Bergenback for collection of the samples and for quantitative X-ray diffraction data), and  $\delta^{18}\text{O}$  bulk was plotted against percentage of calcite, percentage of siderite and percentage of dolomite by fitting a least squares plane to the data (multiple linear regression). Extrapolation of the plane to 100 per cent calcite and 100 per cent dolomite provides the isotopic composition of each of these minerals. The equation is of the form,  $Y = a + bX_1 + cX_2 + dX_3$ , where  $Y = \delta^{18}\text{O}$ ;  $X_1$ ,  $X_2$ , and  $X_3$  = percentage of calcite, siderite and dolomite, respectively;  $a$ ,  $b$ ,  $c$ , and  $d$  are constants. The isotopic composition of calcite, for example, is calculated by setting  $X_1 = 100$ ,  $X_2 = X_3 = 0$ , and solving for  $Y$ . One hundred and thirty-two samples were analysed (by standard techniques, see ref. 5: precision, represented by the standard deviation, is 0.05 per mil) and the data from any given site were discarded if the coefficient of multiple correlation was not significant at the 1 per cent level. Failure to fit an adequate regression plane to the data may arise because of insufficient variation in mineralogical composition among the samples from one site, or

because  $\delta^{18}\text{O}_{\text{ct}}$  and/or  $\delta^{18}\text{O}_{\text{dol}}$  were not constant over the few feet in the stratigraphic section from which the samples were taken.

Even including the results when the multiple linear regression curve did not account for the variation in the four variables ( $Y$ ,  $X_1$ ,  $X_2$ ,  $X_3$ ) to an acceptable degree (that is, the 1 per cent level of significance) the dolomite was invariably enriched in  $^{18}\text{O}$ . Mean values for the six collecting sites where the coefficient of multiple correlation was highest are:  $\delta^{18}\text{O}_{\text{ct}} = -7.87$  permil;  $\delta^{18}\text{O}_{\text{dol}} = -3.04$  permil;  $\delta^{18}\text{O}_{\text{dol}} - \delta^{18}\text{O}_{\text{ct}} = 4.83$ . Both the Flagstaff and the Freeport data support the working hypothesis; the dolomite is relatively enriched in oxygen-18 with respect to modern and Tertiary calcites. If further investigations of freshwater carbonates substantiate these findings, equilibration of the oxygen isotopes of calcite and dolomite prior to lithification may be indicated.

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<sup>1</sup> Epstein, S., Graf, D. L., and Dore, R. T., in *Isotopic and Cosmo Chemistry*, 169 (North Holland Publishing Co., Amsterdam, 1963).

<sup>2</sup> Dore, R. T., and Epstein, S., *Geochim. Cosmochim. Acta*, **28**, 23 (1964).

<sup>3</sup> Weber, J. N., p. 930 of this issue of *Nature*.

<sup>4</sup> Weber, J. N., *Science*, **144**, 1303 (1964).

<sup>5</sup> Keith, M. L., and Weber, J. N., *Geochim. Cosmochim. Acta*, **28**, 1787 (1964).

## CHEMISTRY

### Polythene-backed Paper for High-voltage Electrophoresis

THE improvement of technique achieved in zone electrophoresis through the use of potential gradients up to 150 V/cm has been found to be partially offset by difficulties of manipulation due to the poor wet strength of conventional papers. The marketing of 'Benchkote' (Whatman) sold by H. Reeve Angel and Co., Ltd., 9 Bridewell Place, London, E.C.4, in rolls 45 cm  $\times$  50 m), a polythene-backed filter paper (primarily intended as a protective covering for benches) of very high wet strength, prompted the tests described here, to determine if such a material would exploit the advantages of high-voltage electrophoresis to the fullest extent.

The trials described were performed using a 'Virus' high-voltage electrophoresis apparatus (supplied by Dr. Virus KG, Rosenbergweg 20, Bonn). This instrument requires that the paper medium (dimensions 100 cm  $\times$  45 cm) be soaked in buffer, that excess buffer be squeezed out by passage through rollers and then that it be stretched tightly over a cooled glass plate. The potential is applied through buffer chambers (set 60 cm apart) making contact with the paper through sintered glass moistened with the buffer. Failure to remove excess buffer or to make good contact with the cold glass surface negates the advantages gained by using a high voltage to bring about the resolution desired. The increased wet strength given by the polythene backing of 'Benchkote' makes it a superior material for that series of manipulations, when compared with ordinary chromatographic paper. An added advantage is that the polythene backing prevents water, condensed from the atmosphere on to the cold glass surface, being taken up into the paper.

When 'Benchkote' is soaked with buffer the paper swells but the polythene does not, and it has been found best to blot off most of the excess buffer before squeezing through the rollers. Also the 'Benchkote' should be fed into the rollers paper side down and from above, so that the buffer squeezed out drains off the bottom roller and cannot flood the incoming sheet.

The prepared paper is almost dry to the touch. Electrophoresis has been carried out with potential gradients up to 130 V/cm (the limit of the power pack), but the usual

conditions were 80 V/cm with a maximum current of 40 mA and a cooling plate temperature of 1° C.

Most of the work done using 'Benchkote' in this way has been to study the nucleotides produced by the action of acid on yeast RNA. The resolution has been improved relative to other electrophoretic techniques used formerly and only one-quarter of the time is required for an analysis. Substances which absorb ultra-violet light have been detected using 2537 Å illumination (from a low-pressure mercury arc<sup>1</sup>) with the same ease and sensitivity as on ordinary paper.

Tests of the method have also been made running amino-acid separations, and the gain in resolving power reported previously<sup>2</sup> has been seen. The ninhydrin reagent works effectively for the detection.

Other spray reagents involving heating the electrophoretogram may also be used, as temperatures of 100° C do not damage 'Benchkote' unless the exposure is unduly prolonged, when the softening polythene gives a permanent warp.

Polythene-backed paper has considerable advantages for the kind of work described. The results obtained with 'Benchkote' and the versatility of the technique may be improved by bonding to polythene a range of papers of truly chromatographic quality. (Messrs. Reeve Angel have kindly given me a sample of polythene-backed Whatman No. 3MM paper; polythene-backed papers of chromatographic quality may become commercially available later. They warn that 'Benchkote' is marketed as a bench covering, and that due to the tolerances to which it is manufactured its performance in paper electrophoresis may be variable. A suitable standard ought therefore to be included on each electrophoretogram.)

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<sup>1</sup> Markham, R., *Biochem. J.*, **57**, 9 (1963).

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### Al-Fe Isomorphic Substitution in 3 CaO-Al<sub>2</sub>O<sub>3</sub> and 2 CaO.Fe<sub>2</sub>O<sub>3</sub>, and Interactions between the So-called C<sub>3</sub>A and C<sub>4</sub>AF Phases

THE isomorphic replacement of iron by aluminium in 2 CaO.Fe<sub>2</sub>O<sub>3</sub> has been widely investigated, but much less attention has been directed to the introduction of iron in the structure of 3 CaO.Al<sub>2</sub>O<sub>3</sub>.

A recent investigation by Schlaudt and Roy<sup>1</sup> led to the conclusion that, at 1,389° C, a maximum of 3.5 moles per cent Fe<sub>2</sub>O<sub>3</sub> can be substituted for Al<sub>2</sub>O<sub>3</sub> in 3 CaO.Al<sub>2</sub>O<sub>3</sub>, leading to the limiting solid solution 3 CaO.(Al<sub>0.985</sub>Fe<sub>0.015</sub>)<sub>2</sub>O<sub>3</sub>. This amount is still lower for different (higher or lower) temperatures.

Work carried out in this laboratory by X-ray diffraction and infra-red absorption spectroscopy is not in accordance with this limiting composition.

The observed 2θ diffraction angles have been plotted as a function of composition for 3 diffraction lines of high indices (lines 8.8.0, 12.4.0 and 12.8.4; C.G.R. diffractometer; iron K $\alpha$  radiation). For the 3 lines, a regular decrease of the 2θ values is observed for increasing amounts of iron, up to 10 moles per cent Fe<sub>2</sub>O<sub>3</sub>; these results (Fig. 1) suggest a limiting composition 3 CaO.(Al<sub>0.95</sub>Fe<sub>0.05</sub>)<sub>2</sub>O<sub>3</sub> for the solid solutions prepared at 1,310° C.

These results do not necessarily imply that the limiting composition obtained at 1,389° C by Schlaudt and Roy is in error, but, most probably, the 'low' temperature (1,200–1,350° C) region of their phase equilibrium diagram needs some revision.

By applying the same technique to the isomorphic replacement of iron by aluminium in 2 CaO.Fe<sub>2</sub>O<sub>3</sub>, the

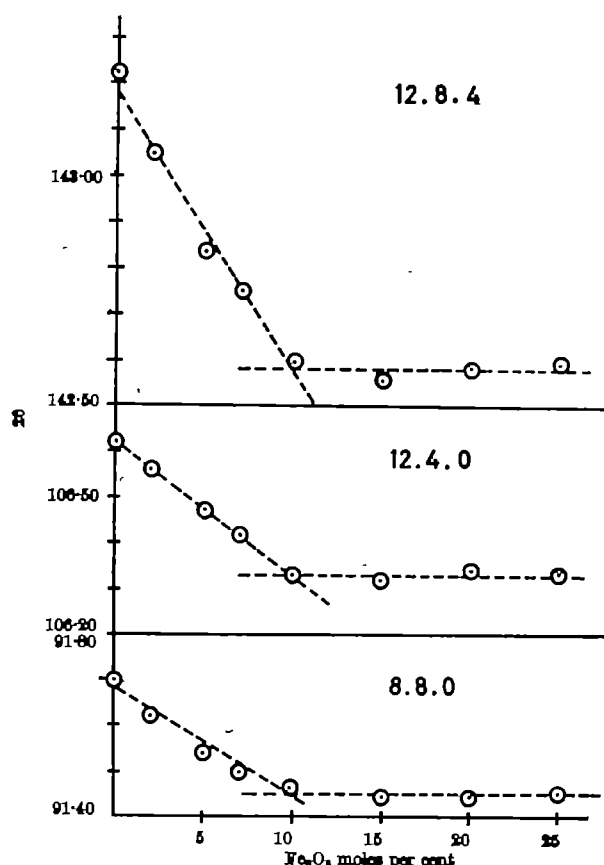


Fig. 1. 2θ diffraction angles of the lines 12.8.4, 12.4.0 and 8.8.0 as a function of  $\text{Fe}_2\text{O}_3$  content in the system  $3\text{CaO}(\text{Al},\text{Fe})_2\text{O}_3$ .

limiting composition of the solid solution is found to be  $2\text{CaO}(\text{Fe}_{0.75}\text{Al}_{0.25})_2\text{O}_3$ , in perfect agreement with previous data of Malguori and Cirilli<sup>2</sup>, but significantly different from the  $2\text{CaO}(\text{Fe}_{0.25}\text{Al}_{0.75})_2\text{O}_3$  limit proposed by Toropov and Boikova<sup>3</sup>.

These results strongly suggest that the phases  $3\text{CaO} \cdot \text{Al}_2\text{O}_3$  and  $2\text{CaO} \cdot (\text{Al}_{0.75}\text{Fe}_{0.25})_2\text{O}_3$  (the so-called  $\text{C}_3\text{A}$  and  $\text{C}_4\text{AF}$  phases in the chemistry of cements) cannot co-exist as such (as it is admitted in the calculation of the potential mineralogical composition of clinkers) since some iron is liable to pass from the  $\text{C}_4\text{AF}$  phase into the  $\text{C}_3\text{A}$  phase—this being counterbalanced by the transfer of an equivalent amount of alumina from  $\text{C}_3\text{A}$  to  $\text{C}_4\text{AF}$ .

Intimate mixtures of  $\text{C}_3\text{A}$  and  $\text{C}_4\text{AF}$  in various ratios were fired at  $1,300^\circ\text{C}$ , then air quenched, and the resulting phases investigated by X-ray diffraction and infra-red absorption spectra. The composition of these phases (as deduced from the 2θ values) is summarized in Table 1.

Table 1. CHANGES IN THE COMPOSITION OF  $\text{C}_4\text{AF}$ - $\text{C}_3\text{A}$  MIXTURES AFTER FIRING AT  $1,300^\circ\text{C}$

Initial composition of the mixture (in moles)		Composition of the solid solutions obtained after firing	
$\text{C}_4\text{AF}$	$\text{C}_3\text{A}$	$\text{C}_4\text{AF}$	$\text{C}_3\text{A}$
0.25	0.75	$\text{C}_4\text{AF}_{0.1-0.2}\text{Fe}_{0.9-0.8}$	$\text{C}_3\text{A}_{0.9-0.8}\text{Fe}_{0.1-0.2}$
0.50	0.50	$\text{C}_4\text{AF}_{0.1-0.2}\text{Fe}_{0.8-0.7}$	$\text{C}_3\text{A}_{0.8-0.7}\text{Fe}_{0.2-0.3}$
0.75	0.25	$\text{C}_4\text{AF}_{0.1-0.2}\text{Fe}_{0.7-0.6}$	$\text{C}_3\text{A}_{0.7-0.6}\text{Fe}_{0.3-0.4}$

The aluminium-iron exchange between the 2 phases  $\text{C}_3\text{A}$  and  $\text{C}_4\text{AF}$  is thus definitely proved. A further, qualitative checking of such exchanges is also given by infra-red absorption investigations, the infra-red absorption spectrum of a simple mechanical mixture of  $\text{C}_3\text{A}$  and  $\text{C}_4\text{AF}$  being fairly different from the spectrum of a mixture of the same gross composition fired at  $1,300^\circ\text{C}$ .

It is also worth mentioning that the high-angle diffraction lines and the infra-red absorption bands of such mixtures are significantly broadened after firing at  $1,300^\circ\text{C}$ : this suggests an imperfect crystallization state. In some of the mixtures investigated, a further firing at

$1,340^\circ\text{C}$  (followed by air quenching) brings out such a broadening of the high-angle diffraction lines that precise position measurements are impossible.

This bad crystallization state, together with the occurrence of  $\text{C}_3(\text{A},\text{F})$  solid solutions containing significant amounts of iron, have important implications in the chemistry of cements, since both factors are likely to affect the chemical nature of the hydrated phases, and the kinetics of the hydration phenomena.

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## BIOPHYSICS

### Ribonucleic Acid Synthesis in Synchronously Dividing Populations of HeLa Cells

A NUMBER of investigations directed towards the description of the events occurring during the reproductive cycle of the mammalian cells have been made in randomly dividing cell populations. For example, the time-course of synthesis of DNA has been elucidated in several systems by use of microspectrophotometric or autoradiographic techniques<sup>1-3</sup>. However, for investigations in which it is difficult to identify the stage of a cell in the mitotic cycle, synchronously dividing populations are of value. Recently, we were able to obtain large populations of mitotically synchronized HeLa S-3 cells by preferential detachment of cells in mitosis from nearly confluent monolayers growing in very low calcium medium. We report here some of our observations on the rate of RNA synthesis in synchronized HeLa cells during one division cycle.

The technique of obtaining synchronized populations is a modification of that of Robbins and Marcus<sup>4</sup>. HeLa S-3 cells were grown in monolayers on a flat glass surface in French square bottles using Eagle's medium supplemented with 10 per cent calf serum. Three to four day stock cultures were trypsinized and  $7.0 \times 10^5$  cells were transferred to each of twelve 32-oz. bottles (surface area  $97\text{ cm}^2$ ). After cells were grown in regular Eagle's medium supplemented with 10 per cent calf serum for three days, growth medium was removed from the bottles, and replaced with low calcium medium. Cells were incubated at  $37^\circ\text{C}$  for 20 h. Three hours before use, the bottles were vigorously shaken and washed with the low calcium medium to eliminate any floating and dead cells. (This procedure was found to be necessary in order to eliminate any interphase cells at the time of collection of the mitotic cells.) At collection time the bottle was grasped by the neck and shoulders, held at eye-level horizontally, and inverted as quickly as possible so that the medium passed rapidly over the cells. The bottle was quickly opened and the cell suspension was poured into a conical centrifuge tube. After centrifugation, the cells were plated in 60-mm plastic Petri dishes in 4 ml. of normal growth medium. All procedures were carried out at  $37^\circ\text{C}$  in a warm room, except for the centrifugation and plating procedures. For the examination of RNA and DNA synthesis, the extent of incorporation of tritiated uridine and tritiated thymidine into RNA and DNA was considered a measure of RNA and DNA synthesis respectively. Cells were incubated for 15 min in media containing the radioactive compounds mentioned here, then washed three times with

saline and removed by trypsinization. 1 ml. of the trypsinized cells was used to count the number of cells per plate by the use of a Coulter counter model B; the rest of the samples were centrifuged and frozen. To determine the tritiated thymidine incorporation into DNA, cells were washed three times in cold 5 per cent trichloroacetic acid, washed once in 80 per cent ethanol, and dissolved in 0.2 ml. of 0.2 N sodium hydroxide. The final sample was counted in a liquid scintillation counter. For the tritiated uridine incorporation into RNA, the separation of RNA from DNA radioactivity was achieved by the method of Kahn<sup>4</sup>. The filters were transferred to counting vials and the radioactivity was counted by the liquid scintillation counter.

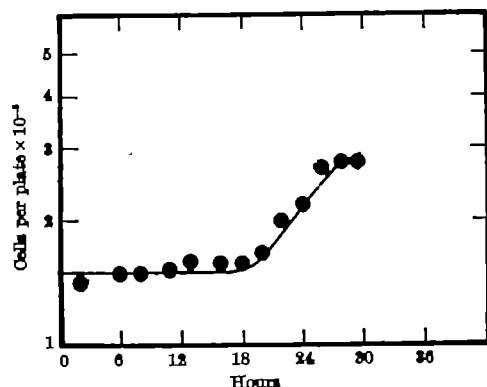


Fig. 1. Growth of mitotically synchronized HeLa S-3 cells. Mitotic cells were collected at 37° C and equal numbers of cells were plated down in plastic Petri dishes. At various intervals, cells were trypsinized and changes in cell number were determined by a Coulter counter model B.

Fig. 1 shows the increase in cell number per plate, after plating down the collected cells. Cytological examinations were made every time the cells were collected. More than 90 per cent of the collected cells were visibly in mitosis. The fraction of cells dividing during the first 20 h constituted less than 10 per cent of the population, after which 85–95 per cent underwent division during a 9–10 h period. In order to determine the degree of the synchrony, we used the index of synchrony ( $F$ ) proposed by Blumenthal and Zaler<sup>5</sup>:

$$F = \frac{N_2}{N_1} - e^{kt}$$

where  $N_1$  is the number of cells at time  $t_1$ ;  $N_2$  is the number of cells at time  $t_2$ ;  $t$  is the time-interval  $t_2 - t_1$ ; and  $k$  is 0.693/doubling time. For the data of Fig. 1, the value for  $F$  is 0.72.

To investigate the timing of RNA synthesis, we determined the rates of incorporation of tritiated uridine into RNA throughout the mitotic cycle (Fig. 2). As a comparison of reference, the rate of the DNA synthesis determined by the extent of the incorporation of tritiated thymidine was made. One sharp peak in the rate of DNA synthesis occurred at 16 h. The rate of RNA synthesis showed two maxima and minima; the maxima appeared at 15 and 20 h; the minima at 18 and 24 h.

The results of the foregoing experiment demonstrate that the rate of tritiated uridine incorporation is not uniform throughout the mitotic cycle but decreases during the mitosis. This finding is, at least qualitatively, in agreement with the recent report by Mittermayer *et al.* in *Physarum polycephalum*<sup>6</sup>, in which two peaks of RNA synthesis, during the mitotic cycle, were shown. Reiter and Littlefield<sup>7</sup>, using partially synchronized mouse fibroblasts, obtained by the use of 5-fluorodeoxyuridine, showed that the synthesis of nuclear RNA, as judged by the rates of incorporation of hypoxanthine-<sup>14</sup>C, decreased somewhat during the period of DNA synthesis, increased as the latter slowed, and remained active before and after mitosis. The use of a metabolic inhibitor, however,

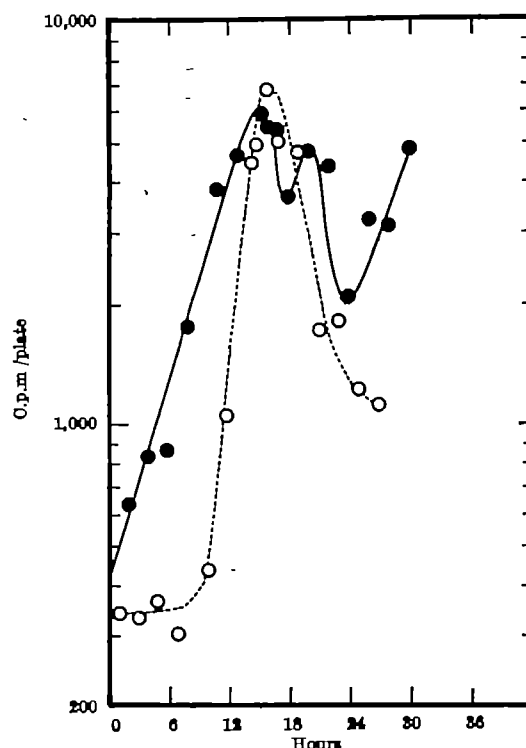


Fig. 2. The rates of incorporation of tritiated uridine and thymidine into RNA and DNA respectively during synchronized growth of HeLa S-3 cells. Approximately  $10^4$  cells per plate were inoculated in regular growth media containing either tritiated uridine (3  $\mu$ Ci/ml, spec. act. 2.0 c/mmole) for RNA synthesis or tritiated thymidine (0.5  $\mu$ Ci/ml, spec. act. 1.9 c/mmole) for DNA synthesis for 15 min. ●, Tritiated uridine; ○, tritiated thymidine.

for study of normal relationships between intracellular metabolic events occurring during the mitotic cycle is limited, as severe temporal distortions may result by the chemical stresses imposed. Our technique of obtaining synchronized population imposes minimal stress on the system and, as demonstrated here, caused no distortion of the normal pattern of DNA synthesis.

At present we are attempting to correlate the cyclic variations of RNA synthesis with RNA polymerase activity in this system.

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### Inhibition of Photo-inactivation of Taka-amylase A by Halogen Ions

RIBOFLAVIN is known as a favourable sensitizer in various photochemical reactions such as photo-oxidation of vitamins<sup>1</sup>, plant growth hormone<sup>2-4</sup> and deoxyribonucleic acid<sup>5</sup>, and photo-inactivation of amylase<sup>6,7,12</sup>.

In the presence of oxygen, riboflavin is decomposed by visible light to lumiflavin<sup>8-10</sup> or humichrome and ribose side chain<sup>11</sup>. Lumiflavin is finally transformed to stable humichrome.

In the presence of riboflavin, taka-amylase A (isolated<sup>13</sup> from taka-diastase 'Sankyo') is rapidly inactivated by



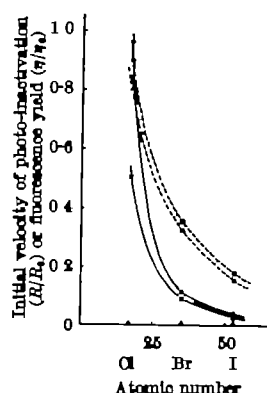


Fig. 1. Effect of halogen ions on initial velocity of photo-inactivation of taka-amylase A and fluorescence yield of riboflavin. Concentration of amylase, 0.005 per cent; concentration of riboflavin,  $8.8 \times 10^{-4}$  M; concentration of halides, 0.05 M; exciting light, light absorbed by 445 mμ-band of riboflavin; pH, 5.6 (acetate buffer); temperature, 20° C.  $R_0$ , initial velocity of photo-inactivation in the absence of halide;  $\eta_0$ , fluorescence yield of riboflavin in the presence of halide;  $\eta$ , fluorescence yield in the absence of halide; curves 1 and 3,  $R/R_0$  and  $\eta/\eta_0$  in the presence of potassium halides; curves 2 and 4,  $R/R_0$  and  $\eta/\eta_0$  in the presence of calcium halides.

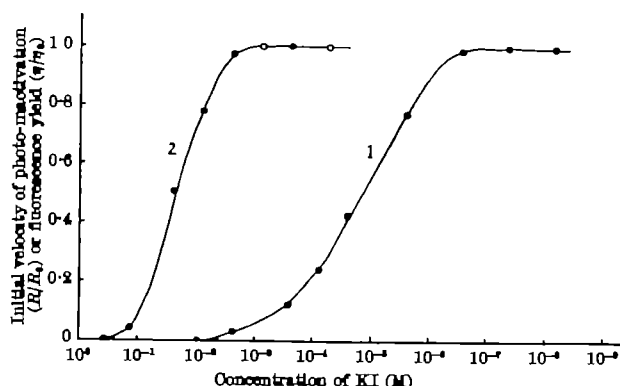


Fig. 2. Dependence of initial velocity of photo-inactivation and fluorescence yield on concentration of potassium iodide. Concentration of amylase, 0.005 per cent; concentration of riboflavin,  $8.8 \times 10^{-4}$  M; pH, 5.6 (acetate buffer); temperature, 20° C; exciting light, light absorbed by 445 mμ-band of riboflavin; curve 1,  $R/R_0$ ; curve 2,  $\eta/\eta_0$ .

the visible light absorbed by riboflavin or its derivatives if oxygen is present. The inactivation is caused by the oxidation of amino-acid residues<sup>12</sup>. The addition of potassium or calcium halide to taka-amylase A solution (pH 5.6) containing riboflavin inhibits efficiently the photo-inactivation. The presence of these halides does not affect the activity of taka-amylase A under the dark condition. The ratio of initial velocity ( $R$ ) of photo-inactivation in the presence of halide to that ( $R_0$ ) in the absence of halide is shown in Fig. 1. The velocity of photo-inactivation decreases with the increase of the atomic number of the halogen atom. The order of effectiveness of the halogen atoms is  $I > Br > Cl$ .

The effect of halogen ions on fluorescence yield of riboflavin in the same system is also shown in Fig. 1. The fluorescence yield decreases with the increase of the atomic number of halogen atom. Fig. 2 shows the dependence of fluorescence yield of riboflavin and initial velocity of photo-inactivation on the concentration of potassium iodide. The velocity of photo-inactivation begins to decrease at an extremely low concentration of potassium iodide, of the order of  $10^{-7}$  M, while the fluorescence begins to be quenched at much higher concentration of potassium iodide, the order of  $10^{-5}$  M. These indicate that the reactive species of riboflavin is the triplet molecule and that the inhibition of photo-inactivation is caused by the quenching of the triplet state of riboflavin by halogen ions.

This consideration is also supported by the strong quenching of orange phosphorescence by the presence of

iodine ion even below  $10^{-4}$  M, which runs parallel to the inhibition of photo-inactivation.

Riboflavin or its derivatives in the metastable triplet state may form a complex with oxygen in the triplet ground state. According to the spin conservation law, singlet, triplet and quintet states are allowed for this complex. The singlet complex dissociates into singlet dye and singlet oxygen (production of reactive oxygen) and the triplet one into singlet dye and triplet oxygen (quenching of triplet state of dye). The metastable and chemically reactive singlet oxygen may oxidize amino-acid residues directly or react with water to produce hydrogen peroxide which may oxidize them. In the presence of halogen ions, the pure triplet state can be mixed with an appropriate pure singlet state as the result of a collisional perturbation of spin-orbital coupling<sup>14,15</sup> in the  $\pi$ -electron orbitals of riboflavin, by halogen ions. The transition probability between singlet and triplet is greatly enhanced.

Perturbation occurs by the overlapping of the  $\pi$ -orbital of riboflavin with the halogen  $p$ -orbitals during the collision. The enhancement of the singlet-triplet absorption band of  $\alpha$ -chloro-naphthalene in intensity by ethyl iodide has been found by Kasha<sup>16</sup> and discussed in terms of the collisional perturbation of spin-orbit coupling. The collisional perturbation gives rise to the enhancement of the dissipation of excitation energy by radiationless transition from excited singlet to ground state via triplet state. From the dependencies of photo-inactivation and fluorescence on halogen ion concentration, it is considered that the collisional perturbation of spin-orbit coupling by halogen ion, in the present case, increases the radiationless transition probability from triplet to singlet state more efficiently than it does that from excited singlet to triplet state, as the result of the mutual relation among the various processes of radiative and non-radiative transitions and heat activation. This results in the shortening of the life-time of the triplet state of riboflavin responsible for the photosensitizing action. The matrix element of the spin-orbital operator included in the singlet-triplet mixing coefficient contains the atomic number as a parameter<sup>14</sup>. For this reason, it is considered that the effectiveness of the halogen atom for the inhibition of photo-inactivation increases with the increase of the atomic number of the halide atoms.

However, we cannot completely neglect the possibility of formation of an unstable charge-transfer complex<sup>16</sup> of riboflavin with halogen ion, especially iodine ion. The fluorescence and phosphorescence states may be deactivated by transfer of charge from halogen ion to riboflavin. This kind of quenching also seems to take part in the inhibition of photo-inactivation to some extent. Further details will be published in a future paper.

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## BIOCHEMISTRY

## Simultaneous Chromatographic Separation and Infra-red Sample Preparation of Indole Acids

In attempting to identify trace amounts of metabolites from biological fluids, the use of one of the most powerful tools of organic chemical analysis, infra-red spectroscopy, presents technical difficulties. The two major ones are: (a) a sample preparation technique for the characteristically polar compounds, and (b) sufficient purity to allow a resolvable spectrum.

Having become involved in the problem of the identification of microgram amounts of indole acids in urine<sup>1</sup>, it was found that conventional chromatography, elution, and KBr micro-pellet sample preparation was frequently accompanied by contamination from cellulose particles and complexes of solvent and cellulose in spite of the use of rather rigorous techniques<sup>2,3</sup>. It was at this time that the work of Sloan<sup>4</sup>, demonstrating the infra-red transparency of membrane filters ('Millipore TH') and their use in the infra-red examination of hydraulic fluids and condensates from tobacco smoke was brought to our attention. About the same time Thomas and Dwyer<sup>5</sup> reported the use of this membrane in the collection and infra-red examination of gas chromatographic effluents. Although both these papers speculated on the use of this material for partition chromatography, there have been no reports of its use in this way. One of the major limitations of the use of this polymer is its solubility in most polar solvents<sup>6</sup>. Another is that with careful compensation using a matched blank piece in a double-beam infra-red system, the strong absorption regions (Fig. 1) of 6.1, 7.8, 9.4 and 11.9 permit too little energy through to allow analysis in these areas. Following the trial of several solvent combinations, it was found that isopropanol-ammonia-water (8:1:1) and butanol-acetic acid-water (12:3:5) (good solvent systems for the indole acids) leave the membrane intact. It should be noted that these two solvent systems are good for amino-acid separations as well. In addition, the compensated membrane has 'windows' in the N—H, carbonyl, and aromatic C—H areas of interest for identification of indole acids.

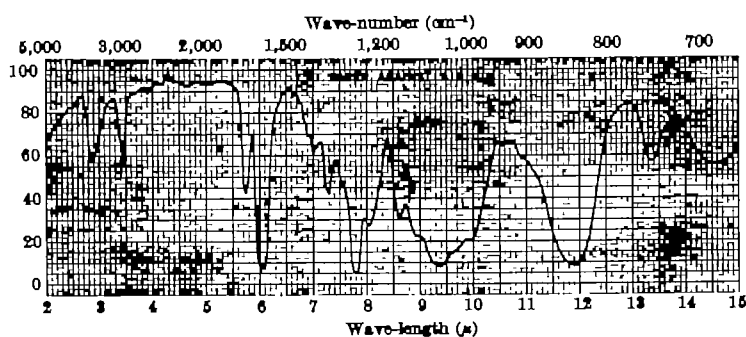


Fig. 1. An infra-red spectrum of the membrane against air

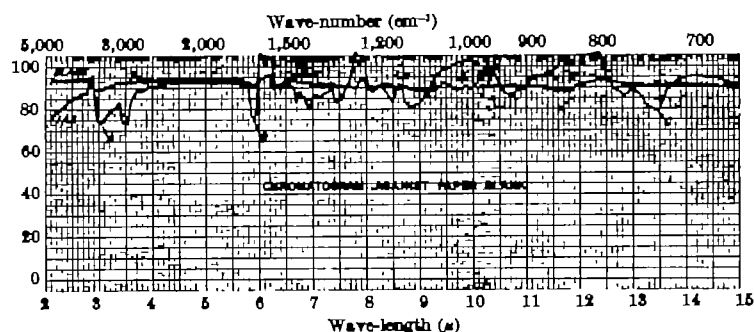


Fig. 2. Two infra-red spectra. 'Blank' is an empty area of the chromatogram against a blank sheet of membrane. 'IAA' is an area of the chromatogram containing indolyl-3-acetic acid against a blank sheet of membrane



Fig. 3. An infra-red spectrum of the  $\nu$ N—H (A), carbonyl (B) and aromatic C—H (C) with a 5  $\times$  increase in IAA concentration

Following spotting, 19 mm by 41 m strips of the membrane were run uni-dimensionally in a conventional ascending chromatography apparatus. An amount of indole acids (indolyl-3-acetic acid, indolyl-3-carboxylic acid, 5-hydroxy-indolyl-3-acetic acid, and tryptophan) equivalent to that contained in 15 c.c. of urine was used. Following chromatography, the solvent was removed by air and/or oven-drying and serial spectra on each chromatogram were run against a blank membrane every 0.4 cm from the origin to the solvent front. After infra-red examination, the membranes were sprayed lightly with acidic alcoholic solutions of *p*-dimethylaminobenzaldehyde or *p*-dimethylaminocinnamaldehyde to locate the substances.

Fig. 2 is a rather typical spectrum that was observed using this technique, with small samples (1 micromole) and a low-resolution instrument (Beckman 'I.R.5'). The 'blank' is the spectrum seen using an area of the chromatogram without sample against a blank sheet of membrane. The 'IAA' is the spectrum of the IAA area of the chromatogram against the blank. Note that the match is slightly undercompensated in favour of the blank so that the strong absorption peaks (\*) of the membrane are reversed (up) leaving the chromatogram's peaks more clearly seen in the conventional down direction. Another feature of the IAA spectrum is the bathochromic shift of  $\nu$ N—H (A) and carbonyl (B) peaks when compared with those seen in KBr pellets. In addition, the  $\nu$ N—H, characteristically sharp, looks 'bottomed out' like the  $\nu$ O—H seen in concentrated solutions due to hydrogen bonding. These peaks

are of importance in the study of indole acids in that they shift characteristically with inductive effects of substituents at various positions<sup>7</sup> and thus are an aid in identification. In order to establish whether the shifts seen in the chromatographic substances were due to intermolecular hydrogen bonding between indole acid molecules or the indole acid and the membrane, increasing amounts of material were placed on the chromatogram (beyond the amounts in which spot size is linearly related to amount) so as to increase the ratio of indole-indole to indole-membrane interactions. Fig. 3 shows both the sharpening of the  $\nu$ N—H (A) and aromatic C—H (C) as well as a shift to lower wave-length of  $\nu$ N—H and the carbonyl (B) peaks consistent with a decrease in hydrogen bonding when the amount is increased to 5 micromoles. This suggests that, with low concentrations, there are bonding interactions of the chromatographed substances with the cellulose ester molecules of the membrane. With a low-resolution instrument, this effect makes the use of  $\nu$ N—H less helpful in discriminating between indolyl-3-acetic acid and indolyl-3-carboxylic acid; the bathochromic shift of the latter becomes less differentiable due to the hydrogen-bonding induced comparable shift in indolyl-3-acetic acid. Having taken this effect into account, however, it was possible to discriminate among these indole acids spectroscopically.

A systematic attempt is being made to improve the separations by systematically varying solvent and solvent ratios. It appears that this method may be quite useful in the application of infra-red techniques to the identification of trace substances from biological fluids.

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### Melanocyte-stimulating Hormone Activity of Synthetic MSH and ACTH Peptides, *in vivo* and *in vitro*

SIMULTANEOUSLY performed *in vivo* and *in vitro* determinations of the melanocyte-stimulating hormone (MSH) activity of a large number of synthetic MSH and ACTH peptides have not been reported previously. The work recorded here compares by both methods the MSH activity of 10 stereoisomeric pentapeptide fragments of MSH and 8 synthetic peptides related to ACTH. The MSH activity of most of the stereoisomers appeared greater *in vivo* than *in vitro*. When these compounds were assayed at various time intervals, some of the pentapeptides revealed differences which may help partially to explain this discrepancy in activity between the two methods. These differences may also help in understanding the alterations of  $\alpha$ -MSH induced by sodium hydroxide<sup>1,2</sup>. In addition, preliminary studies were performed on the inhibitory properties of the stereoisomers.

For the *in vivo* assay, solutions were injected into the dorsal lymph sac of hypophysectomized *Rana pipiens*. Melanin granule dispersal (darkening) was measured in the interdigital web of the foot, as previously described<sup>3</sup>. Doses were given so as to bracket the melanocyte index of 3.0. At the same time, darkening of isolated pieces of frog skin was determined in the *in vitro* assay by reflected light<sup>4,5</sup>. At least 3 hypophysectomized frogs and 2 frog skins were utilized at each of 2 dose levels. For measurement of the synthetic peptides *in vivo*, a mean of 5.2 frogs was used at each of 2.9 doses; for *in vitro* assays, a mean of 4.1 skins was used at 2.5 doses. The 'unit' of activity used in this communication was defined by Shizume *et al.*<sup>6</sup>.

The mean estimates of MSH potency, with 95 per cent confidence limits, are given in Table 1. The *in vivo* assay method gave higher activities for most of the MSH pentapeptides than did the *in vitro* method.

*In vivo*, the 30-min reading of the melanocyte index approximated the maximum degree of melanin granule dispersal. This darkening was significantly diminished ( $P < 0.01$ ) at the final 1-h reading with MSH standard, pure  $\alpha$ -MSH, and the 7 ACTH peptides tested. The darkening was not decreased at 1 h, however, with D-His-D-Phe-L-Arg-L-Try-Gly, L-His-D-Phe-L-Arg-L-Try-Gly, and some of the other MSH pentapeptides tested only in a few frogs before the supplies were exhausted. The same difference in behaviour between stereoisomers and MSH standard when injected into the dorsal lymph sac was observed when the substances were injected directly into the aortic trunk. Changing the pH of the solutions from 6.7 to 2.1, moreover, did not alter this finding.

Table 1. MSH ACTIVITY MEAN ESTIMATES OF POTENCY WITH 95 PER CENT CONFIDENCE LIMITS OF THE ASSAYS GIVEN IN PARENTHESES

Compound	MSH activity (U/mg)	
	<i>In vivo</i>	<i>In vitro</i>
<b>MSH stereoisomers*</b>		
L-His-D-Phe-L-Arg-L-Try-Gly	2.54 × 10 <sup>4</sup> (2.12-3.04 × 10 <sup>4</sup> )	2.30 × 10 <sup>4</sup> (1.92-2.76 × 10 <sup>4</sup> )
D-His-D-Phe-L-Arg-L-Try-Gly	2.09 × 10 <sup>4</sup> (1.55-2.82 × 10 <sup>4</sup> )	0.224 × 10 <sup>4</sup> (0.151-0.344 × 10 <sup>4</sup> )
L-His-L-Phe-L-Arg-D-Try-Gly	7.05 × 10 <sup>3</sup> (5.40-9.16 × 10 <sup>3</sup> )	0.840 × 10 <sup>4</sup> (0.704-0.924 × 10 <sup>4</sup> )
L-His-D-Phe-D-Arg-L-Try-Gly	6.68 × 10 <sup>3</sup> (5.53-7.96 × 10 <sup>3</sup> )	1.05 × 10 <sup>4</sup> (0.748-1.46 × 10 <sup>4</sup> )
D-His-D-Phe-D-Arg-L-Try-Gly	1.06 × 10 <sup>4</sup> (0.617-1.88 × 10 <sup>4</sup> )	0.405 × 10 <sup>4</sup> (0.195-0.842 × 10 <sup>4</sup> )
L-His-D-Phe-D-Arg-D-Try-Gly	0.763 × 10 <sup>4</sup> (0.551-1.04 × 10 <sup>4</sup> )	0.273 × 10 <sup>4</sup> (0.171-0.437 × 10 <sup>4</sup> )
D-His-L-Phe-L-Arg-D-Try-Gly	0.875 × 10 <sup>4</sup> (0.570-0.530 × 10 <sup>4</sup> )	0.143 × 10 <sup>4</sup> (0.0686-0.297 × 10 <sup>4</sup> )
L-His-L-Phe-L-Arg-L-Try-Gly	70.30 × 10 <sup>3</sup>	0.127 × 10 <sup>4</sup> (0.065-0.190 × 10 <sup>4</sup> )
D-His-L-Phe-L-Arg-L-Try-Gly	70.15 × 10 <sup>3</sup>	0.153 × 10 <sup>4</sup> (0.0695-0.337 × 10 <sup>4</sup> )
D-His-D-Phe-D-Arg-D-Try-Gly	7 < 0.10 × 10 <sup>4</sup>	70.04 × 10 <sup>3</sup>
<b>ACTH peptides†</b>		
N $\alpha$ -Acetyl- $\beta$ - <sup>24</sup> -Corticotropin	1.58 × 10 <sup>4</sup> (1.25-2.00 × 10 <sup>4</sup> )	0.513 × 10 <sup>4</sup> (0.399-0.658 × 10 <sup>4</sup> )
$\beta$ - <sup>1-4</sup> -Corticotropin-Lys <sup>14</sup> methyl ester	5.85 × 10 <sup>4</sup> (4.96-6.90 × 10 <sup>4</sup> )	17.0 × 10 <sup>4</sup> (13.1-22.1 × 10 <sup>4</sup> )
$\beta$ - <sup>1-4</sup> -Corticotropin-Pro <sup>24</sup> hydrazide	5.18 × 10 <sup>4</sup> (4.18-6.43 × 10 <sup>4</sup> )	5.59 × 10 <sup>4</sup> (3.72-8.38 × 10 <sup>4</sup> )
$\epsilon$ -MSH-sequence <sup>7-13</sup>	7.13 × 10 <sup>4</sup> (6.00-8.47 × 10 <sup>4</sup> )	6.68 × 10 <sup>4</sup> (4.86-9.15 × 10 <sup>4</sup> )
$\beta$ - <sup>1-16</sup> -Corticotropin	4.35 × 10 <sup>4</sup> (3.98-5.35 × 10 <sup>4</sup> )	1.94 × 10 <sup>4</sup> (1.14-3.30 × 10 <sup>4</sup> )
$\beta$ - <sup>1-18</sup> -Corticotropin	0.407 × 10 <sup>4</sup> (0.318-0.521 × 10 <sup>4</sup> )	0.236 × 10 <sup>4</sup> (0.200-0.292 × 10 <sup>4</sup> )
$\beta$ - <sup>1-20</sup> -Corticotropin	0.309 × 10 <sup>4</sup> (0.225-0.425 × 10 <sup>4</sup> )	0.208 × 10 <sup>4</sup> (0.160-0.270 × 10 <sup>4</sup> )
$\beta$ - <sup>1-24</sup> -Corticotropin	7 < 0.02 × 10 <sup>4</sup>	0.023 × 10 <sup>4</sup> (0.0147-0.0368 × 10 <sup>4</sup> )

\* Synthesized by H. Yajima and K. Kubo, Kyoto, Japan.

† Supplied by D. F. Elliott, Ciba, Ltd.

*In vivo*, 6 ACTH compounds as well as the D-His-D-Phe-L-Arg-L-Try-Gly and the L-His-D-Phe-L-Arg-L-Try-Gly pentapeptides were read every 30 min for 2 h. Each stereoisomer required a significantly ( $P < 0.01$ ) longer time (approximately 2 h versus 1.5 h) to reach its maximum effect than did MSH standard, pure  $\alpha$ -MSH, or any of the ACTH peptides.

Thus, both the *in vivo* and the *in vitro* behaviour of D-His-D-Phe-L-Arg-L-Try-Gly contributed to the discrepancy in MSH values observed for this compound between the two methods. Although L-His-D-Phe-L-Arg-L-Try-Gly manifested similar differences in assay behaviour, it did not reveal a similar discrepancy in MSH activity. No significant deviation from the behaviour of the standard in either assay was noted for any of the ACTH compounds tested.

The differences in darkening shown by some of the stereoisomers, when measured at various time intervals, were similar to the phenomena of 'prolongation' and 'retardation' seen after sodium hydroxide treatment of  $\alpha$ -MSH<sup>1,2</sup>. In an attempt to explain this action of dilute alkali, Lee and Buettner-Janusch<sup>3</sup> obtained evidence that sodium hydroxide caused racemization of 5 of the 13 amino-acids in natural  $\alpha$ -MSH. Of these 5 amino-acids, 4 were present in the synthetic stereoisomeric peptides tested in the assays reported here. The results reported here, therefore, lend support to the suggestion<sup>1,2</sup> that alkali modification of  $\alpha$ -MSH involves racemization of some of its amino-acids.

Inhibitory properties of the MSH pentapeptides were tested only in a preliminary way, due to a limited supply of the compounds. *In vitro*, none of the 10 MSH peptides inhibited the action of MSH standard when given 1 h earlier at a concentration of 1.5  $\mu$ g/ml. However, 83  $\mu$ g/ml. of D-His-D-Phe-D-Arg-D-Try-Gly did result in inhibition *in vitro*, as has been reported<sup>4</sup>. *In vivo*, 600  $\mu$ g of this pentapeptide injected 0.5 h to 2 h before the MSH standard did not cause inhibition. A single dose of 100  $\mu$ g failed to lighten a frog previously darkened by destruction of the hypothalamus.

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## N-Acetylmannosamine Digestion by Human Oral Bacteria

LEACH<sup>1</sup> showed that *N*-acetylneuraminic acid was rapidly metabolized by human saliva and ascribed this to the presence of an inducible aldolase produced by contaminating micro-organisms since such destruction was prevented by the addition of 'Ledermycin' or by heating the saliva in boiling water. This appears to be the second step in a process whereby salivary mucoproteins having *N*-acetylneuraminic acid residues as their non-reducing end groups act as inducer molecules to oral bacteria, producing first a neuraminidase and later an aldolase which cleaves the *N*-acetylneuraminic acid liberated by the neuraminidase into *N*-acetylmannosamine and pyruvic acid. Such a sequence has been found by us<sup>2</sup> to occur when  $\alpha_1$ -glycoprotein, which is present in sputum, is used as an inducer molecule in cultures of *Klebsiella aerogenes*. We wish to report on the third step whereby *N*-acetylmannosamine itself is digested.

Fresh saliva samples (0.5 ml.) were incubated with sterile aliquots of *N*-acetylmannosamine (100  $\gamma$  in 0.1 ml.) at 37° for 2 h, dialysed against water (100 volumes) at 4° and the dialysate freeze-dried before assay with the Morgan-Ellson reagents<sup>3</sup>. From a random group of healthy individuals including smokers and non-smokers, the amounts of *N*-acetylmannosamine remaining were with A, 77  $\mu$ g; B, 34  $\mu$ g; H, 95  $\mu$ g; M, 90  $\mu$ g; P, 84  $\mu$ g and W, 51  $\mu$ g. In a further incubation of saliva from B, only 40, 30 and 10 per cent of the original *N*-acetylmannosamine was left after 1, 2 and 4 h respectively. When saliva from B was assayed with incubation for 1 h before and after a mouth-wash, first with 'Dettolin' (2.5 ml./70 ml. water) and then with hydrogen peroxide (5 ml. 20 vol./70 ml. water), it was found that the first mouth-wash produced arrest of *N*-acetylmannosamine digestion (73  $\mu$ g remaining) compared with saliva before washing (45  $\mu$ g remaining), but this was not increased by the second mouth-wash (73  $\mu$ g remaining).

When glucose (1 mg) was included in the incubation mixture in addition to *N*-acetylmannosamine (100  $\mu$ g), digestion of the latter was again arrested: B with glucose, 63  $\mu$ g; B without glucose, 41  $\mu$ g (2 h incubation); W with glucose, 90  $\mu$ g (2 h), 60  $\mu$ g (4 h); W without glucose, 11  $\mu$ g (4 h). Glucose is widely effective in inhibiting enzyme induction in bacteria.

The micro-organisms responsible for *N*-acetylmannosamine digestion in the saliva of B and W differed in their sensitivity to a range of antibiotics. Each antibiotic (5 mg) was incubated with the saliva for 1 h at 37° before further incubation with *N*-acetylmannosamine (100  $\mu$ g) for 4 h. The amount ( $\mu$ g) remaining with B saliva was with erythromycin, 99; tetracycline, 76; streptomycin, 68; kanamycin, 51; methymycin, 50; without any antibiotic, 37. On one occasion with W saliva the amount ( $\mu$ g) remaining was, with tetracycline, 93; without any antibiotic, 65; while later with another sample no completely effective arrest was shown with streptomycin, 53; erythromycin, 45; methymycin, 26; paromomycin, 25; kanamycin, 22, compared with a control without antibiotic, 14.

Further evidence that the agent responsible for *N*-acetylmannosamine digestion was an enzyme induced in a micro-organism was obtained by comparing the amounts remaining after autoclaving salivas B, 99 W, 100, after Seitz filtration of salivas B, 97 W, 99, compared with no previous treatment of B, 49 W, 64  $\mu$ g.

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## Kinetics of Cyclic Adenosine Monophosphate Changes in Rat Heart following Epinephrine Administration

ALTHOUGH it has become generally accepted<sup>1</sup> that epinephrine causes glycogenolysis in cardiac muscle by a series of enzymatic reactions involving the formation of cyclic AMP, activation of phosphorylase *b* kinase, and finally conversion of phosphorylase *b* to the active *a* form, direct evidence in favour of this mechanism in the intact heart is so far not conclusive<sup>2</sup>. Furthermore, a possible relation between cyclic AMP and the inotropic effect of catecholamines is also tentative.

In view of the recent findings<sup>3,4</sup> that the inotropic response of the rat heart to epinephrine precedes the glycogenolytic effect, it is clearly of great importance to determine the kinetics of the cyclic AMP changes in relation to the elevation of the contractile force and phosphorylase *a* levels in order to gain a deeper understanding of the mechanisms involved.

Hearts were taken from male, fed rats of Wistar strain (220–260 g) and perfused as described by Williamson<sup>5</sup>. Cyclic AMP in 500-mg samples of frozen heart powder was extracted and assayed by the method of Posner *et al.*<sup>6</sup>. Cyclic AMP phosphodiesterase was prepared according to Butcher and Sutherland<sup>7</sup>. Other analytical procedures were similar to those used by Williamson<sup>5,8</sup>.

Fig. 1 compares the change in the level of cyclic AMP with that of the total phosphorylase in the *a* form, and that of the contractile force, following the addition of a single dose of 1  $\mu$ g epinephrine. The contractile force increased to a maximum after 10–12 sec with a half-time of 3.5 sec, and afterwards decreased to a value 30 per cent above the initial at the end of 30 sec. More extensive recordings of the contractile force reported elsewhere<sup>3,4</sup> have shown that the pattern of the contractile force depicted in Fig. 1 is characteristic of a single large dose of epinephrine, and that the force increases again after 30 sec, reaching a plateau 60–70 per cent above the control after about 90 sec. This level is maintained for as long as 10 min. Cyclic AMP levels increased in a manner similar to the force curve ( $t_{1/2}$  = 3.5 sec), and also paralleled it during the declining phase from 15 to 30 sec. At the end

of 30 sec, the cyclic AMP levels were three times higher than the controls. Also seen from Fig. 1, phosphorylase *a* increased from 12 to 65 per cent of the total phosphorylase activity but with a lag of about 2 sec, and the time for a half-maximal increase was 7.5 sec.

The initial cyclic AMP peak may represent an overshoot reaction of the adenylate cyclase system as it is first exposed to epinephrine, or merely a temporary imbalance between an accelerated rate of production and degradation before a new steady state is established.

Fig. 2 shows the results of an experiment in which the hearts were perfused continuously with fluid containing 0.05  $\mu\text{g/ml}$  epinephrine. With this concentration of epinephrine, the force increased to a maximum after 15–18 sec ( $t_{\frac{1}{2}} = 3$  sec) and remained approximately constant for the 2-min duration of the experiment. Cyclic AMP levels increased monotonically from the onset of the augmentation of the contractile force to reach a maximum after about 14 sec ( $t_{\frac{1}{2}} = 5$  sec) and thereafter decreased, in much the same manner as in Fig. 1, to a new steady-state level considerably higher than the controls.

Fig. 2 also shows the changes in the levels of glucose-6-P and fructose-1,6-diP in the same experiment. Glycogenolysis in response to epinephrine produced an increase in the levels of these intermediates after a short lag period. Glucose-6-P increased by 80 per cent to give a broad maximum after 30–40 sec, while fructose-1,6-diP increased three-fold, to reach a sharp maximum after 25 sec and approached a new steady-state level in an oscillatory manner. Fructose-6-P (not shown) followed a pattern similar to glucose-6-P, and the mean ratio of glucose-6-P to fructose-6-P was 5:3.

The work recorded here establishes on a kinetic basis that epinephrine causes a rapid increase of cyclic AMP in the heart, followed by an increase in the level of phosphorylase *a*. Demonstration of glycogen disappearance within the first minute of epinephrine administration to the isolated rat heart has been reported previously<sup>4</sup>. These investigations, therefore, provide the necessary link between the work of Murad *et al.*<sup>6</sup> on particular preparations of heart muscle, and the intact organ, and lend

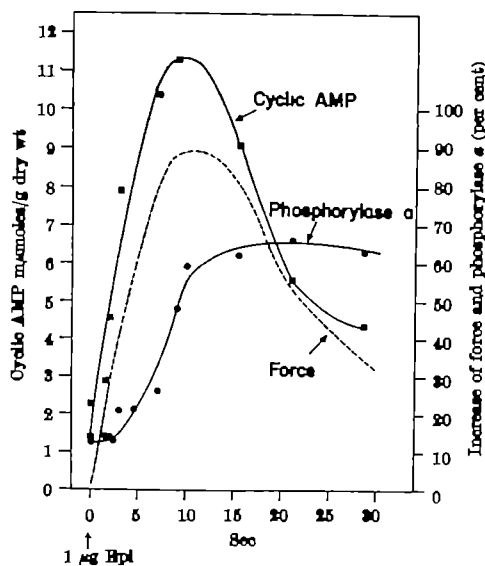


Fig. 1. Effect of epinephrine on the contractile force, cyclic AMP content and percentage of the total phosphorylase as phosphorylase *a* in the rat heart. Hearts were perfused at 37° with non-recirculating medium containing 10 mM glucose. Two determinations for cyclic AMP were made on each sample, once with an aliquot of extract treated with trypsin and trypsin inhibitor<sup>7</sup>, and once with a similar extract after a further incubation for 3 h at 80° with 2 units of cyclic AMP phosphodiesterase. The latter treatment decreased the apparent cyclic AMP to a constant level in all extracts, equivalent to 2.0 nmol/g dry wt. This mean figure was subtracted from each value obtained in the first assay to give the values shown. Recoveries of cyclic AMP added to extracts were essentially quantitative.

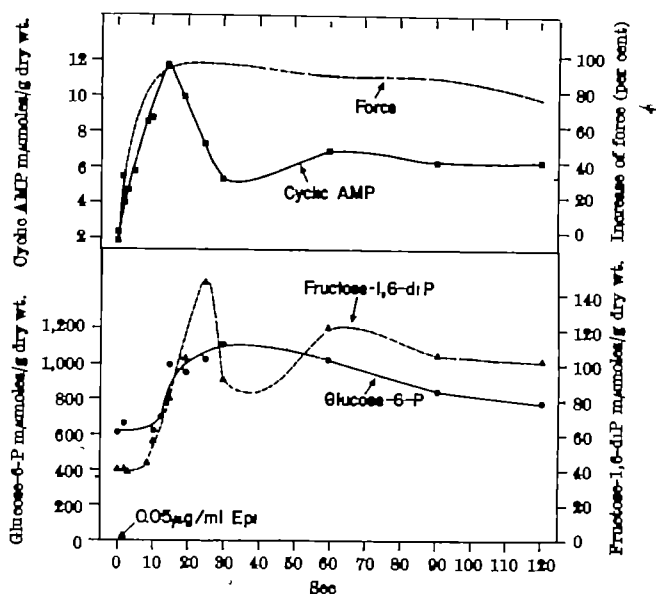


Fig. 2. Effect of epinephrine on the contractile force and the contents of cyclic AMP, glucose-6-P and fructose-1,6-diP in rat hearts perfused continuously at 37° with medium containing 10 mM glucose,  $5 \times 10^{-4}$  M EDTA and 0.05  $\mu\text{g/ml}$  epinephrine. The residual apparent cyclic AMP content after diesterase treatment was 2.8 nmol/g dry wt., and the values were corrected as before.

support to the prevalent hypothesis<sup>1</sup> concerning the mechanism of phosphorylase activation in the heart by epinephrine.

It should perhaps be stressed that the response of cyclic AMP following epinephrine administration is fast and short-lived. The finding of Hammermeister *et al.*<sup>2</sup> that there was no significant change in the level of cyclic AMP 30 sec after epinephrine stimulation is thus explainable in light of the results recorded here.

The short time-lag between the increase of contractile force and cyclic AMP on one hand, and phosphorylase *a* and glucose-6-P on the other hand, indicates that energy produced from glycogen breakdown<sup>10</sup> or the level of glucose-6-P<sup>11</sup> are not involved in the inotropic response. The delay between the stimulation of cyclic AMP production and the conversion of phosphorylase *b* to *a* probably reflects a delay either in the activation of phosphorylase *b* kinase or in the phosphorylation of the serine residues of phosphorylase *b* in its conversion to the *a* form<sup>12</sup>. Danforth and Helmreich<sup>13</sup> have also observed a lag period between the onset of electrical stimulation and the appearance of phosphorylase *a* in isolated frog sartorii, and on the basis of *in vitro* studies, these authors attributed the lag to the time required to activate the phosphorylase *b* kinase reaction.

The fact that cyclic AMP levels in the heart increased more than two-fold within 1.5 sec of the onset of the inotropic response provides kinetic evidence in favour of its also being a mediator in the events leading to the increased force of contraction. The lack of correspondence between the level of cyclic AMP and the contractile force after the peak of cyclic AMP response, however, suggests that it may have a trigger role, possibly by allowing a greater influx of calcium per beat or by interference with the removal of ionized calcium by the endoplasmic reticulum<sup>14,15</sup>.

It has been shown elsewhere<sup>16</sup> that during epinephrine-stimulated glycogenolysis in the perfused rat heart phosphofructokinase is activated by an increase in the levels of inorganic phosphate, AMP and ADP. The kinetics of the changes in the levels of cyclic AMP in relation to those of fructose-1,6-diP shown in Fig. 2 suggest that this nucleotide plays a relatively unimportant part in the activation of phosphofructokinase *in vivo*.

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### Chemical Structure and Biological Activity on *m*- and *p*-Disubstituted Derivatives of Benzene

In an earlier paper on this topic<sup>1</sup> we proposed a four-parameter equation (1), which expresses the quantitative relationship between the structure of *p*-disubstituted derivatives of benzene and the magnitude of their biological activity, for example intravenous  $LD_{50}$ . This report presents our results on a group of compounds of the type



$NO_2$ ,  $NH_2$ . The series includes all possible combinations of groups  $X$  and  $Y$ , with the exception of three substances which were not soluble enough even in 20 per cent aqueous polyvinylpyrrolidone solution to make  $LD_{50}$  measurements possible. The experimental results fitted equation (1):

$$\log \frac{[LD_{50}]_{HH}}{[LD_{50}]_{XY}} = b_X + b_Y + e_X e_Y \quad (1)$$

Constants  $b_X$  and  $e_X$  were obtained by statistical treatment of experimental data under the presumption of the validity of equation (1). Fig. 1 shows the correlation obtained; the values of the substituent constants  $b_X$  and  $e_X$  are given in Table 1.

Table 1

	$NO_2$	Cl	OH	$CH_3$	H	$NH_2$
$b_X$	0.601	-0.248	0.260	0.149	0.004	-0.015
$e_X$	0.90	-0.49	-0.13	-0.23	-0.06	-0.41

Table 2

	$NO_2$	Cl	OH	$CH_3$	H	$NH_2$
$b_X$	0.516	-0.295	0.294	0.191	0.014	0.015
$(e_X)_m$	0.84	-0.45	-0.04	-0.13	0.00	-0.37
$(e_X)_p$	0.68	-0.04	0.57	0.07	0.00	-0.33

A comparison of the constants  $b_X$  for the *m*-substituted compounds with the values of  $b_X$  found for the *p*-substituted compounds<sup>1</sup> showed that for the given group of compounds  $\Delta b_X < 0.1$ , where  $\Delta b_X = (b_X)_p - (b_X)_m$ . We considered it advantageous to recalculate the  $b_X$  constants basing them on all data from both groups. Fig. 2 shows the results of this treatment; the constants  $b_X$ ,  $(e_X)_m$  and  $(e_X)_p$  are given in Table 2.

In our further work we intend to test the relationship between the given equation and linear free energy relationships (L.F.E.R.)<sup>2,3</sup> which have been already applied to biological problems, especially in papers by Zahradnik<sup>4,5</sup> and

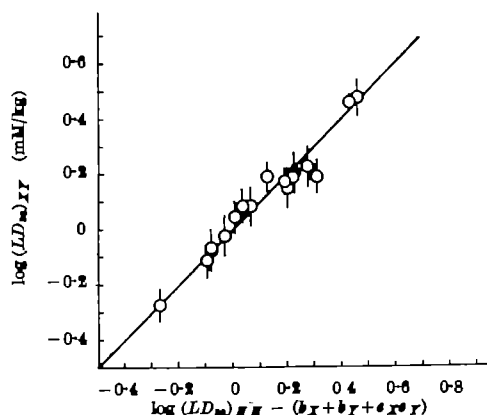


Fig. 1.  $LD_{50}$  were determined on white mice (weighing  $20 \pm 2$  g) by the Thompson method. The substances were administered intravenously in a 20 per cent aqueous polyvinylpyrrolidone solution.

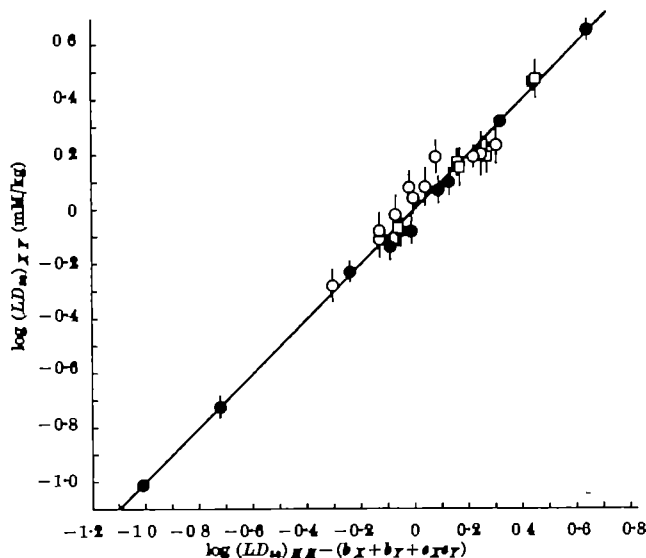


Fig. 2. See caption to Fig. 1.  $\circ$ , *Meta*-substituted derivatives;  $\bullet$ , *para*-substituted derivatives;  $\square$ , mono-substituted derivatives. Hydroquinol and resorcinol are omitted. If they are included the relationship between structure and biological activity becomes more complicated.

lately by Hansch<sup>6-8</sup>. This effort is supported by the surprising fact that the constants  $b_X$  are linearly dependent on the constants of  $f'_i$  obtained in the study of the thyroxine-like activity of 3,3',5,5'-substituted analogues of thyroxine<sup>9</sup>. The constants  $f'_i$  are also linearly dependent on the  $\sigma_m$ -constants.

At present the *o*-disubstituted derivatives of benzene are being studied in the same way, and simultaneously the number of substituents in the *p*-series is being extended to a more complete set of substances.

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### Relation of Magnesium Ions to Calcium and Phosphate Absorption

CHEMICALLY, magnesium and calcium are related since both elements are members of Group II of the Periodic Table. Physiologically, however, in plants<sup>1</sup> or in animals<sup>2</sup> their actions are often antagonistic. It has been shown that magnesium-deficient rats absorb more calcium from the gastrointestinal tract than do control rats<sup>3</sup> and the active transport of calcium ions across the intestinal wall of several species is depressed by magnesium ions<sup>4</sup>. These experiments indicate again a physiological antagonism between these ions and also suggest the possibility of a common absorptive pathway. However, the experimental conditions in the former study<sup>3</sup> are abnormal since magnesium is an integral part of normal diets and magnesium deficiency may have caused changes in the intestinal mucosa which allowed more calcium to be absorbed. *In vitro* type experiments<sup>5</sup> are, by their nature, unphysiological and extrapolations from such isolated systems to interpretations concerning overall calcium absorption *in vivo* may not be valid.

The literature is filled with conflicting reports concerning the relationship between magnesium intake and calcium balance. High magnesium intake has been reported to increase<sup>6-7</sup>, to decrease<sup>8,9</sup> and in some cases to have essentially no effect<sup>10-12</sup> on calcium balance. However, valid comparisons between these studies are impossible since different species were used, dietary regimens were very varied and in some cases the experiments were poorly controlled. Recently, it has been observed that increasing the magnesium intake tended to make calcium and phosphate balance more positive depending on the dietary Ca/P ratio<sup>13,14</sup>. These latter experiments, however, were designed for another purpose and lacked adequate control of dietary calcium and phosphorus. To determine whether magnesium, when administered to rats in amounts greater than required for adequate growth and maintenance, had any significant effect on calcium absorption, a series of experiments was carried out on rats fed diets of varied calcium and phosphorus composition. The animals supplemented with magnesium were pair fed with their controls. Excreta were collected during a 12-day period following a pre-treatment period of 5 days on the different diets and were analysed for calcium and phosphorus. Fig. 1 shows the effects of supplemental magnesium, administered as  $MgCl_2 \cdot 6H_2O$  in 10 per cent glucose drinking water, on faecal calcium. The control rats drank approximately the same volume of a 10 per cent glucose solution. With the exception of those animals fed diets containing only trace amounts of calcium, supplemental magnesium

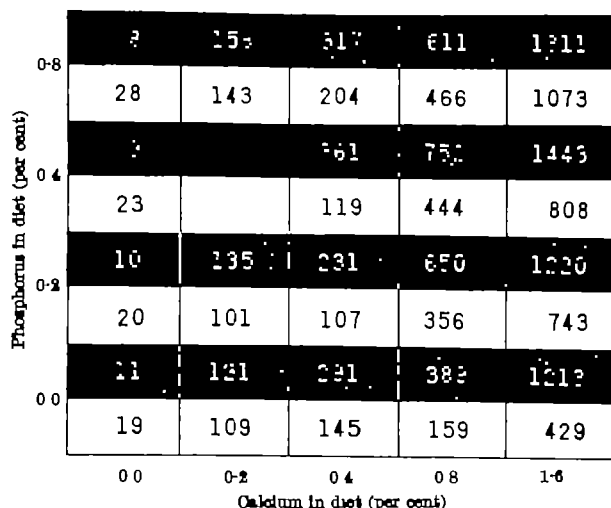


Fig. 1. Faecal calcium (mg). Black, normal magnesium intake; white, supplemental magnesium

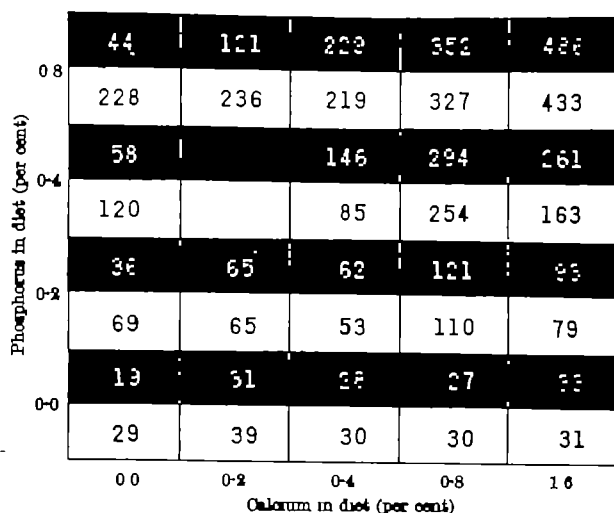


Fig. 2. Faecal phosphorus (mg). Black, normal magnesium intake; white, supplemental magnesium

decreased faecal calcium significantly. This was particularly evident at the higher levels of calcium intake. Thus, it is apparent that under normal dietary conditions, that is, in the presence of calcium in the diet, increasing the magnesium intake promoted calcium absorption rather than inhibited it. However, if the diet was essentially free of calcium (an abnormal condition), increasing the magnesium intake tended to decrease the absorption of calcium.

In Fig. 2 are shown the effects of supplemental magnesium on faecal phosphorus. When the calcium intake was low, magnesium increased faecal phosphorus, but if dietary calcium was 0.4 per cent or greater there was a tendency for decreased faecal phosphorus. Thus, magnesium will either increase or decrease phosphate absorption depending on dietary conditions.

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### Effect of Carcinolipin on the Adenosine Triphosphatase Activity of Isolated Cellular Fractions

CARCINOLIPIN, the carcinogenic lipid isolated from biological materials<sup>1</sup>, was found to stimulate protein synthesis in cell-free systems<sup>2</sup>. However, it was not possible to decide if it affected this process directly or only indirectly, since protein synthesis is an energy-dependent reaction<sup>3</sup>.



Adenosine triphosphatase liberates energy by splitting ATP, which is an indispensable co-factor at all stages of protein synthesis<sup>2</sup>. Therefore, it was decided to elucidate the effect of carinolipin on this enzymatic activity in isolated subcellular fractions, in particular in those involved in protein synthesis.

Liver tissue from adult Wistar rats was homogenized in 0.25 M sucrose in a glass homogenizer with a plastic pestle to give a 5 per cent homogenate. This was centrifuged at 800g for 20 min to remove nuclei and cellular debris. Mitochondria were spun down from the resulting supernatant at 15,000g for 10 min, and microsomes were sedimented at 105,000g for 60 min. From the supernatant fraction, pH 5 enzymes were prepared in the usual way<sup>4</sup>.

Incubation mixtures contained 50  $\mu$ moles tris buffer, (pH 7.4), 2  $\mu$ moles  $\text{CaCl}_2$ , 2  $\mu$ moles ATP (sodium salt), carinolipin suspension (where indicated), and 0.1 ml. of finely homogenized cellular fractions in a total volume of 0.65 ml. They were incubated for 15 min at 37.5° C. Reaction was stopped by the addition of 0.1 ml. 50 per cent trichloroacetic acid, precipitated proteins were centrifuged off and inorganic phosphate was determined in the supernatant by the Fiske-Subbarow method<sup>5</sup>. The protein content of each subcellular fraction was determined as described by Lowry *et al.*<sup>6</sup>, and the results were expressed as  $\mu$ moles inorganic phosphate liberated per hour per mg protein nitrogen.

The mitochondrial fraction showed the highest adenosine triphosphatase activity of all cellular preparations tested. The activity of microsomes was also relatively high, while low enzymatic activities were found in the cell sap and pH 5 enzymes. Average values from five similar experiments with different batches of liver tissue are given in Table 1.

Table 1. ADENOSINE TRIPHOSPHATASE ACTIVITY OF VARIOUS CELLULAR FRACTIONS

Fraction	Phosphate liberated
Whole liver homogenate	0.050
Mitochondria	3.000
Microsomes	0.235
Cell sap	0.054
pH 5 enzymes	0.049

Addition of carinolipin in various doses had no effect on adenosine triphosphatase activity of cellular supernatant and pH 5 enzymes. A stimulation, however, was seen when mitochondrial fraction and microsomes were used. The most effective dose of carinolipin was 5  $\mu$ g, while higher as well as lower doses of this substance were less effective. A summary of these results (mean values of four experiments) is given in Fig. 1.

It appears from these results that a stimulation of energy-yielding reactions by an increased splitting of ATP cannot explain the stimulating effect of carinolipin on protein synthesis. No stimulation of adenosine triphosphatase was found with pH 5 enzymes, which are the only fraction necessary for the first step of protein syn-

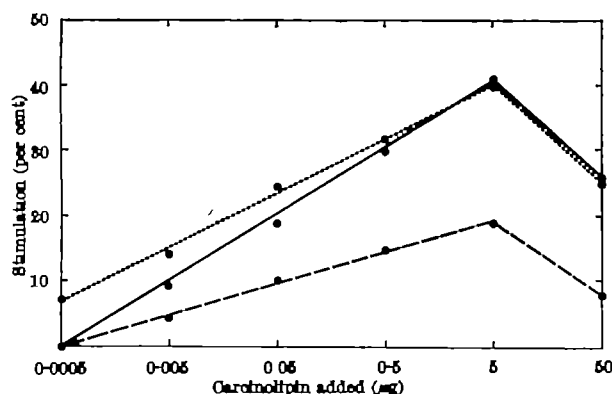


Fig. 1. Effect of various doses of carinolipin on the adenosine triphosphatase activity of rat liver homogenate (full line), mitochondria (dashed line), and microsomes (dotted line).

thesis<sup>4</sup>. It is this first step which is highly stimulated in the presence of carinolipin<sup>4</sup>. Moreover, although the adenosine triphosphatase activity of microsomes is stimulated by this substance, the degree of this enhancement is rather low when compared with the stimulating activity of carinolipin on protein synthesis in a complete cell-free system<sup>3</sup>. From our results it would thus scarcely seem probable that carinolipin affects protein synthesis to any significant extent by increased liberation of energy from adenosine triphosphate.

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### Preparation of an Active Mitochondrial Fraction from the Fruit of *Mangifera Indica*

In connexion with biochemical studies of the effect of growth regulators on the ripening pattern of fruit, it was necessary to isolate a mitochondrial fraction which had respiratory activity with Krebs cycle intermediates as substrates. Considerable difficulty was encountered during the initial attempts at isolation because of the highly acidic nature of the fruit pulp. This problem of low pH has been overcome by using dipotassium phosphate during the isolation procedure for mitochondria from apple<sup>1</sup>, or using an alkaline buffer of pH 9.2<sup>2</sup>. This note reports the isolation of a mitochondrial fraction with tricarboxylic acid cycle activity from the mango fruit. All the operations were carried out at 0°–4° C. Preliminary fruits stored at 21° C were used.

Mitochondria were isolated essentially by the method of Tager<sup>1</sup>, with a few modifications. The homogenizing medium consisted of 0.5 M sucrose–0.1 M phosphate–0.01 M EDTA solution. To 100 ml. of this solution, 77 mg cysteine hydrochloride, 100 mg bovine plasma albumin and 420 mg sodium fluoride were added just before use and the pH was adjusted to 7.4. 100 g mango pulp was homogenized in a blender with 100 ml. of the homogenizing medium. To neutralize the acids released during homogenization, 70–90 ml. of 0.5 M sucrose–1.0 M dipotassium hydrogen phosphate solution (the actual volume being fixed by trial with another aliquot from the same sample) was added at intervals during the one-minute homogenization period. Under these conditions, the pH of the homogenate was always maintained at about 7.4. The mitochondrial pellet sedimenting at 7,000 g was washed twice with 0.5 M sucrose–0.1 M phosphate–0.01 M EDTA buffer solution and suspended in 0.33 M sucrose.

Preliminary experiments indicated considerable increase in succinoxidase activity in the presence of externally added cytochrome c. Hence it was routinely added in all the experiments. In a typical experiment in the present series, oxygen consumption rose from 22 to 43  $\mu$ l./h/mg protein, after addition of cytochrome c. Weiskich and Bonner<sup>3</sup> have recently reported the isolation of mitochondria from sweet potato which did not require cytochrome c addition.

Oxidation of the various substrates was followed manometrically and the results are given in Table 1. Mitochondria prepared in the present experiments had no endogenous oxygen uptake. Succinate oxidation values compared favourably with those reported for another acidic fruit (apple) by Tager ( $\text{QO}_2$ (N), 108–276), but are lower than those reported by Hulme *et al.*<sup>4</sup> when polyvinylpyrrolidone was used in the isolation medium.

Table 1. CITRATE AND SUCCINOXIDASE ACTIVITIES OF MITOCHONDRIAL FRACTIONS FROM LOCAL VARIETIES OF *Mangifera indica* (PEROLIMACTHERIO)

Variety	O <sub>2</sub> uptake/ $\mu$ l./h/mg protein	
	Succinate	Citrate
'Kalapahar'	80	9
'Totapuri'	52	19
'Chittor Yamini'	62	11
'Titar Pasand'	85	8
'Lal Pasand'	30	5
'Pairi'	40	9

(The values are averages of four independent observations)

Each Warburg flask contained phosphate 80  $\mu$ moles, sucrose 800  $\mu$ moles, substrate 20  $\mu$ moles, cytochrome c 4 mg; MgSO<sub>4</sub>·7H<sub>2</sub>O, 10  $\mu$ moles, final volume 3.2 ml., pH 7.4, temp 35° air phase.

Table 2. DEHYDROGENASE ACTIVITIES OF MITOCHONDRIAL FRACTION FROM PULP OF 'PAIRI' VARIETY (PEROLIMACTHERIO)

Dehydrogenase	Isocitrate	Oxaloacetate + NADH	Diaphorase	NADH Cyt C reductase
Units*	0.025	0.676	0.932	1.87

(The values are averages of four independent observations)

\* Optical density change/min/mg protein.

Malate and pyruvate oxidations were also studied for the 'Pairi' variety in the Warburg apparatus and the respiration rates obtained were between 5 and 10  $\mu$ l. O<sub>2</sub>/h/mg protein. The dehydrogenase activities are reported in Table 2. Isocitrate and oxaloacetate + NADH reactions were followed in a Beckman 'DU' spectrophotometer<sup>2</sup>. Diaphorase and NADH-cytochrome c reductase were assayed by the method of Mahler *et al.*<sup>3</sup>

The results presented here have shown the possibility of isolating an actively respiring mitochondrial fraction from the mango fruit. Further, these mitochondria showed respiratory control when tested with the vibrating platinum electrode technique<sup>4</sup> with succinate as substrate in the presence of adenosine diphosphate. Further work on respiratory control and the biochemical properties of mitochondria during various stages of ripening of the fruit is in progress.

I thank Mr. H. Subrahmanyam for providing the fruits.

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### Anthocyanins in Ferns

THE water-soluble red pigments present in young fern fronds do not seem to have been investigated since Price, Sturgeess, Robinson and Robinson<sup>1</sup> in 1938 reported that unusual flavylum salts, resembling 6-hydroxypelargonidin or 6-hydroxycyanidin in their colour properties, occurred in eight ferns. Recent phytochemical interest in the phenolic constituents of lower plants<sup>2</sup> and the discoveries of luteolinidin (I, R = OH) in the moss *Bryum*<sup>3</sup> and of an unidentified anthocyanidin in a fungus<sup>4</sup> suggested that the fern pigments would bear re-investigation.

Two ferns, *Adiantum verticillatum* and *Pteris quadrifaria*, closely allied to those examined by Price *et al.*<sup>1</sup>, were investigated. Extracts of juvenile fronds were hydrolysed with acid and the aglycones produced compared by chromatographic and spectral analysis with authentic anthocyanidins (Table 1). The two aglycones present in *A. verticillatum* were identified as apigeninidin (I, R = H) and luteolinidin (I, R = OH) and that of *Pteris* as luteolinidin. Both these pigments were readily

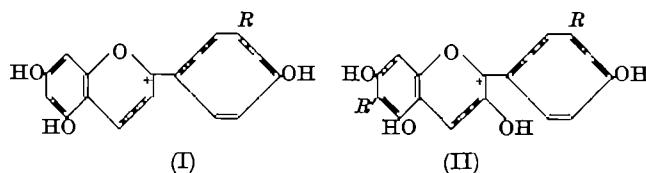
Table 1. CHROMATOGRAPHIC AND SPECTRAL PROPERTIES OF FERN ANTHOCYANIDINS

Pigment	R <sub>F</sub> value in solvent*				MeOH-HCl $\lambda_{max}$ (m $\mu$ )	MeONa $\lambda_{max}$ (m $\mu$ )
	A	B	C	D		
<i>Pteris</i> aglycone	0.61	0.64	0.42	0.43	498†	555
<i>Adiantum</i> aglycones	0.64	0.64	0.42	0.43	498†	555
Apigeninidin	0.79	0.75	0.78	0.56	475	532
Luteolinidin	0.78	0.75	0.78	0.56	476	532
6-Hydroxypelargonidin	0.61	0.64	0.42	0.43	497†	555
6-Hydroxycyanidin	0.57	0.56	0.58	0.24	497	Unstable
6-Hydroxycyanidin	0.30	0.22	0.39	0.21	518	Unstable

\* Solvent A is the Forestal mixture, B is H<sub>2</sub>O-HCl-H<sub>2</sub>O (9:2:3), C is butanol-acetic acid-water (4:1:5) and D is ethyl acetate-H<sub>2</sub>O-2N HCl (85:9:6). Separations using A, B and C were carried out on Whatman No. 1 paper, those with D on silica gel.

† Also gives an AKI, shift of 52 m $\mu$ .

differentiated by R<sub>F</sub> value from pigments having similar colour properties, that is, 6-hydroxypelargonidin (II, R = H, R' = OH) and 6-hydroxycyanidin (II, R = R' = OH), both of which were available for comparison (Table 1). That these two anthocyanidins occur in some unusual combined form and not as simple sugar derivatives follows from the fact that (a) the natural pigments were more resistant than usual to acid hydrolysis, and (b) the pigments in the frond extracts differed in R<sub>F</sub> value from the known apigeninidin and luteolinidin 5-glucosides<sup>5</sup>. The two pigments in *Pteris*, for example, had R<sub>F</sub> values that were higher in both alcoholic and aqueous solvents (0.71 and 0.66 in butanol-2 N HCl (1:1), 0.38 and 0.28 in 1 per cent aq. HCl) than either luteolinidin or its 5-glucoside (0.60 and 0.27 in butanol-HCl, 0.03 and 0.12 in 1 per cent HCl, respectively).



The occurrence in both ferns and mosses of these rare anthocyanidins, which are biogenetically related to the flavones rather than to the flavonols, is of considerable phytochemical interest. It suggests that these plants lack the ability to add a 3-hydroxyl group to a specific C<sub>15</sub>-intermediate to yield the usual type of anthocyanidin such as cyanidin (II, R = OH, R' = H) found in higher plants. The suggestion that apigeninidin and luteolinidin production is a 'primitive' character is not necessarily contradicted by the fact that these rare pigments are also found in the petals of one of the most 'advanced' families of higher plants, the Gesneriaceae<sup>6</sup>. They are presumably produced in this family in response to selection for orange-red flower colour. It should, however, be pointed out that a few ferns do appear to have normal anthocyanins, since cyanidin and pelargonidin glycosides have been reported in *Davallia divaricata*<sup>1</sup> and in *Dryopteris erythrosora*<sup>7</sup>. Furthermore, many ferns contain the flavonols kaempferol and quercetin<sup>8</sup> as well as leucocyanidin<sup>9</sup>. Investigations of the distribution in the Pteridophyta of anthocyanidins, with and without 3-hydroxyl groups, are therefore in progress.

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### Phenolic Compounds in Roots and Leaves of Four Citrus Cultivars

As part of an investigation of the biochemical changes associated with the decline of citrus trees caused by the burrowing nematode, *Radopholus similis* (Cobb) Thorne, a study was undertaken on the isolation of growth promoters and inhibitors in both the roots and young branch terminals. Since a number of phenolics have been identified as co-factors for indolyl-3-acetic acid (IAA) oxidase<sup>1</sup>, a better understanding of the type of growth associated with citrus decline would involve information concerning possible changes in the phenolic constituents. Another reason for consideration is the participation, if any, of phenolics in host resistance either prior to or after pathogenesis. This portion of the investigation is concerned with the extraction, chromatography and identification of the free and acid hydrolysable (bound) phenolics in the leaves and roots of 4 citrus cultivars.

The citrus cultivars used in this investigation comprise two susceptible and two tolerant to *R. similis*. The susceptible cultivars were *Citrus paradisi* Macf. var. 'Duncan' and *C. limon* Osbeck; the tolerant cultivars were *C. sinensis* Osbeck var. 'Ridge Pineapple', and 'Milam', a citrus hybrid of unknown parentage<sup>2</sup>.

Fresh feeder roots and leaves from healthy, two-year-old greenhouse grown seedlings were used for extraction. 100 g tissue was triturated in a Waring blender with hot 95 per cent ethanol, boiled on a water bath for 1 h, and filtered hot through a sintered glass funnel. The solvent was removed under reduced pressure in a rotary film evaporator and the residue was triturated in a small quantity of hot water for subsequent free<sup>3</sup> and acid-hydrolysable<sup>4</sup> (bound) phenolic extraction.

Two-dimensional ascending chromatography was employed using Whatman No. 1 paper 22 cm<sup>2</sup>. Chromatograms were spotted with the equivalent of 250–500 mg plant material. The first direction solvent system was benzene-acetic acid-water (125 : 72 : 3) v/v/v, equilibrated at least 5 h at 17° C prior to use. After drying overnight at 26° C, the second direction was developed in sodium

formate-formic acid-water (10 : 1 : 200) w/v/v, at 26° C. Good resolution and excellent *R<sub>F</sub>* reproducibility were obtained with these solvents at these temperatures. Chromatograms were viewed with and without ammonium hydroxide vapours under ultra-violet light at 3660 Å and at 2537 Å. Further identification was obtained by 2 diazo sprays, *p*-nitraniline and sulphanilic acid. The identity of the phenolic compound was confirmed by co-chromatography with authentic substances.

Fifty-eight phenolics were found in the roots and leaves of the 4 citrus cultivars; 36 and 37 of these were found in the susceptible and 45 and 46 in the resistant cultivars. Twenty-six phenolics were identified. Many of the unidentified phenolics appeared on the chromatograms as small fluorescent spots and were considered as trace amounts (0.5 µg or less). Seven of the unidentified phenolics were present in sufficient amounts (about 10 µg or more) to warrant additional study and identification. The relative amounts of each of the phenolics identified in the free and acid hydrolysed fractions are shown in Table 1. In general, there were 2–3 times as many bound phenolics as free phenolics. Approximately the same number and kinds of bound phenolics were found in the leaves and roots of the 4 cultivars, but the quantities of the bound phenolics were greater in the roots than in the leaves. The free phenolics in the roots were also present in greater numbers and in larger amounts than in the leaves.

Ferulic acid, sinapic acid, *p*-coumaric acid and *p*-hydroxybenzoic acid were present in large amounts in the acid-hydrolysed fraction of both roots and leaves. Umbelliferone and scopoletin were present in large amounts in roots and leaves of *C. limon* and *C. paradisi* and in the roots of *C. sinensis* and 'Milam'. These 6 phenolics plus several closely related phenolics have been shown to influence certain physiological functions in plants, particularly as related to growth<sup>5</sup>, dormancy and photo-induction<sup>6</sup>, and IAA oxidase activity<sup>1</sup>.

Phloretin and esculetin were found only in the acid-hydrolysed fraction of *C. limon* leaves. This is the first reported occurrence of phloretin in citrus, although it was

Table 1. RELATIVE AMOUNTS OF THE ACID-HYDROLYSED (A) AND FREE (F) PHENOLICS IN THE LEAVES AND ROOTS OF FOUR CITRUS CULTIVARS

Type of rootstock	Tolerant								Susceptible							
	<i>C. sinensis</i>				'Milam'				<i>C. limon</i>				<i>C. paradisi</i>			
	Roots		Leaves		Roots		Leaves		Roots		Leaves		Roots		Leaves	
Phenolic	A	F	A	F	A	F	A	F	A	F	A	F	A	F	A	F
Hydroxybenzoates:																
<i>p</i> -Hydroxybenzoic acid	3*	1	1		3	1	1		3		1		3	1	1	
<i>m</i> -Hydroxybenzoic acid													1			
Vanillic acid	1	1	2		1	1	2		1		1		2	1	2	
Vanillin	1				1						1		1		1	
Salicylic acid	1		1		1		1		2		2	1	2	1	1	1
<i>p</i> -Hydroxyphenyl pyruvic	2				2				1				1			
Benzaldehyde													1			
<i>p</i> -Hydroxyphenyl acetic	2		2		1		1		2		2		1		1	
Gentisic acid	2		1		1		1		2		1		1		1	
Syringic acid		1			1						1					
Cinnamic acids:																
<i>o</i> -Coumaric acid	1	1	1		1	1	1		1	1	1		1	1	1	
<i>m</i> -Coumaric acid	1	1							1				1			
<i>p</i> -Coumaric acid	3	1	2	1	3		3		3		3		2		1	
Ferulic acid	2		3	1	2		3	1	1		3	1	1		2	1
Caffeic acid			1				1								1	
Sinapic acid	2		3		3		1		2		2		1		1	
Cinnamic acid													1	1		
Coumarins:																
Umbelliferone	3	1	1		2	1	1		3		2	1	2	1	2	1
Scopoletin	3	1	1		3	1	1		3	2	2		2	1	2	1
Esculetin											1					
Coumarone:																
Bergapten															1	
Flavonoids:																
Naringenin															2	
Quercetin				1							1					
Galangin (?)			2	1			1	1								
Hesperetin			1													
Phloretin																
No. unidentified	8	7	9	5	9	5	7	4	5	4	2	7	4	5	4	2
Total phenolics	22	15	23	9	22	10	20	6	17	7	22	6	20	12	19	7

\* Relative amounts indicated by numbers are: 1 = 1–3 µg, 2 = 4–9 µg; 3 = 10–20 µg.

previously reported in *Micromelum tophocarpum* Turcz. which is in the citrus family, Rutaceae<sup>7</sup>. Esculetin appears to be a natural constituent and not likely to have been pre-formed from *o*is-caffeic acid during chromatography since even small amounts of the latter could not be detected in either the free or bound form<sup>8</sup>. This is also the first report of the occurrence of the hydroxybenzoic acids in citrus, and with the exception of *p*-coumaric acid and sinapic acid, none of the cinnamic acids listed has been previously reported (Table 1).

The identification of galangin is tentative since an unknown phenolic with an *R<sub>F</sub>* close to galangin interfered with separation and identification.

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## PHYSIOLOGY

### An Effect of Sugars on Fluid Entry into Erythrocytes

WHEN human erythrocytes are suspended in a salt solution containing glucose, the sugar enters the cells and establishes an osmotic gradient across the cell membrane. Consequently water follows the glucose into the erythrocytes, causing the cells to swell and ultimately to haemolyse. Some investigators<sup>1-4</sup> have used the rate of swelling or haemolysis as a measure of rate of sugar entry into the cells. An example of the results obtained by this method is shown in Fig. 1. Since the total osmolarity of the suspending medium was not changed on addition of the sugar solutions in this experiment, the observed haemolysis can be ascribed to sugar entry into the cells. However, the kinetic interpretation of such data seems uncertain, for we observed that sugars also tend simultaneously to depress the rate of fluid entry into the erythrocytes.

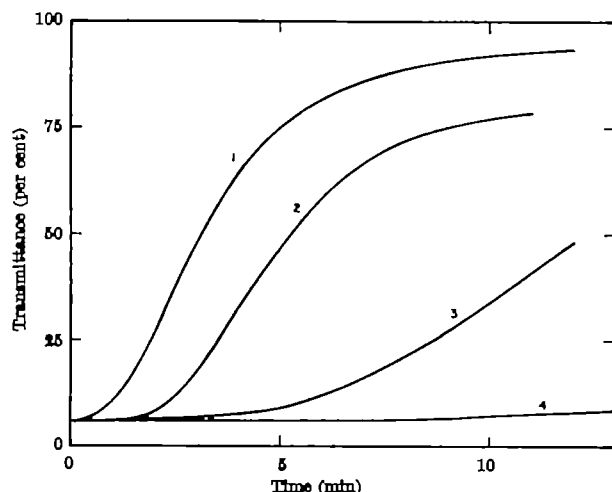


Fig. 1. Haemolysis of human erythrocytes in the presence of different sugars. Two ml. of a 0.1 per cent suspension of erythrocytes was equilibrated in 0.1 M NaCl at 37° C and then mixed at zero time with 1 ml. of 0.2 M sugar solution (1, L-arabinose; 2, D-galactose; 3, D-glucose; 4, L-sorbose). The rate of increase in per cent light transmittance at 550 mμ, measured on a Beckman DK-2 recording spectrophotometer, indicated the rate of haemolysis.

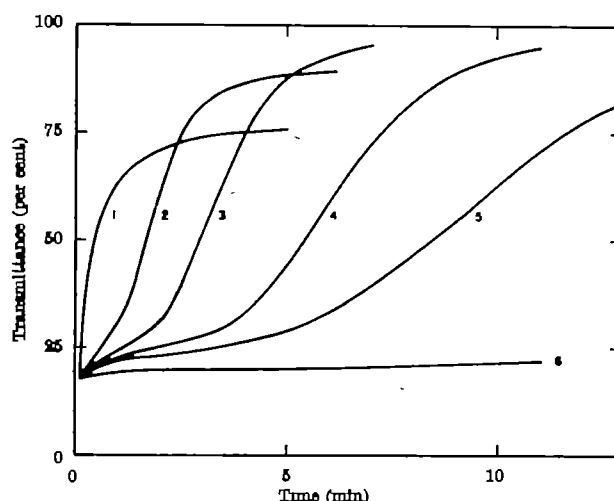


Fig. 2. Haemolysis in hypotonic media. At zero time, 2 ml. of 0.05 M NaCl or of 0.1 M glucose or of mixtures of these two solutions were added to 1 ml. of a 0.1 per cent suspension of human erythrocytes in 0.15 M NaCl. In all cases, the total osmolarity of the added solutions was the same. The glucose concentrations in the added solutions were: 1, 0 M; 2, 0.05 M; 3, 0.025 M; 4, 0.0125 M; 5, 0.00625 M. Sample (6) contained 2 ml. of 0.1 M sucrose. Other details are the same as in Fig. 1.

This depressor effect could be demonstrated clearly when the osmotic gradient was established by lowering the osmolarity of the extracellular fluid (Fig. 2). An appreciable haemolysis occurred rapidly, as might be expected, when 1 vol. of an erythrocyte suspension in 0.15 M sodium chloride was mixed with 2 vol. of 0.05 M sodium chloride. An identical rate of haemolysis was obtained with lithium chloride, potassium chloride and caesium chloride. However, the addition of 2 vol. of sucrose solution of the same osmolarity did not produce haemolysis, while addition of glucose solutions delayed the onset of haemolysis quite markedly as compared with the results observed in hypotonic saline (Fig. 2). This delay in haemolysis increased as the concentration of glucose was increased. Haemolysis did occur eventually in the glucose solutions, presumably because the glucose itself entered the cells in the same manner as illustrated by Fig. 1. Sugars which penetrated the cells most slowly produced the greatest delay in fluid entry under these conditions; thus the time to haemolysis in hypotonic media increased in the series arabinose, galactose, glucose, sorbose, sucrose. Further, the depression of fluid entry by glucose was much more marked with rat erythrocytes, which are reported to be less permeable to glucose<sup>5</sup>, than with human erythrocytes.

Possibly the sugar molecules are adsorbed on to the erythrocyte membrane and thus block the entry of water through water-filled pores in the membrane. This hypothesis is supported by the results obtained with phloretin. Phloretin, which has often been used as a specific inhibitor of entry of glucose into erythrocytes, is known to be strongly adsorbed on to the erythrocyte membrane<sup>6</sup>. We observed that 0.03 mM phloretin not only delayed haemolysis in the presence of glucose, as expected, but also delayed haemolysis in hypotonic sodium chloride solution. Apparently phloretin, too, interfered with the entry of water into the erythrocytes. (Exposure to X-radiation in doses above 1 krad, on the other hand, seemed to facilitate glucose and fluid entry into human erythrocytes, but the mechanism of this effect requires further study.)

The foregoing results suggest that extreme caution should be exercised in the interpretation of results where cell swelling or time of haemolysis is used as a measure of the rate of sugar entry into erythrocytes. Several authors have, for example, reported that the permeability constant for glucose decreases with increasing glucose concentration<sup>1-4</sup>. These results could simply be due to a

depression of the rate of fluid entry at high concentrations of sugar.

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### Cardiovascular and Metabolic Effects of Adenosine 3',5'-Monophosphate *in vivo*

CATECHOLAMINES activate the enzyme, adenylyl cyclase, which forms cyclic adenosine 3',5'-monophosphate (3',5'-AMP) in a variety of animal tissues<sup>1</sup>. This reaction system has been thought to control several metabolic processes, including glycogenolysis, steroidogenesis, ketogenesis, lipolysis and antidiuresis<sup>2-4</sup>. The positive inotropic and chronotropic effects of catecholamines on the heart are generally attributed to catechol interaction with the beta adrenergic receptor site. Rall and Sutherland<sup>5</sup> and Mayer *et al.*<sup>6</sup> have proposed that 3',5'-AMP might directly affect cardiac beta receptors by mediating hormone-induced changes in contractile force without necessarily involving glycogenolysis. During the course of investigations in the isolated perfused rat liver, 3',5'-AMP was observed to induce delayed vasomotor changes unrelated to its glycogenolytic action<sup>10</sup>. This suggested that 3',5'-AMP might have a direct vascular effect on smooth muscle. Although 3',5'-AMP produces hyperglycaemia in intact animals<sup>1,10-12</sup>, correlation of its metabolic action with possible regulation of cardiac activity has not been previously investigated.

Prior to the use of 3',5'-AMP in humans, toxicological investigations were carried out in 59 male Sprague-Dawley rats (250 g), 66 adrenalectomized male rats of the same strain, 12 guinea-pigs, 59 New Zealand rabbits and 27 adult mongrel dogs following single intravenous or intracardiac doses of 3',5'-AMP (4-100 mg/kg). No anaphylactic or adverse clinical reactions were observed. After the safety of the cyclic nucleotide was established in animals, metabolic and cardiovascular responses were also investigated in 14 male and six female human volunteers.

Animals and humans either received food as desired or were kept fasting for 12-36 h. Blood glucose was determined after deproteinization by the glucose oxidation procedure of Cawley *et al.*<sup>14</sup>. Plasma non-esterified fatty acids (FFA) were measured by the method of Dole<sup>15</sup>. Plasma cortisol was estimated as Porter-Silber chromogens by the method of Peterson *et al.*<sup>16</sup>. Plasma osmolality was determined by freezing-point depression with the use of a Fiske osmometer. All blood samples in the rats and rabbits were determined at either 10 or 20 min after injection of 3',5'-AMP. Detailed haemodynamic measurements were performed in dogs and humans. Dogs were prepared with catheters chronically implanted in the right atrium and aortic arch. All measurements in the dogs were made with the animal lying unrestrained, and determinations of cardiac output, heart rate, and mean blood pressure were recorded. 3',5'-AMP (4-8 mg/kg) was administered as a single intracardiac dose into the right ventricle, during cardiac catheterization, to eight human volunteers, by rapid intravenous injection to seven subjects, and by constant intravenous infusion to five others, at the rate of 0.5 mg/kg/min for a period of 1-2 h. The cyclic nucleotide was prepared as a fresh solution, diluted in saline, adjusted to a pH of 7.40, and,

in the case of human experiments, filtered through a monomolecular 'Millipore' filter prior to use. All the animals and humans who received food *ad lib.* demonstrated hyperglycaemia, but there was considerable variation and marked species differences in the FFA response. Table 1 summarizes experiments in the non-fasted state in which glucose and FFA were simultaneously determined.

Table 1. EFFECT OF 3',5'-AMP ON BLOOD GLUCOSE AND FFA

Species†	No. of expts.	Blood glucose* (mg/100 ml.)			FFA* (m equiv./l.)		
		Initial	Response‡	P <	Initial	Response‡	P <
Rat	14	107 ± 14	172 ± 46	0.001	532 ± 183	830 ± 614	ns
Adrenalectomized rat	15	118 ± 13	167 ± 53	0.005	418 ± 137	641 ± 276	0.025
Rabbit	16	92 ± 24	165 ± 19	0.035	883 ± 152	805 ± 584	ns
Dog	24	85 ± 13	118 ± 15	0.001	880 ± 460	509 ± 286	0.005
Man	9	98 ± 12	182 ± 14	0.025	596 ± 304	757 ± 468	ns

\* Results are the means ± standard deviation.

† Rats were anaesthetized with ether anaesthesia while rabbits, dogs, and humans were unanaesthetized.

‡ Response observations represent maximal values after single intravenous or intracardiac administration of 3',5'-AMP (4-12 mg/kg).

In 27 fed and fasted dogs, following single intracardiac doses of 3',5'-AMP (4-8 mg/kg), heart rate increased within seconds. Maximal mean increase in cardiac rate (+57 per cent) and cardiac output (+43 per cent) occurred at 2 and 5 min, respectively. These effects were statistically significant ( $P < 0.001$ ). Cardioacceleration remained significant for 15 min ( $P < 0.025$ ). There was a transient decrease in mean blood pressure, which was statistically significant only during the first 3 min after 3',5'-AMP administration ( $P < 0.001$ ). Calculated total peripheral resistance was significantly diminished at 5 min ( $P < 0.001$ ) and at 15 min ( $P < 0.010$ ). There were no significant changes in stroke volume or mean pulse pressure. Mean glucose increased to a maximum at 7 min (+45 per cent) and then decreased at 10 min (+39 per cent), and 15 min (+24 per cent). As glucose increased, FFA progressively decreased to a maximum at 15 min (-46 per cent). From the time relationships of the maximal changes in glucose and FFA in dogs, it appears that the decrease in FFA is causally related to the 3',5'-AMP-induced hyperglycaemia. No significant cardiac or metabolic changes followed administration of saline or 2',3'-AMP, 5'-AMP, and ATP, which were given in doses equimolar with 3',5'-AMP. Prior treatment with the beta adrenergic blocking agent, propranolol, at dosages of 0.1-0.5 mg/kg, produced a moderate decrease in heart rate, but no biochemical alterations. Pre-treatment with propranolol failed to prevent the cardiac and biochemical responses to 3',5'-AMP, suggesting that 3',5'-AMP does not act directly on beta receptors in order to elicit its action.

Following rapid intravenous or intracardiac administration of 3',5'-AMP to 15 human subjects, cardiac rate increased within seconds. The maximal increase in heart rate averaged 40 per cent above control values ( $P < 0.005$ ) at 5 min. Tachycardia persisted for 15 min. Cardiac output, in the eight subjects studied during cardiac catheterization, increased to a mean of 44 per cent ( $P < 0.025$ ), and total peripheral resistance decreased to a mean of 34 per cent ( $P < 0.005$ ) 5 min after 3',5'-AMP injection. There were no significant changes in stroke volume, systolic or diastolic blood pressure. In those subjects who received a constant infusion of 3',5'-AMP, blood pressure promptly increased an average of 22 per cent above initial values and remained elevated throughout the period of infusion, associated with cardioacceleration (+20 per cent). Hyperglycaemia was consistently observed in all 20 subjects with a maximal mean increase 10 min after 3',5'-AMP administration. FFA response was variable. Plasma cortisol determinations, obtained

on the day preceding the infusion experiments, decreased in all five subjects. On the following day, when 3',5'-AMP was given as a constant infusion, plasma cortisol rose from an initial mean value of  $11.7 \pm 3.5$   $\mu\text{g}/100$  ml. to  $24.6 \pm 9.0$   $\mu\text{g}/100$  ml. ( $P < 0.025$ ) at the end of the infusion. In 15 acute injection experiments plasma cortisol significantly increased in six ( $P < 0.025$ ), remained unchanged in seven, and decreased in two. There were no significant changes in plasma osmolarity in any subject. No electrocardiographic abnormalities or arrhythmias followed administration of the nucleotide in humans or dogs. Several clinical side-reactions to 3',5'-AMP were observed in all five subjects receiving a constant infusion and in four others who received a single dose of nucleotide. These included abdominal pain in eight, headache in seven, nausea in four, fatigue in four, sweating in three, testicular pain in three, vomiting in two, tenesmus in two, and conjunctival suffusion in two.

The results of this investigation indicate that 3',5'-AMP penetrates intact cell membranes in significant concentration to regulate intracellular metabolic events, as reflected by glycogenolysis, steroidogenesis and possibly lipid homeostasis. Experiments in the isolated perfused rat heart have shown that epinephrine rapidly increases cardiac 3',5'-AMP and phosphorylase content associated with a positive inotropic effect<sup>17</sup>. The actions of 3',5'-AMP to increase the output and rate of the heart *in vivo* lend support to the hypothesis obtained from *in vitro* data that 3',5'-AMP may represent the biochemical basis for the myocardial response to catecholamines. These results give no conclusive indication of a positive effect on myocardial contractility (inotropic action) since direct measurements of myocardial contractile force were not performed. From the time sequences of the peak responses of heart rate and blood glucose to the cyclic nucleotide, it would appear that the prompt cardiovascular actions were independent of the observed delayed hyperglycaemia.

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## Hormonal Regulation of Cardiac Muscle Citrate

A CONSIDERABLE amount of evidence indicates that phosphofructokinase (PFK) is of importance in regulating the rate of glycolysis in a number of tissues<sup>1-3</sup>. In turn, glucose-6-phosphate, which accumulates when PFK is inhibited, has an inhibitory effect on the phosphorylation of glucose<sup>4</sup>. Thus, alterations of PFK activity may have a significant influence on carbohydrate utilization. *In vitro*, the activity of PFK may be altered by a multiplicity of factors<sup>5,6</sup>, some inhibitory and others stimulatory. One of the inhibitory substances is citrate<sup>8,11</sup>, and some evidence that citrate may be an inhibitor *in vivo* derives from the observation that citrate is elevated and PFK activity is depressed in hearts of diabetic rats and in normal hearts perfused with fatty acids<sup>8,11</sup>. Likewise, in hearts exposed to fluoroacetate, in which citrate rises to high levels, there is an inhibition of PFK<sup>12</sup>.

Hypophysectomy has long been known to alleviate certain of the metabolic disturbances of diabetes, and in perfused hearts from diabetic animals the slow rate of glucose phosphorylation is greatly improved when the donor animals have also been hypophysectomized<sup>13</sup>. If citrate is in part responsible for inhibited PFK activity and depressed glucose phosphorylation in cardiac muscle of diabetic animals, the citrate-level should return toward normal after the pituitary is removed. Likewise, adrenal cortical hormones are known to aggravate diabetes; thus it might be expected that citrate would be elevated in animals treated with these hormones. The present data show that the level of heart muscle citrate follows these expectations.

Male rats, fed *ad libitum*, were used as the source of tissue in all the experiments. The animals were anaesthetized with sodium pentobarbital and the hearts were exposed and frozen *in situ* between blocks of aluminium previously cooled in liquid nitrogen. In some cases the hearts were quickly excised and then frozen by the same method. No difference was found between the two methods and the results have been pooled.

Normal and hypophysectomized rats were made diabetic by injection of alloxan at a dosage of 60 mg/kg administered intravenously. The serum glucose was in excess of 500 mg/100 ml. serum in all animals given alloxan. The hearts were removed 48 h after the alloxan injection, powdered in a stainless steel mortar at liquid nitrogen temperature, extracted in 10 per cent trichloroacetic acid and assayed for citrate by the pentobromacetone method<sup>14</sup>.

The results are presented in Table I, and, as reported previously<sup>8</sup>, citrate was elevated in the heart muscle of diabetic rats. Hypophysectomy of non-diabetic rats caused some decrease in the citrate concentration, and in diabetic animals hypophysectomy resulted in citrate-levels intermediate between those found in hearts of normal and diabetic rats. When growth hormone and hydrocortisone were injected into hypophysectomized-diabetic rats the citrate-level was significantly elevated beyond that found in hearts from diabetic animals. Likewise, when normal animals were injected with growth hormone and hydrocortisone there was a small increase in heart muscle citrate. Presumably, in the intact animal any large increment in citrate would be prevented by insulin secretion. Insulin previously has been shown to reverse the elevated citrate values in heart muscle of diabetic rats<sup>8</sup>, and the effect is also shown in hypophysectomized animals, which are more sensitive to insulin, in the present data.

The results suggest that diabetes, growth hormone and adrenal cortical hormones may exert a part of their inhibitory effect on glucose phosphorylation via a depression in PFK activity brought about by an elevation of tissue citrate. The hormonal effects on citrate may, in turn, be mediated through alterations in the level of plasma free fatty acids (FFA). The various endocrine conditions

Table 1. EFFECT OF SEVERAL HYPOPHYSECTOMY CONDITIONS ON THE LEVEL OF CARDIAC MUSCLE CITRATE IN MALE RATS

Condition	Cardiac muscle citrate ( $\mu$ moles/g dry wt.)
Untreated	$2.2 \pm 0.17^{\dagger}$ (16) <sup>¶</sup>
Alloxan*	$5.1 \pm 0.34$ (16)
Hypophysectomized	$1.7 \pm 0.14$ (10)
Hypophysectomized plus alloxan	$3.2 \pm 0.2$ (16)
Hypophysectomized plus alloxan plus insulin†	$0.8 \pm 0.05$ (4)
Hypophysectomized plus alloxan plus GH and AOH‡	$8.0 \pm 0.4$ (18)
Intact plus GH and AOH§	$3.5 \pm 0.25$ (6)

\* Alloxan was injected at a dosage of 60 mg/kg, intravenously, 48 h before the hearts were removed.

† Protamine-zinc insulin, 3 units, subcutaneously, 24 and 12 h before killing.

‡ Growth hormone, 1 mg/kg, intraperitoneally, and hydrocortisone, 25 mg/kg, subcutaneously, 24 and 12 h before killing; one half these dosage levels 6 h before killing.

§ Growth hormone, 1 mg/kg, intraperitoneally, 48, 36, 24, 12 and 6 h before killing. Hydrocortisone, 25 mg/kg, subcutaneously, 48, 36 and 24 h, and 12.5 mg/kg at 12 and 6 h before killing.

¶ Standard error of the mean.

¶ Number of animals in each group.

reported here to cause a rise in citrate are accompanied by an increase in plasma FFA<sup>12-17</sup>, and perfusion of normal hearts with fatty acids elevates the citrate level<sup>8,11</sup>. The mechanism for the rise in citrate induced by fatty acids is not yet clear. However, preliminary results<sup>18</sup>, and other data obtained by us, show that the rise in citrate in hearts perfused with fatty acids, or in hearts of diabetic rats, is not limited to this one intermediate of the tricarboxylic acid cycle. Rather, there is an overall increase in the level of the cycle intermediates. Whether there is also an alteration in the rate at which the cycle operates has not been determined.

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## HAEMATOLOGY

## Iodine-125-labelled L Chains of Human Blood Group Antibodies

Cohen and Porter<sup>1</sup> have demonstrated that the light (L) chains of human IgG globulins can be separated into ten subfractions by starch-gel electrophoresis. They also showed that the L chains of guinea-pig anti-ovalbumin and anti-(bovine serum albumin) separated into ten subfractions, but the intensity of the staining of the bands differed from each other and from those of L chains obtained from IgG globulins. The work presented here is concerned with the distribution of <sup>125</sup>I-labelled L chains of human antibody among the ten subfractions.

L chains were prepared from three different sources by the method of Fleischman, Pain and Porter<sup>2</sup>. (1) <sup>125</sup>I-labelled L chains were isolated from six different examples of purified <sup>125</sup>I-labelled anti-D and from two different anti-c antibodies; these antibodies were obtained from women who had given birth to a child with haemolytic disease. Purified <sup>125</sup>I-labelled antibodies were obtained by a method previously described<sup>3</sup>; at least 90 per cent of the iodine-125 in the final preparation represented active antibody. Approximately 20  $\mu$ g of <sup>125</sup>I-labelled antibody was added to 50 mg of unlabelled pooled IgG globulin before isolation of the L chains. (2) <sup>125</sup>I-labelled L chains were isolated from IgG globulin obtained from each of the eight antisera after absorption of the antibody; 20  $\mu$ g of <sup>125</sup>I-labelled IgG globulin were added to 50 mg of unlabelled pooled IgG globulin before isolation of the L chains. (3) L chains were prepared from 50 mg of pooled IgG globulin, all of which had been labelled with iodine-125 prior to isolation of the L chains. The ratio of the optical densities at 280 m $\mu$  of the solutions of separated H and L chains was approximately 75/25 and the ratio of the iodine-125 content in each was approximately 85/15. The iodine/protein ratio was approximately 2/1 (atoms/molecule) except in the case of the pooled IgG globulin where it was 6/1. All preparations of L chains were subjected to starch-gel electrophoresis<sup>4</sup> at pH 9.0. Following electrophoresis, the surface of the gel was stained and the distribution of the stained bands was recorded. The distribution of iodine-125 was then determined by cutting the gel into 2-mm sections and estimating the radioactive content of each section.

A comparison was made between the electrophoretic mobility of <sup>125</sup>I-labelled L chains (preparation 3) and unlabelled L chains obtained from pooled IgG globulin. On staining the starch gel, the <sup>125</sup>I-labelled L chains showed ten 'major' bands which ran in the same position as the L chains obtained from unlabelled IgG globulin. However, between the 'major' bands there were less-intensely staining 'minor' bands which were not present in the unlabelled L chains. These 'minor' bands had moved further towards the positive pole than their corresponding 'major' components. The iodine-125 was found to be distributed in ten 'peaks' of activity, each peak corresponding to the 'minor' bands seen on staining (Fig. 1). These results suggest that not all the L chains are labelled with iodine and those chains that are labelled are more electronegative than the unlabelled chains. Failure to label all the chains may be due to inadequate mixing of iodine-125 and protein during the labelling process, or may be due to heterogeneity of L chains, not all of which may have tyrosine available for labelling. The increased electronegativity of the labelled L chains may be related to the presence of di-iodotyrosine in the peptide chains. The pK (OH) value of L-tyrosine is 10.07, whereas the corresponding value of di-iodo-L-tyrosine is 6.48 (ref. 4).

The distribution of the <sup>125</sup>I-labelled L chains (preparation type 2) obtained from the IgG globulin isolated from each of the eight antisera was closely similar to the



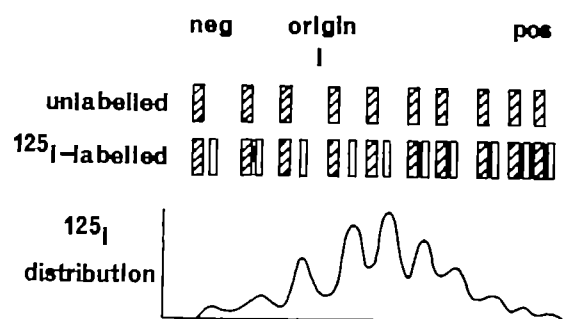


Fig. 1. Starch-gel electrophoresis of  $^{125}\text{I}$ -labelled and unlabelled  $L$  chains. The upper part shows the position of the stained bands (amido-black) and the lower graph shows the distribution of iodine-125. The shaded blocks represent the 'major' bands and the unshaded blocks the  $^{125}\text{I}$ -labelled 'minor' bands. The  $^{125}\text{I}$ -labelled  $L$  chains are more electro-negative than the unlabelled chains.

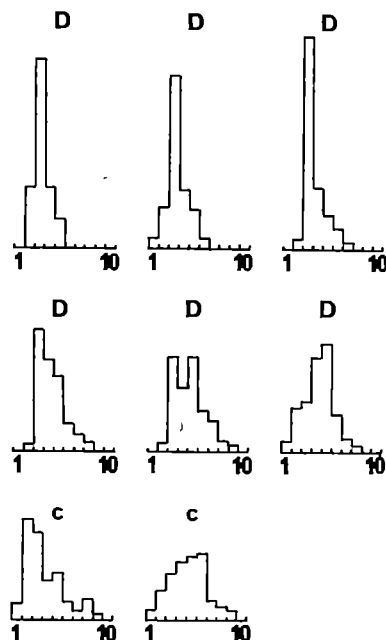


Fig. 2. Starch-gel electrophoresis of  $^{125}\text{I}$ -labelled  $L$  chains obtained from six anti-D (labelled D) and two anti-c (labelled c) antibodies. The histograms give the relative distribution of iodine-125 found in each of the ten subfractions. The numbering of the bands is that used by Cohen and Porter (ref. 1).

distribution of the  $L$  chains from pooled  $^{125}\text{I}$ -labelled  $\gamma$ -globulin (preparation 3).

The distribution of  $^{125}\text{I}$ -labelled chains from the purified antibodies (preparation type 1) differed from each other and from those obtained from IgG globulin from the respective antisera (Fig. 2). The minimum number of  $^{125}\text{I}$ -peaks seen in each example was four and the maximum nine. Three preparations of anti-D had more than 50 per cent of the  $^{125}\text{I}$ -labelled  $L$  chains in peak 3, but the distribution among the smaller peaks was different in each of these examples.

The rate of dissociation of the antibody from red cells was determined for each antibody<sup>4</sup>, and all were found to be heterogeneous with respect to their rate constants for dissociation. One of the anti-D antibodies was separated by differential dissociation into two fractions, one of the fractions having a high and the other a low average rate constant for dissociation. The electrophoretic distribution (alkaline starch-gel) of the  $^{125}\text{I}$ -labelled  $L$  chains from each fraction was found to be identical.

Although iodination of  $L$  chains is unsatisfactory as a method of trace-labelling owing to the alteration in electrophoretic mobility which it produces, these results suggest that the antibody molecules of a given specificity from different donors are heterogeneous with respect to the

type of  $L$  chains. As IgG globulin molecules each contain only two  $L$  chains, the finding that there were between four and nine different types of  $L$  chains present in the antibodies investigated here suggests that there is also heterogeneity of the structure of antibody molecules within any particular antiserum.

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### Ultra-violet Absorption Spectra of Haemoglobins from Various Vertebrates

It is well known that the various vertebrate haemoglobins as well as the individual variants of adult human haemoglobin (Hb A) may be readily distinguished by their different mobility in electrophoresis, adsorbability and stability to thermal denaturation. A possibility of identification, particularly in abnormal haemoglobins, is offered by recombination analysis and, finally, by the determination of the amino-acid composition and sequence. We found the ultra-violet spectrum as recorded between 30,000 and 34,000  $\text{cm}^{-1}$  provided a very simple additional means of differentiation. Little information can be obtained from the literature<sup>1,2,4</sup>. Hb F (foetal human haemoglobin) may be recognized from its well-distinguishable tryptophan band at 34,400  $\text{cm}^{-1}$ , and Matsuda<sup>4</sup> directed attention to the absorption differences between the human  $\alpha$ -,  $\beta$ - and  $\gamma$ -chains. If the ultra-violet spectrum is recorded at high resolution using an appropriate haemoglobin derivative, however, many more differentiated results may be achieved.

Stroma-free haemolysates of red cells, the haemoglobin of which had been oxidized with sodium nitrite and afterwards washed free of plasma and nitrite, served as basic product for the methaemoglobin (Hb(3)) spectrum which is particularly suited for an analysis in the absorption region between 34,000  $\text{cm}^{-1}$  and 30,000  $\text{cm}^{-1}$ . We generally used haemoglobin at a concentration of about  $5 \times 10^{-4}$  val/L. Hb. The concentration was determined by means of the cyanmethaemoglobin absorption at 540 nm. The measurement was carried out with the recording spectrophotometer Unicam SP 700 using the minimum possible slit width. Some results of the measurements are given in Figs. 1a and b.

Each haemoglobin exhibits a specific course of absorption which permits it to be readily identified. This identification may be considerably improved by recording a difference spectrum versus a standard haemoglobin.

In the spectral range investigated, it is, in general, phenylalanine, tyrosine and tryptophan which are respons-

Table 1

Phenylalanine	42,120	41,180	40,330	39,610	38,730	38,200*	37,840	37,330
Hb(3) A	—	—	—	—	38,510	38,000	37,680	37,100
Frequency	—	—	—	—	210	200	210	230

\* This band appears only as a shoulder in the pH region  $>10$ .

Table 2

	$E_{\text{max}}$ $\text{cm}^{-1}$	$E_{\text{min}}$ $\text{cm}^{-1}$	$Q$ $\frac{35,800 \text{ cm}^{-1}}{34,400 \text{ cm}^{-1}}$
Llama	1.55	1.15	1.348
Dog	1.515	1.12	1.343
Rabbit	1.456	1.13	1.314
Hb A	1.467	1.12	1.309
Rat	1.593	1.23	1.293
Guinea-pig	1.52	1.18	1.288
Goose	1.68	1.313	1.25
Weibull	1.42	1.11	1.23
Pig	1.40	1.105	1.237
Hb F	1.56	1.22	1.258

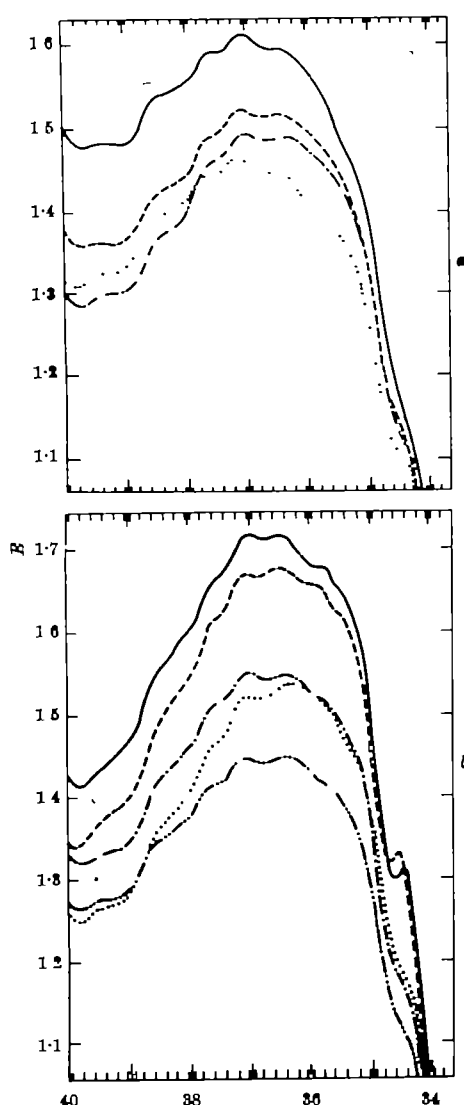


Fig. 1. Ultra-violet absorption spectra of haemoglobin from different vertebrates. Ordinate, extinction; concentration of Hb(3) solution =  $5 \times 10^{-4}$  mol/l. Abscissa: frequency  $\text{cm. } 10^{-3}$ . a, —, Llama; ---, rabbit; ···, Hb A; b, —, Goose; ---, guinea-pig; ···, dog; - · - ·, sheep.

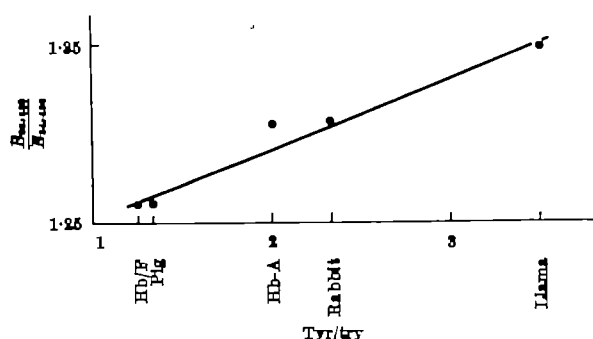


Fig. 2. Dependence of  $\frac{E_{280}}{E_{215}}$  on the proportion of tyrosine/tryptophan.

ible for the maximum in the range discussed. Identification of the individual bands observed, with the corresponding amino-acids is feasible. On various other proteins (ribonuclease, lysozyme, pepsinogen and pepsin, chymotrypsin, various albumins) attempts are made to draw conclusions from the location and the height of the bands, particularly in the case of tyrosine, about the

incorporation of the phenol ring of the tyrosine into secondary bindings within the protein<sup>1,2-4</sup>. It is certainly important that under our conditions the fine structure of the phenylalanine band becomes accessible because, in this case, the theoretical interpretation of the effects observed is easier than in the case of tyrosine. Table 1 indicates the shift of the phenylalanine bands in the protein relative to their position in a corresponding amino-acid mixture (the values were taken from a difference spectrum of human Hb(3) versus an amino-acid mixture free of phenylalanine).

The frequency change by about  $200 \text{ cm}^{-1}$ , with the band fine structure maintained, appears to be clear. It demands further investigation, as does the attempt to determine the content of aromatic amino-acids directly by the modified method of Benze and Schmid<sup>5</sup>. The latter method is not directly applicable, as the haem also contributes to the total absorption in the spectral range examined (Table 2 and Fig. 2).

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### Anti-cerebroside Antibodies in Cerebrospinal Fluid of Rabbits with Experimental 'Allergic' Encephalomyelitis

In the course of experimental 'allergic' encephalomyelitis (EAE), and after injection of brain emulsions, rabbits produce antibodies against cerebroside<sup>1-4</sup>. These antibodies are demonstrable by the complement-fixation test as well as by the agar-gel precipitation test, when emulsions of cerebroside and the auxiliary lipids, cholesterol and lecithin (CCL), or emulsions of ethanolic extracts from spinal cord, are employed as antigens<sup>5</sup>. By ultracentrifugation and immunoelectrophoresis we were able to identify these antibodies as  $\gamma_{1M}$  (19S) and  $\gamma_2$  (7S) globulins<sup>6,7</sup>. The role of these antibodies in the pathogenesis of EAE is not at all clear<sup>8</sup>. It seemed of interest, therefore, to examine whether anti-cerebroside antibodies are also present in cerebrospinal fluid (CSF) of rabbits with EAE, and to ascertain to which variety of globulin they belong.

Rabbits inoculated intradermally with emulsions of homologous spinal cord incorporated in complete Freund's adjuvant showed severe clinical symptoms of EAE approximately 10-12 days after injection. These animals were killed in the tetraplegic state and CSF was carefully withdrawn from the cisterna cerebello-medullaris after complete exsanguination of the animals. Samples with erythrocyte contamination were discarded. Complement-fixation tests were done as formerly described at  $37^\circ \text{C}$  for both phases of the reaction<sup>9</sup>. The antigens used were emulsions of CCL and of ethanolic extracts from bovine spinal cord. For immunoelectrophoretic examination CSF samples were concentrated according to the method of Mies<sup>10</sup>. 12  $\mu\text{l}$ . of the different CSF pools were electrophoretically separated (200 V, 90 min)<sup>11</sup>. CCL emulsion was allowed to diffuse from the middle channel against the CSF protein fractions. In 8 out of 12 cases equal volumes of the corresponding serum pools were tested on the same slides for comparison.

Table 1. EXAMINATION OF CSF AND SERUM POOLS FROM RABBITS WITH EAE BY COMPLEMENT-FIXATION TEST AND IMMUNOELECTROPHORESIS

CSF resp. Serum pool No.	Titre in complement-fixation test:				Immunoelectrophoresis				
	CSF		Serum		CSF		Serum		
	OOL	sp.c.	OOL	sp.c.	Conc. times	$\gamma_2$ -globul. antibody	$\gamma_2$ -globul. antibody	$\gamma_2$ -globul. antibody	$\gamma_2$ -globul. antibody
1	—	—	n.t.	n.t.	30	—	—	n.t.	n.t.
2	2	2	n.t.	n.t.	30	—	+	n.t.	n.t.
3	—	—	n.t.	n.t.	25	—	++	n.t.	n.t.
4	—	—	n.t.	n.t.	35	—	—	n.t.	n.t.
5	—	—	n.t.	n.t.	28	—	+	n.t.	n.t.
6	16	16	512	512	25	—	+	+	+++
7	8	8	512	512	40	—	+	+	+++
8	32	32	n.t.	n.t.	35	±	+++	±	+++
9	128	128	2,048	2,048	100	+++	+++	?	+++
10	16	16	512	512	52	—	±	±	++
11	8	4	1,024	2,048	45	—	—	+	+++
12	64	64	1,024	1,024	48	±	+	+	+++
13 (control)	—	—	—	—	150	—	—	—	—

Antigens applied: OOL emulsion and emulsion of ethanolic extracts from spinal cord (sp.c.); n.t.—not tested.

The protein content of the CSF specimens ranged between 200 and 400 mg per 100 ml., as evaluated by different pools of CSF samples<sup>10</sup>. The normomastix curve dropped deeply in the middle region. In the complement-fixation test we obtained positive results in 8 CSF samples out of 12 pools tested, which were collected from 80 rabbits with acute EAE. The titres ranged between 1/2 and 1/128. Both antigens applied showed about the same reactivity (Table 1). Immunoelectrophoretic examination of the samples showed precipitation in 9 out of 12 cases in the  $\gamma_2$  (7S) globular region. Three of the positive CSF pools precipitated in the  $\gamma_2$  as well as in the  $\gamma_1$  globulin region. The results of the immunoelectrophoretic investigations are summarized in Table 1 and Fig. 1a-c. Control experiments with CSF and serum pools withdrawn from healthy animals gave negative results throughout with both antigens applied.

Similar experiments concerning the demonstration of complement-fixing antibodies directed against ethanolic extracts from rabbit brain were performed by Frick and Scheid-Seydel<sup>11</sup> in 1962. In examining CSF specimens of rabbits with EAE, the authors were able to demonstrate anti-myelin antibodies only in one sample out of 16 tested. It may well be, however, that the discrepancies between Frick's results and our own are due to differences in the sensitivity of the complement-fixation technique applied. It should be stressed that the number of sensitized erythrocytes in the haemolytic system has a great influence on the threshold of sensibility and thereby on the height of antibody titre<sup>12</sup>, in the sense that a higher sensitivity is achieved by applying small amounts of erythrocytes.

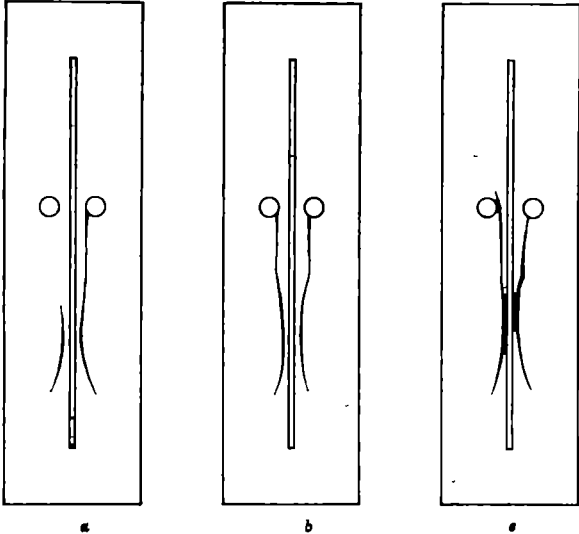


Fig. 1. Immunoelectrophoresis of CSF pools and the corresponding serum pools from rabbits with EAE. Anode above, left side: CSF pool, right side: corresponding serum pool; a, No. 6; b, No. 8; c, No. 9 from Table 1; middle channel: OOL emulsion

It is conceivable that the antibody content in CSF is the result of a breakdown of the so-called blood-brain barrier. It would be interesting to see whether these antibodies enter the CSF spaces before any histological alterations typical for EAE are observed, and which type of antibody can primarily be demonstrated. As a contribution to the barrier problem in EAE, time course studies should be performed to find out whether 7S-antibodies are present in CSF earlier than 19S-type antibodies.

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HISTOCHEMISTRY

Oxidation of L-Ascorbic Acid by Cells of  
Carcinoma of the Human Cervix

THE increased activity of pentose-shunt dehydrogenases, which had been demonstrated histochemically in cancers<sup>1</sup>, has been partly confirmed by biochemical investigations in carcinoma of the cervix in that the cells of the vaginal secretion gave high values for 6-phosphogluconate dehydrogenase<sup>2</sup>. Since this is said to be an oxidative pathway<sup>3</sup>, attempts were made to test if a concomitant rise in cytochrome oxidase activity could be demonstrated. This was done histochemically, to avoid dilution of active cancer tissue by inactive or necrotic tissue and by stroma. A more intense and permanent histochemical procedure for cytochrome oxidase<sup>4</sup> was applied to sections from eight apparently normal cervixes, three apparently normal endometria, ten primary carcinomata of the cervix and seven other carcinomata of the genital tract. The tissues were removed at operation and were frozen and sectioned by a controlled temperature freeze-sectioning method<sup>4,5</sup>.

The cytochrome oxidase-levels were not elevated in the cancers; in some they seemed somewhat depressed.

An attempt has therefore been made to test whether some system other than that involving cytochrome oxidase, but which also may be capable of reacting directly with oxygen, might show the expected increase in activity. Since it is known that the metabolism of some tissues can alter from one using cytochrome oxidase to one dependent apparently on ascorbic acid<sup>6</sup>, it was decided to test whether changes in the ability to oxidize vitamin C occurred in these malignant cells.

Fresh unfixed frozen sections were incubated for 2 h in a medium containing 0.05 per cent neotetrazolium; 0.05 M L-ascorbic acid; 0.1 M sodium azide in 1 M tris-buffer. Although the pH of the buffer began at 7.6, the pH of the final mixture, once the constituents were dissolved, was pH 5.5. In all the experiments, the pH of the incubation medium was measured immediately before use. The medium was added as a drop covering the section, incubation being in a humidity chamber at 37° C. By this procedure, the sections from histologically normal cervixes showed only very weak reactions, almost exclusively in the basal cells, whereas those from the primary carcinomata of the cervix gave strong to intense deposition of the formazan. It was noteworthy that the cancers produced a clearly stronger reaction in each cell than did the basal cells of relatively normal or even slightly hyperplastic tissue.

This reaction occurred only with the L- and not the D-isomer. It was not given if catechol or phenol were substituted for ascorbic acid in equimolar concentration, nor was it given in the absence of L-ascorbic acid. It was lost if the tissue was stored for longer than 4 days at -70° C. The reaction was totally dependent on the presence of a high concentration of azide, being abolished if the concentration was reduced to 10<sup>-3</sup> M. The addition of potassium cyanide, from 10<sup>-3</sup> to 10<sup>-1</sup> M, appeared to have no effect; nor could cyanide take the place of azide in the normal incubation medium. In the absence of azide, no reaction was obtained until the pH was raised to more than pH 7.0; beyond this value, up to pH 8.5, the amount of formazan deposited increased with the increasing pH of the solution. The response of the tissue at the alkaline range was unaffected by the presence of azide.

Similar, albeit very much weaker, reactions have been obtained with sections of rat liver. The response at the alkaline range was not affected appreciably even when the tissue had been previously immersed in boiling water for 10 min. In contrast, the reaction at the acidic pH values was destroyed by this treatment; it showed optimal activity at pH values of 5.5 or 6.0, being obviously weaker at pH 6.5 and 6.0. In later experiments it has been found that the reaction at the acidic pH is readily inhibited by oxygen.

Consequently, sections of carcinoma of the cervix were incubated in the normal medium in Coplin jars. In some either oxygen or nitrogen had been bubbled through the medium for 10 min prior to immersing the slides. Only a very weak reaction was obtained after oxygenation, and this was completely suppressed when oxygen was passed through the medium also during the incubation. Conversely, enhanced reactions were obtained after the preliminary treatment with nitrogen, and these were further enhanced when nitrogen was passed through the medium continuously during the incubation.

Thus it has been shown that the samples of carcinoma of the cervix studied had a very enhanced ability to reduce neotetrazolium to its formazan in the presence of L-ascorbic acid in acidic solution. The inhibition of this reaction by oxygen suggests that the neotetrazolium is replacing atmospheric oxygen in the oxidation of the ascorbic acid, very much as does methylene blue in the conventional biochemical test for oxidases<sup>7</sup>. That this oxidation is probably enzymatic, in the range of pH values of 4-7, is indicated by the following: first, that it shows

distinct optimal activity at about pH 5.5; secondly, that the activity diminishes with storage, as do the activities of many enzymes studied in tissues frozen in this manner; thirdly, that the activity is destroyed completely by boiling the sections; fourthly, that it depends on the presence of the L-isomer.

One of the prime difficulties of detecting cancer at an early stage has been the fact that the biochemistry of malignancy is characteristically that of loss of apparent chemical activity rather than gain of distinctive properties. The discovery of enhanced pentose-shunt metabolism<sup>1</sup> has already yielded promise that the increased activity may be used for the early detection of certain cancers<sup>2</sup>. It is possible that the establishment of further chemical activities which can be used to characterize malignant growths may assist the early diagnosis of this disease.

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## PATHOLOGY

### Attempt to Influence the Increased Solubility of Collagen in Lathyrism by Hydrocortisone

ADMINISTRATION of lathyrogenic agents to growing animals induces a variety of connective tissue malformations such as exostoses, hernias and aneurysms. Some authors claim that it is possible to suppress these malformations of lathyrism by treatment with corticoids, thyroxine and some antirheumatic drugs<sup>1-3</sup>. The basic defect of connective tissue in lathyrism is the alteration of the molecular aggregation of collagen, resulting in increased solubility in acid or neutral salt solutions<sup>4</sup>. In this study an attempt was undertaken to influence the increased solubility of collagen in lathyrism by treatment with hydrocortisone.

For this reason experimental lathyrism was produced in two groups of young rats (initial weight 30 g) by the administration of 60 per cent concentration of sweet-pea seeds (*Lathyrus odoratus*) in the diet for 30 days. One of these groups was treated with hydrocortisone intramuscularly in daily doses of 10 mg/kg. These animals were compared with another two groups of normal rats, one of which was treated with hydrocortisone in a similar manner. The extent of bone changes was checked histologically—the results will be published elsewhere. After 30 days the animals were killed and the dorsal skin was removed, depilated and cut with scissors. In a portion of the tissue the 0.14 M sodium chloride-soluble collagen hydroxyproline was estimated after 24 h extraction at 2° C with occasional vigorous shaking. Dialysable hydroxyproline was assessed after dialysis of a part of this extract against water for 24 h at 2° C; a membrane supplied by Kalle AG., Wiesbaden, was used. Another part of the tissue was extracted with 0.5 M acetic acid for the estimation of

acid-soluble hydroxyproline. These fractions and a sample of the whole skin from each animal were hydrolysed in 6 N HCl at 105° C for 20 h and the hydroxyproline was estimated according to Stegemann<sup>8</sup>.

Table 1 gives the results expressed as  $\mu\text{g}$  of hydroxyproline per 100 mg of dry tissue. It is evident that the animals on *Lathyrus* diet have much higher concentrations of extractable hydroxyproline fractions (neutral and acid soluble). There were high values of dialysable hydroxyproline in lathyritic rats. The treatment with hydrocortisone in intact animals resulted in increased amounts of total hydroxyproline and decreased levels of neutral soluble fraction. This is in good agreement with the results of Smith<sup>6</sup> and those summarized by Kuhn *et al.*<sup>7</sup>. In lathyritic rats the hydrocortisone treatment depressed the elevated amounts of soluble fractions as well as of dialysable hydroxyproline although it did not restore the normal ratio completely.

Table 1

	Total hydroxyproline	Acid soluble hydroxyproline	Neutral salt soluble hydroxyproline	Dialysable hydroxyproline
Control	9 780 $\pm 1 230$	3 660 $\pm 450$	168 $\pm 34$	34.4 $\pm 11.6$
Hydrocortisone	11 160 $\pm 1 370$	4 000 $\pm 780$	109 $\pm 24$	20.8 $\pm 11.7$
Lathyrus	8 390 $\pm 958$	7 000 $\pm 2 180$	2 830 $\pm 615$	3 880 $\pm 543$
Lathyrus + hydrocortisone	10 230 $\pm 1 170$	5 060 $\pm 1 690$	2 010 $\pm 340$	1 590 $\pm 300$

Values indicate statistical average  $\pm$  standard error of the mean.

We suppose that lathyrogenic agents, and hydrocortisone, influence the metabolism of collagen in different ways. It is possible that hydrocortisone promotes the aggregation of collagen molecules, and results in decreased solubility of collagen. In this way hydrocortisone is able to influence the lathyrogenic defect which manifests itself by retardation of collagen maturation<sup>7</sup>. Similar results were obtained when the biological age of lathyritic and hydrocortisone-treated rats was tested by means of chemical contraction and relaxation of tail tendon fibres<sup>9,10</sup>. The possibility cannot be excluded that hydrocortisone inhibits the postulated enzyme which, hypothetically activated by lathyrogens, can sever intra- and inter-molecular cross-linkages<sup>11</sup>.

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## IMMUNOLOGY

### Ultrastructure of Antibody-forming Cells

THE method of Jerne and Nordin for demonstrating individual antibody-forming lymphoid cells has been utilized to obtain quantitative information about the cellular changes during antibody formation<sup>1</sup>, to indicate transplantation immunity<sup>2</sup> and to demonstrate antibody-forming cells in 'tolerant' animals<sup>3</sup>. Recently, this method has been combined with autoradiography to investigate the kinetics of antibody formation<sup>4</sup>. In the present studies the plaque method has been used to identify for study by electron microscopy individual cells which have released antibody.

Cell suspensions were prepared from the spleens of rats at various times after intravenous immunization with washed sheep erythrocytes. Dilutions of the spleen cells mixed with sheep erythrocytes in melted agar were poured into Petri dishes over a solidified layer of agar several mm thick. After the overlayer had solidified, the dishes were incubated in a moist chamber at 37° C for 30 min. Antibody released by individual lymphoid cells during this initial incubation diffused out from the cell and became attached to antigenic sites on surrounding sheep erythrocytes. Addition to the plate of 2 ml. of a 1:3 dilution fresh rat serum supplied complement which lysed antibody-coated erythrocytes to produce a clear zone ('plaque') around the antibody releasing (and presumably forming) cell.

Lymphoid cells which produced plaques can be examined before or after staining with various dyes, but this procedure is only partially satisfactory since cells retain their spherical shape and cytological details are poorly demonstrated. However, a disk of agar containing a plaque can be removed from the plate and processed for electron microscopy with reasonable ease.

With the aid of a binocular dissecting microscope at magnification 10–25 times, plaques were punched from the plate using glass capillary tubing 1.2 mm internal diameter. The agar disks were placed on glass microscope slides and examined at magnifications of about 400 times to verify that a single lymphoid cell was present in the centre of the plaque. The disks were fixed for 15 min in buffered glutaraldehyde, washed in phosphate buffer, and post-fixed in buffered osmium for 10 min. Plaques were embedded in 'Epon' or stained with phosphotungstic acid and embedded in 'Araldite'. The disks in plastic were reoriented with the surface of the plaque parallel with the block face. The block mounted in a chuck was brightly transilluminated. A mechanical device mounted on a microscope made it possible to trim accurate block faces about 0.1 mm  $\times$  0.1 mm around the plaque-forming cell. A larger block face showing a plaque-forming cell partially surrounded by a halo of unlysed sheep erythrocytes is shown in Fig. 1. The sides of the trimmed block were perpendicular to the block face making it possible to view the plaque-forming cell from the side of the block. A microscope with a long-focal-length objective mounted to the side and at a right angle to the microtome made it possible to determine precisely the depth of the cell from the block face during sectioning. Using these procedures,

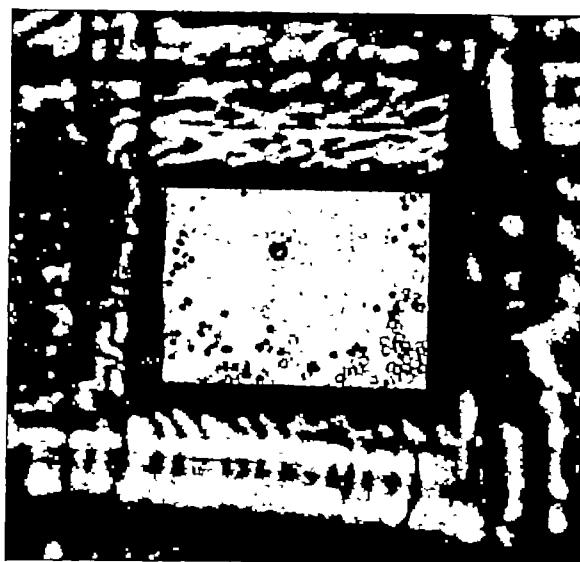


Fig. 1 'Epon' block containing a plaque with a central antibody-releasing cell. The trimmed block face measures 90  $\mu$   $\times$  120  $\mu$ . This block is ready for serial sectioning for electron microscopy.



Fig. 2. Electron micrograph of plaque-forming cell from rat spleen. Rough-surfaced endoplasmic reticulum is abundant. The cell nucleus is not in the plane of sectioning. ( $\times$  9,500)

sections of plaque-forming cells could be regularly obtained. 'Epon' sections were stained with uranyl acetate or lead hydroxide or a combination of the two stains.

A representative example of plaque-forming cells is shown in Fig. 2. The abundant, rough-surfaced endoplasmic reticulum is filled with finely granular material. The nucleus, not seen in all sections, usually contains chromatin material coarsely clumped on the nuclear membrane. Most of the plaque-forming cells have morphological characteristics of plasma cells. Scattered vacuoles of various types in the cytoplasm may be normal cell components or may represent changes of focal cytoplasmic degradation resulting from the rather long interval (up to 45 min) between removal of the spleen and fixation of the developed plaque. However, fine structural details of the endoplasmic reticulum and the mitochondria are preserved, suggesting that cellular morphology is not seriously damaged by the *in vitro* manipulations.

It is not known whether plaque-forming cells release one or several molecular types of anti-sheep erythrocyte antibody. Droplets of purified fractions of either 19S or 7S antibody produce haemolysis of sheep erythrocytes in agar plates, indicating that cells releasing sufficient quantities of either type of antibody would be capable of producing plaques. An exponential increase in the number of plaque-forming cells occurs during the first 4 days after immunization and only 19S antibody is detectable in serum on the fourth day after immunization. Unquestionably, plaques are formed by cells releasing only or predominantly 19S antibody. Spleens of rats killed 4 days after repeated twice-weekly antigen injections contain about 10-fold fewer plaque-forming cells than spleens of animals killed 4 days after a single antigen injection\*. Serum from the repeatedly injected animals had high concentrations of both 19S and 7S antibody. Presumably some plaques obtained from animals receiving repeated antigen injections might contain cells which had released only or predominantly 7S antibody. Up to the present, electron microscopy has not revealed ultrastructural differences in plaque-forming cells from animals in the early primary response when compared with cells obtained from animals receiving repeated antigen injections.

In an attempt to identify the type of immunoglobulin released by cells, plates containing plaque-forming cells were treated with 2-mercaptoethanol. Droplets of serum fractions containing 19S antibody placed on agar plates were inactivated by this treatment while droplets contain-

ing 7S antibody were not. 2-Mercaptoethanol inactivated about the same proportion of plaques in plates prepared from animals in the early primary response as in plates prepared from animals receiving repeated antigen injections.

The foregoing results indicate that plaques are formed by cells in the phase of rapid synthesis of antibody and this phase of synthesis is probably concomitant with synthesis of 19S antibody. However, the results do not exclude the possibility that some plaques were produced by cells synthesizing predominantly 7S antibody. The studies are not sufficient to indicate whether different cell types produce 19S and 7S antibody or whether synthesis of 19S antibody represents only a phase of antibody production by a single cell type. Recent evidence indicates that lymphoid cells usually contain only 7S or 19S immunoglobulin but that some may contain both types\*. No correlation could be made, however, between the morphology of cells and the type of immunoglobulin produced.

In previous investigations of the ultrastructural characteristics of antibody-forming cells ferritin was used as the immunizing antigen\*. The experimental manipulations which are necessary to allow ferritin to penetrate an antibody-containing cell often result in structural alteration. Also, this method indicates only that a given cell contains specific antibody; it furnishes no information about the ability of the cell to release antibody at that time. The plaque method developed by Jerne makes it possible to examine the ultrastructure of cells which have actively released antibody; metabolic activity is required for the development of a plaque<sup>1</sup>.

Electron microscopy of plaque-forming cells together with electron microscopic autoradiography will provide essential information about the morphology of specific antibody protein synthesis.

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### Assay of Insulin Antibodies produced by the Guinea-pig

The antigenic properties of insulin are now well recognized, but no simple method has yet been described for the routine detection and assay of insulin antibodies. The method described here is based on the classical observation by Berson, Yalow, Bauman, Rothschild and Newerly<sup>1</sup>. They showed that during chromatoelectrophoresis on filter paper, <sup>125</sup>I-labelled insulin bound by insulin antibodies in human serum moves with the  $\beta$ - $\gamma$ -globulins, while free insulin remains fixed at the point of application of the serum-insulin mixture on the filter paper. When an excess of unlabelled insulin mixed with a trace of labelled hor-

more is added to the anti-insulin serum, the excess insulin is adsorbed from solution by cellulose and the amount bound by the antibodies is measured in the supernatant solution. We have used this assay system for serum obtained from guinea-pigs treated with bovine insulin<sup>4</sup> and capable of neutralizing insulin at rates ranging from 0.1 to 3.5 units per ml. serum. It can, however, be adapted for the detection and assay of antibody concentrations lower than this; it has not yet been used for anti-insulin sera of other animals or of man.

Anti-insulin serum (0.1 ml.) diluted in phosphate buffer (0.4 ml.; 0.1 M, pH 7.0) is placed in a centrifuge tube to which is then added a mixture of unlabelled bovine insulin (0.6 u.) and a trace of <sup>125</sup>I-labelled insulin (approximately 1 m.u.; 3–10 mc./mg insulin; Abbott Laboratories, Oak Ridge, Tennessee) dissolved in the same volume (0.5 ml.) of buffer. The reaction is allowed to take place at room temperature for 30 min; it is actually complete within a minute. Then a suspension of cellulose in the same buffer (1.0 ml.; 10 per cent w/v; 'MN' cellulose powder, Mecharey, Nagel and Co., Duren, Germany) is added and the mixture shaken intermittently for a further 30 min; again much shorter times are needed. The cellulose is deposited by centrifugation and the supernatant solution (1 vol.) added to Bray's<sup>5</sup> solution (10 vols.) in which the  $\beta$ -emissions are counted in a liquid scintillation counter (Packard Instrument Co., La Grange, Illinois).

In the presence of the same insulin mixture, more than 98 per cent of the added radioactivity was recovered from the supernatant solution when an excess (0.5 ml.) of an undiluted sample of anti-insulin serum was added (Lot 302). Part of this radioactivity (3–8 per cent) was recovered from the supernatant solution when an equal volume of normal serum was used in place of the anti-insulin serum; this probably represents degraded fragments of the labelled hormone and insulin bound non-specifically by the serum proteins. The difference between these two values, or between counts recovered from equal aliquots of supernatant solution under the same conditions, is therefore proportional to the total amount of insulin added initially. Between these two extremes, as shown in Fig. 1, a linear relationship ( $r = 0.971$ ;  $P < 0.001$ ) was found between the volume of anti-insulin serum added and the counts recovered from aliquots of the supernatant solution, but towards the point of neutralization this relationship ceased. Provided an adequate excess of insulin is present, therefore, the potency of a serum sample can be determined from the

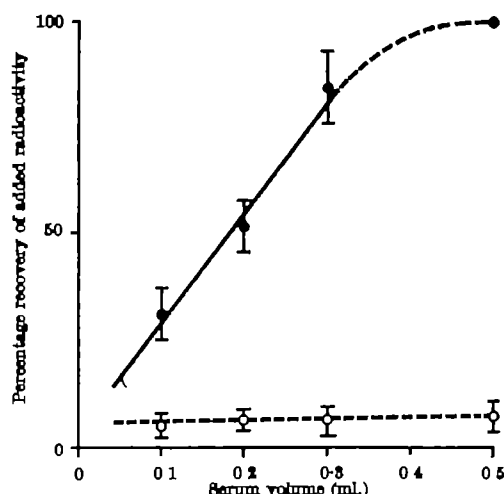


Fig. 1. Percentage of added radioactivity recovered from the supernatant solution after treatment of variable volumes of anti-insulin (closed circles; Lot No. 302) and normal (open circles) guinea-pig sera with a constant amount of bovine insulin (600 m.u.) and a suspension of cellulose. Each point represents the mean ( $\pm 2 \times S.D.$ ) of 5–15 observations. For reasons given in the text, radioactivity recovered using an excess of anti-insulin serum (0.5 ml.) is taken to be 100 per cent

Guinea-pig serum (Lot No.)	in vitro Serum potency	in vivo
196	2.62 $\pm$ 0.3 (13)	2.3
271	1.5 (2)	1.5
301	2.4	2.0
302	1.66 $\pm$ 0.2 (10)	1.5
311	2.0 (2)	1.0
317	2.3 (2)	1.6

Each serum was assayed *in vitro* by the present method and *in vivo* by the method of Armin *et al.* (ref. 4). Mean potencies ( $\pm S.D.$ ) are stated as units bovine insulin neutralized or bound by 1.0 ml. serum, where more than one assay was carried out the number is shown in brackets.

counts obtained from a portion of the supernatant after addition of the cellulose suspension: units insulin bound by serum sample equals:

$$\frac{\text{c.p.m. sample} - \text{c.p.m. blank}}{\text{c.p.m. excess anti-insulin serum} - \text{c.p.m. blank}} \times \text{units insulin added}$$

For any sample of anti-insulin serum assayed an equal volume of normal serum was used as a blank. The volume of normal serum, within the range used here (0.1–0.5 ml.), had little effect on the radioactivity recovered from the supernatant solution which seldom exceeded 10 per cent of that recovered using an excess of anti-insulin serum.

The results obtained by this method, as shown in Table 1, are comparable with those found by the much cruder and less accurate method described previously; potency was then related to the hyperglycaemic response of rats injected intravenously with the serum<sup>4</sup>. From results obtained over a period of 9 months, guinea-pigs were separated into distinct groups according to their ability to produce insulin antibodies. After 4–5 monthly injections of insulin the good producers consistently yielded serum neutralizing 1.5–5.5 u. insulin/ml.; while the poor producers, a very variable proportion, yielded serum neutralizing less than 0.5 u./ml.

Although primarily designed and used to assay these potent sera, the method can be adapted to assay insulin antibodies in much lower concentrations. Thus, by reducing the amount of added insulin, it has been used to assay free antibody concentrations in animals (dogs and rats) injected with guinea-pig anti-insulin serum; normal sera from these animals did not interfere with the assay. Serum samples neutralizing 10–0.1 m.u. insulin have been successfully assayed.

Finally, we would emphasize the empirical nature of this method. Guinea-pig anti-insulin serum contains more than one antibody to insulin and these may have differing insulin-binding capacities. A more accurate and meaningful method of assay will not emerge until homogeneous antibody preparations have been isolated. Meanwhile, this method has proved successful in investigations of the production of insulin antibody in guinea-pigs and should facilitate preliminary analysis of the basic characteristics of the antibody itself.

We thank Prof. R. H. S. Thompson and Guy's Hospital Medical School, London, for assistance in the production of most of the anti-insulin serum used here; the National Institutes of Health for a grant (AM-7211); the Agency for International Development for a fellowship (L. R.-C.); and Mr. L. Norman for assistance.

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## RADIOBIOLOGY

## Fading in Thermoluminescent Lithium Fluoride used for Radiation Dosimetry

LITHIUM fluoride powder is now being used extensively for thermoluminescent dosimetry of ionizing radiations because of its good tissue-equivalence with respect to photon energy, and its reported absence of fading<sup>1-3</sup>. We have found hitherto unreported changes of response with time, predominantly manifested by fading of the read-out by some 10-20 per cent at 3 weeks after irradiation, followed by a tendency to increase towards the initial value at 6-8 weeks. This fading can cause difficulties in practical use unless care is taken to read-out specimens at the same time after irradiation. The fading reported here is other than any occurring in the first 24 h.

We have used commercially available lithium fluoride dosimetry powder, type 'TLD-100' (Harshaw Chemical Co., agents Nuclear Enterprises (G.B.) Ltd., Edinburgh) purchased in several batches between January 1963 and February 1965, and 'type N' (Controls for Radiation, Boston, Massachusetts) from another commercial source. These two types of powder are expected to be similar, and different in composition from TLD-700 and type 7, which we have not tested for fading. Successive exposures followed by annealing for re-use of a given batch of powder result in appreciable changes of sensitivity<sup>4,5</sup>, although the radiation-induced glow peak apparently remains a single peak. Batch-to-batch reproducibility is therefore poor, as is well known, but within one annealed batch the readings are repeatable to within 1-3 per cent. Four different read-out devices were used in these experiments: two were built in our laboratories<sup>6</sup>, while two others are commercially available. The results should therefore not be a function of unusual optics or heating cycles in the read-out apparatus. All four readers measured the integral of the glow curve.

The experimental procedure was to irradiate a capsule containing 1-2 g of LiF powder to usually 1,000 rads, using 15-MeV electrons, 15- or 5-MV X-rays, or cobalt-60  $\gamma$ -rays (with appropriate build-up material, and with subsequent mixing of the powder, to avoid any non-uniformity of dose). The powder was stored in the dark at room temperature unless otherwise stated. One experiment showed that fading was closely similar for doses of 5, 50, 500 and 5,000 rads. At appropriate times after irradiation, 4-9 read-outs using 30 mg of powder each were made for every time point. In some experiments the powder was divided into two parts for a comparative

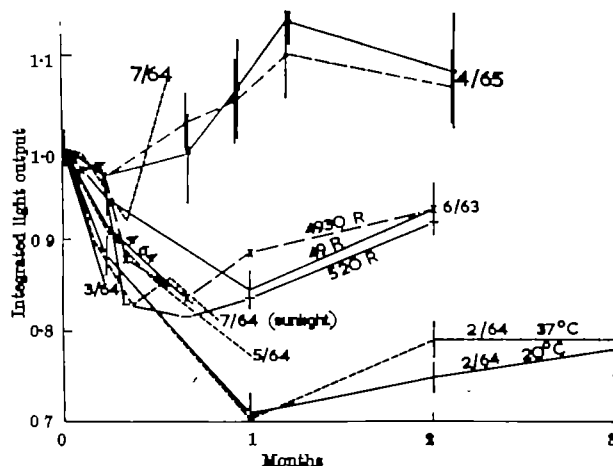


Fig. 1. Measurements of TLD-100 response versus time. Doses of 1,000 rads were given and the powder was stored in the dark at room temperature unless otherwise indicated on the curves, except for the upper pair which are for 100 r. exposure and storage at 24° C (dashed) and -18° C (full line). Month and year of irradiation are given for each curve.

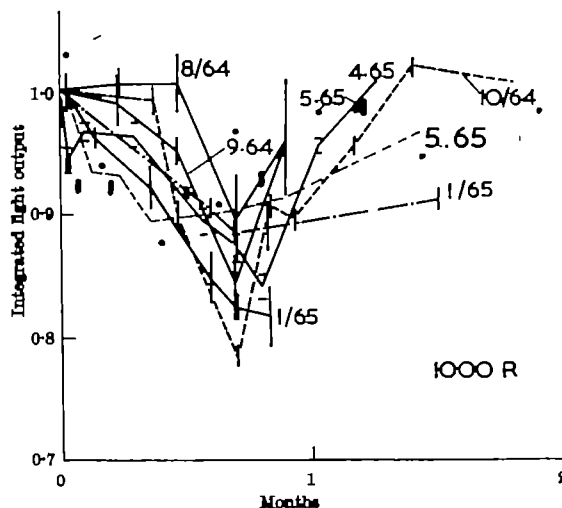


Fig. 2. Fading of LiF given 1,000 rads and stored in the dark at room temperature before read-out. Lines: TLD-100. Dots: Type N phosphor (believed to be similar in composition).

experiment, for example (1) stored in the dark or behind glass exposed to sunlight; (2) stored at 37° C or at room temperature; (3) stored at -18° C or at room temperature; (4) read-out on a laboratory-constructed or a commercial reader. In each such paired experiment the agreement between the two fading curves was within  $\pm 2$  per cent.

Fig. 1 shows eleven curves of response versus time, obtained between June 1963 and July 1964, except for the upper pair at 24° C and -18° C after 100 rads which was obtained in April 1965. During these experiments it was found that the radioactive light source (with  $\text{CaWO}_4$  phosphor) used to standardize the photomultiplier gain was somewhat temperature dependent (that is, 1 per cent decrease in light intensity for 1° C increase in temperature). The nine curves in Fig. 2 have been obtained with precautions to avoid errors due to this variation. We therefore attach most weight to the results in Fig. 2; but the results in Fig. 1 support them.

Fading appears to be independent of dose from 5 to 5,000 rads, and depends less on temperature of storage (between 37° C and -18° C) than on the particular batch, as shown by the pairs of curves in Fig. 1. In three curves a rise of response was seen by about 3 weeks, without a large initial fading, this rise corresponding to the rising part of the other curves in magnitude and in time. Two of the fading curves in Fig. 2 are for the Type N phosphor (dotted) and the results are similar to those for TLD-100. Thus in each of twenty fading curves, obtained at different times over a period of two years, a change of at least 10 per cent was observed during a period of 3 weeks after irradiation.

The temperature of annealing was investigated (nominally 400° C for 1 h, followed by 80° C for 24 h as proposed by Cameron *et al.*<sup>7</sup>). Fading was not eliminated if the temperature was somewhat less than 400° C. The effect of raising it to 420° C, say, or of prolonging the time to 1.5 h was not investigated. No differences were observed when the powder was allowed to cool to 80° C overnight instead of transferring it at 1 h to 80° C.

If the later increase of response often brings the readings close to the initial values, an explanation is provided of the fact that this fading has not been reported by other workers. The results recorded here are, however, consistent with the "absence of fading within  $\pm 7$  per cent" recently reported by Boone, Crosby and Shalek<sup>8</sup>. It is possible that readings taken at 3-, 6- or 12-months intervals would show little change from the initial readings<sup>9</sup>. We have not investigated the accuracy of this recovery to the initial value because each fading experiment requires a relatively large quantity of powder.

Such fading and recovery could be explained in principle by three levels of energy storage, sequential in time, from the second one of which significant optical transitions did not occur on heating for read-out.

It is to be hoped that a solution can be found, and the fading avoided, by different annealing, by pre-heating immediately before read-out, or by reading peak-height instead of area under the glow curve\*. Either of the last two solutions would require a more complicated control of the heating cycle than is generally used. We wish to emphasize that we have only investigated TLD-100 and Type N LiF.

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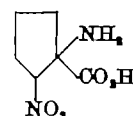
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## BIOLOGY

### An Inhibitor of Plant Growth-produced by *Aspergillus wentii* Wehmer

In the course of an empirical screening programme we found that culture filtrates of *Aspergillus wentii*, grown on Raulin-Thom medium (5 per cent glucose) supplemented with 0.1 per cent Difco yeast extract, have a pronounced inhibitory effect on the growth of 'Meteor' pea seedlings. When seedlings were sprayed to run-off with undiluted culture filtrate, shoot growth almost ceased for a week and then recommenced slowly. Newly formed internodes were abnormally short; leaflets were very narrow, in extreme cases becoming almost linear; flowers were similarly reduced, being almost apetalous; there was a marked loss of apical dominance, several axillary buds producing dwarfed branches. Newly formed stems and leaves were markedly chlorotic. This abnormal growth persisted throughout the subsequent life of the plants. The leaves sprayed, but not those formed after spraying, developed necrotic spots.

The growth-inhibitor was eventually extracted and shown to be 1-amino-2-nitrocyclopentane-1-carboxylic acid<sup>1</sup> (I), referred to hereafter as ANCPA.



(I)

**Effects on plants.** ANCPA produces effects on peas like those described here when sprayed on at 20 µg/ml., by application of 10 µg/plant in a 0.02 µl. microdrop to a single leaf, or by injection of 1 µg into the stem. It causes no necrosis; that observed in preliminary experiments was due to some other constituent, extractable by charcoal, of crude culture filtrates. It has a similar but even more marked effect on dwarf beans (*Phaseolus vulgaris*) and, if bean or cucumber seeds are soaked in solutions of ANCPA (10 µg/ml.) and then transferred to germination chambers, though percentage germination is not reduced, hypocotyl and radicle extension are much reduced. It inhibits extension of lettuce hypocotyls in seedlings growing on filter paper moistened with an aqueous solution, particularly under conditions where growth is maximal in controls, for example in darkness or in white light if gibberellic acid (GA) is supplied in addition\* (Table 2). Surprisingly, ANCPA does not appreciably inhibit extension of pea stem sections or pea root sections, or expansion of bean leaf disks, in buffer-sucrose with or without optimal indolyl-3-acetic acid, but it does inhibit extension of excised pea buds (Table 1) in a medium containing kinetin and gibberellic acid\*.

Table 1. FINAL LENGTH (MM) OF PEA BUDS (Five days growth in buffer/sucrose, in light at 16° C; mean of 20 replicates)

ANCPA µg/ml.	Basal medium	+ Kinetin (2 µg/ml)	+ Gibberellic acid (1 µg/ml)	+ Kinetin and GA
0	4.6	6.2	7.0	10.3
0.01	5.0	5.8	7.8	11.7
0.1	4.7	6.4	7.3	9.7
1.0	4.0	4.6	7.0	5.2
10.0	2.4	2.8	2.9	2.8

Significant difference ( $P=0.01$ ), 0.8

The observations described here do not distinguish between effects on cell division and on cell extension. ANCPA markedly reduces the rate of mitosis in root tips of several species, especially pea (*Pisum sativum*) (Table 3).

In connexion with the chlorosis of plants treated with ANCPA, it is significant to find that at 1 µg/ml. it reduces, and at 100 µg/ml. prevents, chlorophyll synthesis in sections of etiolated wheat leaves exposed to light. It does not delay (op. kinetin) or accelerate senescent chlorophyll loss in sections of light grown wheat leaves.

**Reversal of L-leucine.** Various lines of thought made it seem possible that ANCPA might be acting as an amino-acid antagonist. Of thirty amino-acids and related compounds tested, only L-leucine, and to a lesser extent L-methionine, would reverse the effects of ANCPA on lettuce hypocotyl extension (Table 2); D-leucine and D-methionine were ineffective. Considerable excess of

Table 2. FINAL LENGTH (MM) OF LETTUCE HYPOCOTYLS (Mean of 30 replicates)

ANCPA µg/ml.	Growth in light*			Growth in darkness		
	0	L-Leucine (µg/ml.) 0.25 25.0	100.0	L-Leucine (µg/ml.) 0 100		
0	24.5	25.7	25.0	25.5	24.6	24.1
1	15.3	14.4	16.5	23.7	22.8	24.5
2	13.6	15.1	16.3	21.7	17.2	24.9

Significant difference ( $P=0.01$ ) 2.7

2.1

\* In presence of 10 µg/ml. gibberellic acid.

L-leucine was necessary to reduce the effects of ANCPA appreciably in this experimental system. Similar leucine excess is needed to reverse ANCPA inhibition of root growth of cucumbers or cress seedlings, and it has not been found possible even partially to reverse the inhibition of pea bud growth or growth of intact pea or bean shoots. ANCPA also greatly reduces the rate of multiplication of yeast cells at 20 µg/ml., but this effect is only partially

reversed by a 100 times equimolar concentration of L-leucine.

The effects of ANCPA on mitosis in pea-root tips, on the other hand, can be reversed by very low concentrations of L-leucine. The effect is apparently not competitive (Table 3).

Table 3. NUMBER OF MITOTIC FIGURES PER 1,000 NUCLEI IN PEA ROOT TIPS  
(24 h after treatment with ANCPA)

ANCPA ( $\times 10^{-4}$ M)	Exp. 1 L-Leucine ( $\times 10^{-4}$ M)		Exp. 2 L-Leucine ( $\times 10^{-4}$ M)			
	0	7.6	0	1.5	3.0	6.0
0	92	100	118	—	—	—
0.06	14	90	—	—	—	—
0.6	0	83	—	—	—	—
3.0	0	121	0	18	77	92
30.0	—	—	0	—	—	126
						155

Thus, in some experimental systems at least, ANCPA is a well-defined leucine antagonist. In this connexion its effect on *Nicotiana* plants is interesting. Doses of 10  $\mu$ g/plant injected into the apical buds of young rosette-stage plants delayed stem formation and induced production of narrow, strap-like leaves with pronounced interveinal chlorosis, with increased development of lateral shoots. This closely simulates the physiological condition known as frenching<sup>1</sup>, which can also be simulated by feeding seedlings with isoleucine or *alloisoleucine*<sup>2</sup>. A number of synthetic analogues of ANCPA, including 1-aminocyclopentane-1-carboxylic acid and 1-aminocyclohexane-1-carboxylic acid, have also recently been shown to be amino-acid antagonists<sup>3-5</sup>.

It is not possible at present to characterize the effects of ANCPA on plant metabolism more closely, but it is noteworthy that 2-thiouracil induces comparable morphogenetic changes in *Cannabis*<sup>6</sup> and that this has been interpreted<sup>7</sup> as an inhibition of protein synthesis due to a disturbance of RNA metabolism.

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### Cation-anion Ratios of Plants

Dijkshoorn<sup>1</sup> has recently summarized the results of many analyses for the total equivalents of metals (potassium, sodium, magnesium, calcium) and non-metals (nitrogen, phosphorus, sulphur, chlorine) in *Lolium perenne*, and has calculated the balance of acidity within the plant following on the higher intake of non-metals as anions. From the content (in equiv./kg) of metals as 1.8 and non-metals as 3.2 he concludes that internal acidity must have risen by 1.4, but that this is more than offset by the reduction of nitrate and sulphate, both of which consume acid. If the reduction of nitrate is written as  $\text{HNO}_3 + 8\text{H} \rightarrow 3\text{H}_2\text{O} + \text{NH}_3$ , one obtains a disappearance of 1 or 2 protons per nitrogen reduced, according to whether one treats  $\text{NH}_3$  as a neutral molecule (as Dijkshoorn does) or as an alkali.

This calculation suggests a comparison with the effect on internal acidity of absorbing ammonium ion from solution. For every atom of nitrogen absorbed as  $\text{NH}_4^+$ , one proton disappears from the plant; for every atom absorbed as  $\text{NO}_3^-$  one proton appears in the plant, but the reduction of this ion to ammonium absorbs two protons, so that the net internal effect so far is the same for both forms of nitrogen. However, when the intake of other nutrients is considered, the two forms affect the internal acidity differently. Both these ions in glasshouse cultures are absorbed more rapidly than any others, so that  $\text{NH}_4^+$  cultures become acid and  $\text{NO}_3^-$  cultures become alkaline. But if we calculate the non-nitrogenous ions from such results as those of Cole *et al.*<sup>2</sup>, we find that there remains a net excess absorption of cations in both cases, but far stronger for  $\text{NO}_3^-$  than for  $\text{NH}_4^+$  cultures (Table 1). Thus the nitrate plants consume a greater net total of internal acidity than do the ammonium plants.

Table 1. CONTENT OF MAJOR NUTRIENTS IN PLANTS GROWN IN CULTURE SOLUTION

(Cole <i>et al.</i> ) (in equiv./100 g fresh weight)			
		Nitrate cultures	Ammonium cultures
Maize	Nitrogen as anion	45.0	—
	P, S, Cl	11.9	15.5
	Nitrogen as cation	—	49.0
	K, Ca, Mg	24.5	20.4
	Excess non-N cation	13.6	4.9
Tomato (young leaves)	Nitrogen as anion	55.1	—
	P, S, Cl	11.2	12.1
	Nitrogen as cation	—	75.6
	K, Ca, Mg	40.0	18.6
	Excess non-N cation	23.8	1.5

One may use these results also in considering the doctrine of a constant ratio of metals to non-metals in plants<sup>3</sup>. This has been dealt with by Cunningham<sup>4</sup>, who pointed out that the ratio of metals to non-metals in Italian rye-grass in Britain ranged from 0.33 to 0.93. If the foregoing doctrine rests on the idea that plants absorb cations at about half the rate of anions, then it is opposed by the work just quoted<sup>5</sup> where plants in ammonium cultures absorb cations four times as rapidly as anions. In Cunningham's plants grown with heavy doses of ammonium sulphate, the ratio of metal to non-metal (unfortunately quoted by him as cation-anion ratio) was the lowest of all at 0.33; but the true cation-anion ratio may have been the highest of all since much ammonium was probably absorbed as such. As a further point against the belief in a constant ratio, anion uptake has a higher temperature coefficient than cation uptake<sup>6</sup> and Cunningham's value of 0.93 for winter fits this last-mentioned relation.

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### Genesis of Ethylene in Apples

EYMER since Kidd and West<sup>1</sup> showed that emanations from ripe apples stimulated unripe apples into the respiration climacteric, and Gane<sup>2</sup> proved that the active principle in these emanations was ethylene, search has been made for the origin of this 'ripening hormone'. The search has been intensified in the past few years. Burg and Thimann<sup>3</sup> state that "there is general agreement that ethylene production is restricted to the stage in the life of fruits during which ripening occurs". They were unable to decide precisely the source of the ethylene, but suggested that it may be produced by the mitochondria. Later Burg<sup>4</sup> concluded that the biosynthetic source of ethylene is not to be found in intermediates of glycolysis, acid metabolism

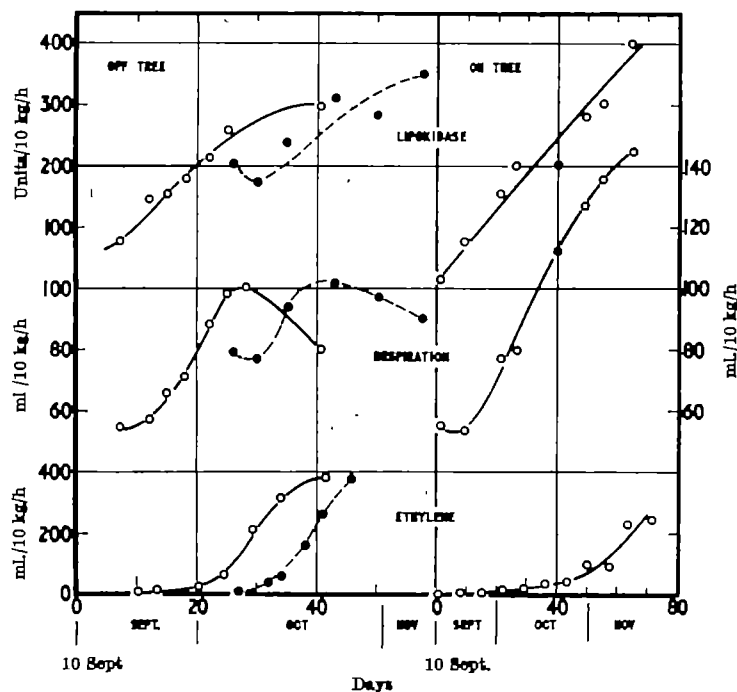


Fig. 1. Ethylene production and respiration rate (carbon dioxide output) of whole fruit, and lipoxidase activity of the peel of 'Cox's Orange Pippin' apples, picked at two stages of development and stored at 12° C ('off tree') and during development ('on tree'). The figures for ethylene production are taken, with the author's permission, from Wilkinson (ref. 9)

or the direct oxidation pathways of sugars, although it may be formed fairly directly from sugars by an, as yet, unknown pathway. More recently, Burg and Burg<sup>4</sup>, using <sup>14</sup>C-labelled, possible precursors of ethylene and apple tissue, concluded that, although ethylene formation is related to the metabolism of Krebs cycle acids as suggested by Wang *et al.*<sup>5</sup>, the relationship does not appear to be a direct one.

Lieberman and Mapson<sup>6</sup> have very recently described a model system which produces ethylene from the unsaturated fatty acid linolenic acid, after activation in various ways including the action of lipoxidase, in the presence of cuprous ions. They suggest that *in vivo* in apples cytoplasmic particles, lipoxidase and a copper enzyme might form a system for the biogenesis of ethylene.

Over the past few years we have been studying the enzymatic systems of senescing apples with special reference to the respiration climacteric. We have found that lipoxidase (determined by a modification of the method of Surrey<sup>7</sup>) appears, especially in peel tissue, during the early climacteric period and increases very rapidly during the course of the climacteric whether this occurs in detached fruit stored at 12° C or in fruit remaining attached to the tree. Wilkinson<sup>8</sup> has investigated the production of ethylene during the development of the climacteric in apples, and in Fig. 1 we have superimposed some of his results on our own for the respiration of whole 'Cox's Orange Pippin' apples and the lipoxidase contents of the peel from similar apples picked at approximately the same time as Wilkinson's apples. The form of the curves for ethylene production and lipoxidase activity in storage is strikingly similar, and increase in lipoxidase activity commences just before the onset of ethylene production and the climacteric. During the development of the climacteric on the tree, increase in lipoxidase activity precedes the appearance of 'measurable' quantities of ethylene. Kidd and West<sup>10</sup> have suggested that the longer time required for the climacteric rise to be attained on the tree is probably due to a greater degree of ventilation in the orchard rather than to a decreased rate of ethylene production.

Burg and Thimann<sup>4</sup> used pulp as well as, presumably, peel tissue in their experiments on ethylene production; we have found that in the pulp tissue also lipoxidase increases during the climacteric period, although the highest values attained are less than one-tenth those in the peel. Meigh<sup>11</sup> has found that ethylene production by the peel of apples is several times as great as that of pulp tissue. In our experiments lipoxidase activity is present in the soluble fraction, and our mitochondrial fraction<sup>12</sup> contains, at the maximum value of overall lipoxidase activity, only one-thirtieth the activity of the 'supernatant' fraction.

One of the most obvious phenomena associated with the climacteric in certain varieties of apples is the development of an oily coating on the skin. The object of this communication is to direct attention to the fat metabolism of the apple as a possible source of ethylene and to suggest that some at least of the enzymes involved in the process are outside the mitochondria. This is in line with Burg's<sup>4</sup> suggestion of a 'fairly direct' route from sugars.

It is suggested that one of the functions, but not necessarily the only one, of lipoxidase is related to the biogenesis of ethylene. Perhaps of relevance here is the recent finding by Freeman<sup>13</sup> that the diene-hydroperoxides found during the action of lipoxidase on unsaturated fatty acids can be decomposed by cytochrome-c.

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## Antibiotics as a Means of Control of Bacterial Contamination of Storage Tissue Disks

DURING the investigation of metabolism of storage tissues of higher plants, it is common to wash and aerate disks of the tissue for periods of several days or weeks. To this end, a variety of techniques has been reported, but in most cases it was not possible to impose rigorous conditions of sterility, although, in analysing the behaviour of tissues treated in this way, it is evidently necessary to ensure that the presence of micro-organisms is not a significant factor. The experimenter has usually used such criteria as retention of pigment if any, maintenance of turgor, and clarity of the suspending liquid. Recently, Bacon, MacDonald and Knight<sup>1</sup> have shown that these criteria may be observed even when substantial contamination of disks by bacteria has occurred, and they found that disks (1 cm diam. and 1 mm thick) of sugar beet showed the presence of about  $3 \times 10^7$  organisms/disk after washing for 4 days. During a programme of research into enzyme synthesis in storage tissue disks<sup>2,3</sup> we have

found that artichoke and carrot disks become contaminated with bacteria to a similar degree, ranging from  $5 \times 10^4$  per disk after 24 h to  $4 \times 10^7$  after 3 days.

Using standard microbiological techniques we have identified micro-organisms which occur on disks of carrot and artichoke, and under our conditions of washing we found only Gram-negative bacilli of the pseudomonad type, for example, *Pseudomonas fluorescens*, *Achromobacter anophage* and *Achromobacter parvulus*; on no occasion was fungal contamination found. Bacon *et al.* imposed sterile conditions in their experiments, but although this is obviously the ideal circumstance it is difficult to achieve consistently and entails considerable labour. Some other workers have used antibiotics, but in assessing their effectiveness they appear to have used the same criteria as above; penicillin (10–50 µg/ml.) has been commonly used, but as it is active primarily against Gram-positive bacteria, it would not seem to be a suitable choice, as soil organisms, the most likely contaminants of storage tissues, are largely Gram-negative. Streptomycin is sometimes used alone (10–50 µg/ml.), or together with penicillin; in neither case does the controlling action appear to have been assayed directly by bacterial counts.

In order to discover suitable controlling agents, we considered it necessary to investigate more directly the effect of these and other antibiotics, and our results show that penicillin and streptomycin are useless under our conditions; the only effective antibiotic, of twelve tested, was chloramphenicol.

These results may be of interest to other workers in the assessment of means of controlling bacterial contamination of storage tissue disks.

Penicillins (potassium salts of penicillins V and G, sodium benzylpenicillin G, ampicillin), dihydrostreptomycin, chloramphenicol, tetracycline, neomycin, terramycin, erythromycin, novobiocin, kanamycin, polymixin, fusidic acid and methicillin were tested for action against the isolated bacteria (4 species) in pure culture using Difco 'Bacto Sensitivity Disks' (Baird and Tatlock, Ltd.) which enable the effect of antibiotics at low concentrations to be determined. Those which were effective by this test, namely, dihydrostreptomycin, chloramphenicol, neomycin, kanamycin, and also the penicillins (which were not effective) were added at various concentrations to the medium at the onset of washing disks: the effects on the growth of the bacterial population are given in Table 1 and the

corresponding effects on a metabolic process of the plant tissue, namely, invertase development, are also given. Chloramphenicol appears to be the only satisfactory compound, as at low concentrations it inhibits bacterial growth to insignificant numbers while not affecting the metabolic processes under investigation; at much higher concentrations, however, it does affect the enzyme development, as might be expected from a known inhibitor of protein synthesis. These conditions must obviously be established for each individual case, but we have found that chloramphenicol at concentrations of 50 µg/ml. has no detectable effect on a range of metabolic activities studied in storage tissues during the washing process. It is noteworthy that the penicillins had no effect in reducing bacterial contamination at the concentrations used, and furthermore they markedly affected invertase development.

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### Transpiration as related to Internal Water Content

THE valuable concept of water movement through plants as a catenary process<sup>1</sup> explicitly assumes no change of plant moisture content. While this assumption is adequate for many purposes, the quantities of water within larger plants are considerable, and variation over a period of time may be important. From Ovington's<sup>2</sup> results for plantations in southern England, the moisture content of the aerial parts of the trees (expressed as the equivalent depth of water) averaged 3.3 in., with a maximum value of about 7 in. (47-year-old *Picea abies*). The needles of young *Pinus sylvestris* in pots well supplied with water had a variation over a 24-h period in summer amounting to 5 per cent of the saturated moisture content<sup>3</sup>. If similar fluctuations occur in larger trees in the field, these would amount to 0.05 in. of water or 1 l./tree at 2,000 trees/ha. in a total water content of the canopy of 40,000 kg/ha. When the soil moisture tension in the experiment with potted pines exceeded an atmosphere, internal drying of the leaves occurred and the lost water was not replaced during the night. Should the moisture tension of soil of the rooting zone in a plantation reach the wilting point, perhaps as much as another 0.1 in. of the canopy moisture might be lost if the leaf water deficits were similar to those for the potted trees.

Probably the best available data on seasonal variation of the moisture content of tree boles are the measurements of Clark and Gibbs<sup>4</sup>, though their methods leave much to be desired<sup>5</sup>. The North American species examined showed, on the average, a seasonal fluctuation of the sapwood moisture content of 40 per cent on a dry-weight basis. The heartwood variation averaged about 15 per cent. The drying of the wood was achieved in approximately 100 days. Comparing these measurements with Ovington's<sup>2</sup> figures for the same genera but different species, it seems possible that a net loss of 0.5 in. or more of internal water could occur by transpiration in the summer.

These highly approximate estimates at least show that the available portion of the internal water content of plants is appreciable and may be quantitatively similar to

Table 1. EFFECT OF VARIOUS ANTIBIOTICS ON BACTERIAL CONTAMINATION AND INVERTASE DEVELOPMENT OF CARROT TISSUE DISKS

Antibiotic	Concentration (µg/ml.)	Bacteria/disk	Invertase development as percentage water control
None (0 h)	—	<10 <sup>3</sup>	—
None (24 h)	(Water control)	$6.2 \times 10^4$	100
Chloramphenicol	10	$1.9 \times 10^3$	100
(Chloromycetin)	50	<10 <sup>2</sup>	100
	100	<10 <sup>2</sup>	100
	500	<10 <sup>2</sup>	89
	1,000	<10 <sup>2</sup>	73
	2,000	<10 <sup>2</sup>	52
Kanamycin	10	$6.3 \times 10^3$	107
(as sulphate)	50	$5.9 \times 10^3$	128
	100	$5.0 \times 10^3$	31
Dihydrostreptomycin	10	$5.0 \times 10^3$	100
(as sulphate)	50	$2.2 \times 10^3$	100
	100	$2.1 \times 10^3$	100
Sodium benzyl penicillin G ('Crystapen')	10	$6.3 \times 10^3$	171
	50	$5.0 \times 10^3$	125
	100	$1.0 \times 10^3$	100
Potassium penicillin G	10	$5.9 \times 10^3$	104
	50	$6.1 \times 10^3$	129
	100	$6.1 \times 10^3$	84
Potassium penicillin V	10	$6.2 \times 10^3$	87
	50	$1.0 \times 10^3$	92
	100	$1.0 \times 10^3$	95
Ampicillin ('Penbrillin')	10	$9.3 \times 10^3$	158
	50	$8.9 \times 10^3$	116
	100	$9.1 \times 10^3$	100

Disks 1 cm diam. 1 mm thick were agitated by passing a stream of filtered air through the washing solution (1 disk/2 ml.), maintained at 25° C for 24 h.

For invertase assay disks were transferred to 0.025 M sucrose (1 disk/1 ml.) and reducing sugar was estimated after 1 h. For bacterial counts, disks were homogenized in sterile Ringer's solution (1 disk/1 ml.); serial dilutions were carried out before estimation by a viable count method<sup>6</sup>.

Results are averages of at least three experiments; intermediate antibiotic concentrations are omitted for brevity.

the differences in water balance between plant associations. Thus it is worth trying to assess accurately the amounts of available water in the various tissues and the resistance to moisture transfer of each fraction to the main sap stream. If sufficient quantitative information could be collected, an electrical analogue method might be used to examine the course of diurnal and seasonal variations of the stored water under conditions of fluctuating absorption and transpiration. Clark and Gibbs<sup>4</sup> have published graphs for *Betula lutea* which seem to indicate that the change in bark moisture content lagged behind the sapwood by one month and the heartwood by three months.

There are certain implications of these considerations. First, when the water use of larger plants is measured by using the heat-flow technique to estimate sap flux in the stem, special care must be taken when calibrating the method by the transpiration from the crown<sup>5,6</sup>. While such a calibration is ideal in that it is non-destructive, the diurnal and other cyclical changes in water storage in the plant cause disparity between the flux of water from the leaves and that up the stem at any instant, and attempts must be made to take this into account. Secondly, some methods of investigating the water balance of large plants fail to take into account that over a given period the amount of internally stored water may have altered appreciably, only to be restored later by a change in the relative magnitudes of absorption and transpiration. Finally, it is possible that the internal moisture reservoir of larger plants may be a contributory cause of the greater water consumption they commonly exhibit compared with other forms of vegetation. Short grass turf, which has been used<sup>7</sup> to obtain an empirical factor to convert a meteorological estimate of evaporation from open water to that from vegetation well supplied with water, probably has minimal reserves of available internal water in the leaves, stem and roots. Other things being equal, it is possible therefore that the maximum rate of absorption of moisture by the roots would be higher than that for plants with considerable tissue storage which is available at a smaller suction than the soil moisture. Higher diffusion pressure deficits may thus be produced in the grass, giving rise to greater stomatal control, and smaller water use relative to larger plants.

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### Reduction of Loss of Moisture by the Cuticle Wax Components of Grapes

THE grape cuticle serves as an effective barrier to the loss of water; but the cuticle layer or layers responsible for this property have not been identified. The chemical composition of the cuticular layers and their morphological structures, which are probably not independent, could both be important in determining the rate of cuticular transpiration, since there appears to be no correlation between the thickness of the cuticular walls and cuticular transpiration rate. This has recently been confirmed by Sitte and Rennier<sup>1</sup> for the transpiration of *Prunus*, *Agave* and *Sonchella*. This would imply that qualitative differences in the composition of cuticles are important in the regulation of water loss.

It was shown<sup>2</sup> that the grape cuticle wax is composed of a low melting 'soft wax' (80 per cent) consisting of long-chain alcohols, aldehydes, esters, free acids and

hydrocarbons, which is readily removed by petroleum ether, and a 'hard wax' consisting mainly of oleanolic acid which is removed by chloroform. Thus the possibility of removing one cuticular layer was of interest in the examination of cuticular transpiration of fruits, particularly so of grapes where the transpiration is not affected by the presence of stomates and their reactions.

Table 1. THE EFFECT OF REMOVING THE 'SOFT WAX' FRACTION FROM SULTANA GRAPES ON THE RELATIVE DRYING RATE\*

Time of exposure to petrol vapour (sec)	—	10	20	60	300
Amount of oleanolic acid removed (%)	0	1	2	5	12
Amount of soft wax removed (%)	0	26	53	63	85
Subsequent relative drying rate	1	1.5	2.0	2.5	4.4

\* The berries contained 136 µg/cm<sup>2</sup> cuticular wax of which about 80 µg were oleanolic acid. (Water loss of untreated control 1.7 mg H<sub>2</sub>O/h/g)

Ripe sultana berries of uniform size (1.5–2.0 g) exposed to the vapour of boiling petroleum ether for times varying from 10 to 300 sec lost increasing amounts of the soft wax fraction, whereas very little oleanolic acid was removed from the fruit under these conditions (Table 1). The rate of drying of untreated and extracted grapes was determined as follows: the berries were dried in wire trays in a ventilated cabinet at 50° and the relative drying rate was calculated from the loss in weight according to the formula:

$$\text{Relative drying rate} = [(W_0 - W_1)w_2]/[(w_0 - w_1)W_2]$$

where  $W_0$  and  $w_0$  = initial weights and  $W_1$  and  $w_1$  = final weights of untreated and treated samples respectively. It appears from the results presented in Table 1 that the drying rate depends on the amount of 'soft wax' fraction on the fruit and a total removal of all cuticular lipids with hot chloroform results in only a small increase of the drying rate (Table 2). Peeling of the fruit, that is, removal of the whole epidermal layer, yields only a comparatively small increase in the drying rate, indicating that the wax-free epidermis is no important water barrier. A similar observation has been made with the cuticles of apples<sup>3</sup>.

Table 2. RELATIVE DRYING RATE OF SULTANA BERRIES AT 50° C

Treatment	Without dipping	After immersion for 2 min in an emulsion of dipping oil	Increase in drying rate by dipping (%)
Control	1	2.5	156
Surface wax extracted with petrol vapour	4.6	5.3	14
Surface wax extracted with hot chloroform	5.7	7.6	20
Peeled	8.3	—	—

The increase of the cuticular transpiration of grapes by dipping solutions has been the object of several investigations. Cold dipping solutions are used in the production of light-coloured sultanas<sup>4</sup> and consist of an emulsion of a mixture of fatty acids, their ethyl esters and sulphonated compounds in alkaline solution. It has been established that very little or no wax is removed by the dipping procedure<sup>5,6</sup>, that the effect of dipping solutions on evaporation is reversible<sup>7</sup>, and that the electrical potential difference across the surface of grapes is not irreversibly changed<sup>8</sup>. Similarly the ultrastructure of the wax, visible under the electron microscope, is retained after removing the dipping solution by washing<sup>9</sup>. Chambers and Poëningham suggested that the surface of the wax plays an important part in the actions of dipping oils.

It was therefore of interest to compare the effect of removal of cuticular components by solvent extraction with that produced by dipping. The dipping oil was a commercial product and was used as an emulsion (2 per cent) in a solution of potassium carbonate (2.5 per cent). The drying rate is increased when the low melting 'soft wax' fraction is present in the grape cuticle (Table 2). Dipping solution has very little effect after removal of the 'soft wax' fraction with petrol or the total removal of cuticular wax with hot chloroform. The 'soft wax' fraction prevents water loss from the fruit probably acting simply as a hydrophobic layer that repels water both from within and without.

The lipids of dipping emulsions used to increase the drying rate of sultana grapes are absorbed and retained by the surface wax<sup>12</sup>. It is suggested that the addition of lipids (that is, fatty acids and their esters) with hydrophilic groups to the cuticular wax establishes a hydrophilic link between the hydrophobic surface of the grape and its watery contents, thus facilitating the flow of moisture through the cuticle.

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### Mechanical Stimulation of Feeding in *Epiactis prolifera*

THE feeding mechanisms of coelenterates were the subject of much interest at the turn of the century, and were investigated by such notable biologists as Loeb<sup>1</sup>, Jennings<sup>2</sup> and Parker<sup>3</sup>. Renewed consideration has been given to these problems in the past few decades largely through the work of Pantin<sup>4</sup>, who used the sea anemone, *Anemonia sulcata*, and more recently through the experiments of Loomis<sup>5</sup>. The latter, who showed that small amounts of the tripeptide reduced glutathione activated feeding in the fresh-water *Hydra littoralis*, directed attention toward the chemical activation of feeding<sup>6,7</sup>. But the question of mechanical stimulation of feeding, although demonstrated in some sea anemones by earlier workers<sup>8,9</sup>, has been neglected in recent years. In this communication, I describe some observations which show that the feeding response of the sea anemone *Epiactis prolifera* can be repeatedly activated by remarkably slight mechanical stimulation with inert objects.

Specimens of *Epiactis prolifera* of approximately 1.2–2.5 cm diameter and 0.75–1.5 cm in height were collected from eelgrass beds in Friday Harbor, Washington. The animals were kept unfed in glass bowls on a sea-table of running fresh sea-water at about 13° C.

When a small piece of Whatman No. 1 filter paper (about 0.5 cm square) was placed gently on the tentacles, those tentacles touching the paper contracted toward the mouth, and the adjacent tentacles both to the right and left bent toward the paper. When the paper was placed forcefully on the tentacles, the 'wave of stimulation' was greater, more tentacles contracting. Usually within 30–60 sec after the tentacles first touched the paper, the lips of the mouth moved toward the paper and ingested it.

The tentacles are required for the reception of mechanical stimuli which activate feeding. The anemone did not seem to be aware of small pieces of paper placed on its oral disk only. If a nearby tentacle were made to touch the paper, however, within a few seconds adjacent tentacles started to envelop the paper and ingestion commenced.

This anemone can ingest a wide variety of inert and plant material. For example, a single specimen of *Epiactis* ingurgitated, in rapid succession during a 45-min span, Whatman filter paper, laboratory 'Kimwipes', paper towelling, pieces of eelgrass (*Zostera*) and the alga *Nucus*, small pebbles (0.2–0.3 g), and a piece of glass rod (0.2 g).

All these objects were regurgitated later, covered with a layer of mucus. The regurgitation time for inert objects varied, some objects being retained for 2–10 h.

Animal food offered to *Epiactis* was usually swallowed within a minute. The anemones were fed flesh from local clams, shrimps, limpets, and sabellid worms. They would not ingest specimens of *Epiactis* half their size, but would ingest specimens of their own species about one-tenth their size. The latter were regurgitated several hours later, unharmed.

Following ingestion of animal tissue, the response to mechanical stimuli became gradually sluggish. For example, 8 h after feeding on the clam *Protothaca* sp., the anemones took from 10 to 30 min to ingest bits of paper, while the fasting control devoured the paper within 30 sec. One day after this feeding, the anemones did not respond to the paper at all and the tentacles seemed to be insensitive to mechanical stimulation. When a fresh piece of flesh was placed on *Epiactis*, however, it was trapped by the tentacles and feeding commenced immediately. After two days, unused food was usually regurgitated, and within one day after regurgitation the anemones were again responding avidly to inert objects.

The rapid triggering of feeding by mechanical stimulation which was so striking in *Epiactis* was not observed among other sea anemones from the Friday Harbor area that were tested, including *Anthopleura elegantissima*, *Anthopleura xanthogrammica*, *Tealia* sp., *Metridium senile*, *Diadumene leucae*, and the zoanthid *Epizoanthus scotinus*.

This response of *Epiactis prolifera* is consistent and repeatable. Furthermore, the anemone is hardy and can be maintained in the laboratory with relative ease. Thus, it may afford a useful experimental system to study in coelenterates questions related to the nature, role and interaction of mechano- and chemo-receptors involved in co-ordinating a single response.

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### Nitrogenous Excretion in Arachnids

ANIMALS are known to excrete ammonia, urea, or uric acid as end-products of protein metabolism. So far as we can ascertain, the problem of nitrogenous excretion in arachnids needs more investigation. Since the work of Gorup-Besanez and Will<sup>1</sup> in 1849, guanine was regarded as taking the place of uric acid as the main nitrogenous end-metabolite in arachnids. The method of synthesis of guanine in these animals is unknown<sup>2</sup>.

Vejropala<sup>3</sup> studied the excrements of the spider, *Epeira diademata*, by the specific enzymatic method of Schmidt<sup>4</sup> and found that guanine accounts for up to 12 per cent of the dry weight of the excrements. Schmidt *et al.*<sup>5</sup> found that guanine accounts for more than 85 per cent of the total nitrogen of the excrements of two species of spiders, the silk spider, *Nephila claviceps*, and the garden spider,



*Argiope aurantia*. Hypoxanthine, xanthine and uric acid were present in not more than negligible amounts. Atkinson and Chorlton<sup>8</sup> found that 40 per cent of the excretory material of the huntsman spider, *Delena cancerides*, was guanine, forming 71 per cent of the total nitrogen excreted and no other purines were present.

Guanine was also found to form the main nitrogenous end-metabolite in the excreta of scorpions. Gregoire *et al.*<sup>7</sup> stated that guanine represented about 90 per cent of the nitrogen excreted in two species of scorpions, *Androctonus australis* and *A. amurensis*. Adenine and hypoxanthine were absent, whereas small amounts of amino-acids and agmatine were present. In another scorpion, *Palamnaeus bengalensis*, Koch *et al.*<sup>9</sup> detected the presence of adenine, hypoxanthine and uric acid in the excreta. As such, animals excrete several intermediates of the same excretory cycle, they considered them to be physiologically less efficient than those excreting a single intermediate. Said<sup>6</sup> found that the excreta of the scorpion, *Buthus quinquestratus*, was mainly formed of guanine as it formed 86.27 per cent of the total nitrogen excreted; xanthine and urea were absent whereas ammonia and uric acid formed 8 and 0.19 per cent of the total excretory nitrogen respectively. Rao<sup>10</sup> and Rao and Gopalakrishnareddy<sup>11</sup>, working on the excreta of some scorpions and certain other arachnids, found that nitrogen is excreted predominantly as guanine. Uric acid accounted for 8–12 per cent of the total excretory nitrogen and was also detected in the poison and coxal glands of the scorpions, *Heterometrus* and *Buthus*.

The work recorded here was undertaken on the excrements of spiders and scorpions in Egypt, in an attempt to add more knowledge to the problem of excretion in arachnids. Excreta from 15 specimens of the scorpion, *Buthus quinquestratus* H.E., were collected in 10 days during September. Excrements from 7 specimens of the spider, *Lycosa urbana*, were collected during 25 days of August and September. The excreta of both animals were white deposits spotted with black or dark faeces.

Excreta were left to dry in a desiccator until weight was constant and then ground in a small mortar. Methods of analysis of excreta for total nitrogen, ammonia, urea and uric acid are similar to those used by one of us<sup>12</sup> for the analysis of urinary concretions of reptiles. Total nitrogen was estimated on the whole excreta; a weighed portion was dissolved in warm water and the soluble portion was used for the determination of ammonia, urea and uric acid. The water-insoluble part was dissolved in 1 per cent lithium hydroxide solution and the soluble portion was used for the determination of uric acid, as lithium hydroxide is a good solvent for uric acid and normally insoluble urates. Another weighed part of the deposit was dissolved in ammonia-water (1:1) to dissolve guanine, which is readily soluble in this solution. Guanine was measured in this solution by the method described by Williams<sup>13</sup> for the determination of guanine in urine.

Constituents / 1 g deposit	Scorpion ( <i>Buthus</i> )		Spider ( <i>Lycosa</i> )	
	As N	As % of total nitrogen	As N	As % of total nitrogen
Total nitrogen	243.000	100	177.419	100
Water soluble constituents				
Ammonia nitrogen	0	0	0	0
Urea nitrogen	0	0	0	0
Uric acid nitrogen	7.496	3.085	0.266	0.150
Water insoluble constituents				
Uric acid nitrogen	0.183	0.065	0.200	0.113
Ammonia water soluble constituents				
Guanine nitrogen	135.200	55.663	167.410	94.390

The results are shown in Table 1. The total nitrogen of *Buthus* and *Lycosa* was 243 and 177.419 mg/g deposit respectively. Ammonia and urea were completely absent from the deposits of both animals. Uric acid nitrogen formed about 3 per cent of the total nitrogen excreted in scorpions, while it was present in very small traces in the excreta of spiders. Such analysis indicates that these arachnids are not grouped under the three main divisions,

ammonoteles, ureoteles, or uricoteles, which excrete respectively ammonia, urea or uric acid as the main nitrogenous end-product of protein metabolism. Scorpions and spiders form a separate group which excrete guanine as the main nitrogenous end-metabolite of protein metabolism. The guanine excreted forms about 55 and 94 per cent of the total excretory nitrogen in the scorpion and spider investigated respectively.

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## MICROBIOLOGY

### Effect of Actinomycin D on the Infectivity of *Trypanosoma cruzi*

PARTIAL immunity against a virulent and lethal strain of *T. cruzi* has been obtained in mice by several procedures<sup>1-4</sup>. All these procedures lead to a limited protection and are characterized by the appearance of parasites in the blood. The use of killed flagellates induces little or no resistance against the virulent strain<sup>1,4</sup>, suggesting that a mild host infection is an indispensable prerequisite for the development of even partial immunity.

During studies of protein and nucleic acid synthesis in *T. cruzi*, it was found that actinomycin D was a potent inhibitor of the growth of the organism *in vitro*. Furthermore, the inhibition of growth remains after the flagellates are washed and resuspended in a medium free of actinomycin D. The earliest detectable effect of the drug was a potent inhibition of ribonucleic acid synthesis, followed by inhibition of protein and deoxyribonucleic acid synthesis. Moreover, the organisms maintained in the presence of actinomycin D, although manifesting normal motility, appeared to lose their potentiality to multiply as indicated by their inability to incorporate radioactive thymidine into DNA. These organisms, however, retained the ability to protect animals against infection by a virulent strain of *T. cruzi*.

The flagellates were cultivated at 28° C either in a medium containing liver infusion, tryptose (LIT medium), salts, serum and haemoglobin or a medium in which the liver infusion and tryptose were replaced by lactalbumin hydrolysate (Lacto medium). The composition of the former medium was made available to us by Dr. Robert G. Yeager, of Tulane Medical School, New Orleans. Intraperitoneal injections of a suspension of flagellates from culture or 200,000 blood forms of the flagellates, collected from mice heavily infected by a virulent strain, were used to inoculate mice.

Microscopic examination of the blood of infected animals and xenodiagnosis were both used to detect parasites in blood. The latter, a highly sensitive procedure in mice, was employed as a check on the microscopic examination to ensure that the blood of animals which had received injections of actinomycin D-treated flagellates did not

harbour the parasite. In this procedure, five *Triatoma infestans*, one of the vectors of *T. cruzi*, were used to feed on each mouse. The mice were bled to death by the bugs. Examinations of the triatomids were carried out two months after the blood meal. Microscopic examination of all mouse blood was carried out before, and 9-15 days after, the inoculation of the virulent flagellates.

The results of the multiplication potentiality of *T. cruzi* measured as a function of the rate of incorporation of tritiated thymidine into DNA are shown in Table 1. It is clear that the actinomycin D-treated organisms lost their ability to incorporate tritiated thymidine into DNA. Other inhibitors, for example mitomycin C and 5-fluorouracil-2'-deoxyriboseide, also inhibited the growth of the organisms *in vitro*, but were not as effective in inhibiting the incorporation of radioactive thymidine under these conditions.

Table 1. RATE OF INCORPORATION OF TRITIATED THYMIDINE INTO DNA OF ACTINOMYCIN D-TREATED *Trypanosoma cruzi*

Pretreatment of flagellates	24 h	48 h	72 h
None	100,100	120,000	165,000
Actinomycin D	0	0	0

Flagellates, cultivated for 3 days in Lacto medium containing 1 µg/ml. of actinomycin D, were centrifuged and resuspended in 3 ml. of LIT medium containing 1 µc. of tritiated thymidine/ml. The final suspension contained approximately  $1.5 \times 10^7$  flagellates/ml. This, together with identical suspensions of flagellates in which actinomycin D was omitted, was incubated at 25° C. At the indicated times, the suspensions were centrifuged and the sediments washed twice with 0.4 M perchloric acid and followed by two washings with 80 per cent ethanol. The final sediments were resuspended in 0.1 N NaOH. The radioactivity was measured in a Packard "Tri-carb" liquid scintillation spectrometer.

Suspensions of the actinomycin D-treated organisms were injected into 20 mice ( $3 \times 10^6$  organisms per mouse) and followed by a similar injection one week later. Two weeks after the last injection, eighteen of the twenty mice and twenty control mice were injected with blood of animals heavily infected with the virulent strain. Each mouse received approximately  $2 \times 10^4$  organisms. The results of this experiment are shown in Table 2. It may be seen that a previous exposure to actinomycin D-treated flagellates resulted in virtually a complete protection against a subsequent exposure to the virulent organisms. The control or untreated mice, on the other hand, all showed very high blood levels of the parasite and died within 13 days of exposure to the virulent flagellates. Particularly significant and perhaps even more important than the high degree of protection offered by the actinomycin D-treated flagellates is the observation that the protection was achieved without the appearance of blood parasites due to the injection of the metabolically inhibited organisms. It should be mentioned that other experiments carried out with groups of twenty mice (without the xenodiagnosis control) showed similar results.

Table 2. PROTECTIVE EFFECT OF *Trypanosoma cruzi* PREINCUBATED WITH ACTINOMYCIN D AGAINST INFECTION BY A VIRULENT STRAIN

Treatment	No. of mice	Blood parasite level		Survival time (days after injecting the virulent flagellates)	No. of survivors
		Before virulent strain	After virulent strain		
Control	20	—	1,000 to 4,230	12 ± 1	0
Actinomycin D	18 2†	0 0	1 to 4 —	* —	18 —

\* All animals in good health were killed after 90 days.

† Animals used for xenodiagnosis tests.

Twenty mice were inoculated with  $3 \times 10^6$  flagellates cultivated for 3 days in Lacto medium containing 1 µg per ml. of actinomycin D. The inoculation was repeated one week after the first injection. Two weeks after the second inoculation 18 of the mice and 20 untreated mice were inoculated with  $3 \times 10^4$  virulent flagellates.

Blood parasite levels are expressed as the number of parasites in 200 microscopic fields ( $\times 230$ ), except when no parasites were found, in which case 4,000 microscopic fields were examined.

The two mice from the actinomycin group that did not receive the virulent strain were used for xenodiagnosis two weeks after the second inoculation, to determine whether a mild infection (very difficult to detect by direct

blood examination) could be detected by this sensitive method. No parasites were found in either the faeces or the intestine of the *Triatoma* bugs, even two months after the blood meal.

Thus it appears that actinomycin D-treated flagellates, though devoid of infective capacity, retain a high degree of antigenic competence. The recent results of Johnson, Neal and Gall<sup>6</sup>, which demonstrate that adjuvants increase the protective effect of *T. cruzi* antigens, suggest a means of improving that of actinomycin D-treated flagellates.

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## VIROLOGY

### Genetic and Non-genetic Factors in Chromatographic Behaviour of Poliovirus

SOME variants of poliovirus ( $E^+$ ) do not adsorb on DEAE-cellulose provided neutral 0.01–0.02 M phosphate buffer is used as a diluent; under identical conditions some other variants ( $E^-$ ) are almost completely detained on the ion-exchanger and can be eluted only with solutions of increased ionic strength<sup>1,2</sup>. The  $E$  character of poliovirus may be considered as a genetic property which has no complete correlation with any other marker investigated so far<sup>3-5</sup>.

In this report we furnish evidence that in some cases the expression of  $E$  phenotype is critically dependent on the virus growth conditions, and particularly on the presence of cystine.

Methods of chromatography and of virus titration were described in detail earlier<sup>1,4</sup>. Briefly, they were as follows: virus, adsorbed on DEAE-cellulose column, was first eluted by 40 ml. of 0.02 M phosphate buffer, pH 7.1, and then by 40 ml. 0.85 per cent NaCl in the same buffer. The virus content of each fraction (10 ml.) was assayed by plaque method. As representatives of  $E^+$  and  $E^-$  variants of poliovirus, the lines M-I-2p and M-III-2p were used, respectively; these lines are plaque-purified derivatives of the Mahoney strain<sup>4</sup>. Viruses were grown in cultures of kidney tissue of *Macacus rhesus* in the medium containing 0.5 per cent lactalbumin hydrolysate in Earle's salt solution. Table 1 shows that the chromatographic behaviour of these variants does not change significantly either after one passage in the cystine-free Eagle's medium or after treatment with cystine *in vitro* by the method of Pohjanpelto (12 h at 37° C in 0.01 per cent cystine in borate buffer, pH 9) (ref. 7). It may be noted that this treatment results in a considerable increase of thermostability of both populations (M-I-2p and M-III-2p).

Quite different results were obtained, however, with another variant derived from M-III-2p by four consecutive passages in the cystine-free Eagle's medium. This procedure resulted in the formation of a population (M-III<sub>4</sub>) which differed from the original M-III-2p, being predominantly an  $E^+$  population. After one passage in the lactalbumin hydrolysate medium, the elution pattern

Table 1. CHROMATOGRAPHIC BEHAVIOUR OF POLIOVIRUS POPULATIONS UNDER DIFFERENT CONDITIONS

Variant	Virus yield (per cent of infectivity loaded on to column)			
	(a) 'Buffer fraction'	(b) 'Salt fraction'	(c) 'Buffer fraction'	(d) 'Salt fraction'
M-I-2p	76	2	82	77
M-III-2p	<1	99	92	<1
M-III <sub>4</sub>	78	8	65	<1

(a) Virus grown on cystine-free Eagle's medium; (b) virus grown on the lactalbumin hydrolysate medium; (c) virus grown on cystine-free Eagle's medium, and treated with cystine *in vitro*. 'Buffer fraction'—the fraction of virus infectivity which is eluted by 0.02 M phosphate buffer, pH 7.1. 'Salt fraction'—the fraction of virus infectivity which is eluted by 0.85 per cent NaCl in 0.02 M phosphate buffer, pH 7.1.

of M-III<sub>4</sub> was changed for E<sup>-</sup>; the modified population again regained its E<sup>+</sup> phenotype after one passage in the cystine-free Eagle's medium (Table 1). We supposed that the change of chromatographic behaviour was due to the presence of cystine in the lactalbumin hydrolysate medium. This assumption was confirmed by experiments with the treatment of M-III<sub>4</sub> with cystine *in vitro*. Such treatment also results in change-over of E<sup>+</sup> phenotype for E<sup>-</sup> (Table 1).

Thus, the chromatographic behaviour of M-III<sub>4</sub> (its E character) is not, strictly speaking, a genetic marker. But the capacity of this variant to react with cystine in a manner that leads to changes in elution properties of the population may be considered as a special genetic character. Such cystine-response variants may be designated E<sup>r</sup>.

We would like to point out that treatment of M-III<sub>4</sub> with some other sulphur-containing compounds (thioglycolate, mercaptoethanol) also results in analogous change of chromatographic behaviour (E<sup>+</sup> → E<sup>-</sup>).

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### Protective Effect of Neonatal Thymectomy on Cystic Disease Induced in Rats by Rous Sarcoma Virus

L. Zilber and I. Kryukova<sup>1</sup> have described a peculiar cystic-haemorrhagic disease caused by Rous sarcoma virus (RSV) in rats infected when newly born. G. Svet-Moldavsky and A. Skorikova<sup>2</sup> have obtained analogous results later on confirmed by other investigators. The morphological investigation revealed that this disease is connected with disease of lympho-reticular tissue of lymph nodes, spleen, lungs and endothelium of small lymphatic vessels<sup>3</sup>. Cysts are formed due to the whole complex of proliferative and destructive processes which change mainly the reticular (reticulo-endothelial) cells and block the lymph-stream.

Various strains of RSV differ in their capacity to cause cystic disease in rats. There is a direct relation of frequency, rate of development and severity of disease on the infecting dose of virus. The neutralization of virus with a specific anti-serum prevents the disease<sup>4</sup>.

Simultaneously the morphological data indicate that the dynamics of cyst formation is connected with cellular reactions and vascular diseases characteristic of a delayed-type sensitivity.

Taking into account the data concerning the important role of thymus in immune reactions of the organism<sup>5</sup>, we

have investigated the influence of neonatal thymectomy on the development of cystic lymphadenopathy induced in rats by RSV.

We used the Carr-Zilber strain of RSV which shows practically 100 per cent cystogenic activity. A 30 per cent extract of chicken sarcoma tissue in saline centrifuged for 20 min at 2,500g was used as the source of virus. The infectious titre of virus was 1:10,000 in 0.1 ml. Rats of the Wistar strain, less than 24 h old, were thymectomized using the technique of Jankovic *et al.*<sup>6</sup>. 1-2 h later the rats were infected subcutaneously (into the interscapular region) with 0.2 ml. of virus.

The inoculations, of 0.5 ml. in dose, were repeated on the second and fourth days of life.

Table 1. FREQUENCY AND RATE OF DEVELOPMENT OF CYSTIC DISEASE INDUCED BY RSV IN RATS THYMECTOMIZED AT BIRTH

Complete thymectomy	Age of rats in weeks when killed	No. of rats killed by this time
0/17*	2	3
	3	3
	4	1
	8	2
	9	3
	11	2
	15	2
	17	1
Partial thymectomy	Weeks after inoculation of RSV, cysts first seen	No. of rats with cysts by this time
(a) Small or microscopic remnants of thymus 9/16	5	1
	7	2
	8	3
	9	1
	13	2
(b) Large remnants of thymus 14/14	2	0
	3	5
Non-thymectomized rats/control 15/16	2	15

\* Numerator—No. of rats affected; denominator—No. of rats infected with RSV.

The results, summarized in Table 1, show:

(1) In non-thymectomized rats (control), the cystic diseases identical to those described before appeared 2 weeks after the inoculation. (2) In rats subjected to complete thymectomy, cysts appeared in none of the cases. (3) When the rats were partially thymectomized the results were quite different depending on the size of the remaining part of the thymus: when the remaining part was large we noticed a certain acceleration of the disease development along with impetuous regeneration of thymus. When the remnants of thymic tissue were small or microscopic, cysts appeared in 9 out of 16 rats and the process of their formation was retarded.

Thus, the results of experiments demonstrate that the neonatal thymectomy suppresses, or at least delays, the development of cystic disease caused by RSV in rats. Since neither the cellular elements of the thymus itself nor the small lymphocytes are the object of the direct pathogenic virus action<sup>7</sup>, we can assume that the immune mechanisms controlled by the thymus play a very important part in the genesis of the cystic syndrome. We propose as a hypothesis the following scheme of cystic disease pathogenesis:

(1) Infection of new-born rats with chicken sarcoma extract causes the alteration of antigenic capacities of lympho-reticular tissue cells affected by RSV (together with enhancement of cell proliferation and possibly also with direct necrotizing virus action).

(2) The further development of the disease is connected mainly with the auto-immune response directed against the cellular elements 'heterogenized' by the virus. This reaction is analogous to the homograft immune response and presumably involves a delayed hypersensitivity mechanism. The serological and morphological data suggest that humoral factors of immunity do not play an essential part in the reaction of rats infected with RSV.

Thus the cystic disease is considered to be due to a virus—autoimmune disease of a rather peculiar type. The whole complex of proliferative and destructive changes due to which the cysts appear can be estimated as a result of

direct pathogenic action of the RSV on the infected cells and immunomorphological phenomena. Neonatal thymectomy brings about a considerable suppression of delayed hypersensitivity reaction<sup>6</sup> and thus excludes one of the links (probably the main one) of the cyst formation process. Apparently an analogous phenomenon occurs in the course of lymphocytic choriomeningitis in mice and neonatal thymectomy has a protective effect<sup>7,8</sup>.

Recently we have obtained similar results concerning the suppressive effect of neonatal thymectomy on the cystic disease in mice (described by us earlier<sup>9</sup>) injected with the mixture of sheep lung adenomatosis tissue extract and polyoma virus; simultaneously under these conditions we have noticed the aggravation of the oncogenic action of polyoma virus.

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## GENETICS

### Polymorphism in the Red Protein Isolated from Milk of Individual Cows

GENETICALLY determined polymorphism of human and other primate transferrins, the iron-binding proteins in blood, has been established by starch-gel electrophoresis<sup>1,2</sup>. The transferrin in cattle differs from that in humans since cattle homozygous for an allele have been shown to contain more than one transferrin<sup>3,4</sup>. The red protein, also called lactotransferrin, is an iron-binding protein found in cow's milk and has now been found to be polymorphous by gel electrophoresis. Although serum transferrin resembles the red protein in size, absorption spectra, and amount of iron bound per mole of protein, it differs in electrophoretic mobility and amino-acid content<sup>5,6</sup>.

Red protein isolated from the milk of individual cows in the experimental herds at Beltsville, Maryland, was examined by zone electrophoresis. Since the red protein is a minor protein in milk, it must be isolated before electrophoretic typing can be carried out. Also, it should be reasonably pure, since a number of other minor proteins with similar mobilities are found associated with the red protein fraction.

Zone electrophoresis by the Raymond method<sup>7</sup> at pH 9.0 has recently been applied to milk proteins<sup>8</sup> and was found to give good resolution of the red protein when used at 5 per cent gel concentration. For most comparisons shown here the red protein was isolated from the casein fraction<sup>9</sup> or the whey fraction of milk<sup>9</sup>. Good agreement in electrophoretic patterns was found for proteins prepared by either method.

Variation in gel-electrophoretic patterns at alkaline pH of the isolated red protein is shown in Fig. 1, numbers 1 through to 5. The photographic reproduction does not show some of the minor bands as clearly as they could be seen in the original gel. The bands are marked according to their mobility—A for the fastest and D for the slowest. Band D generally stains heavily, with each succeeding zone becoming lighter; however, with number 3 the D band is faint. Number 5 is typed (C), D, the parenthesis indicating little protein in the C zone. Numbers 1, 2, and 4 are typed B, C, D, while 3 is A, B, C, (D). The protein in 1 and 2 was isolated from the whey and casein fractions of milk from a single cow. A tabulation of the types according to breed is shown in Table 1.

Table 1. A COMPARISON OF THE ELECTROPHORETIC TYPE OF RED PROTEIN WITH BREED

Breed	Total	Electrophoretic zone*			
Ayrshire	2†, ‡		(C)	D	
Ayrshire	1†	B	C	D	
Brown Swiss	2†		(C)	D	
Brown Swiss	2(2†, 1†)	B	C	D	
Guernsey	3(1†, 2†)	B	C	D	
Holstein	1†, ‡	(A)	B	C	D
Jersey	1†	(A)	B	C	D
Jersey	1†			C	D
Jersey-Brahman	1‡	B	C	D	
Holstein-(mixed breed)	1‡, †	A	B	C	(D)
	16				

\* Letters in parenthesis indicate a very small amount of protein.

† Red protein prepared from casein fraction.

‡ Red protein prepared from whey fraction.

§ Red protein prepared from  $\beta$ -lactoglobulin fraction.

|| Red protein prepared from colostrum milk of this cow.

As some of the bands might result from the binding of varying amounts of iron, electrophoretic determinations were made on protein solutions to which excess iron ions

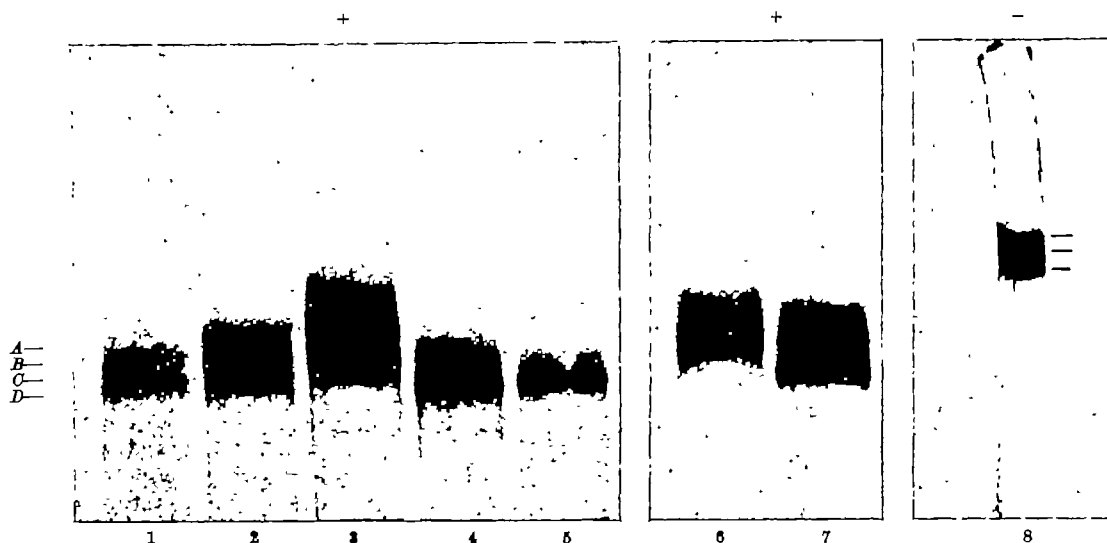


Fig. 1. Gel-electrophoretic patterns of the red protein. Vertical gel, 9.0: 1-5, protein from individual cows; 6, apoprotein; 7, control. Disk gel, pH 4.3. 8, protein from individual cow.

were added. The resulting electrophoretic patterns were unchanged. In another experiment the iron was dissociated from the red protein by adjusting a protein solution to pH 2.0 and removing the iron with a 'Dowex 50' resin in the chloride form. An electrophoretic comparison (Fig. 1, numbers 6 and 7) shows that the zones for the apoprotein, although less sharp, have a greater mobility and are out of phase with those of the control. The consistency of the mobilities of the various red protein types and the fact that the addition of iron does not change the electrophoretic patterns suggest that a small variation in iron will not significantly alter the patterns.

Polymorphism in the red protein has also been found at acid pH, as shown in Fig. 1, number 8. Disc electrophoretic determinations were made in 7.5 per cent gel concentration, pH 4.3, according to Reisfeld *et al.*<sup>10</sup>. A current of 60 m.amp (12 tubes) was applied long enough for the protein to move through about 70 per cent of the lower gel. In contrast to the variation found at alkaline pH, all the proteins examined show a major fraction of 3 closely-related bands.

Electrophoretic differences at alkaline pH in the red protein isolated from milk of individual cows suggest the existence of a genetically controlled polymorphism. Final proof will require a study of larger numbers of individual animals than is practical with the present isolation procedures.

Preliminary peptide maps indicate differences in a few peptides, and this will be the subject of future work.

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### Method for demonstrating the Chromosomes in Adult Human Spleen Cells

To obtain chromosome preparations from *in vitro* cultures of human peripheral blood is now a routine laboratory procedure. Baker and Atkins<sup>1</sup> and Bain and Gauld<sup>2</sup> have extended this technique to human foetal and infant spleen and adult lymph nodes. The present communication describes the further extension of this method, to demonstrate the chromosomes of adult human spleen cells following culture *in vitro*.

In all, five spleens and one spleniculus have been studied as shown in Table 1. Each was freshly obtained from a patient undergoing splenectomy for therapeutic reasons.

Approximately 0.5 g of splenic tissue was taken aseptically and placed in a Petri dish, moistened with 'Medium 199' (Glaxo Laboratories, Ltd., Greenford, Middlesex). The capsule was removed, and the remaining tissue was cut into small fragments with scissors, and then further reduced to a cell suspension in 'Medium 199', using a glass piston blender, after the method of Woodruff<sup>3</sup>. This suspension was washed by centrifuging at 580g for 5 min, and resuspended to a total volume of 10 ml. in fresh 'Medium 199'.

The washed cell suspension was transferred to a siliconized, heparinized 'Universal' container and 0.4 ml. of phytohaemagglutinin (Burroughs, Wellcome and Co., London) was added, the whole being placed in a refrigerator at 4° C for 80 min. At the end of this period the erythrocytes present were seen to have sedimented, and the process was further accentuated by centrifugation at 180g for 3 min. The supernatant was then removed and a nucleated cell count was performed on it. From this cell suspension aliquots of 6-8 ml. were prepared for incubation in further 'Universal' containers as follows.

The cell suspension was diluted with further 'Medium 199', and human group AB Rh +ve serum was added to a concentration of 20-25 per cent, so that the final cell concentration, after addition of the serum, was between 1 and 1.5 million cells per ml.

The culture was incubated at 37° C for 4 days. Any alkalinity in the culture was corrected with a jet of 5 per cent CO<sub>2</sub> in 95 per cent O<sub>2</sub>, initially, and thereafter at 12-hourly intervals.

On the fourth day, 0.1 ml. of a 0.02 per cent (w/v) solution of 'Colcemid Pure Substance' (Ciba Laboratories, Ltd., Horsham, Sussex) was added to each ml. of the culture and incubation was continued for a further 3 h.

The further processing of the material closely followed the method used for cultures of peripheral blood and will therefore be only briefly summarized.

The cells collected from the culture were treated with hypotonic (0.95 w/v per cent) sodium citrate at 37° C and then fixed in a mixture of 3 parts ethyl alcohol to 1 part of glacial acetic acid.

Immediately before making the slides the fixative was changed to one of 3 parts methyl alcohol to 1 part glacial acetic acid, the cells being suspended in one drop of this solution, so that it possessed a 'milky' opalescence.

Slides were cleaned with absolute alcohol, dried, and then cooled by placing on a block of carbon dioxide snow after the method of Fox and Zeiss<sup>4</sup>. The cells were dropped on to the cooled slide, using a Pasteur pipette, and the slide was then rapidly heated in the flame of a spirit lamp. Finally, the slides were stained with 1 per cent aceto-orcein and mounted in 'D.P.X.'.

Table 1. CLINICAL DETAILS OF THE FIVE PATIENTS STUDIED

Patient	Age and sex	Spleen size	Disease and operation
1	Male, 54	Normal	Carcinoma of head of pancreas Splenectomy and removal of tail of pancreas with intestinal anastomosis
2	Male, 20	Normal	Rupture of spleen Splenectomy
3	Female, 50	Increased	Carcinoma of stomach Oesophago-gastroectomy
4	Female, 2	Increased	Spleroerythosis Splenectomy
5	Male, 54	Increased	Carcinoma of stomach Oesophago-gastroectomy

The method described was found to yield a good collection of well-spread mitotic figures in replicate cultures of the spleens from Cases 3, 4 and 5 where the spleen showed evidence of hyperplasia. It was observed, however, that cell division was most rapid in those cultures which showed a frequent change to an alkaline pH.

Only scanty mitotic figures were obtained from the cultures of spleens from patients 1 and 2 and from the spleniculus in Case 5. In these cases there was no splenic hyperplasia.

A similar method applied to the normal rat and mouse spleen, using rat serum, previously heated to 56° C for 1 h, to enrich the culture medium, was found unsuitable for the demonstration of chromosomes (Symes, unpublished observation).

These findings, together with those cited here<sup>1,2</sup> wherein chromosomes were only demonstrated in the foetal and infant spleen, suggest that prior splenic hyperplasia may be important for success. Nevertheless, the method would seem to be of value as a means of assessing the viability of spleen cell suspensions used as haemopoietic

replacement following chemotherapy<sup>1</sup>, and *per se* in the treatment of malignant disease<sup>2</sup>.

We thank Dr. G. H. Tovey for generous supplies of human serum, and Mr. J. Wegrzyn for his assistance.

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## AGRICULTURE

### Significance of Differences between Variety Yields under Experimental and Farm Conditions

PREVIOUS work has shown that if farm yields are plotted against experimental yields they lie along a curve which has a slope of 45 degrees at low values and is almost horizontal at high values<sup>1</sup>. This occurs because, in years favourable to the crop, farm yields do not increase by the same proportion as experimental yields.

This result suggests that it may be erroneous to assume that significant differences between yields of plants obtained under experimental conditions will also be significant on farms. This becomes obvious when the differences between two points on the curve are measured on the abscissae and the ordinate of Fig. 1. In unfavourable years the difference between the yield of two crop varieties in an experiment is  $A_1B_1$ . On farms it would be the smaller but still substantial differences  $A_1B_1$ . Although a much larger difference in the yield of the two varieties  $A_1B_1$  may be obtained in favourable years in an experiment, the difference in yield on farms  $A_1B_1$  will actually be smaller than in unfavourable years. If farm yields fail to reflect the large differences between the yields obtained under experimental conditions in favourable years, it is possible that significant differences due to varieties, fertilizers, insecticides and other cultural practices obtained on an experimental scale would not be significant on a farm scale.

It is possible to examine the differences in the yields of varieties of sugar cane in experimental plots and on farms. In Queensland (Australia) the Bureau of Sugar Experimental Stations has carried out variety trials of commercial strains of sugar cane in all major sugar areas using the latin square or randomized block techniques over a period of years. Differences in the yields of varieties are not published unless they are significant at the 5 per cent level. The cultural and manual practices used in the experiment are similar to those used on farms in the mill

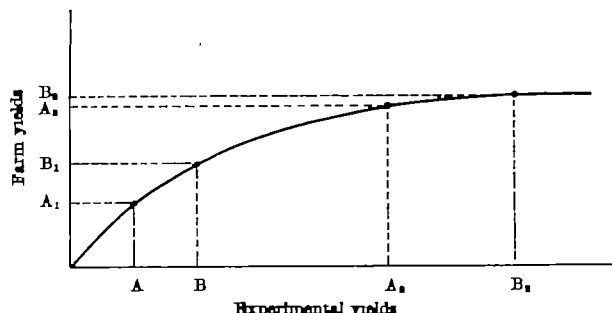


Fig. 1. Differences between yields on farms and in experiments in favourable and unfavourable years

Table 1. COMPARISON OF HIGH- AND LOW-YIELDING VARIETIES OF SUGAR CANE IN EXPERIMENTAL TRIALS AND ON FARMS

Variety pair	Yields (tons cane per acre)				Total area of farm crops in mill area (acres)	
	Experimental plots High-yielding variety	Experimental plots Low-yielding variety	District average High-yielding variety	District average Low-yielding variety	High-yielding variety	Low-yielding variety
A	22.26	20.97	17.6	15.7	6698	1011
B	23.34	25.12	23.2	23.5	5325	2296
C	16.65	13.99	16.9	17.3	2065	5886
D	40.25	22.28	19.8	16.6	2873	1807
E	40.26	26.28	19.8	24.4	2873	808
F	43.28	33.66	20.0	31.8	1873	6966
G	43.28	23.45	20.0	26.2	1873	309
H	46.85	39.90	20.0	29.6	1856	8729
I	35.22	27.81	23.4	23.3	4648	1201
J	33.65	22.43	31.8	26.2	6966	309
K	33.28	26.28	16.6	24.4	1607	808
L	41.87	39.06	20.6	32.5	1400	4122
Mean	36.11	28.35	23.8	24.3	3328	2538
Mean difference	+7.8		-0.5			

area in which the trial is conducted; the concurrent yields of individual varieties of sugar cane produced on farms are collected for each mill area by the Bureau of Sugar Experimental Stations (N. J. King, personal communication).

Using this information, a comparison can be made between any two varieties of different yield in a particular trial in a particular year. Similarly the average yields of the same high-yielding variety can be compared with the average yield of the same lower-yielding variety in the mill area in which the trial is located. Unfortunately mill areas are restricted to a few varieties and, if areas where trials and farm crops are partially or fully irrigated are excluded, only twelve pairs of varieties can be compared with farm data. This comparison is made in Table 1.

In the experiments the mean yield of the high-yielding varieties was 7.8 tons (27 per cent) higher than that of the lower-yielding varieties and this difference was significant at the 1 per cent level. On farms there was no significant difference between the yield of the high- and low-yielding varieties. The lack of difference on farms between high- and low-yielding varieties cannot be explained by the total acreages of each variety grown on farms. Substantial acreages of all the varieties studied were produced, and there was no correlation between acreage of crop grown in the mill area and difference between the yield of high- and low-yielding varieties on the farms.

This investigation was restricted to a comparison between experimental yields and average farm yields of high- and lower-yielding varieties of sugar cane, but it does suggest that, although a variety may give significantly higher yields than other varieties under experimental conditions, this difference may not exist on farms even when climate, soil and cultural practices are similar to those in the experiment. It is possible that this failure of farm yields to reflect experimental trends may also be true of comparisons between different levels of fertilizer or different cultural practices. Further comparisons between differences in yields in experiments and on farms would be necessary to establish the magnitude and significance of differences which exist on farms for a given difference established in an experimental trial at a particular level of significance. For the data presented here a difference in the average yield of 27 per cent, which is significant at the 1 per cent level in experiments, is not reflected in average farm yields. It is possible that the reduction in the difference in yield, which occurs when a practice is translated from experiment to farm, changes with the variable or the type of crop being examined, and that the actual degree of reduction in yield for particular variables and different crops must be found before the results of experiments can be applied to farming.

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## SOIL SCIENCE

## Role of Exchangeable Potassium and Magnesium on Caesium Absorption on Marine Sediments

The potassium ion fixed into the interlayer spacing of a clay mineral is not easily replaced, and the results of chemical analysis cannot distinguish these interlayer potassium and the structural potassium. Therefore, the total potassium obtained in the chemical analysis includes the gross structural potassium and the edge-site exchangeable potassium. One may assume, therefore, for the comparison of the adsorbability of various clay minerals, that the smaller the ratio of structural potassium, or alternatively the larger the ratio of exchangeable potassium to the total potassium, the greater will be the adsorption of metal ions, especially of rubidium and caesium, which are adsorbed in the dehydrated states having rather small cationic radii and can easily penetrate into the interlayer opening.

This assumption was exemplified in our investigation of the adsorption of caesium on Pacific pelagic and terrigenous sediments, containing illite, chlorite, and montmorillonite, the details of which will be published elsewhere. Samples were fractionated into three portions according to the size of particles. The plot of the distribution coefficients versus the ratio of exchangeable potassium to the total potassium ( $R_K$ ) resulted in straight lines intercepting at the origin; the slopes of them differ from one sample to another. Such relation was also found for the ratio of exchangeable magnesium to the total magnesium ( $R_{Mg}$ ), but not for the respective ratios of sodium and calcium.

For the purpose of assigning to which clay mineral is worked either one of the ratios,  $R_K$  and  $R_{Mg}$ , cristobalite, kaolinitic montmorillonite, illitic chlorite, and two illitic kaolinites were fractionated into two or three portions according to the particle sizes ranging from 1 to 44 $\mu$ . The distribution coefficients of caesium-134 were determined in triplicate in water and in N sodium chloride solution, both at pH 8.0. It was found that linear relation held only for kaolinitic montmorillonite when  $K_d$  versus  $R_K$  plots were made (Fig. 1), while  $K_d$  versus  $R_{Mg}$  plots resulted in straight lines, intercepting at the origin, only for illitic chlorite (Fig. 2). From the clay mineralogical data of the samples used, the role of  $R_K$  can be attributed to montmorillonite, whereas that of  $R_{Mg}$  can be attributed to

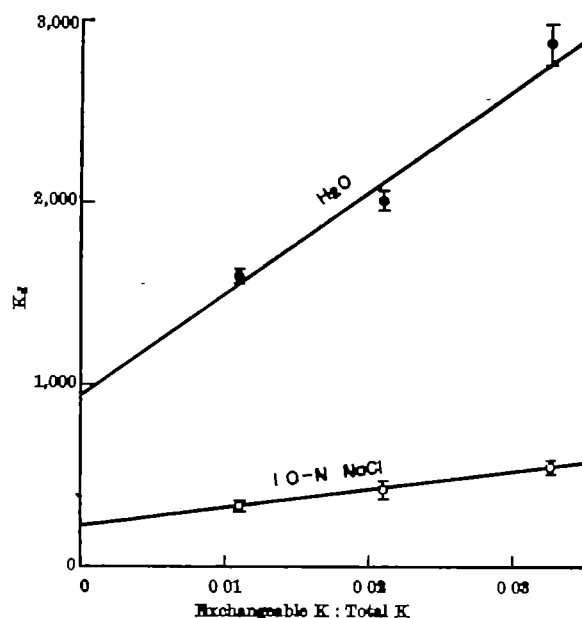


Fig. 1. Linear relation between  $K_d$  and  $R_K$  for the adsorption of caesium on kaolinitic montmorillonite

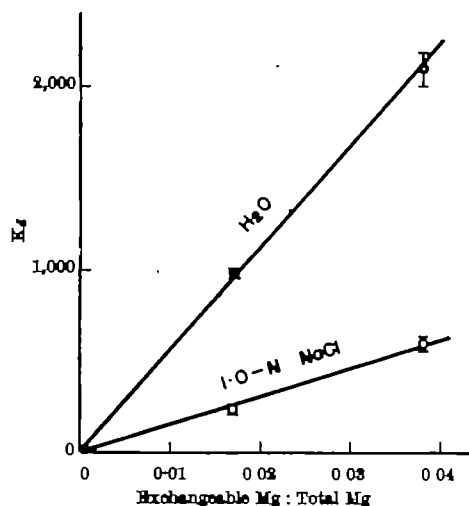


Fig. 2. Linear relation between  $K_d$  and  $R_{Mg}$  for the adsorption of caesium on illitic chlorite

chlorite. These results are consistent with previous reports<sup>1</sup> showing that potassium fixation occurs in montmorillonite and magnesium fixation occurs in degraded chlorite.

In the case of marine sediments, the straight lines obtained in the plot of  $K_d$  versus  $R_K$  and  $K_d$  versus  $R_{Mg}$  exclusively intercept at the origin, while in the present cases  $K_d$  versus  $R_K$  plots do not pass the origin. This situation can be understood because the marine sediments are completely saturated by the metal ions composing sea-water, so that the unbalanced charges in the structural units due to the lattice substitution are completely balanced by these ions. On the other hand, in the present montmorillonite, charges given by the lattice substitutions are available for the adsorption of caesium and the definite magnitude of adsorption even at  $R_K = 0$  will result. In the presence of N sodium chloride solution, the plot still does not intercept at the origin, though the distribution coefficients markedly decreased as compared with those in water, presumably due to the incomplete balancing of the charges emerging from the lattice substitution by sodium ions of this concentration and also due to the competition of both cations.

The major cause of cation exchange property of chlorite arises in the broken bonds on non-cleavage surfaces. That the plot of  $K_d$  versus  $R_{Mg}$  passes the origin without observable adsorption on the broken bonds at  $R_{Mg} = 0$  may be the result of complete saturation of these bonds in the case of marine sediments.

For the other metal ions tested, namely, strontium, cobalt, and europium, no such relations could be found, as was in the case of the marine sediments. Therefore, exchangeable potassium and exchangeable magnesium, respectively, in montmorillonite and in chlorite, play an important part in the adsorption of caesium. Furthermore, two other montmorillonite samples found from different localities, for which particle fractionation was impossible, were missing on the straight lines obtained in Fig. 1. This fact suggests the importance of the clay mineral structure, especially the number of broken bonds, and the chemical composition.

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## PSYCHOLOGY

## Vocalization-at-presentation, Auditory Presentation and Immediate Recall

PREVIOUS work<sup>1</sup> has shown that if subjects performed increasing degrees of vocalization on a visually presented list of 8 consonants, recall of that list improved monotonically as vocalization level increased. Thus voiced lists were better recalled than whispered lists, whispered lists than mouthed lists and mouthed lists than silently perceived lists. The relative efficiency of voicing as regards its effect on recall was greatest for lists presented at fast rates (4 letters/sec); written recall was also found to be superior to spoken recall, although there appeared to be no significant interaction between vocalization level at presentation and the mode of recall (spoken against written).

At first sight these results might seem to indicate that the 'more' one responds to a list at presentation, the better will be recall. However, there is an alternative hypothesis. It might be that auditory presentation *per se* is particularly good for immediate recall purposes, and that the advantage of vocalization may be put down to the addition of an auditory message to the visual message given at presentation. One way to test this would be to reduce the subject's ability to hear himself, by concurrently presenting white noise. Such an experiment will shortly be reported; but we also need some more objective evidence concerning the efficiency of auditory presentation on immediate recall. In the work to be described, we shall compare the recall of lists merely seen with that of lists merely heard, and in addition compare the recall of voiced lists (self-produced auditory stimulation) with that of lists seen and simultaneously heard as spoken by the experimenter (externally produced auditory stimulation). If it were true that the degree of overt activity were the main determinant of vocalization's advantages for recall, we should expect self-produced auditory stimulation to give superior recall to that for externally produced auditory stimulation. On the other hand, if simply obtaining an auditory message were the main determinant of recall, there should be little difference between the two conditions, and in addition lists merely heard should be better recalled than lists merely seen. It should be noted that examination of the available literature<sup>2,3</sup> does not give a clear-cut answer to the question of whether visual or auditory presentation is superior for immediate recall: their relative efficiencies appear to vary, for example with age or intelligence<sup>4</sup>, although more recent work by Buschke<sup>5</sup> has suggested that auditory presentation is superior to visual presentation for immediate memory as measured by a recognition method. The results of Henmon<sup>6</sup> should be particularly noted, however: he found that the recall of 10-item lists of digits or nonsense-syllables was best if they were merely heard, and that there was little difference between self-produced and externally produced auditory presentation as regarded immediate recall, but only 6 subjects were used.

The technique used in the present experiment has been described previously<sup>1</sup>. Subjects saw lists of letters presented one at a time through the viewing-slit of a screen covering a continuously moving roll of paper on which the letters were printed. In each list there were eight consonants (all different, selected according to a random procedure), and the presentation-rate was set at 1.33 letters/sec. for all lists. A typical list was LVMKTNOS.

Two presentation-conditions used previously were used again. As the letters were presented, subjects either: (i) merely looked at each letter as it appeared (condition *R*—read) or (ii) looked at, and also voiced aloud, each letter (condition *V*—voice). In addition, three new conditions were used: (iii) *S* covered up the viewing-slit and merely heard the letters read out to him by *E* (condition *A*—auditory); (iv) *S* both saw the letters and simul-

taneously heard them read out by *E* (condition *RA*); (v) *S* first saw each letter and, about 0.4 sec later, heard it read out by *E* (condition *RA*<sup>+</sup>). The purpose of *RA*<sup>+</sup> was to make the situation of seeing and hearing more analogous to that of voicing; when a subject voices a letter, he hears his voice a vocal reaction-time (approximately 0.4 sec)<sup>6</sup> behind his initial perception of the letter.

Concerning these various presentation-conditions: *R* and *V* have been described. Condition *A* was achieved by simply covering up the viewing-slit, while *E*, concealed behind a screen, read out the letters as he himself saw them through a set of mirrors arranged about *S*'s viewing-slit. For condition *RA*, *E* also read out the letters, though this time they were in addition visible to *S*. For condition *RA*<sup>+</sup>, the delay of *E*'s voice was arranged by preliminary trial-and-error to coincide as exactly as possible with *S*'s own vocal reaction-time. The interval was found by moving a bar across one of the mirrors to a position such that if *E* read out the letters as they crossed the bar, his voice coincided with *S*'s own voice as *S* read out the letters. (In the actual test, of course, *S* did not voice on condition *RA*<sup>+</sup>.) With a presentation rate of 1.33 letters/sec, *E*'s voice never coincided with *S*'s perception of the next letters in the list.

Recall was given immediately after having perceived the eight letters, *E* having switched off the machine. The recall was either spoken into a tape-recorder, or written on prepared answer-slips; *S* could recall in any order, provided he specified the original positions of all letters recalled. If a letter could not be recalled, *S* could omit or guess as he preferred.

Twenty male adult subjects (mainly students) were used, all of whom had taken part a year previously in an experiment in which conditions *R* and *V* had been investigated. Thus they were highly practised at the general task, but were not familiar with conditions *A*, *RA* or *RA*<sup>+</sup>.

Table 1 shows the percentage correct of the total possible number of letters on each of the conditions. Each letter was marked as correct only if it appeared in its correct position in the presented list.

Table 1

	<i>R</i>	<i>RA</i>	<i>RA</i> <sup>+</sup>	<i>V</i>	<i>A</i>	Mean
Spoken recall	57.27	64.37	66.90	65.63	68.44	64.50
Written recall	69.45	78.06	77.81	77.27	79.09	76.45
Combined recall	63.36	71.21	72.45	71.45	74.06	70.45

Analysis of variance applied to the raw data showed that the vocalization, recall-method, and 'subject' factors all gave variance-ratios significant at  $P < 0.001$  ( $F=8.08$ , 132.0 and 12.76 respectively). In addition, the interaction between vocalization and subjects was significant at  $P < 0.05$  ( $F=1.56$ ); the interactions, vocalization with recall-method and recall-method with subject, were not significant. Tests by the method of orthogonal comparison<sup>7</sup> showed that the difference between the 'combined' totals of *R* and *RA* was significant at the 0.01 level ( $F=22.78$ ) and that the difference between *RA*<sup>+</sup> and *A* was not significant ( $F=1.14$ ). It can be seen that there were no significant differences between *RA*, *V*, and *RA*<sup>+</sup>; and that the difference between *A* and *R* was significant at a level of at least  $P < 0.01$ .

It therefore appears that: (i) it made little difference for recall-purposes whether the auditory stimulus was self-produced (*V*) or externally produced (*RA*, *RA*<sup>+</sup>); (ii) merely hearing the list (*A*) gave recall insignificantly superior to both hearing-and-seeing and voicing, and significantly superior to merely seeing (*R*); and (iii) in the *RA* conditions, it made little difference whether the heard letter coincided with, or was slightly behind, the seen letter. These results are very similar to those of Henmon, and they hold both for spoken and written recall, although the latter gave significantly superior recall overall.

The implication of the findings, we suggest, is that although adding cues to the visual message was of assistance

tance, optimum performance was obtained when cues were presented entirely in the auditory modality. The advantage of vocalizing may therefore be hypothesized to rest partly on the fact that more cues were in general available to *S*, but more particularly on the fact that *S* was presenting himself with a cue in a preferred modality, that is, auditory. Introspective evidence obtained from the *S*'s supported this, and also suggested that *A* gave (insignificantly) superior recall to *RA*, *RA*<sup>+</sup> and *V* because *A* provided less 'distraction' during the task of perceiving and storing the presented list. The fact that *S*'s were already highly practised at *R* and *V* lends greater force to the present finding that pure auditory presentation gave recall at least as good as voicing-at-presentation. Why auditory immediate memory should in fact be so efficient—at least under the conditions described here—remains to be answered. It should be noted that the significant interaction of subjects with vocalization suggests that there might be an important element of individual variability as regards preference for one presentation-method or another.

This work was done while I held a studentship at Trinity College, Cambridge. I thank A. J. Watson and Prof. O. L. Zangwill of the Cambridge Psychology Laboratory for their advice and encouragement, and Dr. A. G. Worthington of the Psychology Department, Queen's University, for statistical advice.

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## Effects of a Drug and Immediate Memory

A NEW test of immediate memory was recently suggested by Buschke<sup>1</sup>. *N* - 1 items drawn from an ensemble of *N* are presented, and the task is to name the missing item. An experiment is described in which this technique, the 'missing scan method', was used to evaluate the effects of sub-anaesthetic doses of nitrous oxide on immediate memory. The results seem to cast light on underlying processes. It is suggested that two forms of storage may be distinguished—that used depending on the presentation-rate of items—and that one form is especially susceptible to drug effects.

Random auditory sequences, each consisting of seven letters drawn from the ensemble A-H, were presented at two rates: two items per sec (fast), and one item every 2 sec (slow), to subjects fully equilibrated under low doses of nitrous oxide. Practice sequences were given during induction of the gas. Sixteen student and technician volunteers (aged 18-33), used as their own controls, received 15, 25 or 35 per cent nitrous oxide and air (control) on successive days according to a 4 × 4 factorial design. Dose-effect curves for errors and latencies of correct responses (geometric means) are shown in Figs. 1a and b respectively.

There was no evidence of impairment of performance accuracy in the 'fast' condition up to and including the 25 per cent dose, but in the 'slow' condition as little as 15 and 25 per cent nitrous oxide increased errors significantly (2-tailed Wilcoxon tests, *P* < 0.01; *P* < 0.05 resp.). Thus,

since disruption of stimulus registration by the drug should result in a greater drug effect at faster rates, there was no evidence of a drug impairment of this stage. A drug effect on retention is therefore suggested. There were no significant differences between the input conditions under any dose of nitrous oxide. But under air there were fewer errors at the slower rate (2-tailed Wilcoxon, *P* < 0.01). The results are therefore not consistent with a simple trace decay theory, since under air control subjects did better when they had to wait longer before responding. The drug appears to have disrupted some process in the 'slow' condition. The results suggest some sort of interference with memory traces and resemble Fraser's<sup>2</sup> findings on the effects of ageing.

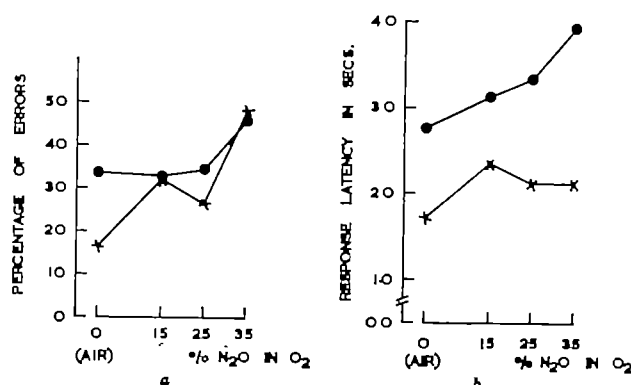


Fig. 1. a, Dose-effect curves for errors; b, dose-effect curves for response latencies. Rates of presentation. ●, fast; ×, slow

As Figs. 1a and b suggest, accuracy of performance in the 'slow' condition was prone to drug impairment, response latency was not. In contrast, accuracy in the 'fast' response was resistant to impairment (except at the highest dose), but latency increased progressively with dose (Jonckheere trend test; *P* = 0.012). The longer response latencies for the 'fast' rate should be noted: the difference between the conditions was very highly significant (analysis of variance on mean log latencies; *P* < 0.001).

Introspective reports seem relevant in accounting for the latency difference between the 'fast' and 'slow' rates of presentation, and in explaining why the two input conditions were differently affected by nitrous oxide on the two performance measures. Subjects reported that in the 'slow' condition they formed a spatial image of the ensemble and had time to cancel each item as it arrived, so that at the end of presentation they had only to 'read out' the missing item. Many reported that the drug tended to interfere with this image. In the 'fast' condition, subjects reported that the items arrived too fast to cancel as they occurred, and were first stored as sound, being transferred to the spatial image at the end of presentation.

Assuming the introspective evidence of two separate stores is valid, the latency data imply that the time for transfer of the items from the first to the second store was normally about a second, and that it was progressively increased by nitrous oxide. The error data suggest that the second store may normally be the more efficient, but is especially vulnerable to drug effects.

Further work is in progress to evaluate this two-store hypothesis of immediate memory. Drugs promise to be a useful tool for this purpose.

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<sup>1</sup> Buschke, H., *Nature*, **200**, 1139 (1963).

<sup>2</sup> Fraser, D. C., *Nature*, **180**, 1163 (1958).

## SCIENCE, THE CREATIVE ART

IN scope and in calibre Sir Cyril Hinshelwood's presidential address to the British Association for the Advancement of Science (see p. 1055 of this issue) resembles his earlier presidential addresses, to the Chemical Society at its centenary meeting, and at the tercentenary of the Royal Society. Indeed, to read it in the context of those addresses quickens one's appreciation of Sir Cyril's understanding of the wider audience which he is addressing at Cambridge, the skill and imagination of his exposition, and the challenge of his message: "Science, whatever else it may be, is a form of creative art". Sir Cyril seeks to commend this view of science to society at large by brief glances at some outstanding scientists of the past generation and finally by looking at the way in which scientists to-day are facing the oldest and most absorbing questions of all: the nature of the cosmos, the nature of matter, the nature of life. Passages in his address recall an address on "Science for its Own Sake", which his predecessor, the late Sir Edward Appleton, delivered twelve years ago (*Nature*, 172, 424; 1953). However, Sir Cyril's approach and treatment are all his own, and he does not pursue the theme of science as an artistic and human value further than to illustrate the nature of scientific activity.

He begins at the level of ordinary life. Science, he reminds us, is no longer a private affair but a public concern, and he touches surely on some of the practical implications of the new situation, particularly of the wider general interest in applied science than in pure science. The relation between the two is exceedingly subtle and complex and Sir Cyril attempts no definition of either pure or applied science. He is content to remind us of their interdependence and of the folly of insisting that all scientific effort should be devoted to demonstrably practical ends. We cannot be reminded too often that Nature in her own time reveals her secrets to the patient questioner, and is infinitely cleverer than man. Science cannot be dismissed as a polite amusement, and it would indeed be a tragedy if people with a real vocation for science were seriously diverted in the supposed interest of utility.

What does disturb Sir Cyril is that few of the large numbers of recruits to science seem to evince the interest in technology for which the future calls. For this situation Sir Cyril suggests two factors have been important. First, failure to give science its proper status, either in the Civil Service or elsewhere, has tended to deter all except the minority with a real vocation. Secondly, while the achievements of this dedicated minority have to some extent rectified this position, society has largely withheld its due esteem from practical skills. Furthermore, with this failure to understand the real nature of either science or technology, our educational system has tended to direct into other more remunerative fields precisely those young people whose minds are disposed to ask not so much "Why does this happen?" as "How could this be done?" or "What use could be made of this?"

At this point there is a passage which is liable to be misunderstood. Sir Cyril condemns the unjustifiable primacy which, in our traditional methods of education, selection and promotion, we have accorded to skill in purely verbal expression. It is quite wrong to suppose that all thought is through the use of words: non-verbal

images can be just as important. Some people think with their hands, and Sir Cyril reminds us that we need to give much greater credit to manual skill than we do—except, perhaps, in athletics.

Now this timely reminder of the immense importance of manual skill as a form of expression, especially for the craftsman and the myriad kinds of technician required to support the scientist or technologist, is not to be interpreted as repudiating the need for accuracy of expression. Sir Cyril is too fine a scholar to give any countenance to the disturbing prevalence of illiteracy or slovenliness of expression to be found to-day in universities as well as in schools. He is too skilled an expositor himself to deny to the gifted expositor or interpreter the honour that is his meed, and later in his address he quotes the *obiter dicta* attributed to Rutherford that no physical theory is worth much if it cannot be explained to a barmaid.

What Sir Cyril is concerned with here is not the problem of communication at all but our failure to accord to those who work with their hands the status and prestige that are their due—our tendency to exalt the academic over the practical, to place applied science second to pure science, instead of on an equality. The adventure of controlling Nature, he reminds us, is no less great than that of understanding its deep secrets. The whole climate of opinion in society must change if we are to find the recruits for technology that we need: not all of them will come from science itself; many may well be found in quarters where society has hitherto discouraged the development of any interest in science at all.

Here Sir Cyril's pragmatism is addressed to us all and in it we discern the real motif of his address—an interpretation to the ordinary man or woman of the way in which the scientist works and thinks. He passes then to the organization of research, and here his pragmatism is aimed at the administrator and politician; his words are not always comfortable. In the grand perspective of world history, the interplay of discovery and application has been largely an affair of chance. Those who now wish to guide and plan the process, to rationalize and accelerate it, have to remember that inspiration comes only to rare individuals, floating up intuitively from the subconscious. It is communicable from a leader to a team only if the members have confidence in him, both as a man and as a scientist. There is little place in scientific research for philosopher kings, prescribing broad human objections to teams of docile scientists.

Almost any debate on science or its applications in the House of Commons will indicate how much our legislators need Sir Cyril's warning, how disposed they are to believe that the planning and direction of research is a logical process, proceeding by obvious stages from point to point to a predetermined goal after an expenditure of time and money calculable in advance by accountants. On the contrary, as Sir Cyril says, if we wish to plan research we can only do so by assembling a community of people with varied and mutually complementary talents, operating with strategic flexibility, and in an atmosphere of curiosity in which the members know and understand what the others are talking about and respect their leaders. At a time of considerable change in our organization of civil science, it is particularly easy to forget that an

honoured place must be found for the dedicated thinker and that no committee structure can replace such devotion.

Sir Cyril's warning here is emphatic and is reiterated. A scientific community cannot be led either by people uninterested in human relations or by professional humanists without real personal understanding of what science involves. His prescription for future success in applied science is humane enough: a well-assorted community of human types, including the analytical, the creative, the enterprising, and the manually dexterous, all enjoying equal status, so that each part of the task may be taken up by some when others have made their contribution, and all familiar with the rudiments of the same language—the general idiom. Nevertheless, the advance of discovery and science cannot be speeded up merely by administrative elaboration or by imposing political doctrines. Creative scientific research remains a very difficult business and its application is both adventurous and laborious, demanding not only courage and skill but also faith. If its spirit is to spread widely from the relatively few individuals of the past then the whole climate of society must be favourable.

Once again we hear Sir Cyril's leitmotif and now it leads him to discuss how scientists work. Just as firmly as Sir Edward Appleton did, he insists that science is one of the great activities of the human mind and soul, and he adds that, in the last resort, society was made for man and not men for society. It is in this connexion that, like Sir Edward, he quotes the words which Dante long before put into the mouth of Ulysses when he encouraged his companions to venture with him, beyond the furthest point of the known world: "You were not born to live like the beasts, but to strive after virtue and knowledge". Sir Edward, however, quoted more fully and also applied to the scientist seizing his opportunities the words with which Tennyson closed his picture of Ulysses:

"... yearning in desire  
To follow knowledge like a sinking star,  
Beyond the utmost bound of human thought . . ."  
"To strive, to seek, to find, and not to yield."

Sir Cyril, however, passes on to make his point from a brief reference to the lives of three Nobel laureates in physiology—to Pavlov, Ramón y Cajal and Sherrington. He shows that with differing background, temperament and tastes, the trio shared a common passion for knowledge, for discovering things previously unknown, and for unravelling mysteries, and that they also shared an essentially aesthetic appeal in their work. He pauses also to note, just as Appleton did before him, that this is true of most other scientists, and then goes on to drive home his point that science is a creative activity by glancing briefly at the lives of some great physicists of roughly the same generation—at Max Planck and Einstein, at Eddington and Rutherford.

The creative scientist, Sir Cyril observes, is usually more concerned with the relations of things one to another than with the precise verbal analysis of what these things are. He seeks a representation of the world which grows continually by an extension or transformation of what is there already. What many scientists are really after is the adventure of discovery itself, and, referring to Rutherford's belief in the imminence of wonderful new revelations about to occur as amply warranted at the present time, Sir Cyril brings us back to Newton himself, not indeed to:

"... a mind for ever  
Voyaging through strange seas of Thought, alone".

but rather to his words when asked how he made his discoveries, and he replied: "By always thinking unto

them. I keep the subject constantly before me and wait till the first dawnings open little by little into the full light".

That quotation might well be regarded as the key passage in Sir Cyril's address, for when finally he refers briefly to some of the fundamental problems of science which are of the deepest significance in their own right, it is more to display the approach of the scientist than to illumine or even expound the problems themselves. Let it also be noted that Sir Cyril reminds us that the study of these problems, if history has any lessons to impart, will in the long run have the greatest effect on human life; however, he is careful to make no reservation as to the urgency of other work of shorter range. Even with Hamlet's wise warning to Horatio, and without straying into the abstruse, he shows how lively imagination continually plays on a stream of new discoveries wherever man is thinking about the problems of existence—of the structure of the universe, of the structure and properties of matter, or of the physics and chemistry of living matter.

At all the boundaries of science, he reminds us, we confront what are probably the inherent limitations of human understanding. At the edge of biology we meet the chasm between what science describes and what the mind experiences. In the physical sciences we encounter insoluble contradictions if we try to contemplate the limits of space, or the beginning of time. Nevertheless, the men of science, as depicted by Sir Cyril, are not perturbed by the limitation of their possible understanding. There seems to be no foreseeable terminus to their adventure. If the canvas on which they represent the world is bounded, it has room enough for them to paint pictures which can inspire the enquiring mind, can delight those who have the sense of wonder, and can show the way to benefit humanity for many centuries to come.

The question is whether the natural perversity of man may quench that inspiration or spirit of enquiry, crush or kill the sense of wonder, or block the way, and it is on that cautionary note, with its implications, perhaps especially for the educationalist, the administrator and the politician, that Sir Cyril closes. It is probable that only those who have themselves been concerned with scientific research will appreciate all the fine nuances of Sir Cyril's address, but the picture he paints of the scientist as a creative worker, of the need for freedom of expression and appropriate conditions of work, and of public understanding if his work is to be fully effective, is intelligible to any layman. It is no picture of a scientist working and living in some 'ivory tower', or even of Thomson's Newton, "stemming alone vast eternity's unbounded sea", but rather of a happy voyager of strange seas of thought, in company with others trained in the same or many other disciplines. Nor is the picture the less sharp in that Sir Cyril paints it with scarcely a reference either to the particular discipline of which he is himself so eminent a practitioner or to the research group or team. It is above all a picture of a thinker, but not of one to whom Pascal's words apply: "Le silence éternel de ces espèces infinies m'effraie", but rather of the Pascal who added, on reflection, "Toute notre dignité consiste en la pensée". If science is an essential means to an understanding of the forces of the universe or an element in forming any worthy and adequate view of life, it must be understood and treated as such, for only as it flourishes can man know and do aright and steer onward to some purposed haven.

## THE CAMBRIDGE REGION

## The Cambridge Region 1965

Edited by Prof. J. A. Steers. (Prepared for the Meeting of the British Association held from 1 to 8 September, 1965.) Pp. xv + 249 + 12 plates. (Cambridge: Cambridge Local Executive Committee of the British Association for the Advancement of Science, 1965.) n.p.

*THE Cambridge Region 1965* has been specially prepared by the Cambridge local committee of the British Association for the Advancement of Science and edited by Prof. J. A. Steers. Its aim is to provide background information on the region for those who are visiting Cambridge for the annual meeting of the Association. The Publications Committee considered that many people, in addition to members of the Association, would be interested in having a book giving an account not only of new work which has produced different ideas about the interpretation of the district (rural and urban), but also of new views ranging, for example, from those concerned with education to those on the economics of farming or on the scientific investigation of the raw materials of pre-history, since the Association last met in Cambridge in 1938. Essentially, then, the emphasis is on change.

Eleven chapters have been provided by twenty-two contributors and should give the visitor a good picture of the Cambridge region. First to be described are the geology and ground water, then in logical sequence: relief and drift deposits; flood protection and Fen drainage 1939-64; natural history; soils; agriculture; archaeology; Cambridge and its regional setting; education in Cambridgeshire; the University of Cambridge; aspects of development 1938-65; research and industry in and around Cambridge.

Of special interest to the geologist are the outcrops of Jurassic and Cretaceous rocks; however, results of bore-hole work carried out in the early 'fifties have thrown much interesting light on the hidden Palaeozoic floor. Within the region representatives of the Oxfordian and Kimmeridgian zones of the Jurassic are present, while for the Cretaceous there are the Lower Greensands, the Gault Clay and Lower, Middle and Upper Chalk. Concomitant with these the ground waters of interest are the chalk waters and the Lower Greensand waters. Throughout the chapter extensive references are given to the relevant Geological Survey New Series 1-in. sheets. There is, perhaps, a case for presenting a general geological map of the region in these handbooks because many of the amateur geologists will not necessarily have access to the Survey maps.

The major elements of relief within the basin of the Cam and that of the Great Ouse below Huntingdon are the Fen islands (now drained) and the associated Fen margins. Spread over these are sheets of boulder clay (till) and ribbons of river silt (roddons). These are described and discussed in Chapter 2, together with the problem of the age of the widespread till. Also outlined are the evidence for, and history of, glacial events in the region, including pre-Gipping relief and deposition, the Gipping glaciation, physical events of the last interglacial period, the last glaciation and its effects, and, finally, the post-glacial period.

Only 2 million acres of England and Wales (or just more than 5 per cent of the total area) is classified as first-class arable land. The majority of the Fenland area comes within this classification. Chapter 3 briefly outlines the history of the Fens since their reclamation during 1630-52 by the fourth and fifth Earls of Bedford—"A doleful catalogue of disasters, interspersed by brief intervals of triumph". The story for the 1939-64 period is certainly a sorrowful one of floods, war and Government red-tape, but one which gradually brightens as contract work gets under way and is completed on the relief channel, the widening and deepening of the Ely Ouse and Ten Mile Rivers, and the out-off channel with follows around the

eastern margin of the Fens. It is interesting to learn that there is scarcely any gravity drainage from the peat lands and that the rivers flow in normal times some 6-12 ft. above the Fens behind flood banks.

The Cambridge area had always offered much of interest to naturalists because of marsh and aquatic habitats associated with slow-moving rivers, flooded clay- and gravel-pits, and poorly drained and seasonally flooded meadow and pasture. While engineers, builders, agriculturalists and town planners have been vigorously concerning themselves with land reclamation, flood protection and Fen drainage, the naturalists have been frantically trying to preserve the Fen from drying out, from bush colonization and especially from threat of total drainage and destruction. S. M. Walters, in his chapter on the natural history of the region, emphasizes the frightening phenomenon to the naturalist of taming and urbanizing south-east England and the associated losses of flora and fauna, and he puts forward a strong case for carefully planned conservation.

Three principal soil types determine the main range of surviving semi-natural habitats for plants and animals in the county. The most important of these are the woodlands on heavy clays, the grassland and scrub of the chalk, and the Fenlands. Important examples of all these are protected as Nature reserves or sites of special scientific interest, such as Hayley Wood, Holme Fen, Wicken Fen and Wood Walton Fen. Much of the concern of naturalists has inevitably been with the rarer species, particularly when its continued existence in a particular locality is threatened. However, it now seems that, besides the red squirrel, the frog, the rabbit and the kestrel are becoming increasingly scarce. Newcomers to the region include the grey squirrel, the coypu and the collared dove, and among the plant kingdom, the willow herb, *Epilobium adenocaulon*, and the moss, *Orthodontium lineare*.

The present regional handbook has been carefully prepared so as not to repeat what was written in the survey prepared for the 1938 meeting. In the latter the soils of the area were viewed from a mainly agro-geological point of view. Chapter 5, "Soils", gives emphasis to the genesis of the soils and their relationship with broad world groupings. In 1950 the Soil Survey of England and Wales undertook the preparation of a detailed map of the country immediately north-east of Cambridge and this was published in 1963. The present chapter is accompanied by a coloured soil-map of the region at a scale of 1:250,000. Soils of the district are classified into seven groups: podzolized soils; brown earths; calcareous soils; grey soils, warp soils; immature soils; organic soils. Of special interest are the Fen peats and Fen silts. Essentially these are artificial, for the water-level has been controlled by the drainage measures of the past 300 years. Drainage of the peat led to considerable shrinkage, which reached a maximum of about 10 ft. Fen silts are marine and estuarine deposits which at present are being laid down most rapidly on the southern margin of the Wash where the silt is trapped by salt-marsh vegetation. For purposes of land reclamation a new sea-wall is constructed when a reasonable area of marsh of a suitable surface texture has accumulated to a height of about 10 ft. O.D.

Agriculturally the Cambridge region is characterized by high dependence on crops for sale, as compared with live-stock products. Chapter 6, by means of plentiful illustrations and tables, makes it obvious that cash crops in the region are about ten times as important as grazing livestock. It shows that the region provides a major percentage of the total production in England of, for example, brussels sprouts (25.1), celery (42.1), gooseberries (25.4), carrots (18.7), sugar-beet (14.0). For descriptive purposes the region is divided into six farming areas: (1) south-east Cambridgeshire (40 per cent of the region's crops and grass); (2) east Bedfordshire (market gardening); (3) west Cambridgeshire and Huntingdonshire (crops, beans and peas); (4) Ely Black Fens (potatoes and

sugar-beet); (5 and 6) Cottenham and Wisbech (fruit-growing). Since 1938 the agricultural picture has developed as one of greater output, produced by fewer men but more machinery and fertilizer and with more land under the plough—the barley acreage has increased nearly four-fold and now represents 28.2 per cent of the region's total crops and grass.

The region is well endowed with organizations concerned with agricultural research and advice—including the Agriculture and Veterinary Medicine Departments in the University of Cambridge, the National Institute of Agricultural Botany, the Veterinary Investigation Centre, the Agricultural Research Council Institute of Animal Physiology and the Plant Breeding Institute, the Ministry of Agriculture's Experimental Husbandry Farms, and the Poultry Research Station—all of which play an important part in the agricultural efforts of the region, and of the nation as a whole. The final section of the chapter deals with some current developments and problems, including high cereals rotation, mechanization, maintaining healthy crops and farm management.

In the chapter on the archaeology of the Cambridge region, only discoveries made and work done since 1940 are mentioned specifically; however, an attempt has been made to incorporate more traditional material in discussions on particular points. The first part of the chapter deals with "Prehistory" and here the emphasis is on results gained from the application of various sophisticated scientific aids which have become available to help the prehistorian in dating, identification and interpretation. Radiocarbon dating is being undertaken on the Upper Palaeolithic industries near Mildenhall. Recent work on the Mesolithic of the British Isles has clarified the picture of this phase of early settlement and has been greatly assisted by the work of Prof. H. Godwin in the zoning of post-glacial deposits by pollen analysis. An important site at Peacock's Farm, Shippea Hill, known to have a Neolithic occupation, has been studied and pollen and radiocarbon analyses carried out. (This particular piece of work has helped to show that the British Neolithic is older (3400–3000 B.C.) than was previously assumed.) Metallurgical studies of artefacts and copper sources from Bronze Age sites have been carried out and various metal objects have been subjected to optical emission spectroscopy: results gained have thrown much new light on the origin of the various ores used. Several Iron Age sites, including the Wandlebury hill-fort, have been surveyed by means of proton-magnetometers and successfully excavated on the basis of this. The second part of the chapter deals with "The Romano-British Period". In this is described recent work carried out in tracing the Roman road and water systems of the area. Numerous successful excavations are reported on settlements, defences and farmsteads. Much new material has been unearthed including mosaics, pottery, pewter tableware, bronze and glass jugs, figurines, bracelets, etc.

"Cambridge in its Regional Setting", by far the longest chapter in the handbook, is divided into three sections which deal with: (1) "The Cambridge Region: Settlement and Population"; (2) "Cambridge: its Origin and Growth"; (3) "The Modern City: Future Trends". The first section constitutes almost half the chapter. The problems of Cambridge are intimately bound up with those of south-east England as a whole, and the relationship with London is of particular importance and significance at the present time. Cambridge has unquestionably taken dominance as the regional centre. Surrounding it is a ring of small market towns; but the clarity of this pattern is lost in the Fenland and around its margins. The variety of rural settlement is considerable and ranges from dispersed and substantial farmsteads, standing alone in fields of a large holding, or the isolated bungalow of a Fenland smallholder to the big villages and market towns which are to be found near the margins of the Fenland. The various patterns of settlement in the area not only

depend on the way in which the area was initially colonized from Anglo-Saxon times, but also on subsequent changes in population and economy. In the Fenland the difficulty of finding firm sites for building and the chronology of reclamation are important factors affecting the pattern of settlement.

Cambridge is situated on a meander of the River Cam and, as the second section of Chapter 8 shows, it is the particular situation of the site that has given Cambridge its especial importance. It lay in the angle between the prehistoric bundle of tracks, the Icknield Way and the Roman Road, Ermine Street. East Anglians journeying from Colchester or Ipswich or Norwich to the Midlands found their easiest way skirted the southern edge of the Fenland and crossed the Granta (Cam) Valley in the neighbourhood of Cambridge. It was the Romans who, in building their roads, gave pre-eminence to this crossing. In times of the Anglo-Saxon invasion Cambridge gained strategic importance. An entry in the *Anglo Saxon Chronicle* for A.D. 875 records the name 'Grantebryce': this indicates that the ford had been replaced by a bridge (it is the earliest known recorded use of the word 'bridge'). It was this bridge that enhanced the importance of the little settlements beside it, and in due course 'Grantebryce' became the administrative centre of the region and a market town. To this thriving centre came first the mendicant orders, then the teachers and scholars. The origins of the University are obscure. In 1209, however, a group of scholars arrived from Oxford, probably attracted by teachers already famous. Little land was at first needed, teachers and pupils lived as and where they could; but as their numbers increased hostels, halls, then colleges were founded—land being purchased or acquired by gift. During the thirteenth-sixteenth centuries, and in the nineteenth century, more and more colleges were founded and little by little Cambridge took up the form that it has to-day.

In his section on the "Modern City", A. A. L. Caesar discusses a problem only too well known to many present-day town planners in Britain. The problem is one of planning for a growth of population and a car-owning population, but this related to the lay-out of a historic centre. The twentieth century has brought to Cambridge, as elsewhere, a great acceleration of change, and one of the most conspicuous forces responsible for this has been the great development in transport. While facilities in Cambridge were sufficient until about the 1920's, a crisis point has now virtually been reached. Mr. Caesar puts forward his case with great care and clarity and sees long-term planning as the most important aspect of the remedy. Planning, he feels, should be in terms of 2065 rather than of simply answering the immediate problems of 1965. Commercial and domestic buildings may well have a life-span of 100 years, but street lay-outs tend to be less durable. He discusses the pros and cons of pedestrian precincts, road development and a new main shopping area. He describes the projected redevelopment of the City Road and Lion Yard areas. Cambridge as he sees it stands poised—choosing, weighing new ways. However, as he states, it cannot pause for long: plans must be made and implemented if growth unplanned is not to be wholly destructive.

The various facets of the Cambridgeshire educational scene are described by eight authors in Chapter 9. As a result of the Education Act of 1944, the Cambridgeshire County Council became the Local Education Authority for the whole county. In 1965 the two Authorities for Cambridgeshire and the Isle of Ely were amalgamated. So far as an adequate supply of teachers is concerned, Cambridgeshire, because of its many amenities, is fortunate compared with many parts of Britain. The distinctive feature of secondary education in the region is the Village College—a combination of secondary modern school and a community centre providing for the education, social and recreational life of the rural area. The first of these



was founded in 1930, and since then ten in all have come into being.

Recently, considerable effort has gone into putting into practice many of the recommendations of the Newsom and Robbins Reports. Audio-visual aids, including very-high-frequency radio, television, record-player, projector and tape-recorder, are having an ever-increasing role in school teaching. The Authority has endeavoured to encourage an atmosphere of experiment and enquiry in the junior schools. Developments within the rural area and the City are described together with the role of technical colleges, farm schools, youth employment schemes, and psychological and remedial services. Also described are the direct-grant and independent schools, University extra-mural teaching, and the parts played and the work done by the Eastern District of the Workers' Educational Association, the University Department of Education, and the Cambridge Institute of Education.

In the twenty-seven years since 1938, numerous changes have occurred in the University of Cambridge. New buildings have been erected to accommodate overflowing departments and house new research efforts. Two new colleges, New Hall for women, and Churchill for men, have been founded. A third, Darwin College, has recently been established: this is an innovation in that it is restricted to graduate students. The undergraduate population has increased 50 per cent since 1938, and this has been accompanied by a corresponding growth of the teaching staff. The strain on the Colleges has been threefold: accommodation, facilities and adequate teaching for more undergraduates; adaptation to meet the needs of research and other graduate students; expansion of the number of fellowships. Since the Second World War a new structure of university stipend has been introduced, and the holder of a university office no longer simply depends on the stipend from his College. A number of new forms of tripos have been introduced, and many have been, or are in the process of being, revised. New post-graduate diplomas relating to agriculture, criminology, estate management, mathematics, etc., have been introduced. A movement is at hand to introduce a new degree (M.Phil.) for award on an examination based on a 2-year course of advanced study in a specialist field. Chapter 10 ends by briefly directing attention to some aspects of research at Cambridge, including computer work, radio-astronomy and macromolecular biology.

Cambridge remains a university town and market centre for one of the least-industrialized regions in Britain. However, a number of science-based industries and industrial or government research stations have grown up or been attracted to the district. Chapter 11 gives brief accounts of the history and work of the various organizations. First, the industrial concerns are dealt with under: chemicals and plastics; electronics and instruments; metallurgical and engineering; other research organizations. Under the heading "Government Science" are described the various units in the region which are the responsibility of the Agricultural and Medical Research Councils. These have relationships of various closeness with the corresponding departments within the University.

It is pleasing to note that most of the chapters have been provided with a list of references and that an adequate index has been prepared—the latter is something which has been absent from many of the British Association regional handbooks in recent years. The one criticism that can be levelled at the present publication is that a general map has not been provided. Certainly this would have helped considerably to bring together the various themes which are presented. In any event, Prof. Steers and his colleagues are to be congratulated on preparing a well-balanced account of the Cambridge scene and its development over the past twenty-seven years.

R. J. FIFIELD

## THE COMPLEAT PHYSICIST

### Encyclopaedic Dictionary of Physics

Editor-in-Chief: J. Thewlis; Associated Editors: R. C. Glaze, D. J. Hughes (deceased) and A. R. Meetham. Vol. 2: Compensator to Episcadmium Neutrons. Pp. ix+880. Vol. 3: Epitaxy to Intermediate Image. Pp. ix+804. Vol. 4: Intermediate State to Neutron Resonance Level. Pp. ix+836. Vol. 5: Neutron Scattering to Radiation Constants. Pp. ix+782. Vol. 6: Radiation Continuous to Stellar Luminosity. Pp. ix+883. Vol. 7: Stellar Magnitude to Zwitter Ion. Pp. ix+866. Vol. 8: Subject and Author Indexes. Pp. viii+498. Vol. 9: Multilingual Glossary. Pp. xvi+988. (Oxford, London, New York and Paris: Pergamon Press. Vols. 2, 3, 4, 1961; Vols. 5, 6, 7, 1962; Vol. 8, 1963; and Vol. 9, 1964.) £108 net per set of 9 volumes.

AN encyclopaedic dictionary of physics is fundamentally a new concept. The only comparable work is that produced by Glazebrook and called the *Dictionary of Applied Physics*; but the present work is a great deal larger than this: it has tried to cover all concepts in physics. Individual editors have been chosen for the various parts of physics, but ultimately, of course, there are no sub-divisions in the completed work, since the entries are arranged in alphabetical order. They form a series of articles of not more than 2,000 words, each written by a specialist whose name is given at the end. Many of the entries are, of course, very much shorter than this; for example, a rheostat is defined as "a resistor whose resistance can be varied".

Almost all the articles are excellent. Since it is known who has written each one they are readily acceptable as authoritative, and those that I have had occasion to refer to have proved to be so. They are written at a very reasonable level. Nowadays most authoritative works, such as the *Reports of Progress in Physics*, tend to be too long and detailed; the articles in this *Dictionary* come straight to the point, are short enough for the authors not to be tempted to become too sophisticated, but are long enough for quite specific information to be given. They are certain to be extremely useful for practising physicists (for example, external examiners) who are called on to deal occasionally with subjects outside their own fields. For example, I consulted an article more or less at random: the entry "Shock Wave" describes the nature of these waves, including a historical discussion; it is purely qualitative, and contains no mathematics, although the relevance of mathematics is indicated. Different types of shock waves are then discussed; there are sections on oblique shock waves, and on conical and spherical shock waves. The entry is illustrated by eight figures, gives references to other relevant entries in the dictionary, and concludes with a bibliography of six other works that could be consulted. In addition, one finds that the previous entry—"Shock Tubes"—is also relevant. Throughout the work associated subjects tend to be together for this sort of reason.

It is not easy to discover any real defects in the work. One cannot examine every entry for accuracy. The policy on definitions does not seem to be consistent; some are included, but others are absent; for example, the Maxwell is defined, but not the Weber or the Newton. The older physicist is sometimes at a disadvantage because so many new terms have been introduced since his undergraduate days, and concise definitions would not have taken up a great deal of space.

Some articles—for example, theodolites and complexes (chemical)—seem rather out of place in a work on physics; but one appreciates that it is difficult to draw a hard-and-fast line at the borders of the subject. Certainly, a casual glance through the various volumes does not indicate any undue emphasis on non-physical concepts.

There is little that appears to be quite wrong. The article on the Schmidt lens is useless; it is not a lens but



a correcting plate for a spherical mirror, and no mention is made of the important fact that it is placed at the centre of curvature of the mirror. The article on mirage is rather absurd, and can only be understood if one knows what the author is trying to say; several physicists completely misunderstood it. Obviously an entry in a dictionary is useless unless it can be immediately understood.

The *Dictionary* is not intended to act as a work of reference for values of physical quantities. Only a few tables are given—for example, constants of the solar system and the periodic table—but generally the work must be regarded as a set of explanations rather than of details. This point is illustrated particularly by the various mathematical functions included; they are, of course, generally of interest to physicists, and the emphasis is on explanation rather than on numerical values. For example, those interested—as most physicists are—in probability theory will find compact articles on such topics as the Poisson distribution, stochastic processes, regression line and the error function.

On the other hand, there are also articles on severely practical matters. There is an article on photographic darkrooms, and this shows plans of a standard darkroom with layout of benches and sinks, and gives an indication of the ancillary apparatus required. Another entry deals with laboratory design; this is quite short, but gives a useful collection of eight references at the end.

The complete encyclopaedia consists of nine volumes. Seven of them are of the form which has been described; Volume 8 is an index which gives the subjects of the encyclopaedia and also the authors of all articles longer than 500 words; and Volume 9 consists of a glossary of scientific terms designed to help the non-English-speaking users of the *Dictionary*. An ingenious system enables translations of terms to be made from and into five other languages. Each entry in English is numbered in alphabetical sequence, and in five following columns are given the numbers of equivalent terms in French, German, Spanish, Russian and Japanese. These terms are then given in alphabetical sequence in five later sections of the volume. The information will enable a person who knows no Japanese to find the equivalent of a given English entry, but will not enable him to carry out the reverse process unless he knows the order of the Japanese characters.

Publication of this *Dictionary* must be regarded as an important event in the world of physics. No departmental library should be without it. The price is rather high; it comes to about £13 a volume of 1,000 pages; but perhaps this is inevitable when so many different people have been involved. The editors and publishers are to be congratulated not only on producing this handsome and well-balanced work, but also on persuading such an authoritative body of people to write for them.

The first volume of this work was reviewed in *Nature* (193, 411; 1962).  
H. LIPSON

## CENTENNIAL CELEBRATION OF THE NATIONAL ACADEMY OF SCIENCES

### The Scientific Endeavor

Contributed by Melvin Calvin and 23 other authors. (Centennial Celebration of the National Academy of Sciences.) Pp. vii+331. (New York: The Rockefeller Institute Press, 1966.) 2.50 dollars.

THE settlers from Europe went to America when the new age of scientific endeavour of the seventeenth century was being fostered by the young scientific academies of Italy, England and France. When a more social way of living was established in America they also organized societies for discussions of their observations and experiments.

The American Philosophical Society at Philadelphia was established before the Revolution, and the American Academy of Arts and Sciences was founded in Boston soon after. Benjamin Franklin, Benjamin Thompson (Count Rumford) and Thomas Jefferson were among many colonial Americans who studied science for its own sake as well as for its usefulness. Literate farmers, artisans and tradesmen gathered in scores of local societies for the discussion of science.

By the middle of the nineteenth century there was a growing acceptance of natural science as a suitable subject for teaching in the colleges of liberal arts, and there were increasing numbers of young men who wished to devote all their time to scientific study, teaching and research. The Morrill Acts of 1862 made available grants for the support of colleges of agriculture and engineering and thus emphasized the need for wider diffusion of scientific knowledge as the essential foundation for the development of the national economy. The Civil War showed the needs of the Federal Government for technical advice, and accordingly in 1863, Congress, with the co-operation of fifty scientists, founded the National Academy of Sciences. Promising young scientists went to Europe for advanced study and returned to poorly equipped laboratories and heavy teaching duties that left little time for research. (In less than a century this position has tended to reverse itself!) The second half-century of the Academy began just before the First World War, and again the Academy was called on to satisfy the technical needs of the armed forces. The growth of universities and the multiplication of industrial laboratories stimulated the increasing demand for scientists. As the United States emerged from international isolation, the Academy developed closer ties with its sister academies of other countries and acted as a centre of the integration of scattered and narrow specialisms. To symbolize the national significance of the Academy, the Carnegie Corporation of New York provided the funds for a monumental building in the capital. The centennial of the National Academy of Sciences was celebrated in the autumn of 1963. To mark the occasion many of the six hundred and fifty members of the Academy gathered in Washington with representatives of scientific bodies from other parts of the world. A number of foundations and corporations announced their generous intention to complete the House of the Academy that had been started forty years previously.

The President of the United States, the late John F. Kennedy, and twenty-three distinguished members of the Academy delivered memorable addresses in which the history of the universe, the nature of the matter, the determinants and evolution of life and the spirit of the scientific endeavour were described in a scholarly manner. Prof. I. I. Rabi summarized the proceedings by saying: "Day after day the mysteries of life were laid bare and, antecedent to life, the structure of matter, and indeed of the universe, were presented in dramatic and fascinating clarity. . . . The juxtaposition of topics did a very great deal to show the essential unity of scientific disciplines however different their techniques. . . . We listened to great wisdom".

The lectures were collected to form a book of considerable substance with adequate illustrations. The volume, even in its cheap paper-back version, is a worthy tribute to the occasion. The style of all the writers is clear and simple, and there has been no attempt to write down to the uninitiated in the popular style which nearly always leads to ambiguous statements, bad analogy and florid writing. These faults have been avoided absolutely.

*The Scientific Endeavour* divides naturally into five sections which are in the historic order of cosmic development. The history of the universe is considered in five chapters: "The Origin of the Elements", by William A. Fowler; "The History of Stars and Galaxies", by Jesse L. Greenstein; "The History of the Solar System", by Fred L. Whipple; "The Origins of the Continents, Oceans and

Atmosphere", by H. H. Hess; "The Origins of Life", by George Wald. The second section comprises the chapters on: "Symmetry and Conservation Laws", by Eugene P. Wigner; "Elementary Particles", by Geoffrey F. Chew; "The Structure of Nuclei", by Victor F. Weisskopf; "The Architecture of Molecules", by Linus Pauling; "The Organization of Living Matter", by George E. Palade. The third section deals with "The Determinants and Evolution of Life", which was introduced by Theodosius Dobzhansky and contains the lectures on "Genetic Determinants", by E. L. Tatum; "The Differentiation of Cells", by T. M. Sonneborn; "The Influence of the Environment", by G. Evelyn Hutchinson; "The Evolution of Living Systems", by Ernst Mayr; "The Physiological and Cultural Determinants of Behaviour", by Neal E. Miller. The fourth section was devoted to "The Scientific Endeavour" and consisted of lectures in "Communication and the Comprehension of Scientific Knowledge", by Robert Oppenheimer; "The Role of Science in Universities, Government and Industry: Science and Public Policy", by Jerome B. Wiesner; "Synthesis and Applications of Scientific Knowledge for Human Use", by J. B. Fiak; and "Science in the Satisfaction of Human Aspiration", by I. I. Rabi. The final section was devoted to a lecture by President John F. Kennedy on "A Century of Scientific Conquest". The book concludes with a bibliography of references for each of the lectures.

The eminence of each of the scientists who contributed to the occasion would indicate that each of the chapters is masterly both as an essay in science and in exposition. The whole book is a remarkable achievement, and the amount of consistently connected and developed information which it contains, particularly at so low a price, is remarkable. It should find many readers: the undergraduate, the graduate and the specialist in science. It should prove a means of obtaining a scholarly introduction to broad fields in the sciences.

W. L. SUMNER

## PLANT PHENOLICS

### Methods In Polyphenol Chemistry

Proceedings of the Plant Phenolics Group Symposium, Oxford, April 1963. Edited by J. B. Pridham. Pp. ix+146. (Oxford, London and New York: Pergamon Press, 1964.) 50s.

**A**PART from their intrinsic interest, deriving from the existence of a multitude of variations based on a relatively simple structure and their aesthetic interest as the source of a large proportion of the pigments which provide colour in plants, the plant phenolics have great importance, as a group, as an aid to plant classification by chemo-taxonomic techniques. Yet until quite recently the difficulties involved in their adequate separation and characterization were so great, and the methods so tedious, as to render their employment almost entirely impracticable.

Thus although among the most easily extractable and most visually characteristic of all plant compounds, it was only with the development of paper chromatography over the past fifteen or twenty years that their potential became exploitable. Particular interest therefore attaches to the symposium of the Plant Phenolics Group in April 1963, which is now published under the title *Methods in Polyphenol Chemistry*, and which was particularly concerned with the methods and techniques of analysis and characterization which has made this development possible.

Separation largely depends on chromatography, and as would be expected, a large part of the symposium was devoted to descriptions of the various techniques which have been found useful. These include contributions on paper chromatography by Bate-Smith, which contains a

most necessary plea for standardization wherever possible of solvents; on the use of thin-layer chromatography by Thaller; a particularly encouraging discussion of the use of the new polyamides by Horhammer; and a description of the application of gas-liquid chromatography by Norman and his colleagues. In addition there are two papers on the closely related subject of electrophoresis by Weigel and by Pridham, although it is pointed out that owing to its greater complexity this technique does not provide a substitute for chromatography, but may be a useful complementary tool for particular applications.

Just as chromatography has transformed the problem of separation, so spectroscopic methods have greatly simplified quantitative measurement and identification, and contributions at the symposium included papers by Harborne on the use of ultra-violet spectroscopy, with special attention to the relationship between structure and spectra and the use of the technique in the identification of new compounds; infra-red spectroscopy of flavonoids by Wagner; a description of preliminary attempts to make use of the sensitivity characteristic of spectrofluorimetry by Bridges; and a brief discussion on the basic principles of nuclear magnetic resonance spectroscopy by Abraham, with examples of its application to the determination of molecular structure in phenolics.

The final paper by Swain and Goldstein is concerned with the quantitative analysis of phenolic compounds, and is particularly notable for its cautionary approach, pointing out in respect to a number of possible techniques that while there is comparatively little difficulty in obtaining values of a sort, great care is needed to ensure that these are comparable and therefore meaningful, and the paper makes clear that a considerable amount of effort has been wasted as a result of failure to take this precaution.

Collectively the papers provide an excellent introduction to the range of techniques available for separation and analysis of phenolic compounds, though as a rule the scope of the particular techniques described is less well defined, and their limitations tend to be omitted. Individually the papers are well presented and highly readable, providing a high quota of information without being exhaustive or exhausting, and the volume can be recommended confidently to anyone interested in the subject. The book lacks an index, and even though the need for this is to some extent obviated by its shortness and division into clearly defined papers, this omission makes it unnecessarily difficult to look for information about particular compounds. This is unfortunate when one considers the very considerable amount of physical data relating to individual phenols which is contained in the various tables and figures.

P. A. THOMPSON

## OCEANOGRAPHY FOR ENGINEERS

### Oceanographical Engineering

By Prof. Robert L. Wiegel. (Prentice-Hall International Series in Theoretical and Applied Mechanics. Fluid Mechanics Series.) Pp. xi+532. (Englewood Cliffs, N.J., and London: Prentice-Hall, Inc., 1964.) 108s.

**I**T is realistic and progressive to maintain that hydraulic engineering should reach down river estuaries as far as low-water mark, and that oceanographic engineering should extend up the rivers as far as high-water mark. Prof. Wiegel, who holds the chair of civil engineering in the University of California at Berkeley, has such wide experience, and his new and detailed book *Oceanographical Engineering* can be recommended as a comprehensive guide especially to engineers engaged in coastal and harbour engineering. It gives much information that is essential to structural design but is more particularly outstanding in its description and appreciation of the factors that are likely to influence functional design and

the overall effect of the structure. Quite often the execution of a programme of improvement has created problems as severe as those it was conceived to solve, and differences of professional opinion about how problems should be tackled are often wide enough to demonstrate that basic understanding of the forces and motions involved is quite inadequate. It is too early for any complete theory, even of such an apparently elementary problem as the spacing, height and length of a system of groins, but there are partial and practical answers to many difficult questions, and the body of sound information is growing so rapidly that all designers are likely to gain useful guidance from it if they are prepared to face up to the unavoidably difficult analysis and reasoning. A large part of the book is devoted to waves, since they are important in almost every aspect of oceanographical engineering, and also because it is to this aspect of the subject that Prof. Wiegand's experience is most outstanding. He does not deal in detail with fouling organisms, submarine geology, underwater acoustics and sea-ice in which he has not specialized, or with corrosion and erosion of materials, or with measuring instruments for which the existing literature is already so large. But his 532 pages provide broad coverage and depth of information about most of the things that civil engineers want to know about the sea and which are not easy for them to enquire into.

The wave chapters review our knowledge of the theory of wind waves, much of it the outcome of recent work. They emphasize the need for further advanced theory and experiment because of the growing evidence we have of the importance of non-linear effects which could reasonably be neglected in the first approaches to the subject. Solitary and impulsively generated waves are also considered, with special reference to seismic seawaves, storm surges and harbour oscillations. These are followed by studies of the effect of beaches and structures on waves, including the effects of different kinds of breakwaters and resonant absorbers, of the nature of wave forces on different kinds of beaches and structures, and the longshore drifts and beach changes produced by them. There is a separate chapter on modern wave-forecasting procedures and their reliability, and a short note on tides and other changes in water-level and mean sea-level. Perhaps there should have been a more specific consideration of wave set-up—the inshore changes of sea-level which can be produced by oncoming swell without the agency of local wind. There is a chapter on the functional design of fixed and floating structures, piers, breakwaters, sea-walls, groins, underwater pipelines and sewer outfalls, and another on ship motion and on moorings.

Characteristics of ocean water, currents and mixing processes are described, but there is not much about ocean drilling, deep-sea prospecting, deep submergence vehicles and the increasing range of deep-sea technology, which is now referred to as oceanology.

The index is scarcely adequate, but it would be very difficult to refer to everything in a book so densely packed with information, tables, diagrams and references. It is quite an easy book to find one's way about in, and very rewarding.

G. E. R. DRACON

## PRINCIPLES OF HISTORICAL GEOLOGY

### Stratigraphy and Life History

By Prof. Marshall Kay and Prof. Edwin H. Colbert. Pp. 736. (New York and London: John Wiley and Sons, Inc., 1965.) 74s.

THE student reader of an introductory text on historical geology is faced with several difficulties additional to those of mastering new conceptions and a new vocabulary. In particular, the demands made on his geographical knowledge are considerable; and for this reason the most

useful elementary books are usually those describing the rocks of his homeland. This noteworthy volume by Prof. M. Kay and Prof. E. H. Colbert is concerned principally with the United States. Elsewhere, therefore, it is unlikely to be prescribed other than as collateral reading; but its very existence emphasizes the need for an equally well-illustrated introduction to the geology of Western Europe. Geology, like charity, should begin at home but not remain there.

In the usual fashion the book begins with a description of the rocks of the Pre-Cambrian era and then discusses the Phanerozoic systems one by one; but it is unique in that it essays to explain the principles of stratigraphical, petrographical and geochronological correlation by reference to the non-fossiliferous Pre-Cambrian formations, particularly the ore-bearing rocks of Michigan, Minnesota and Ontario. In fulfilling this difficult task it is remarkably successful. The principles of faunal correlation and faunal provinces are introduced with the description of Cambrian strata; and thereafter appropriate opportunities are taken to bring in various broad concepts on the tectonic control of sedimentation and similar matters. The palaeontology of the various systems, related in very general terms, dovetails with the accounts of the rocks and their palaeogeography; but there are three separate chapters on life in the Palaeozoic, Mesozoic and Cenozoic, and a fourth giving a systematic review of fossil organisms. The emphasis throughout is on the establishment of the principles which guide geological thinking. A chapter on continental drift is lucid reading but it is doubtful if one on the evolution of the crust in North America expresses its ideas sharply enough to be understood by the average undergraduate. A penultimate chapter on the origin of the Earth, by Dean B. McLaughlin, seems too remote to be relevant.

When the authors' writing touches on geochemistry it is much less felicitous and slips are frequent. On pp. 66–69 we are told that "there are two forms of isotopes of uranium", that  $^{238}\text{Pb}$  is primary lead, and that  $^{40}\text{K}$  forms 1 per cent of natural potassium (although the accompanying diagram gives the correct value of 0.012 per cent); and it is wrongly implied that an age determination can be derived from the ratio  $^{235}\text{U} : ^{238}\text{U}$ . On pp. 82–83 it is not made clear that the figure of 2,700 m.y. for the oldest rocks relates solely to North America—there are much older formations in Russia and southern Africa; or that the age of 1,700 m.y. given for the Gunflint and Biwabik iron formations is their metamorphic date—the date of sedimentation is much older, as shown on p. 49. Similarly on p. 594 it is said that "there are two isotopes of carbon having atomic weights of 14 and 12: the latter is common carbon". On p. 91 the uraniferous hydrocarbon in the Cambrian shales of Sweden is wrongly referred to as pitchblende; and a reader could pardonably gain the impression that dolomites do not appear before the Silurian (all earlier references are to limestone) and that all dolomites are penecontemporaneous. Students should not be encouraged to use the old term Proterozoic for the Pre-Cambrian in view of potential confusion with Proterozoic. It does not appear in the American Geological Institute's dictionary.

In its wealth of illustrations, this text-book is the most lavish of its kind yet produced. A large page size (30 cm × 24 cm) has permitted about half the total space to be occupied by photographs and diagrams, accompanied by lengthy explanations; but only a persistent reader will finally locate a description of the frontispiece photograph on p. 436, and of the dust jacket on p. 176. A coloured geological map of North America is printed inside the jacket. Reproductions of dioramas of palaeontological habitat groups, mostly from exhibits in American museums, present a vivid representation of life through the ages. Some other diagrams of fossils (particularly of microfossils) could have been improved by the addition of a scale.

O. F. DAVIDSON

### The Theory of Order-Disorder In Alloys

By M. A. Krivoglaз and A. Smirnov. Pp. x+427. (London: Macdonald and Co. (Publishers), Ltd., 1964.) 90s.

IN an ordinary metallic solid solution of, say, a two-component system, the two species of atoms are arranged at random on the points of the crystal lattice, and at a composition  $AB$ , for example, any particular lattice point may be occupied by either  $A$  or  $B$  atoms. In certain systems, however, below a critical temperature, segregation of one kind of atom to a certain set of lattice points can occur, leaving the other kind to the remaining positions. This phenomenon is known as 'ordering'. The possibility of ordering had been suggested by Tammann in 1919, and was confirmed by the X-ray work of Bain in 1923. Since that time many metallic systems have been discovered to have this property at certain simple ratios of  $A$  to  $B$ , the best-known examples occurring at compositions  $AB$  and  $A_2B$ , such as  $Cu_3Au$  and  $Ag_3Zn$ . Clearly such a modification of the structure of the alloy as the temperature falls is expected to influence in turn many of the physical and mechanical properties, and a considerable theoretical structure has been erected to relate the observed changes in properties to changes in the models of the electronic structure.

The present volume is a translation from the Russian, and the approach is largely theoretical. It consists of eight chapters. The first chapter is a general review of the properties of ordered alloys, and the second, third and fourth deal with the thermodynamic and statistical theories of ordering, and with the theory of diffusion. A discussion of the effect of ordering on the behaviour of the valence electrons appears in chapter five, and the effect of ordering on wave-motion in chapter six. Chapters seven and eight cover the theory of residual resistivity and the magnetic, galvanomagnetic, optical and mechanical properties of metals and alloys.

The book is aimed at graduate and post-graduate workers and is a fairly comprehensive treatise on the theoretical aspects of ordering. However, there are a number of points of criticism. The treatment is excessively formal, and the explanation of the relation between the mathematical and physical models is disappointing. The first chapter of ninety or so pages is well illustrated with eighty-six figures, but the remaining three hundred pages have only twenty-eight illustrations between them. It is pleasant to have many references to Russian work in the text, but the overall balance of the book suffers. For example, in the chapter on the theory of the residual resistivity of alloys the names of Mott and Friedel do not appear. The book has three hundred and thirty-seven references, but since it was published in Russian in 1958 the most recent reference is 1957. In the copy sent to me for review the quality of the printing and the pages was somewhat uneven. There is no index, and in view of the price one can make only a cautious recommendation.

J. A. CATERALL

### Ultrasonic Cutting

By L. D. Rozenberg, V. F. Kazantsev, L. O. Makarov and D. F. Yakhimovich. Translated from the Russian by J. E. S. Bradley. Pp. xi+142. (New York: Consultants Bureau, 1964.) 17.50 dollars.

THIS carefully translated text is based primarily on the experience of the authors in the Ultrasonic Laboratory at the Acoustics Institute of the Academy of Sciences of the U.S.S.R., and in the Special Designs Office of the Moscow City Economic Council. The authors have, however, attempted to make due note of work done elsewhere—both inside and outside the U.S.S.R.—and the inclusion of more than 220 literature references is one result of this.

Since this book should be just as useful in creating interest in, and potential users for, this technique, as in providing a reference text for those already expert, it may be as well to give a brief reminder of what ultrasonic

cutting is. It is a manufacturing process that has developed over the past twenty years to facilitate the cutting of, or drilling of holes in, materials normally too brittle for working by conventional methods. A slurry containing extremely hard abrasive particles (for example, 'Carborundum' or boron carbide) is fed to the working position, where an acoustically designed tool of relatively soft material vibrates at ultrasonic frequency, hammering down on the trapped particles. In this way an extremely rapid succession of intense 'micro-blows' is imparted to the working surface which thus erodes away, leaving a cavity which reflects the shape of the acoustic tool. Accordingly, and depending on the manner in which the tool is vibrated (for torsional vibrations have as much usefulness as longitudinal vibrations), it is possible to produce round holes, non-circular holes, twisted holes, slots, curved holes, disks and internal and external screw threads, among other forms. Versatility is limited only by the nature of the workpiece material, which must be brittle.

The text covers basic principles as well as practical development. The first chapter discusses elastic wave propagation and vibration for various media, both solid and fluid. Subsequent chapters show how these concepts lead to a detailed understanding of ultrasonic machining, so that—making use of piezo-electric or magnetostrictive excitation—the acoustic tool head may be logically designed to achieve the desired effect. Discussion thereafter widens to deal with different types of ultrasonic machine tool, and indeed with the whole technology of the process.

The book is remarkably readable: the mathematical analysis employed in the early chapters is a digestible minimum and is well presented. Apart from the numerous references already mentioned, the text is finally supported by 31 well-chosen tables of numerical data, and 155 clear illustrations.

B. N. COLE

### Contributions to Sensory Physiology

Vol. 1. Edited by William D. Neff. Pp. x+274. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 60s.

THIS is the first volume in a new series of publications which has two main objectives, to bring together reports of current research on sensory physiology, and to enable individual research workers to present some particular theory of the physiological basis of sensation. They are definitely not intended to be review articles.

Of the five articles which it contains, three are concerned with vestibular function. The first is a study of the organ of Corti at a cellular level, another concerns the functional anatomy of the ciliated end organs of the labyrinth and lateral line and the third is a psychophysiological study of vestibular responses. If the editors continue to bring together articles like these, which are not normally found in the same volume, the series will certainly fulfil a useful function.

The remaining two articles are on vision and are of particular interest from the comparative point of view as one gives an account of work, some unpublished, on the vision of various primates, while the second is a translation from the French of a paper by Piéron on vision in intermittent light. It is a sad fact, but nevertheless true, that many English-speaking biologists have little time to digest the details of longer papers written in other languages. The publication of translations of this type could do a great deal to help in the dissemination of results that otherwise might not be read in the detail they deserve.

The whole volume is well bound, and has a good reference list and author and subject indexes. It is only unfortunate that the price is so high for it adds to the ever-increasing burden that libraries must bear in subsidizing the costly production of volumes of this type.

G. M. HUGHES

OBSERVATIONS OF RADIO GALAXIES WITH THE  
ONE-MILE TELESCOPE AT CAMBRIDGE

By PROF. M. RYLE, F.R.S., B. ELSMORE and ANN C. NEVILLE

Mullard Radio Astronomy Observatory, Cavendish Laboratory, Cambridge

ON July 8, Lord Bowden and other distinguished guests visited the Mullard Radio Astronomy Observatory, Cambridge, to inspect the new, large radio telescope<sup>1</sup> which has recently come into use. This instrument, which was constructed with the aid of a grant from the Science Research Council, makes use of an advanced form of aerial synthesis to provide a resolution and sensitivity considerably greater than have been achieved hitherto.

The telescope was designed primarily to further our understanding of the physical processes occurring in radio sources of different types, both galactic and extragalactic. In each case one of the chief requirements is a detailed knowledge of the structure for different wave-lengths and polarizations. Although the large baseline interferometers at Jodrell Bank<sup>2</sup> have set lower limits on the surface brightness occurring in many sources, they have not provided much information on their shape. More detailed interferometric observations, but with less-extensive baselines, have been made at the California Institute of Technology<sup>3</sup> and the results have been interpreted in terms of various simple models of the brightness distribution; these models may not, however, be unique, and information has not been obtained for more complex sources.

From what is already known about the intrinsic radio luminosities of radio galaxies and quasars, it is clear that

an investigation of the numbers of sources occurring in different ranges of flux density, and the examination of the spectra and other features of sources of different flux density, may allow powerful tests of different cosmological models. The present observations<sup>4-6</sup> have already imposed important restrictions on the possible models, and these restrictions should be increased if the observations can be extended to weaker sources; such an extension requires an instrument having both greater angular resolution and greater sensitivity.

The new telescope has been designed with these needs in mind. In the earlier large instruments at Cambridge, a long, thin aerial has been used in conjunction with a smaller movable one to carry out a one-dimensional synthesis. In the new telescope, the method is extended to two-dimensional synthesis using relatively small circular elements the spacing and orientation of which are varied to include all the relative positions present in the large equivalent aerial. Use is made of the rotation of the Earth to alter the projected position angle of an east-west axis on the sky; by using aerial elements capable of tracking a given point in the sky for 12 h all the information appropriate to an elliptical ring of a large equivalent instrument is obtained<sup>1</sup>. By repeating 12-h runs at each of a number of east-west separations, it is then possible to

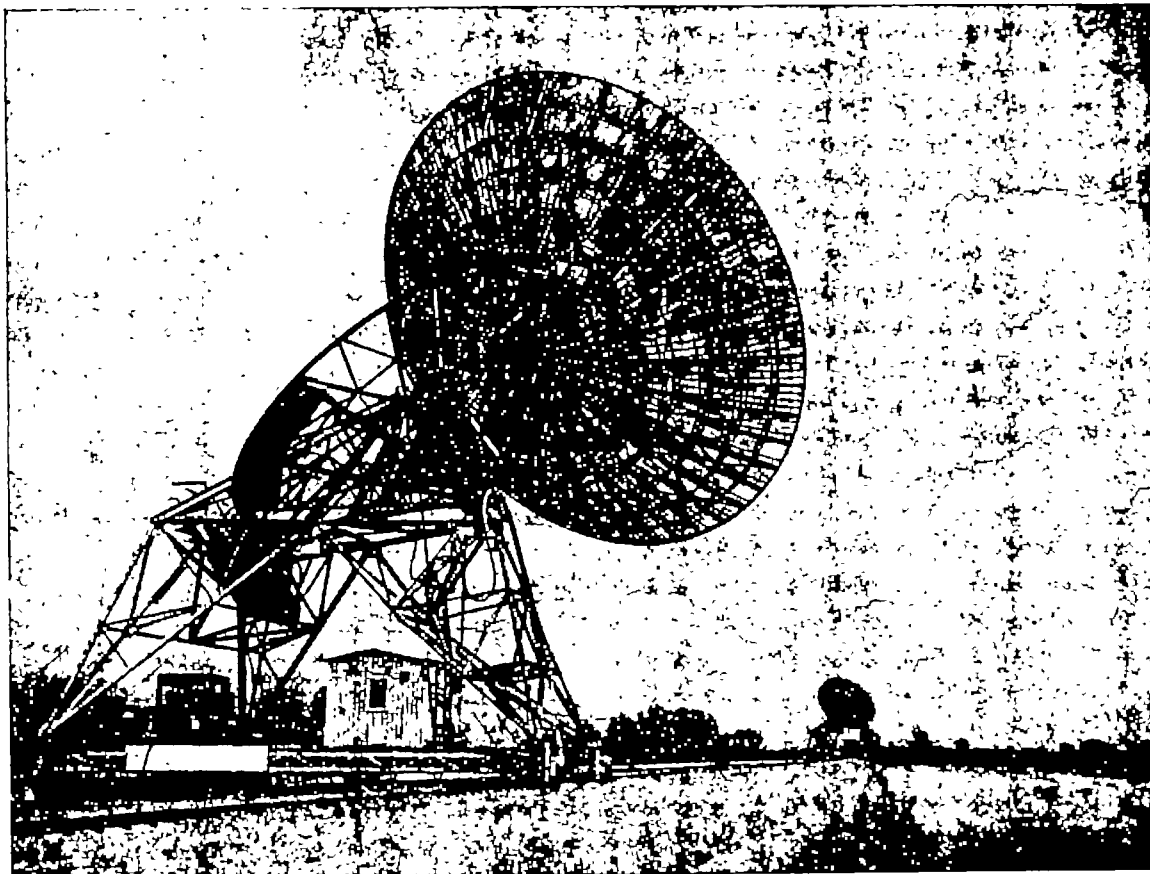


Fig. 1. The three 60-ft. paraboloids of the new telescope. The moving aerial and rails can be seen in the foreground.

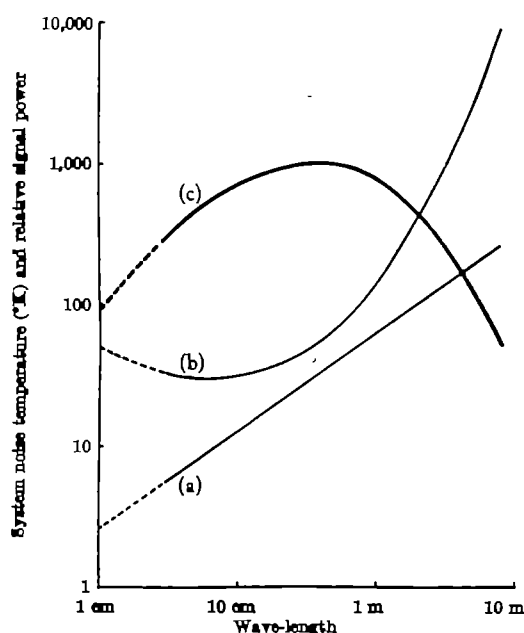


Fig. 2. Curves showing as a function of wave-length: (a) the variation in flux density of a typical radio galaxy; (b) the total system noise of a typical installation using low-noise amplifiers; and (c) the ratio of (a) and (b).

build up an equivalent elliptical aerial the major and minor axes of which are  $D$  and  $D \sin \delta$ , where  $D$  is the maximum separation of the aerial elements and  $\delta$  is the declination of the area of sky under observation. A maximum separation ( $D$ ) of about 1 mile (1,550 m) can be used in the new telescope.

Owing to the high cost of the stable rail foundations, two fixed paraboloids are used in conjunction with a movable one on 0.5 mile of rail track; this arrangement permits two different separations to be recorded simultaneously and halves the observing time. The use of an even shorter rail track and a larger number of fixed elements would have reduced the observing time still further but would have resulted in an increased cost.

The three elements may be seen in Fig. 1. Each is an equatorially mounted paraboloid 60 ft. in diameter. The instrument is being used to observe simultaneously at two wave-lengths, 74 and 21.3 cm, at which the final beam-widths are 80 and 23 sec arc respectively.

It is interesting to compare the signal-to-noise ratio achieved with this system, with those of more conventional instruments of the same resolution when used to observe a given area of sky in a given total time. If  $d$  is the diameter of each of the aerial elements and  $D$  is the maximum separation, it can be shown<sup>1</sup> that the signal to noise in the final map is equivalent to that of an instrument with a collecting area of approximately  $3d \times D$ , or  $10^6$  sq. ft. In addition, the ability to use wave-lengths considerably greater than those which would have to be used in a feasible paraboloid to obtain the same resolution confers a further improvement in the relative sensitivity. In Fig. 2 curves are shown of the relative flux density of a typical radio source at different wave-lengths together with typical values of the overall system noise (receiver noise and radiation from the ground and sky). It can be seen that the ratio of these two curves shows a well-defined maximum in the neighbourhood of 50 cm. If, for example, a resolution of 23 sec arc were to be achieved by the use of a 200-ft. paraboloid operating

on a wave-length of 8 mm, the relative sensitivity would fall below that of the new instrument operating at  $\lambda = 21$  cm by a factor of about 300.

Apart from the question of the engineering feasibility of constructing a paraboloid with sufficient accuracy to provide a resolution better than 1' arc, it is therefore clear that if such an instrument were to provide maps having a comparable signal to noise, then the observing rate would have to be very much slower than that of the new telescope.

The aerials may be controlled individually but, normally, they are all operated from the Central Control Room shown in Fig. 3. This building also contains the receivers, digital converters and paper-tape punches on which the signals are recorded. When observations are made away from the meridian, large path differences to the different elements occur and it is necessary to introduce a path correction which varies continuously as the observations proceed. This correction and the control of the punches and telescope tracking motors are made automatically by means of a control tape previously prepared in the computer.

During the first seven months, the instrument has been engaged in three main observing programmes:

### (1) Accurate Positions

In order to make full use of the resolution available at  $\lambda = 21$  cm, the relative positions of the three instruments must be established with an accuracy better than  $\sim 0.5$  cm.

Attempts have been made to determine the necessary quantities (a) by observing small-diameter radio sources associated with stellar objects the positions of which have been accurately measured<sup>10,11</sup> and (b) by direct survey methods. The former method has been used by Adgie<sup>11</sup> and, as he points out, it suffers from the rather small number of sources for which the identity of optical and radio positions can be assumed; most of the sources also lie in a limited range of declination. For the second method we are indebted to the Ministry of Public Building and Works for the final survey of the instrument with an accuracy believed to be 1 in  $10^6$  and 0.5 sec arc.

A comparison of the two methods has revealed discrepancies greater than the uncertainty expected from either of the survey methods.

Although the cause of this discrepancy has not yet been discovered, it is possible to use the present results to determine the positions of sources having  $S_{21} > 2.5 \cdot 10^{-26}$  Wm<sup>-2</sup> (c/s)<sup>-1</sup> with an accuracy of  $\pm 3$  sec arc in  $\alpha$  and  $\pm (2.5 \cos \delta)$  sec arc in declination.

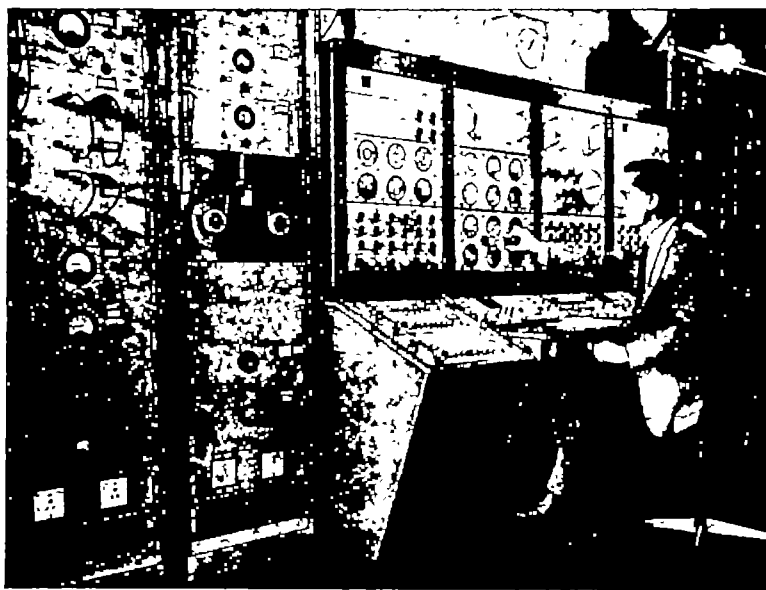


Fig. 3. The control desk and part of the receiving system.

Some 50 sources have been observed, about half being apparent point sources. The positions of a number have been examined on the prints of the 48" Sky Survey and some possible new identifications have been noted. These include blue stellar objects close to the positions of the following sources: 3C 270-1, 3C 277-1, 3C 309-1 and 3C 454.

## (2) Source Structure

When observing intense sources, the angular size of which is considerably less than the primary beam of the individual aerials, it is unnecessary to make observations with all separations of the aerial elements. Instead, a relatively small number of positions of the moving aerial along the full length of the rail track can be used to synthesize a grating instrument. This method was employed in the observation of the intense sources in Cygnus and Cassiopeia already reported<sup>12</sup>. Some of the sources investigated have been observed previously by Moffet and Maltby<sup>3</sup>, who fitted simple source models to their observed amplitude-spacing curves. In several cases the distribution of radio brightness has been found to be more complex, while in others the sources have not previously been observed with sufficient resolution to show the detail now seen.

The results are presented automatically as contours on a curve plotter attached to the *Edsac II* computer.

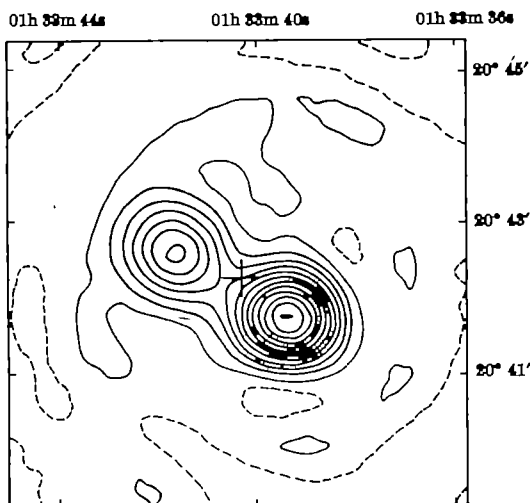


Fig. 4. 3C 47. The optical quasi-stellar object is marked by a cross

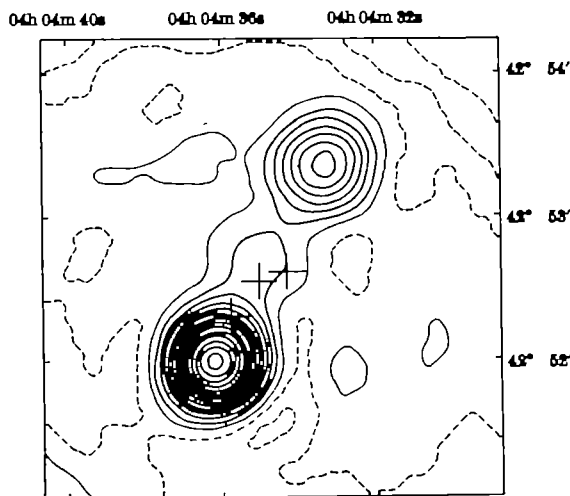


Fig. 5. 3C 103. The central of the three objects shown is probably a star with  $m_{\text{vis}} = 16$ . Two diffuse red objects ( $m_{\text{vis}} = 18-19$ ) are also visible

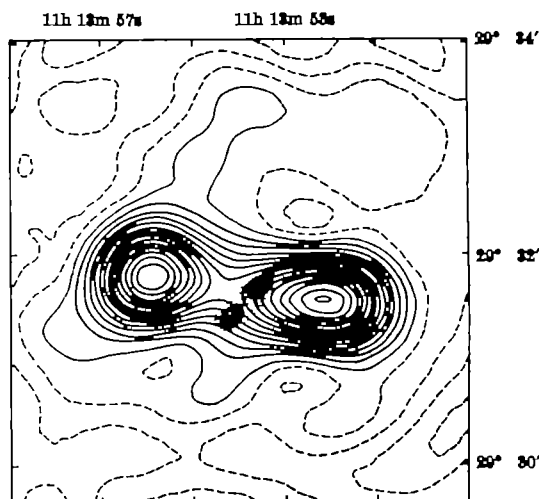


Fig. 6. 3C 29 41. The two 15 15" galaxies in a common halo are shown

Observations have been made on both 74 and 21 cm; the maps obtained for some of the sources on a wave-length of 21 cm are shown in Figs. 4-7. In order to simplify the examination of the structure of the sources, the scales of all the maps are such that the beam appears circular; the scale of the maps is therefore the same in right ascension, but varies as  $\sin \delta$  in declination.

(a) 3C 47. This source was one of the original group of quasars<sup>13</sup>. The new observations (Fig. 4) show that it consists of two components having flux densities in the ratio 1.8 : 1. The components have an angular separation of 62 sec arc and are disposed nearly symmetrically either side of the optical object which lies about 8 sec arc north of the line joining them. The more intense component is just resolved with an angular extent of  $\sim 10$  sec arc in R.A.; the weaker component cannot be resolved, but could be of the same angular size. The interferometric observations of Anderson *et al.*<sup>14</sup> and the absence of interplanetary scintillations<sup>15</sup> have shown that there is little structure as small as 2 sec arc.

The optical object has a red-shift  $\Delta\lambda/\lambda = 0.452$  (ref. 13), and the observed angular separation of the two components corresponds to a physical separation of 200-300 kpc depending on the cosmological model adopted. It is therefore physically one of the largest radio sources known, with a component separation some three times that of Cygnus A; its radio luminosity is nearly the same as that of Cygnus A.

This result is important in relation to theories of quasars and radio galaxies, since, even if the two components are supposed to be ejected at speeds close to that of light, and in a line perpendicular to the line of sight, the radio source must be at least  $3 \times 10^6$  years old; a life in excess of  $10^6$  years seems more likely. The presence of intense ultra-violet emission from a stellar object associated with such an old source indicates either that there is a continuing source of energy for the ultra-violet source which does not give rise to intense radio emission, or that a second release of energy can occur in such objects.

The large physical dimensions of the two emitting regions (7-35 kpc) also require a total energy in the form of fast particles and magnetic field, which is comparable with that of Cygnus A ( $\sim 10^{44}$  ergs). As discussed by Fowler<sup>16</sup>, the relatively short life-time which has been associated with quasars only requires sources of energy of  $10^{43}$ - $10^{44}$  ergs; the association of a quasi-stellar optical object with a radio source having energy requirements some 100-1,000 times larger may place greater emphasis on those mechanisms of energy release in quasars which are capable of supplying this greater energy.

(b) 3C 103. This source (Fig. 5) consists of two components separated by 88 sec arc, each having an angular



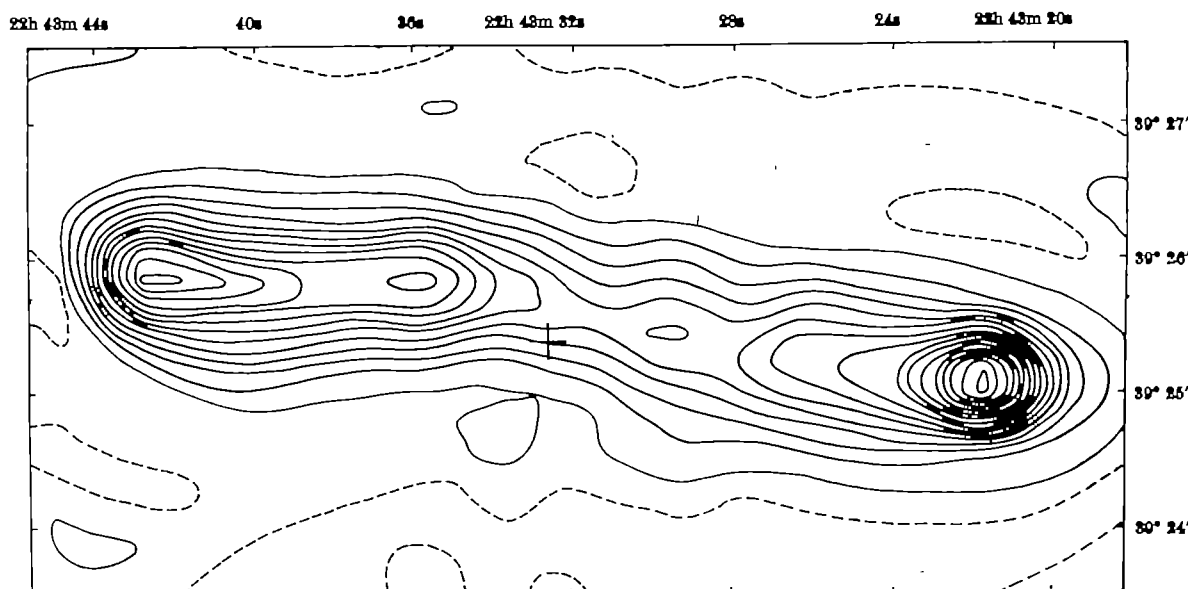


Fig. 7. 3C 452. The 16<sup>m</sup> galaxy suggested as an identification (refs 18, 19) is shown at  $\alpha = 22^{\text{h}} 43^{\text{m}} 32^{\text{s}}$ . This galaxy has a red-shift  $\Delta\lambda/\lambda = 0.062$ , implying a separation between the outer peaks of 280 kpc.

diameter  $< 10$  sec arc; a faint bridge joins the two components. The south following component has a flux density some 1.9 times greater than the other. No associated optical object has previously been suggested, and the low galactic latitude ( $b_{\text{II}} = 7^\circ$ ) may involve considerable obscuration; some possible objects are marked in Fig. 5.

(c) 4C 29.41. This is a source from the 4C catalogue<sup>17</sup>. Its flux density at 21 cm is  $1.8 \times 10^{-26} \text{ Wm}^{-2} (\text{c/s})^{-1}$  and it consists of an extended or double major component, separated from a second, unresolved component, by 50 sec arc (Fig. 6). Two 15<sup>m</sup> elliptical galaxies in a common halo are probably associated with the radio source.

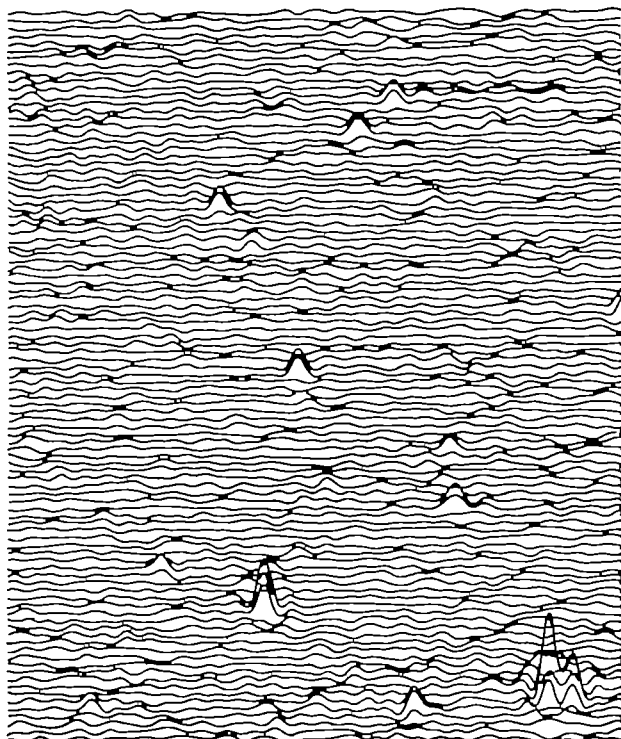


Fig. 8. Part of the first deep survey at  $\lambda = 74$  cm obtained with the new instrument; the figure covers an area of about 1 sq. degree. The source near the centre has a flux density about 1/25th that of the weakest sources in the 4C catalogue (ref. 17).

(d) 3C 452. The contour map is shown in Fig. 7 and reveals a remarkable ridge of emission 4 min arc in length and 20 sec arc in width; it also shows an intense and elongated source at either end of the ridge, neither of which is completely resolved in declination.

The position of a galaxy which has been suggested by Dewhurst<sup>18</sup> and Matthews<sup>19</sup> as the identification is shown in Fig. 7.

### (3) Deep Survey

A test survey has been made using the instrument to synthesize a full 1-mile aperture in order to provide a detailed map of an area of sky which is some  $3^\circ$  in diameter at 74 cm and 50 min arc diameter at 21 cm. The amplifiers used in these observations were only of moderately low noise-level ( $T_R = 450^\circ$  at 74 cm and  $400^\circ$  at 21 cm), yet nevertheless extremely faint sources can be distinguished. Part of the 74-cm map (of area approximately 1 sq. degree) is shown in Fig. 8. The more intense component of the double source in the lower right-hand corner has a flux density  $S_{74} = 0.17 \times 10^{-26} \text{ Wm}^{-2} (\text{c/s})^{-1}$  and the source near the centre of the map has  $S_{74} = 0.04 \times 10^{-26} \text{ Wm}^{-2} (\text{c/s})^{-1}$ .

We thank Dr. S. Kenderdine who was responsible for the reduction of the deep survey observations, Miss Judy Bailey for help in the programming, E. A. Parker for permission to use the results of his search for optical identifications before publication, and Prof. M. V. Wilkes for the use of the *Edsac II* and *Titan* computers.

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## PROFESSIONAL OBSOLESCENCE AND THIS RAPIDLY EXPANDING TECHNOLOGICAL ERA

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IF you have graduated prior to 1959, there is a strong probability that you are becoming 'technically obsolescent'. Technical obsolescence means this: a competent technical person with an excellent formal education finds himself, after some years of practice, unable to discharge his technical responsibilities effectively. Young scientists, entering employment, have only a 'breather' before they must plan to refresh their knowledge and techniques, because at the present rate of research discovery, their training will be adequate for about five years. But professional obsolescence can deliver a real knockout blow to older scientists if they do not follow new developments and ideas; they may reach a dead-end in their careers by the time they are forty-five years of age.

The problem can be summarized as follows. Graduates of ten years ago have to spend a minimum of 10 per cent of their time acquiring technical knowledge beyond their original academic training if they hope to compete in value with more recent graduates. This statement is, of necessity, predicated on the assumption that they have retained most or all of their previous training. However, if the loss of unused knowledge is 10 per cent per annum, and the advance in new knowledge is also 10 per cent, scientists must then grow in knowledge at a rate of 20 per cent per annum to remain of equal value to their employers. In the past, scientific meetings, conferences and seminars were relied on as the means of keeping scientists informed about new advancements, but this is no longer adequate.

### Technical Obsolescence not a New Problem

Technical obsolescence is a problem, a very serious problem, but it can scarcely be considered a new one. It has always been hazardous for a scientist to permit himself to operate indefinitely on the training and education which was acquired formally. The modern problem is complicated by the fact that scientists can no longer update their technical knowledge without assistance from many sources. Help is needed. Basically, this is because, when technical obsolescence does occur, it appears at the foundations of the scientist's training rather than in the day-to-day problems which he encounters. The rapid accumulation of new technology, especially in advanced engineering and in the various phases of weapon design, space and other aspects of technical power politics, makes technical obsolescence and updating of technical skills a very serious matter.

The late Eger Murphree listed the threat of technical obsolescence as "at least a hundred billion dollar problem". The American Chemical Society has estimated that 23 per cent of all the research in chemistry throughout the entire history of the world was conducted over a four-year period between 1957 and 1961. Similar comments can be made about the other physical sciences. Small wonder, then, that there is a desperate need for updating the technical backgrounds of our scientists.

### Definition of a Professional

A professional is paid for making value judgments. These judgments affect life, property and money. The

better the quality of these judgments, the greater the remuneration should be. The compensation should also vary directly with the magnitude of the difficulty involved in making the judgment.

Some examples of professional personnel are: traditionally, the medical doctor and the lawyer; and, more recently, the aircraft pilot and the personnel manager. It would, therefore, appear that this designation of 'professional' is somewhat arbitrary; however, they do have one aspect in common, that is, they are required to make judgments. By this definition, the scientist would also be classified as a true professional. He must acquire information on which to base a judgment, and then reach a decision. This he does since he must decide which way a research programme should be oriented; what experimentation should be conducted; evaluate the results; plan new approaches, and report to management.

### Pre-requisites for Practising a Profession

Now what does it take to prepare a professional to perform his functions? In order to practise his profession, a scientist must learn the rudiments of a particular science and its techniques of practice. After graduation, and during the period that he is practising his profession, it is necessary for him to keep informed of the changes in knowledge relative to the practice of his profession.

Since the making of a judgment is a step, regardless of how small, into uncharted territory, and not a rehearsal of already known ideas or fact, the professional scientist must have at his finger-tips the latest knowledge and facts concerning the area where the judgment is to be made. This, in conjunction with previously acquired knowledge, will only then provide the foundation for a valid judgment.

### Stimulation of the Professional Scientist

The scientist must be stimulated in order to provide an essential growth atmosphere in which his curiosity and thirst for knowledge are continuously nourished and his sense of values maintained. A yardstick must be provided so that he can periodically measure the degree to which he is falling behind. This can very aptly be illustrated by an over-simplified analogy. A person fluent in French will have a difficult time maintaining this linguistic dexterity in an English-speaking environment. In fact, in such an environment, there exists no challenge or yardstick for gauging whether his fluency in French has slipped to any degree. Motivated by his deep love for the language, he can resort to the use of language records and other teaching aids in an effort to retain or augment his knowledge of the language. Nevertheless, he is destined to forget, and forget he will, for two reasons: nobody challenges him to demonstrate what he has remembered, and as he associates with the people in his immediate vicinity, he is not provided with a means which enables him to gauge the degree to which he is becoming obsolete.

Now, suppose that this individual elects to aspire to a higher level of recognition, and suppose that this level requires that he learn German. If the rewards for such recognition appear of sufficient significance to him, he will

have to modify his sense of values, and seek such recognition. In doing this, he is confronted with a more complicated situation since he now runs the risk of forgetting his skill in French at an even more rapid rate. This risk is very real, and has been labelled the 'risk of management', since the risk of accelerating technical obsolescence occurs in the pursuit of higher management responsibilities.

### Types of Professional Obsolescence

(1) *Keeping up with the literature.* There are two different types of professional obsolescence. At one end of the spectrum there is an obsolescence resulting from an inability to keep abreast of the present literature in a particular scientific area. That is, an inability to keep up with the newly obtained results and changing postulates. In this age of the so-called publication explosion, many insist that such an effort is, at best, a losing battle, and it must be conceded that, in these busy times, the battle requires great personal sacrifice in time and effort. Some claim that it is physically and intellectually impossible to keep abreast in any particular scientific field.

Keeping up with the present scientific literature is a 'must' in order to minimize the risk of individual obsolescence. In its essentials, the problem is how to ensure that the individual's fixed amount of reading time is used on that particular selection from the available present flow of new information which is optimum for him. Selectivity is the keynote, as it has always been, and its crucial importance increases with the steady rise in volume of output available.

Proliferation of the literature, at the present time, appears to be proceeding faster than the rate of improving the know-how to cope with it. This means that unremitting effort is required from the scientist, and initiative is required to achieve a relative gain and not decline in the mastery of a reading programme.

(2) *Preventing erosion of the scientist's basic discipline.* At the other end of the spectrum is the failure to keep up with the fundamental structure of the scientist's basic discipline. In this situation, the job of keeping up is really much easier. In fact, the ready availability of well-written text-books, review articles, symposia, etc., places this achievement within the grasp of almost every scientist. Publishers and outstanding scientific writers are aware of this deficiency, and an increasing number of text-books specifically designed to teach concepts are now appearing. From this point of view, this latter kind of technical obsolescence warrants attention because it exists when, in fact, it should be very rare.

### Examples of Advances In Modern Technology

Since the Second World War, the rising level of expenditure on research by the Federal Government and industry (from 5.2 billion\* dollars in 1953 to an estimated 19 billion dollars in 1965) has produced a host of major new technological advances which will have a profound influence on the future of all scientists and non-scientists. Technological obsolescence is not restricted to a particular scientific discipline. The defence-space programmes have been the sources of a large number of new advances which overlap many scientific disciplines. This can be most appropriately illustrated by the impact these advances have had on the steel industry. The competitive pressures for aluminium, plastics and reinforced concrete, combined with the domestic and foreign market pressures in this industry, have rendered economically obsolete much of the industry's present steel-making facilities. These pressures are forcing companies to install new facilities that embody an entirely new steel-making technology. As a consequence, the scientists in this industry are confronted with the need of acquiring newer skills required for these more modern methods of production.

\* 'Billion' mean a million millions in Britain and elsewhere, but a tenth of that (a thousand millions) in the United States and some other countries.

Another example can be garnered from the field of electronics. This field has rapidly outgrown its initial role in radio, television and communications. It has not only developed into a basic scientific discipline in its own right, but has also broad potential application in virtually all areas of scientific endeavour. As greater knowledge and understanding are attained, it is becoming increasingly possible to accomplish many tasks by electronic techniques rather than by mechanical or electromechanical devices. A list of such inroads would include these significant accomplishments: electronic telephone switchboards; electrostatic printing; electrochemical machining; electrical discharge machining; numerically controlled metal-working; computerized typesetting; photocomposition printing.

Another example, and one that is of particular interest to this group, can be labelled as 'engineered materials'. The traditional approach to materials research was segmented into numerous specialized areas of interest, such as ferrous metals, non-ferrous metals, plastics, rubber, ceramics, etc. This concept is rapidly being superseded by research based on the concept that the same fundamental principles underlie the behaviour of all materials. With a more thorough comprehension of the underlying relationship between the basic structure of materials and their physical properties, the synthesis of 'engineered' materials, such as propellants, which have properties that are best suited for a particular application becomes a reality. The more important recent outgrowths of this new science of engineered materials include transistors, tunnel diodes, masers, lasers, piezoelectric devices, etc.

The final example, which warrants some attention, is numerical control. This is regarded as a most significant development in manufacturing technology and is comparable with Henry Ford's development of the assembly line. It brings to the field of small-volume production many of the manufacturing economies which are at present inherent only in highly automated production systems.

Although numerical control has attained its highest state of development in the machine tool industry, this new concept has many potential applications. These include the inspection of finished products, metal flame-cutting, wiring and assembly of electronic printed circuit boards, and the preparation of engineering drawings.

### Programmes for bringing Scientists up to Date

The fight to keep up with fast-moving scientific developments requires the resources of the individual, industry, professional societies, government and academic institutions. Some employee education programmes exist, but are fairly new and still in the trial stages. Their effectiveness is, therefore, difficult to assess.

(1) *Projected educational changes in night schools.* Despite the changes that are projected for high schools and colleges during the coming decade, the greatest impact will probably be felt in adult night school education. Although business and Government will be forced to increase their re-training activities for their scientific personnel, it is most probable that a substantial portion of the re-training and bringing up to date will have to be provided in adult night-time programmes. Night school is expected to become an accepted part of the weekly activities of a large segment of a country's technical force. The growing need for adult night-school education poses several serious problems. This will be particularly so for the educational systems in urban areas because it will be here that the impact of obsolescence will be more deeply felt. Not only will school systems be faced with the challenges of providing adequate facilities and qualified educators to meet the expanding needs of the night school programmes, but also these challenges will be compounded further with the more difficult problem of trying to provide training in entirely new scientific fields

which are not well defined and which are in a constant state of flux.

(2) *Projected changes in college training.* I need scarcely point out the serious deficit of college facilities for handling the educational needs because of the exploding population. The offering of refresher courses to the technically trained would further tax the facilities of these institutions. In addition, the evening courses which are at present being offered are generally a portion of degree-oriented programmes, and are, therefore, not readily available, nor geared for, those interested only in furthering their knowledge in a particular field. Colleges and universities, especially those in urban areas, will thus be confronted with the necessity of markedly increasing the number of non-degree educational programmes which are offered. Because of the lack of adequate facilities, they will find it necessary to develop new relationships with the local schools so that college-level programmes can be provided through existing school systems. The acquisition of an adequate professorial staff who are fluently conversant with the newer and constantly changing scientific disciplines is going to be a problem of major magnitude.

Another approach would consist of developing intensive courses from its undergraduate and graduate curricula which would then be taught by regular faculty members during the summer session 'in-plant'.

(3) *Projected changes in the activities of professional societies.* The role of professional societies in updating the technical training of their members has been frequently debated. The American Medical Association has pioneered one approach. Various practising groups in the medical profession are required to hold frequent clinical conferences and programmes designed to keep their members abreast of new developments. For example, the American Academy of General Practice with 27,000 members (about one-third of the general medical practitioners) requires its active members to engage in and complete 150 hours of postgraduate education acceptable to, and outlined by, its Commission on Education in each three-year period. If a participant fails to comply, active membership is not renewed. Similarly, the American Institute of Chemists has recently introduced the regulative principle of "Accreditation of Scientific Modernism". The intention is to stimulate the individual chemist or chemical engineer to protect himself against the outdating of his technical skills. For accreditation, the individual's recent scientific updating efforts are reviewed by local examining boards, which then recommend whether the candidate should be accredited.

Further, considerable modification of the methods for conducting the national meetings of the professional societies is a necessity to cope with the changing times. Present emphasis at these meetings is on the presentation of detailed research discoveries in highly specialized areas of a particular science. More appropriately, with the secondary goal of providing the opportunity of bringing those attending up to date, some summary or review papers which discuss the more recent developments should be liberally interspersed among these fundamental papers.

(4) *Efforts by industry and Government to bring their scientists up to date.* Certain industrial organizations have already taken several important steps in supplying the tools which are needed to ensure that their scientists can keep up to date. The plans for such endeavours are in the form of advanced degree programmes, including educational leaves of absence for its scientific employees, and co-operative plans with universities where facilities and staff are made available for providing advanced and refresher courses on the job.

Most of these employee educational programmes are new, and are still in the trial stages, and therefore their success is difficult to assess. Consequently, it is not possible to estimate the number of scientists who are already engaged in updating their technical backgrounds.

What little experience is available indicates that many professional personnel have not taken the matter of personal obsolescence seriously as yet.

Some comments about the more important stimuli for updating follow.

### Tuition Payment Plans

Payment of part or all of the tuition costs for employees seeking advanced degrees has existed within the United States for a considerable time. These plans have been especially motivated in providing the incentives for advanced study. A limited survey, conducted by the Outboard Marine Corporation in 1961, suggests that these programmes are growing in popularity with employers but not necessarily with employees. According to the survey, an average of only 6 per cent of the eligible employees have seen fit to take advantage of the tuition-paid educational plan.

### 'In-plant' Programmes

Additional 'in-plant' educational opportunities for technical employees are needed. The lecturers can be recruited from either within or outside the organization. These lectures should comprise survey courses provided by qualified university staff members or by senior employees of the organization's own scientific staff who can present such courses to their younger associates and to members of other departments. Employees should have full-time assignments to attend these courses in company time.

### Newer Tools for fighting Technical Obsolescence

There are three principal kinds of newer tools, namely, computers, copying and recording devices, and rapid communication systems that can be used in meeting the educational crisis facing the country.

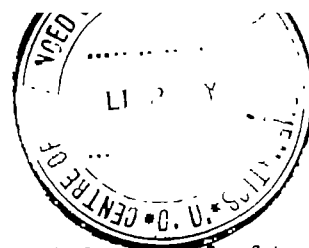
The use of the computer and associated technology will make the literature more readily accessible to each scientist. This mechanical slave can accomplish the tasks of extracting, assembling and coarsely organizing whatever information is in the literature stockpile which corresponds to the questions which are posed. For the start-up of such a new method, however, large tasks are involved, such as the organization of the total information accumulation into suitable fashion, and the provision of pre-tested operational techniques.

There is little doubt that the new schemes should provide an exceptional service in the treatment of facts, such as molecular structure, mechanical properties, etc., which appear in the literature. The computers can manipulate these results to effect correlations among these, and this would be extremely difficult and tedious without machine aid. However, when it involves literature components which are primarily interpretative, theoretical or speculative rationalizations, less satisfactory results will have to be acceptable. The reasons are rooted in the difficulties of precise indexing of the less-specific and less-precise pieces of information. Conceptual themes weave in and through large aggregates of facts, and are, consequently, difficult to index for effective mechanical manipulation by the mechanical rigour which computer programmes demand.

The adoption of computerized information storage and retrieval techniques will interfere with the ability to browse. Browsing is a desirable capability, and is useful and essential, especially to those engaged in truly fundamental research.

Newer communication developments, such as television, tele-lecture, 'blackboard by wire' and conference calls, are additional techniques which, when used in combination and in conjunction with present systems, can be projected to alter greatly teaching methods.

## OBITUARIES



## Sir John Russell, O.B.E., F.R.S.

SIR JOHN RUSSELL, director of Rothamsted Experimental Station during 1912-43, and for more than half a century a dominant figure in world agriculture, died at Goring on July 12, 1965. He survived Lady Russell, who constantly supported him through an exceptionally long and active career, by only three months, and he finished his last book, a history of agricultural research in Great Britain, only a few weeks before his death.

Edward John Russell was born in 1872 at Frampton, Gloucestershire, the son of a school teacher who later became a Unitarian minister. His family was unconnected with either agriculture or science; but his father had unusually wide interests, extending from religion and social work not only into politics and philosophy but also into chemistry and biology, and these clearly influenced young John. His first formal contact with chemistry was at the Birmingham Technical School and so fascinated him that, when he had to leave the school to earn his living at the age of fourteen, he was "full of a desire to study chemistry" but "saw no hope whatever of doing so". However, to quote further from his autobiography, *The Land Called Me*, "thinking that chemistry was practised in chemists' shops, I decided to try and get into one and save up money to go to college". He got into one only to be disillusioned, but he was not easily put off and, with the persistence and energy that typified his whole life, he continued to study by attending evening classes and gained scholarships that took him in turn to the Presbyterian College, Carmarthen, to the University College of Wales, Aberystwyth, and, in 1894, to Owens College, Manchester.

Russell failed at his first attempt at the London B.Sc. degree, because his physics and mathematics were not up to standard. He then contemplated entering a theological college, but Prof. Dixon appointed him a research assistant at ten shillings a week, which allowed him to continue at Manchester, where to make ends meet he did other jobs as diverse as organist, reporter, lecturer, and coaching "some dull people through examinations that they never ought to have passed". At his second attempt he passed in the first division both the B.Sc. and the honours chemistry examinations, and then did research on various subjects, of which the most important was the oxidation of phosphorus, for which he was awarded the London D.Sc. degree in 1901. In addition to his chemistry, he was active as a social worker in the slums of Manchester, and, paradoxically, it was there that he first considered turning his attention to agriculture. He thought that many of those who failed to make good in cities would be happier and more prosperous in the country, and he had the idea of founding and leading a rural settlement where such people could be trained in agriculture. There were two major obstacles to implementing the idea: he had no money to found a settlement, and he knew nothing about agriculture. The second was the easier to overcome, so, against the advice of his seniors, who saw no future for a chemist in agriculture, when a lectureship became vacant at Wye Agricultural College, he applied for it, was appointed and started working there in January 1901. His social work played another important part in his life, for it was through it that he met Elnor Oldham, whom he married in 1903. They had six children, of whom five survive.

At Wye, Russell started the fruitful collaboration with A. D. Hall, principal of the College until 1902 when he moved to Rothamsted, that led among many other things to *The Soils and Agriculture of Kent, Surrey and*

*Sussex*, which provided the model for future surveys. As Russell saw more of practical agriculture, he increasingly appreciated not only the impracticability of his ideas about a rural settlement, but also that agriculture itself was woefully backward and urgently in need of research. His interests then changed and he turned his missionary zeal to getting scientific methods applied to agriculture; the career he was to follow so successfully had now begun. The course of lectures he gave at Wye on agricultural chemistry also formed the framework for his classic book *Soil Conditions and Plant Growth*, first published in 1912, which has passed through nine editions, the last two by his son, Prof. E. W. Russell, and has had a world-wide influence.

In 1907, Russell followed Hall to Rothamsted, where he was appointed Goldsmith's chemist, and in 1912 he succeeded Hall as director—a post he held with increasing distinction for thirty-one years. Hall did much to revive Rothamsted, where research had sadly declined from its peak in the earlier years of Lawes and Gilbert; but it was Russell who fashioned the comprehensive programme of research which now characterizes the Station. Russell was an able chemist and research worker and did some outstanding work on soils, particularly on partial sterilization, but his great achievements lie much more in the way he influenced the development of agricultural science than in his personal research. As a chemist interested in soils, it was to be expected that he would maintain and extend the work on crop nutrition that had made Rothamsted famous in the nineteenth century, but there were probably few other chemists who would have appreciated, as he did, the need for adding such diverse subjects as botany, physics, microbiology, entomology, pathology, biochemistry, statistics and the study of pesticides. Not the least of his qualities that helped to make his ventures succeed was his ability to judge people shrewdly and select able ones to act as leaders.

The extent to which he developed Rothamsted was all the more notable considering the spirit of the time when he did it. Except for a brief period after the end of the First World War, when neither the British Government nor people could fail to see the need for agriculture and the economic value of agricultural research, he got little official support for his proposals. In the 1930s when the Rothamsted Estate was up for sale, the Government was so uninterested that it did nothing to prevent it from being bought by a speculative builder; but Russell was undeterred by this lack of official backing, and with characteristic determination and enthusiasm raised the money to buy the Estate and preserve it for agricultural research. Similarly, he organized the centenary fund, which paid for the urgently needed new laboratories and glasshouses built in 1939. For these activities, all those who have since worked at Rothamsted and those still to come owe him an immense debt of gratitude.

In both World Wars, Russell served on many Government committees, dealing not only with agricultural subjects but also many others. His services as an adviser were also often sought by, and always willingly given to, many foreign Governments and organizations overseas. The value of these services to both agriculture and science is amply indicated by the list of honours and awards he received from British and foreign universities, academies, Governments and scientific societies—a list far too long to recount here. In all these, he took a due pride, but they left him free from conceit, never pompous or self-important, and always friendly and courteous.

Russell's memory, both for facts and people, was great. He seemed never to forget anyone he had

met or anything he had seen, read or heard. As he loved meeting people, travelled extensively and read widely, his knowledge was encyclopaedic, as is evidenced by his notable survey, *World Population and World Food Supplies*, published in 1954. An excellent lecturer and prolific writer, he dispensed information as readily as he acquired it. Always a keen supporter of the British Association, he rarely missed a meeting; he was its president in 1949, the first agricultural scientist to be elected to this position and perhaps of his many distinctions the one in which he took most pride.

A serious illness incapacitated him for a year shortly after he retired from Rothamsted; but he soon resumed a fully active life, not only continuing his writing and lecturing, but also his travelling. His interest in, and knowledge of, agricultural science was undimmed by age, and at ninety and more he was still asking the most penetrating questions, often dealing with subjects that had barely been born when he retired. His last visit to Rothamsted, to celebrate his ninetieth birthday, was an unforgettable occasion. He was his usual, lively self and without a note made a speech lasting more than forty minutes that was as memorable for his stimulating ideas about the current and future needs of agricultural research as it was for the wit and humour with which he recalled its history.

Only a man of exceptional ability, determination and energy could have achieved what he did, for during most of the first sixty years of his life he was continually battling to overcome difficulties and indifference to his aims. To have left school at fourteen and be elected to the Royal Society at forty-five is possibly unique, for self-made success is much rarer in research than in business or industry. But to have promoted agricultural research successfully in a period when a secretary of the then Board of Agriculture could say: "I cannot conceive the circumstances in which the board will be at all interested in scientific work", was a still greater achievement. The Royal Society of Arts awarded Russell the Albert Gold Medal—its greatest honour. The inscription reads, "For researches and leadership in agricultural science and services to husbandry in many lands"; although it fails to show that he also led a successful rebellion against ignorance and indifference, it is difficult to think of a more fitting epitaph.

F. C. BAWDEN

#### Dr. R. A. Alexander

RAYMOND ALBERT ALEXANDER died in Pretoria on July 8 at the age of sixty-six. He was born in Benoni, Transvaal, on July 29, 1899, and was educated at Jeppe High School, Johannesburg. After matriculating in 1915 he studied at the Potchefstroom School of Agriculture and was afterwards accepted for military training. In 1919 he went to the Transvaal University College and obtained the degree of bachelor of science, agriculture, in 1922. He then enrolled as a student in the newly formed Faculty of Veterinary Science under the renowned Sir Arnold Theiler and was awarded the degree of bachelor of veterinary science in 1925. In 1928 he joined the staff of the Veterinary Research Institute, Onderstepoort, and started his career as a virologist.

Having been awarded a fellowship by the Empire Marketing Board of Great Britain in 1931, Dr. Alexander studied at the Strangeways Institute, Cambridge, where he worked on tissue culture techniques under Dr. (now Dame) Honor B. Fell. During this time he also represented South Africa at the first Commonwealth Veterinary Conference in London. On his return to South Africa he continued his research work on virus diseases and produced original studies on the neurotropic virus of horse-sickness. As a result he was awarded a D.V.Sc. degree in 1935. In 1938 he again went overseas and worked for varying periods at the Rockefeller Institute, Harvard University, the Federal Bureau of Animal Industry and

the Canadian Veterinary Laboratory at Ottawa. When an extensive outbreak of horse-sickness occurred in Egypt and the Middle East during 1944, Dr. Alexander went to the assistance of the affected countries and was able to help control the scourge by means of the vaccine which he had developed at Onderstepoort.

He was appointed deputy director of veterinary services in 1949 and in that year took a leading part at the African Rinderpest Conference held in Nairobi, and the International Rinderpest Conference convened by the Food and Agriculture Organization of the United Nations. Later he served on a Commission appointed by the British Colonial Office to investigate veterinary problems and research in East African territories. In 1953 he was invited by the United States Government to visit America and to advise on the control of blue-tongue in sheep which was causing devastating losses.

Dr. Alexander was appointed director of veterinary services of the Union of South Africa in 1950 and served in this capacity for 11 years until he retired on superannuation.

He devoted his life to furthering veterinary science in general and virology in particular. As a result of his success in this respect he held many important posts, among which were the following: chairman of the Inter-African Bureau of Animal Health (1955-58); life vice-president of the South African Veterinary Medical Association; member of the Committee for Research into Medical Sciences of the Council for Scientific and Industrial Research; chairman of the Committee of the Virus Diseases Research Unit of the University of Cape Town; chairman of the Veterinary Board; member of the Technical Advisory Committee of the Poliomyelitis Research Foundation and of the Advisory Committee on Virology to the South African Minister of Health; consultant to the Food and Agriculture Organization of the United Nations/Office International des Epizooties at a world conference on emerging diseases held at Ankara.

Several honours came to Dr. Alexander. In 1955 he was awarded a D.Sc. (*honoris causa*) by the University of Cape Town and in 1957 he was elected a Fellow of the Royal Society (South Africa). In 1961 he was elected an honorary member of the Section of Comparative Medicine of the Royal Society of Medicine.

As a virologist, he gained a world-wide reputation and produced more than sixty scientific publications. He will be remembered for his enthusiastic participation in scientific meetings and his practical approach to animal disease problems.

B. C. JANSEN

#### K. A. Vlasov

KUZMA ALEXANDROVICH VLASOV, a prominent Soviet petrologist and geochemist, died on September 29, 1964. He was born in the Ryazan district of Russia on November 14, 1905. In 1931 he graduated in geology at the Timiryazev Academy and soon began field work on the Kola Peninsula under the guidance of A. E. Fersman. Starting with a study of beryllium minerals, Vlasov went on to investigate beryllium-bearing pegmatites, and this led in turn to an intensive study of granite-pegmatites in general. The results of his field observations were summed up in a comprehensive genetic-textural classification of granite pegmatites. His *magnum opus*, however, was his detailed work on the geology, petrology and geochemistry of the Lovozero alkaline massif on the Kola Peninsula. This was published in 1959. In 1953 Vlasov was elected a corresponding member of the Academy of Sciences of the U.S.S.R. and in the same year he was appointed director of the newly founded Institute of Mineralogy, Geochemistry and Crystallography of Rare Elements. The work of the Institute is being published in a three-volume edition (two volumes have already appeared), entitled *Geochemistry, Mineralogy and Deposits of Rare Elements*.

S. I. TOMCHENKO



## NEWS and VIEWS

Physical Chemistry in the University of Cambridge :  
Prof. R. G. W. Norrish, F.R.S.

ON September 30, Prof. R. G. W. Norrish will retire from the chair of physical chemistry in the University of Cambridge, a post which he has held with distinction for the past twenty-eight years. There can be few whose careers have been so completely linked with one city and its institutions. Norrish attended the Perse Grammar School in Cambridge, from which he entered Emmanuel College as a Scholar in 1915. He has since held a fellowship of that College for many years. Following war service, he took a double first in the Natural Sciences Tripos. He gained a Ph.D. in 1924 and an Sc.D. in 1936. He was appointed a demonstrator in physical chemistry in Cambridge in 1926, and later became Humphrey Owen Jones lecturer—a post which has been held by several physical chemists now occupying distinguished positions. He was elected a Fellow of the Royal Society in 1936 and in the following year became professor of physical chemistry at Cambridge. The great bulk of Norrish's work has been in reaction kinetics, much of it with a strong photochemical emphasis, and has been notable for a pronounced flair for clear experiments. Another interesting feature is the way it has taken the impact of the application of quantum mechanics in its stride. Norrish's attachment to photochemistry showed itself in early papers (1923) on potassium permanganate, and was followed by distinguished work on the effects of light on nitrogen dioxide and on chlorine. This led him into the fields of photosensitization and of chain reactions, in which he made outstanding investigations. His classical series of papers in the late 1930's on photolysis of aldehydes and ketones must also be mentioned.

All these lines have been pursued more recently, but the early 1950's saw the introduction of a remarkable new phase of activity based on flash photolysis and high-speed spectroscopic techniques, which enabled very rapid reactions and the details of exchange of molecular energy to be elucidated. This has been one of the most fruitful fields in which Norrish has worked, and to which, at the moment of retirement, he is contributing with distinction. Norrish's work has been recognized by foreign honours, among them an honorary doctorate of the University of Paris (Sorbonne), foreign membership of the Polish Academy of Sciences, and corresponding membership of the Academy of Sciences of Göttingen and of the Royal Society of Sciences of Liège. He has been president of the Faraday Society and vice-president of the Royal Institute of Chemistry. The quality of his research was recognized very early with the Meldola Medal of the Royal Institute of Chemistry in 1926, and confirmed by the awards of the Davy Medal of the Royal Society (1958) and the Faraday Medal of the Chemical Society (1964). He will be wished a long and active retirement by past members of his Department, who include eleven Fellows of the Royal Society, and by an enormous number of colleagues all over the world.

Prof. J. W. Linnett, F.R.S.

DR. J. W. LINNETT, reader in inorganic chemistry in the University of Oxford, who succeeds Prof. R. G. W. Norrish as professor of physical chemistry in the University of Cambridge in October, was educated at King Henry VIII School, Coventry, and at St. John's College, Oxford, where he was a pupil of Prof. H. W. Thompson.

After graduating in 1935 with first-class honours in chemistry he spent two further years in Oxford on research work for a D.Phil., and then went to Harvard for a year as a Henry Fellow. He returned to Oxford as a Junior Research Fellow of Balliol, and in 1945 was elected to a fellowship at The Queen's College. His first research interests were infra-red spectroscopy and molecular force fields, and later he turned to the application of quantum mechanics to problems of molecular structure. But although the author of numerous theoretical papers, he has also been actively involved in the experimental study of chemical kinetics, first of gaseous chain reactions by conventional methods, then of reactions in flames, and more recently of atom-recombination reactions. He has been prominent in administration, both in Oxford and elsewhere, being a vice-president of the Faraday Society and a secretary of the Chemical Society, and he has travelled and lectured widely. He was elected a Fellow of the Royal Society in 1955. Cambridge therefore gains, at Oxford's expense, a very talented physical chemist of wide interests and considerable experience.

Materials Technology in the College of Advanced  
Technology, Birmingham : Prof. H. J. Pick

DR. H. J. PICK has been appointed professor of materials technology in the Department of Metallurgy in the College of Advanced Technology, Birmingham (University of Aston in Birmingham designate), and will take up his new duties on September 1. On leaving Torquay Grammar School, Dr. Pick attended the University of Liverpool where, after a break of three years during the Second World War when he served as a pilot in the Royal Air Force, he obtained in 1949 a B.Sc. degree in engineering with honours in metallurgy. After a further two years at the University of Birmingham he was awarded a Ph.D. for research on industrial metallurgy. Dr. Pick is at present chief metallurgist with Joseph Lucas (Electrical), Ltd., Birmingham, and he previously held appointments as Research Fellow and as lecturer in industrial metallurgy at the University of Birmingham. He has carried out a considerable amount of research on casting and electro-deposition.

## Social Anthropology in the University of Hull :

Prof. I. G. Cunlison

PROF. I. G. CUNLISON has been appointed to the newly established chair of social anthropology in the University of Hull from January 1, 1966. Prof. Cunlison read for Part I of the Modern Languages Tripos at Cambridge before serving with the Royal Artillery during 1942–45. When he returned to Cambridge he took Part II of the Anthropology and Archaeology Tripos, graduating with first class honours. In 1952 he was awarded a D.Phil. degree by the University of Oxford, where he had been a research assistant in the Institute of Social Anthropology. After three years as research officer at the Rhodes-Livingstone Institute, Northern Rhodesia, he became an assistant lecturer in social anthropology in the University of Manchester. In 1952 he was appointed social anthropologist to the Sudan Government, and returned in 1955 to the University of Manchester, where he was Simon Research Fellow and later lecturer in social anthropology. He became head of the Department of Social Anthropology and Sociology in the University of Khartoum in 1959, where since 1964 he has been professor of social anthropology.



### Commonwealth Students in Britain

In a written answer in the House of Commons on July 16, Mr. A. E. Oram, the Parliamentary Secretary to the Ministry of Overseas Development, gave the number of students from each of the Commonwealth countries taking full-time courses in the United Kingdom in the academic years 1959-60-1963-64. To the total, which had risen from 30,169 in 1959-60 to 35,664 in 1960-61, 39,122 in 1961-62, 42,094 in 1962-63, and 42,322 in 1963-64, Nigeria, with 6,000, 6,800, 7,836, 8,954 and 8,630, respectively, was the largest individual contributor, followed by India, with 3,510, 3,400, 3,496, 4,281 and 4,129. Malaya, with 2,200, 2,220, 2,428, 2,496 and 2,882; Ghana (1,840, 3,793, 3,348, 1,885 and 1,991); Jamaica (1,940, 2,980, 3,151, 4,083, and 3,841); followed by Hong Kong (1,550, 1,580, 1,559, 1,619 and 1,715) and Pakistan (1,570, 1,245, 1,470, 1,711 and 1,924) came next. Trinidad and Tobago (1,100, 1,370, 1,622, 1,858 and 1,844) and Kenya (1,030, 1,272, 1,592, 1,532 and 1,593) were the only other countries to send more than 1,000 a year until 1960-61 when this figure was exceeded by Barbados; in 1961-62 it was also exceeded by Uganda, in 1962-63 by British Guiana, and in 1963-64 by Ceylon and Cyprus.

### Postgraduates at Universities in Britain

In a written answer in the House of Commons on August 4, Mr. A. Crosland, the Secretary of State for Education and Science, stated that the number of postgraduates at universities in Britain had risen from 1,400 in 1920-21 and 2,237 in 1930-31 to 11,327 in 1950-51, 12,668 in 1955-56, 17,836 in 1960-61 and 24,255 in 1964-65.

### Medical Research and Work Study

In a written answer on July 16, Mr. A. Crosland stated that expenditure by Government agencies, including the Medical Research Council, the Health Departments, Ministry of Defence, and the proportion devoted to medical research from the general grant to the universities, is estimated at £5.5 million in 1954-55, £6.5 million in 1955-56, and £7 million in 1956-57, rising to £14.5 million in 1961-62, £16 million in 1962-63, £18.5 million in 1963-64 and £23 million in 1964-65. No reliable estimate could be given of expenditure on medical research by other bodies including the pharmaceutical industry and voluntary organizations. During the academic year 1964-65, 623 students attended the Work Study School at the Royal College of Aeronautics, Cranfield, and, of its income of £55,165, £55,131 was from fees paid by sponsoring firms and organizations. For the same year, the College received a recurrent grant of £865,000 and non-recurrent grant of £96,005, as well as a capital grant of £28,995; for 1965-66 these were estimated at £825,000, £66,500 and £103,500, respectively. The College was the only exclusively post-graduate technological teaching institute of its kind in Britain, and the net cost per student had been reduced to a little more than £2,000 in the current year.

### Industrial Training Boards

In replying for the Government on July 29 to a Motion to annul the Industrial Training Levy (Engineering) Order, of June 11, 1965, Mr. R. Marsh, the Joint Parliamentary Secretary to the Ministry of Labour, said that the Engineering Board Levy of 2.5 per cent was likely to yield about £80 million in the first year. Direct Treasury assistance to the boards up to March 31 was about £135,000, apart from other assistance, such as senior staff, and during the current year direct Government grant to the boards would be about £1.5 million, compared with approaching £100 million from the levies, and none of this money would leave the industry. The Motion was withdrawn. In a written answer on August 2, Mr. R. J. Gunter, the Minister of Labour, who had previously given

the names of the chairman and deputy-chairman and members of the Ceramics, Glass and Mineral Industry Training Board, stated that, in January-June 1965, 35,434 boys and 5,717 girls gained apprenticeships on first entering employment, or 37.2 per cent and 6.5 per cent, respectively, compared with 34,221 (32.6 per cent) and 6,030 (6.1 per cent), respectively, in January-June 1964.

### In-Service Training for Teachers of Mathematics

THE serious shortage of teachers of mathematics will not be remedied by any single stroke, but one contribution to the solution of the problem lies in the development of in-service training, whereby those already teaching may be encouraged to revise and extend their knowledge of mathematics and so to fit themselves for coping with modern trends in mathematics and for undertaking greater responsibilities. Several institutes of education have provided excellent courses to this end, but much remains to be done, and the Joint Mathematical Council now offers a short report by its working party, in which a national scheme is propounded (*Report on In-Service Training for Teachers of Mathematics*. Pp. 12. London: Prof. J. G. Semple, Hon. Secretary, Joint Mathematical Council of the United Kingdom, c/o King's College, 1965). It suggests: (i) the setting up of mathematics centres to provide the necessary facilities, under local education authorities; (ii) the establishment by each institute of education of an advisory unit; (iii) that the Schools Council for the Curriculum and Examinations should set up a permanent sub-committee to recruit and train key personnel and to obtain necessary funds; (iv) the establishment of a national information centre to link together all people concerned and to provide information on all relevant topics. The report does not waste time and paper on deploring the present situation, but concisely and forcefully makes precise suggestions for the development and organization of a valuable constituent in any final solution of an urgent problem.

### Information and Communication

*Focus on Information and Communication*, recently issued by Aslib and edited by Barbara Kyle, includes seven papers (Pp. viii+113. London: Aslib, 1965. 14s.). It incorporates Barbara Wootton's two papers on problems in communications (reprinted from *Aslib Proceedings*, November 1963 and November 1964); A. R. Meetham's paper on preliminary studies for machine-generated index vocabularies (*Language and Speech*, January-March 1963); W. T. Williams's paper on "Computers as Botanists" (*Nature*, 179, 146; 1963); R. Crawshaw-Williams's paper on "The Double Criterion of Empirical Judgment" (previously circulated privately in duplicated form); Prof. P. Meredith's "Documents, Programmes and Topics—Some Observations on Topic Analysis" (based on two earlier papers on topic analysis and an analysis of relations); Prof. J. K. Feibleman's "The Integrating Levels in Nature" (a revised version of an earlier paper in the *British Journal for the Philosophy of Science* in 1954).

### Scientific Information Notes

THE June-July 1965 issue of *Scientific Information Notes* (7, No. 3; 1965), issued by the National Science Foundation, includes further information on the two-year experimental programme to provide a rapid source of chemical information for which the Science Adviser to the President has announced a 2-million dollar contract between the Foundation and the American Chemical Society. It provides for the establishment, by the Society's Chemical Abstracts Service, of a computer-based registry system for chemical compounds based on chemical structure and for selected research. It is expected that

some 400,000 references will be fed into the system each year, of which 75,000 will be to new compounds, and the system will identify any compound which has previously been processed and assign the same number to it each time it appears. The Federal Council for Science and Technology has made a task group of its Committee on Scientific and Technical Information responsible for developing a long-range plan for improving national scientific and technical information systems. The Atomic Energy Commission and the National Bureau of Standards are jointly sponsoring an Atomic and Molecular Processes Information Centre at the Oak Ridge National Laboratory to collect, store, evaluate and disseminate information from all over the world relating to atomic and molecular cross-section and other particle collision processes in the three areas: interaction of heavy particles; particle penetration through matter; excitation, dissociation, ionization and detachment by external electric and magnetic fields.

#### The Royal Ontario Museum, University of Toronto

THE annual report of the Royal Ontario Museum for the year July 1963–June 1964 is of special interest in that it records the reorganization that has been undertaken by the new director, Prof. W. E. Swinton, late of the British Museum (Natural History) (Pp. 16. Toronto: Royal Ontario Museum—University of Toronto, 1965). In brief, the director now takes a larger place in the University, becoming a member of the Senate, and of the Council of the Faculty of Arts and Sciences. In the Museum he is responsible for general policy, curatorial matters, display and academic publication. The Associate Director is responsible for the non-curatorial staff, and for day-to-day administration. The abolition of the divisional structure gives a large measure of autonomy with, of course, increased responsibility to each of the departments. All curators have direct access to the Director, and each has inaugurated research programmes of a high order. This new upsurge of activity has already borne fruit in the form of publications completed during the year and now in the press. In several cases some of this work has been done jointly with other institutions, for example, with McGill University in ichthyological researches in Barbados, and with the National Museum of Canada in an anthropological project.

#### The Museums and Art Gallery, Leicester

THE fifty-eighth report of the Committee of the City of Leicester Museums and Art Galleries, for 1963–64, records that much thought has been given to the establishment of a museum of science and technology (Pp. 13. Leicester: The Museums and Art Gallery, 1965). The popular interest shown in industrial archaeology indicates that this is a growing study, the importance of which should be reflected in the Museums. However, material is being collected and this eventually will form the nucleus of the collections. The new Museum of Archaeology on the Jewry Wall has been completed and the Committee has plans to tidy up the Newarke Gateway. An interesting appointment was that of Mr. G. J. Bemrose, late director at Stoke-on-Trent Museums, to advise on the purchase of ceramics—a branch of applied art in which the Museum's collections were curiously deficient. Although the Committee is continuing to train student-assistants and appoint trainee-assistants, there is still a dearth of officers for the more senior posts, and most of the departments of the Museum are understaffed. The report is well illustrated, has an arresting cover, and concludes with an impressive list of acquisitions.

#### Atlas of the Antarctic

THE weekly journal of the Russian book trade, *Novye Knigi*, announces the forthcoming appearance, late in 1965, of a major *Atlas of the Antarctic*, to be published by

the Main Administration for Geology and Cartography (GUGK) of the U.S.S.R. The work will incorporate the results of the cartographical, geological and geophysical researches of the twelve nations which have collaborated in Antarctic work during and since the International Geophysical Year. It is planned in two volumes, the first of which (Pp. 300, size 38 × 60 cm., price 30 roubles) will comprise maps of all kind, and the second (price 5 roubles) an explanatory text. Orders placed through the national agencies for Russian books should quote *Novye Knigi* 25–1965, item 21.

#### Scottish Field Studies

THE Scottish Field Studies Association opened its Field Studies Centre at Kindrogan, Perthshire, in November 1963, and, in its annual report for 1964, reviews the Centre's activities to date (Pp. 25 + 4 plates. Glasgow: The Scottish Field Studies Association, 1965. 5s.). During the first season, 502 students attended, 134 taking courses in geography, 72 in biology, 175 in botany and 121 in a combined course of biology and geography. 214 of the students came from schools, 110 from universities and technical colleges, 54 from training colleges and 124 paid 'unattached' visits. With its attractive facilities, Kindrogan should gain increasing support; however, although it has received generous financial support from educational bodies, the complete lack of response to an appeal from Kindrogan to Scottish industry is not only lamentable but shows how short-sighted industrialists can sometimes be.

#### Comparison of Colorimeters

THE Association of Clinical Biochemists has published Scientific Report No. 1, entitled *Colorimeters Fitted with Flow Through Cells* (Pp. 54. Liverpool: J. T. Ireland, Biochemistry Laboratory, Alder Hey Children's Hospital, 1965. 13s. 6d.; Association members, 8s. 6d.). In these instruments, the optical densities of a number of solutions, for example a batch of blood haemoglobin solutions, can be measured without removing the optical cells from their holders. The report gives a detailed and critical appraisal of four commercial colorimeters of this type. All four contained a selenium photocell and the wave-length band was selected by use of colour filters or an interference wedge. The report also covers such topics as maintenance, faults developing during use and the adequacy of the instruction booklets. The methods developed for assessing the performance of the instruments should be helpful to anyone wishing to test the performance of any colorimeter. The report should also be helpful to manufacturers anxious to improve the design and reliability of their instruments.

#### Progress In Concrete Research

As forecast in the previous report for 1963 (*Nature*, 204, 233; 1964), the Hon. Leo Russell, director-general of the Cement and Concrete Association, in his introduction to the report for 1964 confirms that the increase in the financial resources of the Association has been reflected in each of its main fields of activity during that year (Pp. 116. London: Cement and Concrete Association, 1965). In research and development projects, special mention is made of the work on factors affecting skidding resistance of concrete roads at high speed, carried out in close collaboration with the Road Research Laboratory; on investigations of surface appearance of *in situ* concrete, culminating in the symposium organized jointly with the Royal Institute of British Architects in October 1964; and on the introduction of 'Standard Mixes' by the committees responsible for the preparation of the new *Code of Practice for Precast Concrete* (CP 110) and for amendments to the *Code of Practice for Reinforced Concrete* (CP 114). Further developments in the matter of standard prestressed precast concrete beams for bridges are also

noteworthy. The continuing research on materials, design of concrete structures, and on methods of construction is adequately catalogued. On the materials side are discussed fundamental properties of unstressed concrete; properties of concrete under stress; durability of concrete; X-ray diffractometry of cement minerals; the system  $\text{CaO-Al}_2\text{O}_3\text{-SO}_3$ ; hydration of cement; problems of chemical analysis of cement and concrete; research on concrete under stress; elasticity of high-strength concrete; damping capacity; concrete strength testing; relationship between bending strength of reinforced concrete beams and concrete cube strength; perlite aggregate concrete; and dimensional changes in unloaded concrete. In the section recording research on design of structures, among other subjects, mention is made of the work on bridges; doubly curved shell roofs; ultimate load behaviour of framed structures; analysis of shear walls in multi-storey structures; and model testing of structures. Methods of construction include work on factors affecting appearance of concrete surfaces; on omission of expansion joints from concrete roads; on skidding resistance; and on no-fines concrete for paving. Looking ahead, there is immense scope for development of precast concrete structural units based on standard mould sizes; the increased capacity planned for production of high-quality concrete facing blocks should materially help to relieve the present shortage of building bricks.

### Prestressed Concrete and Machinery Structures

A SYMPOSIUM on the "Application of Prestressed Concrete to Machinery Structures" was held during January 14-15, 1964, at the Conference and Training Centre of the Cement and Concrete Association, Wexham Springs, Slough, Buckinghamshire, on behalf of the Committee set up to examine this subject by the Fédération Internationale de la Précontrainte. The *Proceedings* of this symposium have recently been published by the Prestressed Concrete Development Group (Pp. 40. London: Prestressed Concrete Development Group, 1965. 25s.). Following a brief address of welcome by A. R. Collins (director, Research and Technical Services, Cement and Concrete Association), the opening address was delivered by Y. Guyon (président, Fédération Internationale de la Précontrainte) wherein he described the subject as "... certainly a fascinating one, and the field so far is almost virgin, with a tremendous potential for development". The papers which followed were: "Prestressed Concrete and its Relevance to Machine Frames" by A. J. Harris; and "Local and Bearing Stresses" by R. E. Rowe. Under the general heading "Applications of Prestressed Concrete to Machinery Structures", six papers were presented: "Power Stations" by C. D. Crosthwaite; "Dynamic Loading" by J. H. A. Crockett; "Some Structures subject to Shock Loading" by F. Walley; "Foundations for Heavy Plant" by Th. Hinckeldey and R. Oppermann (West Germany); "Presses and Plant for Heavy-Metal Cutting" by J. T. Lewis; and "Triaxially Prestressed Bedplates or Heavy Frames" by I. G. Ludkovsky (U.S.S.R.). The summing up and general conclusions reached at this symposium were left to A. J. Harris and are given at the end of this publication, which certainly breaks new ground and for this reason alone is an important contribution to our knowledge of the use and scope of prestressed concrete.

### Magnetism Group

THE Council of the Institute of Physics and the Physical Society has announced the establishment of a Magnetism Group. The aim of the new Group is to further interest in magnetism by holding regular discussion meetings and in other ways. It is intended that these meetings shall be of relevance to physicists from industry, Government establishments and academic institutions and co-operation will be maintained with

other specialist groups of the Institute and Society, and with similar groups at home and abroad. The first meeting of the new Group will be held in December 1965 and further details will be announced later. The following have been appointed as the provisional Committee and granted power to co-opt additional members: Dr. W. M. Lomer (chairman); Prof. E. B. Wohlfarth (honorary secretary); Dr. G. G. E. Low; Mr. R. D. Lowde and Dr. W. Marshall. Further information, including details of membership of the Institute and Society and of its Magnetism Group, can be obtained from the Registrar, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

### Announcements

MR. R. ORGAN, formerly chief experimental officer in the research laboratories of the British Museum, has been appointed curator of conservation at the Royal Ontario Museum.

THE bicentennial celebration commemorating the birth of James Smithson will be held at the Smithsonian Institution during September 16-18. Further information can be obtained from the Smithsonian Institution, The Mall, Washington 25, D.C.

A SYMPOSIUM on "Insect Behaviour", arranged by the Royal Entomological Society of London, will be held at the Imperial College of Science and Technology during September 23-24. Further information can be obtained from the Registrar, Royal Entomological Society of London, 41 Queen's Gate, London, S.W.7.

A SYMPOSIUM on "The Soil Resources of Tropical Africa", organized by the African Studies Association of the United Kingdom, will be held at University College, London, on September 29. Further information can be obtained from Prof. J. D. Fage, Centre of West African Studies, University of Birmingham, Birmingham 15.

A CONFERENCE on "Optics in Space", arranged by the Optical Group of the Institute of Physics and the Physical Society, will be held at the University of Southampton during September 27-30. Further information can be obtained from the Meetings Officer, Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

A CONFERENCE on "Non-metallic Thin Films", arranged by the Electronics Group of the Institute of Physics and the Physical Society, will be held at Chelsea College of Science and Technology during September 23-24. Further information can be obtained from the Meetings Officer, Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

THE twentieth annual "Electronics, Instruments, Controls and Components Exhibition and Convention" of the Institution of Electronics (Northern Division) will be held at Belle Vue, Manchester, during September 28-October 2. Further information can be obtained from Mr. W. Birtwistle, Institution of Electronics, 78 Shaw Road, Rochdale, Lancashire.

**CORRIGENDUM.** In the article entitled "Mitosis in Hybrid Cells derived from Mouse and Man" by Prof. H. Harris *et al.*, which was published on p. 606 of the August 7, 1965, issue of *Nature*, the blocks for Figs. 1 and 2 should be interchanged. The legends should stand as at present.

**ERRATUM.** In the article entitled "Correlation between Coding-triplets and Amino-acids" by Dr. S. R. Polo, which appeared on p. 597 of the August 7, 1965, issue of *Nature*, lines 8 and 9 of the second paragraph should read "... No amino-acid in these columns contains a methyl group".

## LIBRARY AND INFORMATION SCIENCES EXHIBITION

By D. L. SMITH, Librarian  
Oxford College of Technology

IN Great Britain library work is still generally considered to be one of the gentler professions, even more perhaps among the dreaming spires of the "home of lost causes" than in industrial cities. Librarianship has not yet been recognised as the library science of the United States. It may seem surprising, therefore, that Oxford should be the centre for an exceptionally comprehensive exhibition of literature devoted to library and information sciences. It is less surprising that a large proportion of the material displayed should have been American, especially in the documentation and information retrieval aspects which were particularly emphasised.

The exhibition was arranged by the Documentation and Supply Centre of Robert Maxwell and Co., Ltd., in their Oxford bookshop below the new buildings of Magdalen College, during July 29-31. Its opening on the first afternoon was attended by a representative cross-section of the academic, technical and scientific library, information officer and book-selling professions and, in the absence of Mr. Robert Maxwell on parliamentary duties, the objectives were explained by Mr. Cadmus Page, who has recently become managing director of the firm's book-selling service. Mr. Page briefly outlined the new imperative need of research workers in every field for rapid, accurate and thoroughly comprehensive literature searches, and said it was fortunate that the much-publicized information explosion had been followed by the introduction of computers, automatic control and punched-card techniques into the processes of documentation and information retrieval. This was the first exhibition of its kind to be held in Britain, and as such was a vital contribution to scientific research. A special value of the occasion lay in the opportunities for informal discussion of present-day problems among the participants.

Tribute was paid to the work of Mr. Hans Zell, in charge of Maxwell's Documentation and Supply Centre, who was responsible for collecting the extremely wide range of literature on display. The office of the Centre, with its permanent collection of bibliographical tools, unrivalled except in the larger university libraries, was also open to visitors; likewise the new warehouse (with storage space for more than three million volumes) and offices, now nearing completion half a mile away at Headington Hill Hall on the site shared with Pergamon Press, which have been planned for the improvement of the firm's nationwide and international service for the supply of books to libraries. Mr. Zell is already well known as editor of a number of other useful lists and bibliographies distributed to librarians, including *New Reference Tools for Librarians* of which the third annual volume has just appeared; subject bibliographies on a wide range of topics, such as cartography, nutrition, graphic arts and industries, quality control and biophysics, all international in their coverage; and the new 300-page *International Bibliography of Non-periodical Literature on Documentation and Information*, the publication of which coincided with the opening of the exhibition. More specific in its content, the latter gives full details of more than 1,500 items published since 1930 (Russian material being excluded), with appendixes devoted to relevant periodicals and associations; arrangement is alphabetical, with a manually produced, permuted title index, and it is planned that a more useful subject index, produced by computer, will be incorporated in a later edition.

Another service operated from the same Documentation and Supply Centre, and now firmly established, is the punched-card system giving advance information on new books, batches of cards being distributed free to individual libraries on the basis of their own subject coverage.

In support of the exhibition the same editor has issued an attractive and clearly laid-out catalogue of its contents, with a supplement to bring it right up to date—nearly 800 books and pamphlets from eighteen countries altogether. While the emphasis is on modern methods of storing and speedily retrieving information in print and in every recorded form—the mechanized systems available for cataloguing, indexing, storage, location and extraction—the exhibition also embraced every branch of library science and librarianship in the more traditional sense, and includes a comprehensive range of recently published bibliographies, encyclopaedias and other reference books in every field likely to be of use to the librarian or reader in search of information. All the material has appeared since the beginning of 1963, and there were a few proof copies of books to be published shortly, such as the next two volumes in the "How to Find Out" series from the Commonwealth and International Library of Science, Technology, Engineering and Liberal Studies, of Pergamon Press. One section of the exhibition was devoted to specimen copies of recent journals dealing with information processing, storage and retrieval.

Perhaps the most notable feature of the display and its accompanying catalogue was the success of the efforts made to include a great deal of ephemeral, obscure and out-of-the-way material, such as bibliographies or specialized papers issued by libraries, schools of librarianship, research establishments and some of the less-well-known Government organizations. Many of these are unlikely to be listed in regular national bibliographies or publishers' catalogues. The means by which so many obscure pamphlets had been traced and assembled was a mystery to at least one librarian, and the opportunity of examining so much material of this nature in itself made the whole operation well worth while. This achievement was all the more remarkable in view of the fact that some of the libraries originally approached for details of their publications apparently did not reply at all. Of the catalogue there can be only one small criticism: a contents list of the broad subject headings governing its arrangement would have been helpful, unless it is realized that they are in Universal Decimal Classification order.

From such a wealth of material it is impossible to pick out any items more worthy of notice than others; the smallest and least conspicuous were often as valuable as the largest tomes. Among the latter J. G. Davis's two interesting volumes on *Chaos* seemed at first sight out of place, until it was realized that Volume 2 consists entirely of an annotated bibliography with subject index.

It was perhaps a little unfortunate that the exhibition should have been held at the peak of the holiday season, when many librarians and information scientists were far away; and undoubtedly in London it would have attracted even more attention. The sponsors have announced, however, that it is being repeated at the Library Association Conference at Eastbourne in September, and in Scotland at Maxwell's Glasgow bookshop and Cairns Brothers of Edinburgh; possibly also at Aslib headquarters in London.

## PUBLIC EXAMINATIONS AT OXFORD

THE report of the Committee on the Structure of the First and Second Public Examinations of the University of Oxford, issued in March 1965 as Supplement No. 3 to the *University Gazette* (and now published separately\*), has thirty-two principal recommendations. The first of these is that, since the normal period of study for the first degree cannot be extended beyond three years, more of the top third of the students should be encouraged to stay on, either for a second Honours School or for a B.Phil. or Diploma course. No form of the First Public Examination should be based on less than three terms' work in the University, and the B.A. course should normally be divided so that each undergraduate without senior status has the opportunity of taking two examinations, each of medium size. Nevertheless, any faculty which so desires should be allowed to maintain an undivided Honour School at which candidates can satisfy the requirements of the First Public Examination and the Second Public Examination together at one time.

Provision is also recommended for new combinations of subjects and for "high level conversions" without reducing the standard of honours work and without multiplying separate examinations or special arrangements between faculties. New joint Schools linking the natural sciences and the humanities should be instituted where desirable by special arrangement on the patterns of the Final Honour School of Engineering and Economics. Teachers of the natural sciences in the university are recommended to consider together the possibility of establishing a comprehensive and integrated form of First Public Examination. This would give all students of science what they need in their first year while facilitating

the choices and changes which may be desirable before undergraduates are committed to their Final Honour Schools. The offer of supplementary subjects in a natural sciences course should be further encouraged and Honour Moderations in Classics and Theology retained as examinations to be taken after five terms.

On evidence of ability and industry supplied by his college, an undergraduate, unable by reason of sickness to sit Honour Moderations at the normal date, should be allowed to proceed with his studies as though he had passed, and the normal period of study for a single subject Final Honour School in modern humanities should be four terms for candidates who have already taken an Honour Moderations course of five terms. However, in appropriate cases a candidate who has already taken one such Final Honour School should be encouraged to offer himself for examination in another one year later. It should be possible for selected undergraduates to prepare themselves for examination in two short Final Honour Schools together seven terms after Honour Moderations, and the administration of the new arrangements suggested in the report should be entrusted to two new Boards, one for science and one for humanities.

Abolition of the Pass School is recommended, but an undergraduate who has to abandon hope of finishing a full Honours course should be given credit for what he has already done and be able to qualify for a degree, either by a reduced version of what he originally intended or by a wider and more elementary course constituted from pieces of the First Public Examination. Discussion of the structure of the University's examinations should proceed on the assumption that the University year will not be changed, and the University should negotiate with other British universities for greater uniformity in the titles of higher degrees.

\* University of Oxford. Report of the Committee on the Structure of the First and Second Public Examinations. (Supplement No. 3 to the *University Gazette*, March 1965.) Pp. 141. (Oxford: The University, 1965) 10s.

## CODING RESPONSE OF N-FORMYL-METHIONYL-sRNA TO UUG

By DR. B. F. C. CLARK and DR. K. A. MARCKER

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RECENTLY, it was established that methionyl-sRNA is partly converted to N-formyl-methionyl-sRNA in a cell-free system from *Escherichia coli*<sup>1</sup>. The function of N-formyl-methionyl-sRNA in relation to the biosynthesis of protein has not been elucidated so far. We have investigated the coding characteristics of methionyl-sRNA and of N-formyl-methionyl-sRNA by using them as sources of amino-acids for polypeptide synthesis in a cell-free system and by binding them to ribosomes in the presence of specific triplets.

Studies of the T-1 ribonuclease digestion products of unfractionated partially formylated methionyl-sRNA showed that at least two methionyl-sRNA species exist in *E. coli*, and at least one of them cannot be formylated<sup>2</sup>.

Nirenberg's group first reported that poly UG stimulated the incorporation of methionine into protein-like material<sup>3</sup>, but their later preparations of poly UG failed to do so<sup>4</sup>. Because preparations of poly UAG and of poly UAGO stimulated methionine incorporation, the codeword UAG, of unspecified sequence, was assigned to methionine<sup>4,5</sup>. In recent months, most possible ribonucleotide triplets of specified sequence have been assigned to one or other of the 20 naturally occurring amino-acids<sup>6,7</sup>,

using the elegant technique of Nirenberg and Leder<sup>8</sup>. In particular, methionyl-sRNA was found to be coded for by the triplet AUG<sup>7</sup>.

We have been able to repeat the earlier observation that poly UG stimulates the incorporation of methionine into polypeptide using a partially purified *E. coli* cell-free system with negligible formylation activity and a polymer with a base analysis of U/G=1.8/1. When N-formyl-methionyl-sRNA or the means of formylation were added to the cell-free system, there was a clear increase in the rate of incorporation of methionine into polypeptide (Fig. 1).

We investigated the nature of a polypeptide product synthesized *in vitro* in a system involving an S-30 fraction<sup>10</sup> from *E. coli* MRE 600, poly UG, and a mixture of unfractionated methionyl-sRNA and N-formyl-methionyl-sRNA. The amount of <sup>35</sup>S-methionine incorporated in this system was about 12 per cent that of <sup>14</sup>C-phenylalanine. Interestingly, the analysis of the TCA-insoluble material revealed that 80-90 per cent of the incorporated methionine occurred as N-terminal N-formyl-methionine. This suggests that only the methionyl-sRNA which can be formylated responds to poly UG in a protein-synthesizing system.

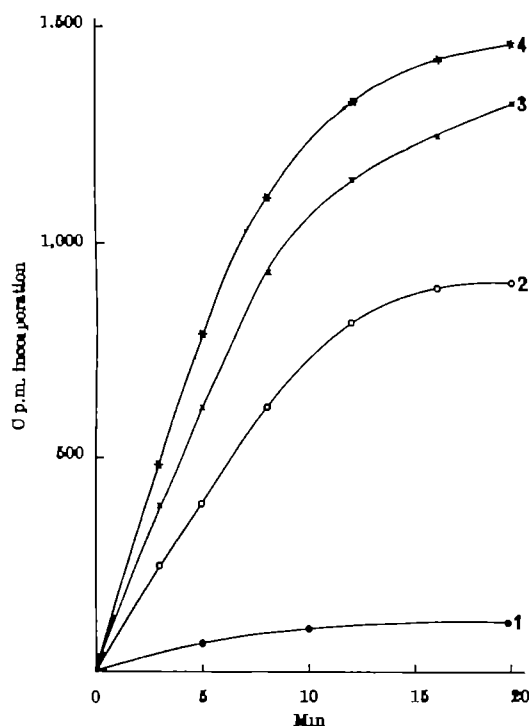


Fig. 1. Incorporation of  $^{35}\text{S}$ -methionine from unfractionated  $^{35}\text{S}$ -methionyl-sRNA. The reaction mixture (0.5 ml.) contained 80  $\mu\text{moles}$  *tris*,  $\text{HCl}$  pH 7.4, 80  $\mu\text{moles}$  ammonium acetate, 5  $\mu\text{moles}$   $\text{MgCl}_2$ , 2  $\mu\text{moles}$  phosphoenolpyruvate, 0.5  $\mu\text{moles}$  ATP, 0.1  $\mu\text{moles}$  GTP, 5  $\mu\text{g}$  pyruvate kinase, 2  $\mu\text{moles}$  mercaptoethanol, 11.2  $A_{260}$  units of *E. coli* *MRE* 600 ribosomes, 150  $\mu\text{g}$  of a partially purified transfer enzyme fraction and 200  $\mu\text{g}$  of  $^{35}\text{S}$ -Met-sRNA ( $1.6 \times 10^5$  c.p.m.;  $1.6 \times 10^5$  c.p.m. = 1  $\mu\text{mole}$ ) which was charged in the presence of the  $^{14}\text{C}$ -amino acids minus methionine<sup>1</sup>. The ribosomes were purified further, after the usual washing, by chromatography on a DEAE-cellulose column<sup>2</sup>. During the incubation at 30° C, 50  $\mu\text{l}$ . samples were removed at the times indicated and added to tubes containing 100  $\mu\text{l}$ . each of 0.5 per cent bovine serum albumin solution. The polypeptide products were precipitated with 10 per cent TCA and then heated at 90° C for 15 min before being collected on 'Oxoid' filters. The radioactivity on the filters was assayed in a Nuclear Chicago thin-window gas flow counter. The curves represent: (1) No polymer added. (2) The presence of 20  $\mu\text{g}$  of poly UG and unfractionated Met-sRNA. When 125  $\mu\text{g}$  of purified transformylase<sup>3</sup> was added the same results were obtained as for curve 2. (3) As for curve 2 but with the addition of 100  $\mu\text{g}$  of *N*-formyl-tetrahydrofolic acid and transformylase. (4) As for curve 2 without transformylase but with about 60 per cent of the Met-sRNA converted into *N*-formyl-Met-sRNA.

Using the binding assay described by Nirenberg and Leder<sup>4</sup>, we obtained the results shown in Table I. Briefly, both a mixture of all methionyl-sRNAs labelled with

Table I. BINDING OF AMINO-ACYL-sRNA TO RIBOSOMES STIMULATED BY TRI- AND POLY-NUCLEOTIDES

Addition ( $A_{260}$ units)	$^{35}\text{S}$ -Met-sRNA ( $\mu\text{moles}$ of Met)	$^{35}\text{S}$ - <i>N</i> -formyl-Met-sRNA ( $\mu\text{moles}$ of Met)
Exp. I		
None	0.14	0.16
UUG (0.20)	1.51	1.74
AUG (0.25)	1.99	2.00
Poly UG (0.40)	0.32	0.34
Poly AUG (0.12)	0.44	0.37
Exp. II		
None	0.06	
UUG (0.24)	0.70	
AUG (0.20)	1.50	
Poly UG (0.20)	0.48	

Conditions for binding were similar to those described by Nirenberg and Leder<sup>4</sup>. The sRNA from *E. coli* B was charged according to Marcker and Sanger<sup>5</sup> but in the presence of 19  $^{14}\text{C}$ -amino-acids. In experiment I, 9.7  $\mu\text{moles}$  of  $^{35}\text{S}$ -*N*-formyl-Met-sRNA or 12.5  $\mu\text{moles}$  of  $^{35}\text{S}$ -Met-sRNA with specific activity of 900  $\mu\text{Ci}/\mu\text{mole}$  were added to the reaction mixture which contained 3.25  $A_{260}$  units of *E. coli* D21/4 ribosomes. Analysis of the polymers gave U/G = 2/1 for poly UG and U/A/G = 4/1/1 for poly UAG.

In experiment II, 6.3  $\mu\text{moles}$  of  $^{35}\text{S}$ -Met-sRNA with specific activity of 2,000  $\mu\text{Ci}/\mu\text{mole}$  were added to a reaction mixture, 0.01 M in  $\text{Mg}^{++}$ . The ribosomes (1.8  $A_{260}$  units) and poly UG were samples of preparations described in Fig. 1.

$^{35}\text{S}$ -methionine (not formylated) and a preparation consisting mostly of *N*-formyl-methionyl-sRNA responded significantly to the triplets UUG and AUG. Recovery and hydrolysis at pH 10 of the bound *N*-formyl-methionyl-sRNA yielded *N*-formyl-methionine, showing that the binding process did not remove the formyl group.

Although the codeword UUG has been suggested for leucine by the binding assay<sup>11</sup> and by the finding that the amber codeword, which is thought to be UAG, spontaneously reverts to a leucine codeword<sup>12</sup>, we propose that in certain circumstances UUG is a codeword for methionine. To clarify our results, investigations using fractionated sRNA are in progress.

<sup>1</sup> Marcker, K., and Sanger, F., *J. Mol. Biol.*, **8**, 835 (1964).

<sup>2</sup> Marcker, K., *J. Mol. Biol.* (in the press).

<sup>3</sup> Martin, R. G., Matthaei, J. H., Jones, O. W., and Nirenberg, M. W., *Biochem. Biophys. Res. Comm.*, **6**, 410 (1962).

<sup>4</sup> Matthaei, J. H., Jones, O. W., Martin, R. G., and Nirenberg, M. W., *Proc. U.S. Nat. Acad. Sci.*, **48**, 666 (1962).

<sup>5</sup> Jones, O. W., and Nirenberg, M. W., *Proc. U.S. Nat. Acad. Sci.*, **48**, 2116 (1962).

<sup>6</sup> Trupin, J. S., Rotiman, F. M., Brimacombe, R. L. C., Leder, P., Bernfield, M. R., and Nirenberg, M. W., *Proc. U.S. Nat. Acad. Sci.*, **53**, 807 (1965).

<sup>7</sup> Nirenberg, M. W., Leder, P., Bernfield, M. R., Brimacombe, R. L. C., Trupin, J. S., Rotiman, F. M., and O'Neal, O. H., *Proc. U.S. Nat. Acad. Sci.*, **53**, 1161 (1965).

<sup>8</sup> Nirenberg, M. W., and Leder, P., *Science*, **145**, 1399 (1964).

<sup>9</sup> Stanley, jun., W. M. (personal communication).

<sup>10</sup> Nirenberg, M. W., and Matthaei, J. W., *Proc. U.S. Nat. Acad. Sci.*, **47**, 1588 (1961).

<sup>11</sup> Leder, P., and Nirenberg, M. W., *Proc. U.S. Nat. Acad. Sci.*, **53**, 1521 (1964).

<sup>12</sup> Khorana, H. G., *U.S. Fed. Proc.* (in the press).

<sup>13</sup> Weigert, M. G., and Garen, A., *Nature*, **206**, 902 (1965). Brenner, S., Stretton, A. O. W., and Kaplan, S., *Nature*, **206**, 904 (1965).

## RNA OF LOW MOLECULAR WEIGHT IN RIBOSOMES OF MAMMALIAN CELLS

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THE presence of RNA of low molecular weight linked to ribosomes in *E. coli* has been reported by several authors (Elson<sup>1,2</sup>, Roesset and Monier<sup>3</sup>, Roesset, Monier and Julien<sup>4</sup>) and its presence in *Blastocladiella emersonii* has been reported by Comb and Katz<sup>5</sup>. According to Roesset *et al.* and Comb *et al.*, this nucleic fraction is heterogeneous when chromatographed on methylated albumin columns (MAK), and is composed of transfer RNA and another fraction called '5S' RNA by Roesset *et al.* We noted in preparations of KB cells and rat liver the constant presence, on the methylated albumin chromatograms, of a peak eluted under the same conditions as those described by these authors. This report describes some properties of this RNA.

KB cells (human epidermoid carcinoma line) were cultivated in Roux bottles and grew exponentially in

monolayers with an average generation time of 24 h, in a casein hydrolysate medium supplemented by 15 per cent of calf serum. The cells were gathered by scraping the wall. Preparations were also made starting with liver cells of male Wistar *OF* rats weighing about 15 g. The animals were killed by decapitation after fasting for 24 h.

Labelling of the KB cells with  $^{32}\text{P}$  was obtained by cellular growth with 2  $\mu\text{Ci}/\text{ml}$ . in a casein hydrolysate medium buffered with *tris* and supplemented by 15 per cent of calf serum dialysed against 0.15 M NaCl.

Total RNAs were extracted from cells disrupted by 0.5 per cent of purified sodium dodecylsulphate in  $2 \times 10^{-3}$  M sodium acetate buffer, pH 5.1, and then deproteinized with an equal volume of freshly distilled and water-saturated phenol.

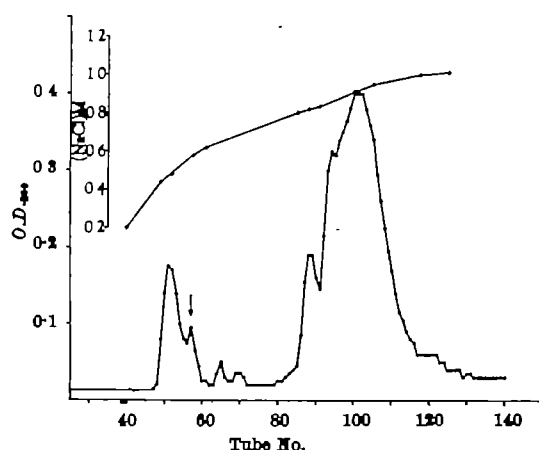


Fig. 1. Chromatographic pattern of total RNA of KB cells: 1.5 mg of phenol-extracted RNA was loaded on MAK column and eluted by linearly increasing NaCl (from 0.2 M to 1.2 M) with phosphate buffer at pH 6.9 (3 ml./tube). The peak of '5 S' RNA is indicated by an arrow

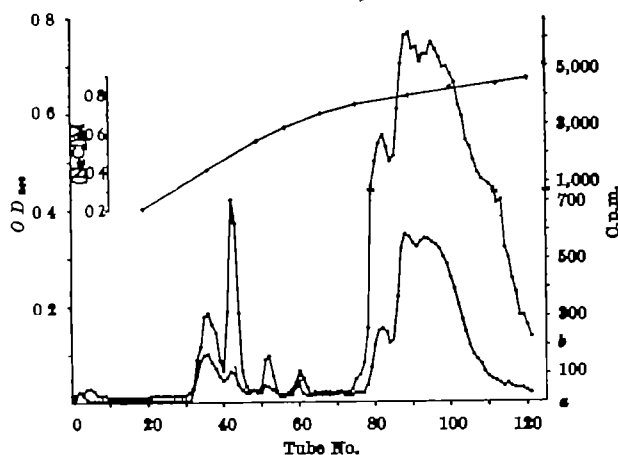


Fig. 2. Chromatographic pattern on MAK column of RNA from ribosomes of KB cells labelled with  $^{32}\text{P}$ . Cold KB cells were added as a carrier before extraction (a, optical density; b, radioactivity)

Soluble and ribosomal RNA were extracted respectively from 105,000g supernatants and centrifugation pellets from disrupted cells in Hoegland medium<sup>8</sup> plus sodium deoxycholate (0.6 per cent). All manipulations were carried out at 4° C.

The methylated albumin columns were prepared and loaded according to Mandell and Hershey<sup>7</sup>, and were eluted by a sodium chloride gradient from 0.2 M to 1.2 M in 0.05 M phosphate buffer at pH 6.9.

Base composition was determined by the procedure of Katz and Comb<sup>9</sup>. The assay of the methylated bases and of pseudo-uridylic acid was carried out in accordance with Roesset *et al.*<sup>4</sup>.

The total RNA chromatograms of the KB or rat liver cells showed the usual fractions already described by several authors (see Philipson<sup>6</sup>): one peak of transfer RNA and two peaks of ribosomal RNA respectively eluted by 0.42 M, 0.80 M and 0.90 M NaCl (Fig. 1).

The DNA was eluted by 0.65 M NaCl. The size of this peak varied somewhat but was always low under the extraction conditions used.

With an average molarity of 0.50 M NaCl, a peak which overlapped that of transfer RNA and represented about 10 per cent of the latter appeared for both types of cells. Since there was a reasonable agreement between the ribose determination and the ultra-violet absorption at 2600 Å, and since the material of this peak was sensitive to the action of the RNase and resistant to the action of the DNase, we concluded that it was certainly composed of RNA.

The intracellular localization of this fraction could be illustrated by differential extraction. Chromatography of RNA extracted from ribosomes showed that the importance of this new fraction increased considerably in relation to the transfer RNA (Fig. 2). Moreover, this fraction was absent in the chromatograms of RNA extracted from 105,000g supernatants (Fig. 3).

Since we could obtain, after extraction from ribosomes and chromatography on methylated albumin, a '5 S' RNA containing less than 5 per cent of transfer RNA, but with a poor recovery, the purification of the '5 S' RNA was successfully obtained by subjecting the total RNAs to repeated chromatography on methylated albumin. An alternative method was by gel filtration on 'Sephadex' G100 (190 × 1.8 cm columns-elution by 0.05 M ammonium acetate, pH 5.1; Virmaux *et al.*<sup>10</sup>). Under these conditions, the '5 S' RNA was eluted between ribosomal RNA and transfer RNA (Fig. 4).

A few separation tests by ultracentrifugation in a 5–20 per cent sucrose gradient (in acetate 0.01 M buffer, pH 5, containing 0.1 M NaCl) showed that the sedimentation coefficient was slightly greater than that of the transfer RNA. These results agreed with the gel-filtration experiments.

Although the analysis of the base composition showed a great analogy between the two RNAs '4 S' and '5 S', a

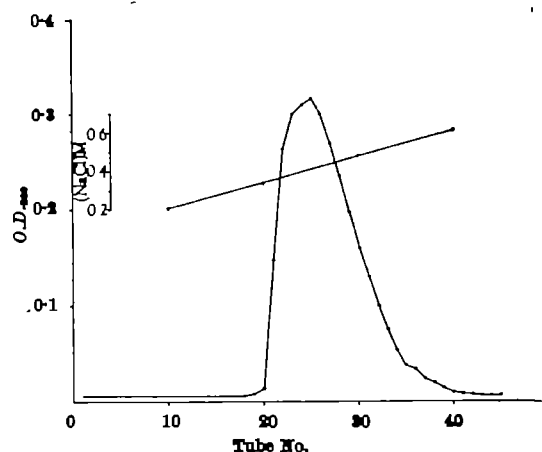


Fig. 3. Chromatographic pattern on MAK column of RNA extracted from 105,000g supernatants of disrupted KB cells (two one-hour centrifugations at 105,000g)

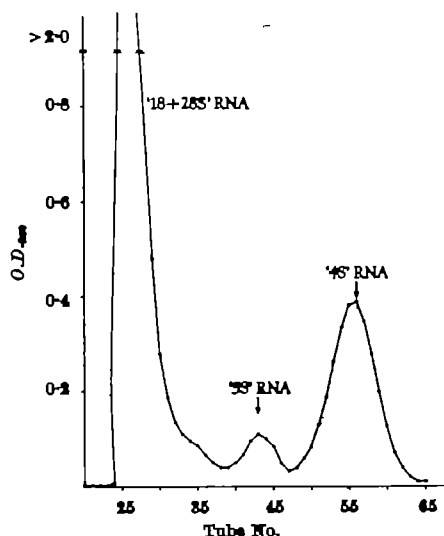


Fig. 4. Chromatographic pattern of total RNA (2 mg) of KB cells on 'Sephadex' G100 column (190 cm × 1.8 cm) eluted by 0.05 ammonium acetate pH 5.1 (4 ml./tube)



statistical study of the results by means of Student's *t* test confirmed that the base composition was significantly different for U, C and A ( $P < 0.01$ ). Moreover, there were no methylated bases or pseudo-uridylic acid (which are characteristic of the transfer RNA) in the '5 S' RNA (see Tables 1 and 2).

Table 1

	U (+pseudo U) (%)	G (%)	C (%)	A (%)
Transfer RNA	27.16	30.97	25.33	16.53
'5 S' RNA	24.68	31.01	25.33	17.66

Means of four transfer RNA and five '5 S' RNA experiments. Samples of each peak were collected, dried on planchets and counted with a Packard gas flow counter.

Table 2

	Methylated bases (%)	Pseudo-uridylic acid (%)
Transfer RNA	1.5	4.60
'5 S' RNA	0	0.25

The aminoacyl acceptor capacity of the two soluble RNAs was tested *in vivo*. KB cells in exponential growth were labelled with  $^{14}\text{C}$  protein hydrolysate ('Algal') for 5 min. The RNA after deproteinization were chromatographed on an MAK column. The residual radioactivity was localized in the peak of transfer RNA and there was none in the RNA '5 S' peak.

The new fraction of soluble RNA found in the KB or rat liver cells shows a number of properties identical to the RNA '5 S' described by Rosset *et al.*<sup>4</sup>: solubility in M NaCl, molecular weight, behaviour on MAK columns,

linkage on the ribosomes, base composition approaching that of transfer RNA although different, absence of methylated bases and pseudo-uridylic acid, and absence of aminoacyl acceptor capacity.

Elson has also described a '4-5 S' particle, linked with ribosomes, the aminoacyl acceptor properties of which are lower than those of transfer RNA. This can be explained by a simultaneous liberation of a mixture of transfer and '5 S' RNA. Rat liver ribosomes, dialysed under conditions identical to those of Elson, release into the supernatant of centrifugation two ribonucleic fractions the behaviour of which on MAK columns is the same as that of the transfer RNA and the '5 S' RNA (unpublished data).

Comb and Katz<sup>5</sup> have found in *Blastocystis emersonii* a fraction which might be identical with ours. By pulse labelling and chase experiments they have been able to attribute to it the role of a precursor of the transfer RNA. Experiments are in progress to determine the biological role of the fraction we have isolated.

This work was supported by research grant 61 FR 137 from the Délégation Générale à la Recherche Scientifique, Paris.

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## ROLE OF HISTONES AND OTHER PROTEINS IN GENE CONTROL

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SINCE the original suggestion of Stedman and Stedman<sup>1</sup> that the basic proteins present in the chromatin of animal tissues (histones) act as gene inhibitors, much effort has been devoted to their characterization and a number of fractionation procedures have been worked out<sup>2</sup> which give rise to proteins of characteristically different compositions. There is some doubt as to how far these fractionations can be continued since most of the fractions obtained still show at least one subsidiary band on starch electrophoresis. It has been suggested that the various fractions obtained may still be complex, so that each contains a large number of distinct proteins of a similar character. However, the evidence available does not support this. When 'finger-prints' are made of tryptic digests of the various fractions the number of peptide 'spots' observed is not greater than is compatible with the very limited degree of heterogeneity seen in the starch electrophoretic pictures<sup>3</sup>. If the histones are responsible for gene repression, we could expect that different tissues would show marked differences in their histone components. A number of investigations have been made on this point<sup>4,5</sup>, and they do not reveal any significant qualitative differences in the histone fractions from diverse animal tissues. A number of substitutions in the amino-acid sequences may be found<sup>6</sup>, but they do not appear to be much more extensive than occur in other proteins from different sources, for example, insulin or haemoglobin, and are not sufficient to change the character of the protein.

Similar changes of gene activity would, of course, occur in this theory if certain histone fractions were lacking in particular tissues. At present few investigations have been made of the relative amounts of different histone fractions present in particular cells, owing to lack of reliable

methods. It can only be said that starch electrophoresis pictures of the whole histone from different animal tissues are remarkably similar<sup>3</sup>. If each gene were associated with a distinct histone, there would have to be some highly specific types of interaction which would be capable of associating each DNA with a particular histone molecule. Johns and Butler<sup>7</sup> found no evidence of specific interactions between histone fractions and different parts of DNA.

On the other hand, the gene inhibitor theory of histones has been supported by experiments *in vitro*<sup>8</sup> which show that histones do inhibit the synthesis of RNA on the DNA template, and that native chromatin can be made to support RNA synthesis to some extent by removing histone (although the removal of the lysine-rich histone F1 has been reported<sup>9</sup> to have no effect) and that active chromatin fractions which are deficient in histone have been obtained<sup>8</sup>.

On the other hand, histones have not been found associated with DNA in bacteria, although complex types of gene regulation occur. It has been demonstrated that the bacterial chromosome may consist of a single continuous circular DNA fibre and that in replication the DNA polymerase moves around the fibre beginning at a specific attachment site<sup>10</sup>. It has been suggested<sup>11</sup> that the DNA polymerase is a double ring which moves down the two strands of the DNA. A single ring of this kind would suffice for the RNA polymerase and it is significant that electron micrographs indicate this shape<sup>12</sup>. Berg *et al.*<sup>13</sup> have described experiments which indicate that while DNA synthesis can only begin at a single attachment site, RNA polymerase action can begin at any point, or at least at a number of points on the circular chromosome.

It has been shown in *B. megaterium* that the RNA polymerase is firmly attached to the DNA<sup>14</sup>, and can only be removed by disrupting the DNA. This is only compatible with Kornberg's observation if there are a number of entry sites at which the RNA polymerase can become attached to one of the strands of the circular chromosome. According to the theory of Jacob and Monod<sup>15</sup> the structural genes, which actually produce messenger RNA, are controlled by regulator genes which normally produce repressor substances, and activation occurs only when the flow of the latter to the operator genes is interrupted. However, no repressor substances have been isolated. The entry sites for the polymerase would naturally be associated with the regulator genes the function of which would be to permit or prevent the attachment of the polymerase to the DNA fibre. Once attached it could be supposed to be able to move down one of the strands of the DNA of the operator gene, presumably determined by an end structure, although the different polarity in the two strands might be sufficient for this purpose.

If such a mechanism is postulated it is evident that the combination of the appropriate substrate with the protein on the regulator gene could produce a conformational change which would 'open the gate' and permit the RNA polymerase to attach itself to the operator genes.

A similar mechanism can be extended to apply to the chromosomes of complex differentiated organisms. It has been suggested by Hotta and Basell<sup>16</sup> that in these also the DNA consists of circular loops, joined together by some form of backbone, possibly also consisting of DNA. As the amount of histone is usually insufficient to cover the whole of the DNA, non-histone proteins, the 'residual proteins', which may well be of a more varied character, are also present. If this is so the ring parts of the chromosome may well be associated with different proteins from the 'backbone', and while the major ring portions would naturally be associated with the histones, the backbone might well be associated with residual proteins and function as regulator genes. As in the bacterial case this would provide more varied proteins than histones provide. As in the bacterial situation they could be sensitive to appropriate substrates or to hormones.

This type of control is indicated by the experiments of Talwar *et al.*<sup>17</sup>, who isolated from rat uterus a protein which by itself was inhibitory and when combined with a specific hormone produced a stimulation of RNA synthesis.

The chief advantage of this hypothesis is that it provides greater opportunities of specific gene control by cell constituents than is provided by histones. The difficulty of explaining how a particular protein is associated with a particular piece of DNA remains. While histones are

bound to DNA almost entirely through ionic bonds, the non-histone proteins which are non-basic and predominantly acidic in character may not be bound in this way. With these proteins there is undoubtedly more scope for specific interactions through hydrogen bonds with the free  $-NH_2$  and  $-OH$  groups of the DNA; but it seems more likely that the regulatory proteins are made on or close to the site to which they must become attached.

This would mean that the regulator genes must code for their own proteins. This would not present any difficulty even if synthesis occurred on ribosomes, since it has been shown (in the bacterial case in any event)<sup>18</sup> that ribosomes are present in close proximity with the genes and are probably required to pull newly formed messenger RNA off the template. This means that the combination of messenger RNA and ribosome could begin to synthesize protein at once, even before the RNA was completely separated from the template, and the attachment of the protein formed to the template would follow. It is interesting to note that Patel and Wang<sup>19</sup> have suggested that 'residual' protein can be formed directly on the DNA template. If confirmed this would get over the difficulty of finding mechanisms to ensure that the regulatory proteins reach their proper sites.

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## FREE AMINO-ACIDS ON HUMAN FINGERS: THE QUESTION OF CONTAMINATION IN MICROANALYSIS

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FEW quantitative studies using modern techniques have been made concerning the amino-acids present on human skin<sup>1,2</sup>. With the exception of the work of Hamilton<sup>3</sup> we know of no studies which have dealt specifically with the amino-acids on the skin of the fingers. The handling of samples and experimental apparatus usually involves contact with fingers. Even in cases where handling is done by mechanical devices, there is a probability that during the history of a given sample (rock, meteorite, etc.) such a contact has taken place. Since it is possible that contamination by the ninhydrin-positive compounds present on the surface of fingers may affect

the results of amino-acid analyses at the micromolar level, we have made a qualitative and quantitative study of these compounds as they are normally found on human hands and fingers. In this article we report primarily the amino-acids found on the fingertips of seven individuals. The analyses were carried out by ion-exchange column chromatography using the Beckman-Spinco model '120B' automatic amino-acid analyser.

The experimental procedure was as follows. The samples were taken at random with regard to the time of the day and to the age and sex of the individuals. Any foreign particles which might have been present on the

Table 1. AMOUNTS OF INDIVIDUAL AMINO-ACIDS CORRESPONDING TO CHROMATOGRAMS IN FIG. 1  
(Values shown represent  $\mu\text{M} \times 10^3$ )

Sample	Urea	Asp	Thr	Ser	Glu	Pro* Cit	Glu	Ala	Val	Met	Ileu	Leu	Tyr	Phe	Orn† Lys	Hist	Arg
<i>S</i> <sub>1</sub>	5	6	7	40	2	14	19	11	4	—	4	6	3	2	23	9	1
<i>S</i> <sub>2</sub>	7	9	9	40	2	5	25	14	7	1	5	5	3	3	2	—	—
<i>R</i> <sub>1</sub>	4	6	3	21	5	—	11	5	1	—	2	2	1	1	12	2	—
<i>R</i> <sub>2</sub>	8	3	3	19	1	—	13	6	3	—	1	2	2	2	10	2	—
<i>I</i> <sub>1</sub>	4	8	9	53	2	—	20	14	—	—	2	2	—	—	21	7	—
<i>I</i> <sub>2</sub>	6	12	9	55	3	—	34	16	2	—	4	4	2	2	19	7	—
<i>N</i>	1	7	5	23	—	—	16	6	—	—	2	3	1	1	15	6	—
<i>O</i>	16	35	23	123	8	17	74	25	16	2	12	12	13	7	68	26	4
<i>K</i>	3	11	9	47	3	—	31	12	7	—	3	4	3	2	16	4	—
<i>U</i>	6	9	9	41	2	5	23	12	—	—	3	4	2	2	19	7	—

\* This peak contains both proline and citrulline. The value shown was obtained from the absorbance at 440 m $\mu$  to which the citrulline contributes, but not to a large degree. † This peak contains both ornithine and lysine.

surface of the index finger were removed with tissue paper. The tip of the finger was placed in contact with 0.5 ml. citrate buffer, pH 2.2, for 15 sec in a small, clean beaker. Exactly 0.25 ml. of the resulting solution was applied to the chromatographic column for analysis.

Fig. 1 shows the ion exchange chromatograms obtained from seven individuals, identified by the letters at the left of the figure. The chromatograms in the left-hand column (A) correspond to the acidic and neutral amino-acids and those in the right-hand column (B) to the basic amino-acids. Two analyses were made on different days from each of the first three subjects (*S*, *R*, *I*). This was done in order to determine the reproducibility and the possible variation of the results from day to day.

Other related analyses are shown in Fig. 2 (chromatograms 1-5). In order to detect amino-acids which may be present only in trace amounts, the hands of one subject were washed in 100 ml. of distilled water which was then evaporated to dryness at 1 mm Hg pressure and 25° C. The resulting residue was dissolved in 4 ml. citrate buffer, pH 2.2, 1 ml. of which was analysed as before. The results are shown in chromatogram 1. Chromatogram 2 is a blank run showing the results obtained from the analysis of 0.25 ml. of citrate buffer taken from a clean beaker which was handled in the same manner as in the previous analyses. Chromatogram 3 shows the results obtained from a sample taken from a subject's fingertip shortly after the individual had washed his hands with soap and ordinary tap water. Because dust which is present in the air is also a possible source of contamination, a 0.002-g sample was obtained from the top of a high shelf, extracted with citrate buffer and analysed for amino-acids in the usual way. The results are shown in chromatogram 4. A similar sample of dust was refluxed for 18 h in 6 N HCl, evaporated to dryness and dissolved in citrate buffer. This solution was then analysed for amino-acids, and the results appear in chromatogram 5.

The observations can be summarized as follows. The composition of free amino-acids on the surface of human fingers appears to be essentially constant, not only qualitatively but also in terms of their relative abundance. This becomes evident by looking at the chromatograms of Fig. 1. Each chromatogram contains urea, aspartic acid, threonine, serine, glutamic acid, glycine, alanine, isoleucine, leucine, tyrosine, phenylalanine, lysine, ornithine, histidine, and ammonia. In addition, some show smaller amounts of taurine, proline plus citrulline, valine, methionine, arginine, and one or two unidentified compounds.

Table 2. RATIOS OF AMINO-ACID PAIRS

Sample	Ser	Gly	Ser
	Thr	Ala	Ala
<i>S</i> <sub>1</sub>	5.70	1.72	3.68
<i>S</i> <sub>2</sub>	4.45	1.78	2.85
<i>R</i> <sub>1</sub>	7.00	2.20	4.21
<i>R</i> <sub>2</sub>	4.50	2.17	3.00
<i>I</i> <sub>1</sub>	5.90	1.43	3.78
<i>I</i> <sub>2</sub>	6.11	2.27	3.66
<i>N</i>	4.90	2.67	3.84
<i>O</i>	5.80	2.11	3.48
<i>K</i>	5.23	2.58	3.92
<i>U</i>	5.12	2.33	3.41
Average	5.39	2.13	3.58
S.D.	0.77	0.37	0.38

The most impressive similarity between the analyses is the apparent constancy in the relative concentrations of amino-acids. Obvious examples of this are the amino-acids which appear together in pairs such as threonine-serine (peaks 5 and 6), glycine-alanine (peaks 9 and 10), isoleucine-leucine (peaks 13 and 14) and tyrosine-phenylalanine (peaks 15 and 16). Although the similarity of these pairs is most obvious, there is actually a constancy in the relative abundances of most of the other amino-acids as well (for example, serine-alanine). In some cases, however, the concentrations are too low to permit measurements accurate enough for quantitative comparison. This correlation of relative abundances can also be extended to include the basic amino-acids. As an example the ratio between lysine and histidine (peaks 17 and 19) appears to be fairly constant. A cross-correlation between basic amino-acids on one side and neutral and acidic amino-acids on the other is not possible from these data, because the chromatograms in columns A and B of Fig. 1 were not necessarily obtained from the same samples. In order to ascertain how constant the relative abundances are, the amounts of individual components were quantitatively determined (Table 1) and ratios were obtained for the three most significant pairs of amino-acids. These ratios appear in Table 2.

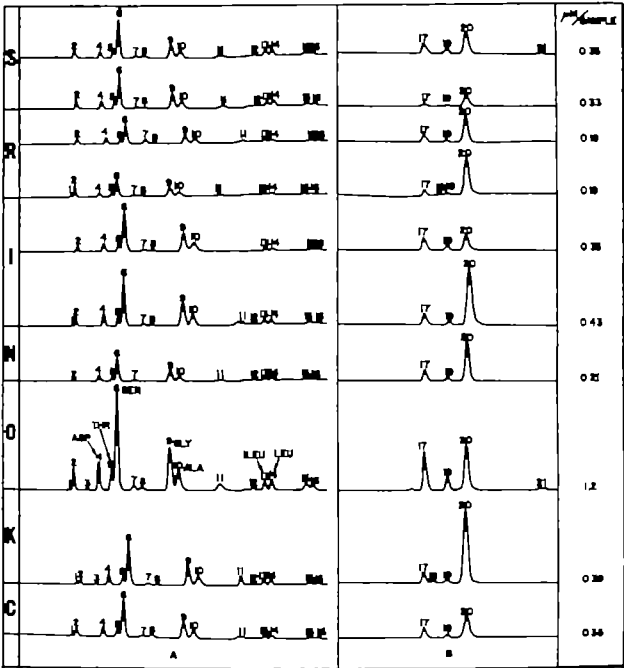


Fig. 1. Chromatograms of amino-acids present on the fingertips of various individuals, *S*, *R*, *I*, *N*, *O*, *K*, and *U*. Column A shows neutral and acidic amino-acids. Column B shows basic amino-acids. (1) Taurine, (2) urea, (3) unidentified, (4) aspartic acid, (5) threonine, (6) serine, (7) glutamic acid, (8) proline plus citrulline, (9) glycine, (10) alanine, (11) valine, (12) methionine, (13) isoleucine, (14) leucine, (15) tyrosine, (16) phenylalanine, (17) lysine plus ornithine (the presence of ornithine was confirmed by Hamilton), (18) unidentified, (19) histidine, (20) ammonia, (21) arginine. The last column shows the amino-acids of ninhydrin-positive compounds present in each sample excluding ammonia.

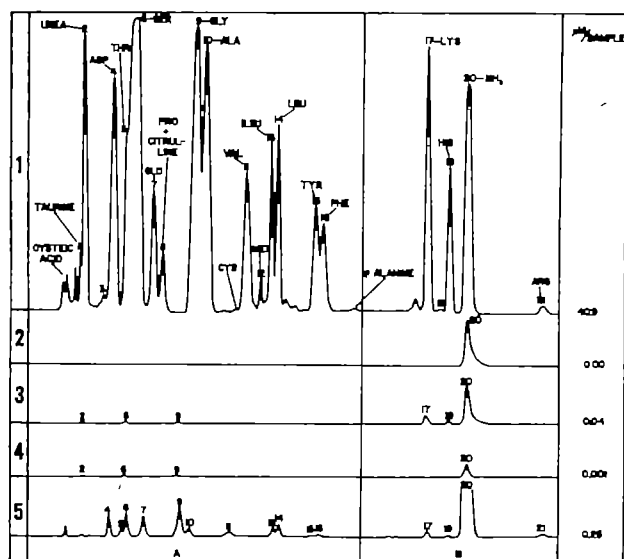


Fig. 2. Chromatogram 1 is an analysis of the ninhydrin-positive compounds from the hands of an individual. Column A shows neutral and acidic amino-acids. Column B shows basic amino-acids. Chromatogram 2 is a buffer-glassware blank. Chromatogram 3 is an analysis of a sample like those in Fig. 1 except that the subject's hands had been recently washed. Chromatogram 4 is a dust extract analysis. Chromatogram 5 is the analysis of a dust hydrolysate. Peaks are numbered as in Fig. 1. The last column shows the  $\mu$ moles of ninhydrin-positive compounds present in each sample excluding ammonia.

The analysis of the hand-wash sample (chromatogram 1 in Fig. 2) agreed well with the analyses in Fig. 1. In addition, several compounds were observed at lower levels of concentration than the previously mentioned amino-acids. These include cysteic acid, cystine,  $\beta$ -alanine, and about ten unidentified components. The buffer-glassware blank showed complete absence of amino-acids or other ninhydrin-positive compounds with the exception of ammonia. The appearance of ammonia in the chromatograms is difficult to avoid because of its presence in the atmosphere. The analysis of the compounds obtained from the surface of a recently washed fingertip (chromatogram 3) showed a marked decrease in the presence of ninhydrin-positive compounds, with only traces of urea, serine, and glycine appearing in the neutral and acidic analysis and with the basic amino-acid analysis little changed.

The analysis of the dust extract (chromatogram 4, Fig. 2) revealed only traces of urea, serine, and glycine in addition to ammonia. On the other hand, the dust hydrolysate (chromatogram 5) showed most of the protein amino-acids with traces of a few additional compounds. It should be pointed out that cystine and methionine were not observed at the low concentration-levels of this analysis but a significant peak corresponding to cysteic acid was apparent. There appeared to be similarities in the relative abundances of this chromatogram and those of Fig. 1. However, there were definite differences such as (1) higher than usual concentrations of cysteic acid, aspartic acid, glutamic acid and ammonia, and (2) a lower than usual concentration of urea.

The consistency of the relative abundances of the amino-acids present on human fingers is not surprising since it is probably a reflexion of the steady-state concentration of the free amino-acids in epidermal cells. The metabolism of the epidermis of normal subjects would be expected to be basically the same.

The fact that a typical pattern of relative abundance is evident, and that certain amino-acid pairs are so apparent, can act as a guide in detecting contamination in microanalysis of amino-acids. In other words, there is now a 'contamination picture' of sorts for analyses at the micromolar and lower levels, and data that fit such a picture should be scrutinized carefully. In some cases

where only minor contamination is indicated, it may be possible to make the necessary subtractions to obtain more correct analytical data.

A more complete discussion of these results in relation to data obtained from amino-acid analyses of carbonaceous chondrites and other meteorites<sup>4-6</sup> and some synthetic processes<sup>7</sup> will be published elsewhere. At this moment we would like to note the following.

(1) The amino-acid composition of meteorites<sup>4,6</sup> is very similar to that reported here. This is shown by a visual comparison of the chromatograms in Fig. 1 of this paper and the third chromatogram (free amino-acids) in Fig. 1 of reference 6. The ratios for three pairs of amino-acids (Ser-Thr, Gly-Ala, Ser-Ala) from meteorites (Table 6 of reference 5) is given in Table 3. A good agreement with Table 2 can be observed. The larger values for the standard deviations in Table 3 are to be expected on the following grounds: (a) the analytical method used in this case was paper chromatography; (b) some of the samples were hydrolysed in hydrochloric acid; (c) the histories of the samples were different<sup>8</sup>.

Table 3. RATIOS OF AMINO-ACID PAIRS TAKEN FROM METEORITE ANALYSES<sup>5</sup>

Sample		Ser		Gly		Ser	
		Thr	Ala	Thr	Ala	Thr	Ala
Orgueil	free	5.4	2.9	2.9	2.6	1.8	1.8
	comb.	3.1	1.8	1.8	1.0	1.1	1.1
Cold Bokkeveld	free	—	0.6	2.3	0.7	1.1	1.1
	comb.	1.2	0.9	1.1	0.7	1.1	1.1
Migdal	free	—	1.8	2.6	2.6	2.0	2.0
	comb.	2.1	1.7	1.6	1.2	1.2	1.2
Murray	free	4.6	1.6	1.6	1.6	1.6	1.6
	comb.	5.7	1.1	1.1	1.1	1.1	1.1
Warrenton	free	—	1.1	1.1	1.1	1.1	1.1
	comb.	3.2	1.1	1.1	1.1	1.1	1.1
Lancé	comb.	4.7	1.6	1.6	1.6	1.6	1.6
Felix	free	7.4	1.2	1.2	1.2	1.2	1.2
	comb.	6.2	1.2	1.2	1.2	1.2	1.2
Mokola	free	8.0	2.4	2.4	2.4	2.4	2.4
	comb.	4.6	2.1	2.1	2.1	2.1	2.1
Average		4.7	1.6	1.6	1.6	1.6	1.6
S.D.		3.0	1.7	1.7	1.7	1.7	1.7

(2) From a quantitative point of view, the amino-acid levels in Fig. 1 are of the same order of magnitude (micromolar) as those reported in meteorites<sup>4,6</sup>. Compare, for example, the micromoles of amino-acids per sample given in Fig. 1 of this article with the corresponding values for meteorites given in Fig. 1 of reference 6. A more correct comparison of results would be possible if the values had been reported per sample instead of per gram. Thus, the higher values (527.7  $\mu$ g/g) reported for the Lancé meteorite (Table 6 of reference 5) may in part result from the fact that less than 1 g (0.32 g) of this meteorite was analysed, necessitating a multiplication of the actual amount of amino-acids found by a factor of about 3.

(3) The situation with regard to the sulphur amino-acids appears to be similar in the present analyses and in those of meteorites. These amino-acids are practically absent at low concentration levels<sup>9</sup> and show up in traces or small amounts at higher concentration levels<sup>9</sup>. Therefore, the argument often adduced<sup>4,7</sup> that the absence of sulphur-containing amino-acids is an indication of the absence of contamination cannot any longer be accepted.

(4) Since  $\beta$ -alanine is not normally present in protein hydrolysates, its presence in a given analysis might also be taken as an indication of the absence of contamination. That this is not so is indicated by the fact that small amounts of this compound have been detected in the hand-wash sample (Fig. 2). Additional amounts of  $\beta$ -alanine can conceivably be formed as a result of bacterial decarboxylation of aspartic acid. Indeed, common bacteria and other micro-organisms are known to decarboxylate aspartic acid into  $\beta$ -alanine<sup>10</sup>.  $\beta$ -Alanine is also distributed by the common housefly (Hamilton, private communication).

(5) It should also be pointed out that, depending on the conditions of the analysis, the positions of certain amino-acids on the chromatogram may be sufficiently changed to cause ambiguity in identification (for example, allo-leucine and methionine). With samples other than

protein hydrolysates the identification is further complicated by the possible existence of other ninhydrin-positive compounds with elution times essentially identical to those of protein amino-acids, as shown by Zacharius and Talley<sup>8</sup> and by Hamilton<sup>10</sup>. Therefore, whenever possible, identification by at least two independent methods should be carried out in these cases.

From the data presented here, and from other studies on carbonaceous chondrites<sup>11</sup>, it would appear that the bulk of the free amino-acids found in the analyses of these meteorites are the result of contamination. This would confirm the results obtained by Calvin and Vaughn<sup>12</sup>, Briggs<sup>13</sup>, and Awapara (personal communication), who were unable to detect amino-acids in carbonaceous chondrites. Using a different approach, Hamilton<sup>8</sup> obtained results similar to those presented in this article.

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## REVERSAL OF POTENTIAL ACROSS A LIQUID NON-AQUEOUS MEMBRANE WITH REGARD TO MEMBRANE EXCITABILITY

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PREVIOUS reports<sup>1,2</sup> established that the electrical potential of a pentanol membrane was caused by the appearance and disappearance of the head charges of amphipathic molecules at the oil/water interface. It was also established that the sign of the potential was determined by the sign of the head charge at the interface, and the size of the potential decreased with increasing electrolyte concentration and consequent binding of the counter-ions on the head charges.

The rapid change of sign of the electrical potential of nerve membranes is a feature of the action potential, the mechanism of which, however, is not fully understood. As the oil membrane was found<sup>1,2</sup> to produce a spike of obvious biological interest, this article delves further into the mechanism of the events which may cause the change of sign of the potential.

In my early experiments with pentanol membranes<sup>1</sup> I observed a sudden reversal of potential some time after injecting the amphipathic molecules at the oil/water interface. The explanation was: the amphipathic molecules, after migrating from the spreading interface, overturned and showed the head charge at the opposing interface, thus causing the reversal of potential.

While illustrating that first mechanism I wish to show that the reversal of potential can be brought about not necessarily by a real migration of the amphipathic molecules but alternatively and significantly by either or both of two main ways: consecutive appearance of head charges of the same sign (events  $+a$ ,  $-b$  and  $-c$ ,  $+d$  in Fig. 1) at opposing interfaces, and consecutive appearance of head charges of opposite sign at the same interface (events  $+a$ ,  $-c$  and  $-b$ ,  $+d$  in Fig. 1); and in both theory and practice, the entire cycle of events corresponding to resting spike-resting potential can be made to occur and repeat by any adsorption, ion exchange, film penetration, phase inversion and charge inversion mechanism which is able to operate the successive appearance and disappearance of the head charges at the oil/water interface.

The experiments were performed at 25° C in a modification of the 'hanging drop' cell<sup>1,3,4</sup>. Two saturated calomel electrodes connected the lower and upper aqueous compartments at interfaces A and B (Fig. 1) respectively

to the plus and minus poles of a high-impedance electrometer, Keithley model 610A. The oil phase between A and B was *n*-pentanol. Fig. 1 presents a summary of the signs of the changes of potential as related to the signs of the head charges at the interfaces.

In Fig. 2 the potential in mV is plotted versus time over a total period of 50 sec. At about 6 sec, from left to right, sodium decyl sulphate (SDS) was applied on  $10^{-3}$  M potassium chloride at interface A; the potential rose rapidly and then decayed a few mV between 7.5 and 19 sec as the SDS desorbed from interface A. Molecules of SDS migrated through the oil, and on reaching interface

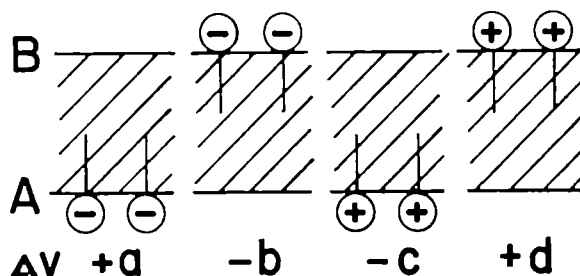


Fig. 1. Signs of potential changes. When potassium chloride concentrations are  $10^{-3}$  M at A and  $10^{-4}$  M at B,  $|b| > |a|$  and  $|d| > |c|$ .

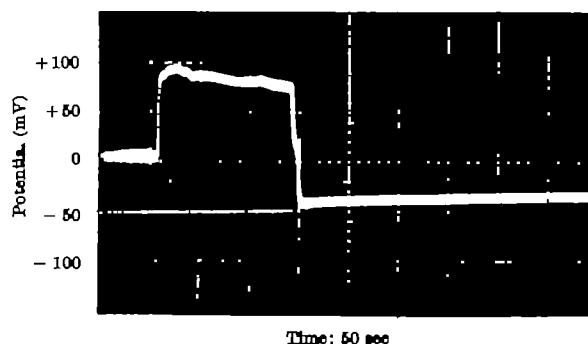


Fig. 2. Oscilloscope (Tektronics 535-A) tracing of a typical reversal of potential across the pentanol membrane. SDS, 200  $\mu$ g in  $10^{-3}$  M aqueous solution, was applied at interface A on  $10^{-3}$  M potassium chloride. Membrane thickness, 3 mm.

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*B* overturned and acquired the expected orientation; hence, the appearance of the negative head charges at interface *B* caused a negative ( $-120$  mV, from  $75$  to  $-45$ ) change of potential, which is much larger than the positive one at interface *A* primarily because the electrolyte concentration ( $10^{-3}$  M potassium chloride) at *B* is ten-fold as small as at *A*. The resulting picture of the membrane at  $20$  sec is the combination of  $+a$  and  $-b$  of Fig. 1. Analogously, a reversal from negative to positive potential and a final picture of superimposed  $-c$  and  $+d$  of Fig. 1 followed injection of cetyltrimethylammonium bromide (CTAB) at interface *A*.

The rate and extent of recovery of the potential (between  $20$  and  $50$  sec in Fig. 2) varied from system to system. But actually, in every system investigated, rapid return of the potential to the value preceding the reversal was brought about by restoration of the charged interfaces as illustrated later. In line with a previous observation<sup>3</sup>, the time between spreading and reversal was obviously the shorter the thinner the membrane. Migration of the amphipathic molecules through the oil was confirmed with both anionic and cationic surfactants by testing oil samples at the given interface for the given surfactant; and the reversal of potential occurred only when the surfactant reached that interface. As predicted from Fig. 1, no reversal was obtained when the concentration of the electrolyte at the second interface was larger than or equal to that at the spreading interface; for example, after migration of CTAB from  $10^{-3}$  M potassium chloride at *A* to  $10^{-3}$  M potassium chloride at *B* the potential changed only from  $-150$  to  $-70$  mV and not through zero to a positive potential.

Relevant to these studies and Fig. 2 is the drop, though not reversal, of potential observed by Dupeyrat<sup>4</sup> in experiments with a nitrobenzene membrane. When the stream of surfactant (dodecyltrimethylammonium bromide) migrating under a concentration gradient reached interface *B*, where the electrolyte concentration was the same ( $1$  M potassium bromide) as at *A*, the potential suddenly dropped to nearly zero. At variance with Dupeyrat, the concentration or distribution potential is of no avail to account for the sudden drop of potential. Indeed, with the proper electrode<sup>5</sup> in the stream of surfactant (CTAB) I measure a new concentration potential, and yet there is no drop of potential so long as the surfactant does not appear at the opposing interface. Vice versa, injection of the surfactant at the opposing interface causes the sudden drop of potential independently of the bulk concentration of surfactant; and in both cases, as expected, the concentration potential does not change until the migrating surfactant touches the electrode.

Unlike that in Fig. 2, where migration of SDS from *A* to *B* occurred, the same reversal of potential though without migration of SDS was experienced after spreading SDS successively at *A* and *B* at any desired time-interval, in a system equivalent to the consecutive appearance of charges of the same sign at two opposing interfaces. Previously<sup>3</sup>, duplication of the rise and fall of the spike potential was shown when acetylcholine was applied to an oil membrane; the mechanism of the pertinent changes, however, was not fully explained.

In another system, illustrating the successive appearance of charges of opposite sign at the same interface,  $10^{-3}$  M potassium chloride was put on both sides of the pentanol membrane; a reversal of  $+870$  mV from  $-150$  to  $+220$  mV followed injection of  $600$   $\mu$ g of CTAB or SDS consecutively at interface *A* while no change was made at *B*. Apparently, the incoming SDS removed CTAB by penetration and/or adsorption (ion pairing) and produced a negatively charged surface at *A* responsible for a positive potential.

Furthermore, anionic and cationic liquid membranes were prepared by dissolving the surfactant in the oil. In a picture resembling the combination of  $+a$  and  $-b$

of Fig. 1, a solution of  $10^{-3}$  M SDS in amyl alcohol between  $10^{-1}$  M potassium chloride at *A* and  $10^{-3}$  M potassium chloride at *B* produced a stable potential of  $-72$  mV. Sudden reversal of the potential (without migration of surfactant across the membrane) from  $-72$  to  $+70$  mV followed injection of CTAB at interface *B*: removal of SDS and establishment of CTAB at *B* caused the negative potential to vanish and a net positive potential to appear, which is about the combination of  $+a$  and  $+d$  of Fig. 1.

Similar results were repeatedly obtained with a number of organic molecules and with various preparations of cephalin and lecithin contaminated with acidic lipids at the oil/water interface<sup>1</sup>. Conditions leading to the reversal of potential are numerous. A difference in electrolyte concentration across the membrane is not required to produce a potential and a reversal of potential. Injection of different quantities of surfactant at *A* in contact with electrolyte of the same concentration as at *B* (Fig. 1) caused a potential which increased with the concentration of that surfactant, meaning that really the charge density of the surfactant at the interface gives the measure of the potential. The reversal of potential may therefore be brought about by the concerted appearance and disappearance of given quantities of fixed head charges at the interface. That could be made to occur in a number of ways, among which ion exchange in the double layer and alternate admission (appearance and disappearance) of different elements of a mosaic-like structure may be worthy of attention.

The action potential, actually, consists of the sequence of two reversals, that is, from resting to spike and back to resting. Thus far I have described only the first reversal. In all the cases investigated I produced the second reversal by restoration of the surface charge to the state preceding the first reversal. For example, a pentanol membrane containing  $10^{-4}$  M SDS between  $10^{-3}$  M potassium chloride at *A* and  $1$  M potassium chloride at *B* produced a potential of  $+125$  mV. Injection of  $600$   $\mu$ g CTAB at *A* caused a reversal to  $-145$  mV. Thereafter, that same membrane experienced consecutive reversals of potential to  $+125$  and  $-125$  mV six more times (so far as I observed) back and forth following the consecutive injection of the same quantity of SDS and CTAB three times each at interface *A*. No migration of surfactant was involved here; film penetration, adsorption and ion exchange may be surmised; in Nature, that general mechanism of change of the surface charge may be provided by ion exchange and phase and charge inversion mechanisms operating near or within the membrane.

Regardless of the form, be it a real physical migration or overturning of head charges through a fluid structure, or, more likely, be it a mechanism of phenomenological migration (of head charge) associated with the appearance and disappearance of the head charges on the surface of the membrane, the concept of migration of the head charges across the membrane is relevant to the suggestion made by Hodgkin and Huxley<sup>6</sup>. They maintained that only the movement, across the membrane, of species possessing a large charge or dipole moment could account for the observed changes of ionic permeability during the excitation of the nerve membrane. The transport of the electrolyte across the living membrane may therefore just coincide with, or follow, the development of the electrical potential by the charged interfaces rather than by itself be the immediate cause of the potential.

Inasmuch as no flux of electrolyte was required for the dramatic changes of potential observed across the thick ( $0.3$ – $10$  cm) oil layer, the oil membrane exemplifies for the first time the unequivocal separation between potential of the fixed head charges and potential of the electrolyte. It is not known if and to what extent this new element of non-concentration and non-diffusion potential of the oil membrane could be operating in biological

membranes. Simulation of a change of surface charge by the alternate admission of anionic and cationic solid exchange membranes ('Amphion A-80 and C-89'), in place of the oil membrane in Fig. 1, also caused the typical reversal of potential where there was no change of electrolyte concentration; a fundamental ingredient in the mechanism of resting and action potentials may therefore have to be sought directly in the charged

components of the membrane rather than in the electrolyte bathing the membrane.

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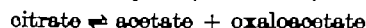
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## METAL-CATALYSED DECOMPOSITION OF $\alpha$ -HYDROXYPOLYCARBOXYLIC ACIDS

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IN connexion with work on the reaction catalysed by the enzyme citrate lyase (EC 4.1.3.6):



we have made a detailed examination of the magnesium and manganese complexes of citrate, these two metal ions being activators of this enzyme. The magnesium complexes were straightforward<sup>1</sup>, but unusual results were obtained for the manganese complexes.

Patnaik and Pani showed that, in the presence of manganese (II)<sup>2</sup>, cobalt (II)<sup>3</sup>, zinc<sup>4</sup>, and nickel<sup>4</sup>, four protons ionized from citric acid below pHs near 10, and Warner and Weber<sup>5</sup> obtained similar results in the presence of copper (II) and iron (III). The extra ionization in the presence of manganese was also observed by Shnarevich<sup>6</sup>.

From potentiometric titration of citric acid in the presence of manganese chloride we have confirmed that one extra proton is liberated below pH 10 from citric acid per metal atom present. Thus from 1:1 citric acid:MnCl<sub>2</sub> mixtures four protons were liberated per citric acid molecule, while from 2:1 citric acid:MnCl<sub>2</sub> mixtures 3.5 protons per citric acid molecule were titrated (Fig. 1). However, we observed that in the region of the fourth ionization (pH 7-10) the pH, which rose rapidly on addition of alkali, fell slowly to an equilibrium value. This observation suggests that a process other than a simple ionization occurs. Attempts to calculate the acid dissociation constant of the MnHCit<sup>-</sup> complex from the equilibrium pH values failed to yield a constant value.

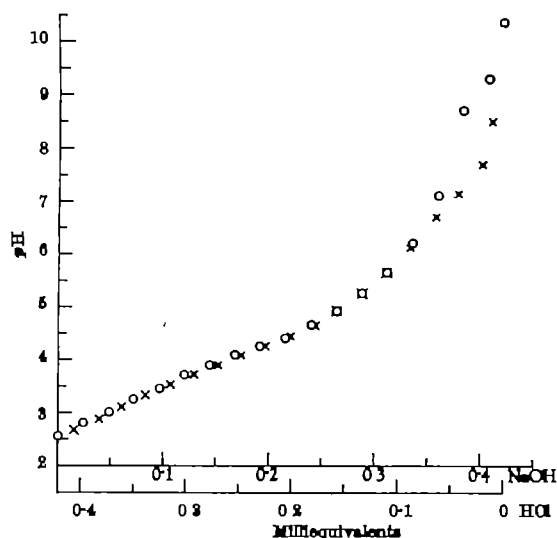


Fig. 1. Titration of citric acid (0.18 mmole) with NaOH in the presence of MnCl<sub>2</sub> (0.06 mmole) and back titration with HCl. O, Forward titration with NaOH; x, back titration with HCl.

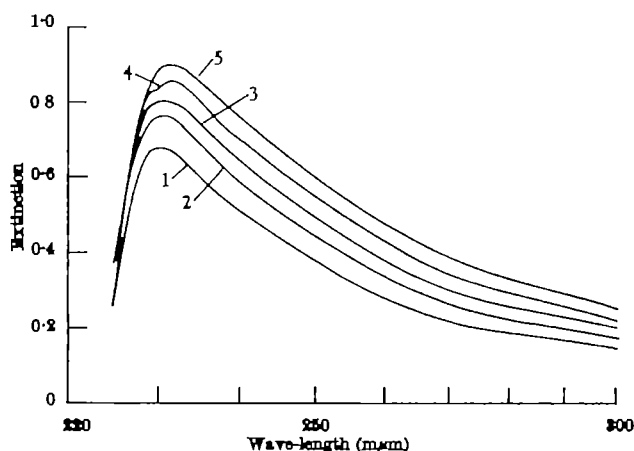
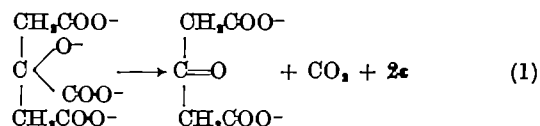


Fig. 2. Time variation of the optical density of a solution containing 0.0464 mmole trisodium citrate + 0.006 mmole MnCl<sub>2</sub> and excess alkali in a total volume of about 3 ml. The times at which the scan passed 250 mμm were: curve 1, 90 s; curve 2, 215 s; curve 3, 340 s; curve 4, 465 s, and curve 5, 590 s.

Fig. 1 shows a titration curve of a 2:1 citric acid:MnCl<sub>2</sub> mixture, sodium hydroxide solution was the titrant in the forward direction and hydrochloric acid solution in the back titration. The clear difference between the forward and the back titration curves shows that a change had taken place in the acid-base system.

The occurrence of another process was confirmed by spectrophotometric measurements. Fig. 2 shows the change with time of the ultra-violet absorption of a solution of trisodium citrate (adjusted to pH 11 with alkali) after the addition of MnCl<sub>2</sub>. A new species with an absorption band near 230 mμm was evidently formed. There was no visible sign of manganese hydroxide precipitation; this is not surprising since the ligand:metal ratio was > 9.

It appeared possible that the unknown process might be the non-enzymatic cleavage of citrate to oxaloacetate and acetate. Tests on alkaline citrate-manganese solutions with 2,4-dinitrophenylhydrazine showed that a keto-acid was present<sup>7</sup>. However, attempts to demonstrate that the keto-compound was indeed oxaloacetate, by its reduction to malate with NADH in the presence of malate dehydrogenase, were unsuccessful. The oxidative decarboxylation of citrate tetra-anion could give acetone dicarboxylate according to the reaction:





The keto-acid dinitrophenylhydrazones from the reaction mixture were therefore extracted by McArdle's method<sup>6</sup> and examined by paper chromatography using the solvent system of El Hawary and Thompson<sup>7</sup>. The chromatogram showed spots corresponding to the dinitrophenylhydrazones of both acetone dicarboxylic acid and acetoacetic acid. The latter could have been formed by the decarboxylation of the former. The results were, however, not always reproducible, possibly because in some circumstances the acetone dicarboxylic acid was completely decarboxylated to acetone the dinitrophenylhydrazone of which is not extracted by the method used.

To test whether this is a general reaction of  $\alpha$ -hydroxy-polycarboxylic acids with transition metal ions, we titrated isocitric, citramalic, malic, isomalic and tartronic acids in the presence of  $Mn^{2+}$ ,  $Co^{2+}$  and  $Cu^{2+}$ . The ionization of an extra proton was unambiguously demonstrated in the presence of all these metal ions for isocitric, isomalic and tartronic acids, whereas with citramalic and malic acids the extra ionization was only seen definitely in the presence of  $Cu^{2+}$  ions. With these last two acids and  $Mn^{2+}$  or  $Co^{2+}$  ions the evidence was less definite, possibly due to the low stability of the complexes. Malonic, succinic and tricarballic acids did not give rise to extra ionizations in the presence of any of these metal ions, which suggests that the source of the extra proton is the hydroxyl group and not a co-ordinated water molecule. With all these hydroxy-acids, titrated in the presence of one of these metal ions, keto-acids were detected in the solutions after titration of the extra proton. By analogy with citric acid, the following conversions would be expected:

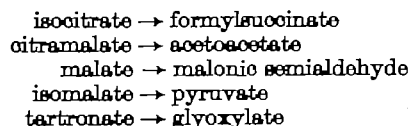


Fig. 3 shows the increase with pH in the amount of keto-acid (presumably glyoxylate) formed in solutions containing tartronate and  $MnCl_2$  in a 5:1 ligand:metal ratio, estimated as the 2,4-dinitrophenylhydrazone<sup>7</sup>. The enhancement of keto-acid formation by the passage of oxygen through the solution and its suppression when oxygen was excluded by passing oxygen-free nitrogen (nitrogen bubbled through Fieser's solution) through the

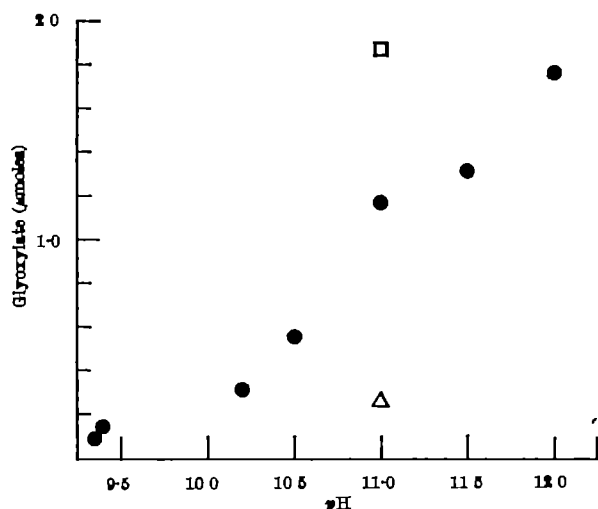


Fig. 3 pH-dependence of keto-acid (glyoxylate) formation from tartronate in the presence of  $MnCl_2$  at 25°. ● Each solution contained 1 ml. 0.05 M tartronic acid + 1 ml. 0.01 M  $MnCl_2$  + 5 ml. water. The pH was quickly brought to the required value with 0.108 M NaOH and maintained there for 15 min. At this time 5 ml. of the mixture was pipetted into 1 ml. M. HCl and the keto-acid estimated by the method of Friedmann and Haugen (ref. 7).  $CO_2$ -free nitrogen was passed through the solution during incubation; Δ, same conditions except that oxygen was rigorously excluded; □, same conditions except that oxygen was passed through the solution during incubation.

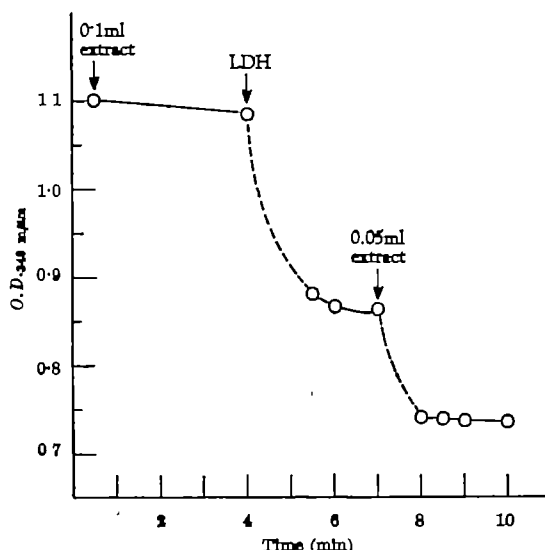


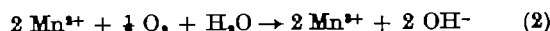
Fig. 4. Confirmation of the formation of pyruvate from isomalate in the presence of  $MnCl_2$ . The reaction mixture, volume 8.0 ml., initially contained 2.5 ml. 0.03 M  $KH_2PO_4$  (adjusted to pH 7.4 with NaOH) + 0.1 ml. of extract prepared as described in the text + approximately 0.5  $\mu$ moles NADH. Approximately 1.2 units of lactate dehydrogenase (Boehringer) were added as shown.

solution are also indicated. The formation of pyruvate and glyoxylate from isomalate and tartronate, respectively, was confirmed by paper chromatography of the dinitrophenylhydrazones.

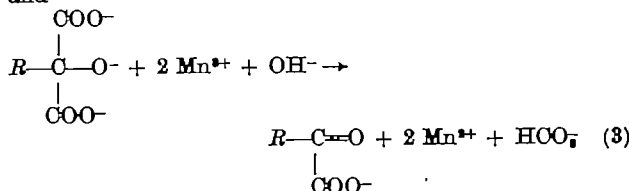
The formation of pyruvate from isomalate in the presence of  $Mn^{2+}$  ions was further confirmed as follows. A mixture of 1 ml. 0.1 M isomalic acid + 5 ml. water + 0.8 ml. 0.1 M  $MnCl_2$  was adjusted to pH  $\approx$  10 with 0.98 M NaOH, incubated for 15 min, acidified, and extracted with ether. The ethereal layer was re-extracted with about 2 ml. of 0.03 M  $KH_2PO_4$ , adjusted to pH 7.4 with sodium hydroxide solution. This extract was used to oxidize NADH in the presence of lactate dehydrogenase as is shown in Fig. 4, the reaction being followed by the fall in optical density at 340 m $\mu$ . Fig. 4 shows that 0.1 ml. of the extract caused the oxidation of only a part of the NADH present and further addition of 0.05 ml. of the extract caused the oxidation of a further amount of NADH. Isomalate (50  $\mu$ moles) did not oxidize NADH in the presence of lactate dehydrogenase.

Further experiments with alkaline isomalate solutions containing  $Fe^{2+}$ ,  $Fe^{3+}$ ,  $Zn^{2+}$  and  $Ni^{2+}$  ions showed that keto-acid was formed in the presence of  $Fe^{2+}$  and  $Fe^{3+}$ , but  $Zn^{2+}$  and  $Ni^{2+}$  proved inactive in this respect.

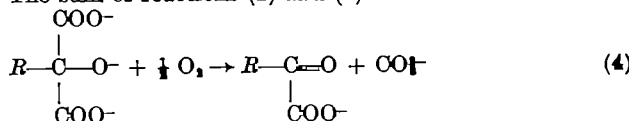
It is difficult to visualize the oxidation of  $\alpha$ -hydroxy-polycarboxylic acids in the presence of reducing species such as  $Mn^{2+}$ . The experiments shown in Fig. 3 indicate that oxygen may be involved. The following reactions may therefore take place:



and



The sum of reactions (2) and (3) is:



assuming that  $\text{HCO}_3^-$  reacts with  $\text{OH}^-$  to form  $\text{CO}_3^{2-}$  and water.

When oxygen was rigorously excluded from the solution (Fig. 3), the fall in pH with time in the region of the extra ionization was still observed but no keto acid formation could be detected. This would indicate that reactions (2)–(4) here are not the only ones which occur in this system.

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## WEBER RATIO IN GUSTATORY CHEMORECEPTION; AN INDICATOR OF SYSTEMIC (DRUG) REACTIVITY

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THE exponential nature of the perceptual process has been amply documented through the years from Fechner's discovery in 1860 to Stevens's<sup>1</sup> proposed modification of it in 1961. In order to propagate quantitative information, the nerve cells cannot increase the intensity of the impulses: they only increase their frequency. Katz<sup>2</sup> and Adrian<sup>3</sup> found that this frequency is proportional to the logarithm of the 'intensity' of the stimulus. It appears now that Stevens's power law describes sensation magnitude before adaptation to a test stimulus, whereas the Fechnerian discriminability law describes it after adaptation<sup>4</sup>.

### Re-examination of Data and Models

Let us examine the logarithmic function implied in the Weber-Fechner law in one sense modality: gustatory chemoreception. We focus specifically on the data of Lemberger<sup>5</sup> since they have been repeatedly used to support models and mechanisms in gustatory chemoreception.

Lemberger obtained 40 successive 'just-noticeable differences' (jnd) for sodium saccharinate. Beidler<sup>6</sup> plotted these data according to his "fundamental taste equation", an adaptation of the Langmuir adsorption isotherm, with the assumption that "each jnd is proportional to an equal increment of neural activity"; specifically  $C/R$  was plotted against  $C$ , where  $C$  is the concentration and  $R$  is the number of the successive 'just-noticeable differences'. A closer inspection reveals that the linear relationship applies for the concentration range between the 11th and the 36th jnd alone.

Duncan<sup>7</sup> also re-examined Lemberger's data for sodium saccharinate and re-plotted her first 22 jnd's; he found a pronounced curvature in the range of the first nine jnd's instead of a linear relationship implied by Beidler. It is apparent, therefore, that Lemberger's data in this lower range do not fit the fundamental taste equation nor is there a linear relationship beyond the 36th jnd.

In summary, Lemberger, Beidler and Duncan used the same set of data to fit their three different models. (1) Lemberger<sup>5</sup> (p. 308) showed that the taste response closely follows a logarithmic curve deviating from the Fechner law only in the lower and higher ranges. (2) Beidler<sup>6</sup> used Lemberger's data to fit his fundamental taste equation, whereas (3) Duncan<sup>7</sup> used them for the demonstration of a "hyperbolic rather than a logarithmic relationship between the magnitude of the response and the stimulus intensity".

It was of interest to us to plot Lemberger's data without trying to fit them to a model. Such an 'assumptionless'

plot of the 40 successive jnd's of Lemberger for sodium saccharinate relates the concentrations of the lower limits on the abscissa to their corresponding upper limits on the ordinate. Our plot (Fig. 1) illustrates a closely linear relationship between the lower and upper limits of successive jnd's within the range spanning jnd 7 to jnd 38. Our 'assumptionless' plot apparently illustrates a non-linear relationship for the first 6 successive jnd's followed by a linear relationship to the 38th jnd—just 2 jnd's from the maximum response.

We may define a jnd in taste as a 'signal' and its lower concentration limit 'noise' as Barlow<sup>8</sup> did for light stimuli. The well-known Weber ratio or signal/noise ratio<sup>9</sup> in these terms is independent of the units of measurement and thus is a dimensionless number. (Instead of equal increments of neural activity, we prefer to define Weber ratios as 'dimensionless numbers', that is, 'similarity criteria' or 'dimensionless ratios' rather than 'units'.) We plotted the size of each successive jnd,  $\Delta S$ , of Lemberger against the concentration of its lower limits,  $S$ ; the Weber ratio,  $\Delta S/S$ , is illustrated in Fig. 2 on a log-log scale. A distinctly non-linear relationship exists for the first six successive jnd's resembling a 'half moon'; a linear one for the range between the 7th and the 36th jnd revealing the prevalence of a steady signal/noise ratio. The non-linearity of the 'half-moon' portion of the curve, that is, the first six jnd's with a lack of steady signal/noise ratio, does not support models based on the Langmuir adsorption isotherm.

We proceeded to calculate the mean Weber ratio for sodium saccharinate—between the 7th and the 36th jnd—and found it to be  $11.02 \pm 0.08$  per cent (S.E.); the corresponding value for sucrose, again using Lemberger's data, between the 7th and the 22nd jnd, that is, the linear portion of the curve, was larger, namely,  $15.64 \pm 0.14$  per cent (S.E.).

The obvious hypothesis suggested itself: the larger Weber ratio for sucrose may be due mainly to its being a less-effective competitor than sodium saccharinate for receptor sites in the presence of tap-water electrolytes. Our subsequent experiments, however, did not substantiate such a hypothesis.

### A Comparison of Methodologies In Jnd Determination

Lemberger's criteria of taste testing are fully described in her paper<sup>5</sup>. The main features of her procedure are recapitulated only to enable us to compare them with ours. Specifically, she was her own subject, used tap-water as a rinse, a temperature of about 19° C for solutions and

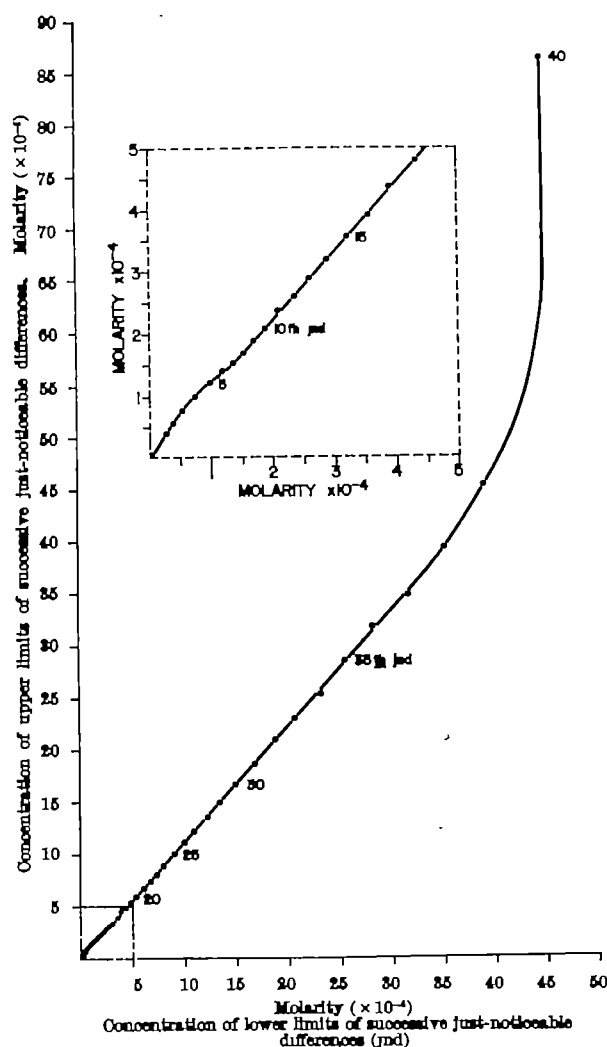


Fig. 1. Relation between upper (ordinate) and lower concentration limits (abscissa) of successive just-noticeable differences (jnd) in the taste sensation of sodium saccharinate calculated from Lemberger's data (ref. 5, Table 8). The plot of the first 18 jnd's is magnified in the upper left corner for better visibility.

rinses and a tediously slow sorting procedure—with one jnd determination apparently requiring 2–3 days. Lemberger's limits of accuracy for determining a jnd were based on obtaining at least 75 per cent differentiations.

Our modification of the Harris-Kalmus threshold determination procedure was adapted to just-noticeable taste difference testing and involved the use of distilled water for solutions as well as for rinsing between cups and the final 100 per cent correct sorting technique<sup>11</sup>. The following adaptations were introduced in order to make the method, originally designed for the determination of taste thresholds, suitable for jnd measurements. The original procedure involved taste thresholds, represented by solution numbers each of which corresponded to a concentration the double of the previous concentration. In our adaptation: (1) a threshold—the baseline or lower limit of the first jnd—was determined to a resolution of 1 per cent of a whole solution number; the same was true during subsequent jnd determinations, that is, also for testing the upper limit of each jnd (the standard errors of our procedure are to be found in Tables 1, 2 and 3); (2) during the determination of the upper concentration limit of each jnd we used—instead of distilled water placebo—the upper concentration limit of the preceding jnd; (3) the temperatures of all solutions and rinses were thermostatically controlled to either 19 or 22  $\pm 0.1^\circ\text{C}$ , the former corresponding to Lemberger's experimental conditions and the latter to our standard laboratory procedure. Our

subjects were trained, that is, 'learned', laboratory personnel constantly exposed for from 1 to more than 2 years to taste testing practice, and one additional subject who was a novice in taste testing. Lemberger's procedure required a criterion of 75 per cent correct sorting against our requirement of 100 per cent; this difference, however, cannot account for more than 0.5 per cent change in the values of the concentration limits for jnd's; (4) the concentrations are expressed linearly as molarities in Tables 1, 2 and 3, whereas, in fact, the resolutions of the upper and lower limits of the jnd's are determined to 1 per cent of a concentration corresponding to the next higher solution number, a member of an exponential series with a base of 2 (ref. 12).

The first eleven successive jnd's are well within the linear portion of a Weber ratio plot as evidenced by the fact that even up to the fifteenth jnd the plot remains linear. Therefore, we restricted ourselves to the determination of the first eleven successive jnd's.

#### Attempts to reproduce Lemberger's Data

Our attempts to reproduce Lemberger's data, specifically the 'half-moon' part of the  $\Delta S/S$  plot (Fig. 2), were unsuccessful. We set out, therefore, to determine the influence of each variable in the procedure on the outcome of the experimental data to account for our inability to reproduce her data. We studied the influence of three main variables possibly involved in the initial 'half-moon' feature of Fig. 2, namely, temperature, type of rinse water and chemical structure of the compound.

The importance of the difference in temperature of the compound solutions, rinses and placebo was determined by comparing Weber ratios obtained at a temperature of 19 $^\circ\text{C}$  (as used by Lemberger) and at a temperature of 22 $^\circ\text{C}$ . Table 1 presents the first eleven jnd's for sodium saccharinate obtained with solutions, rinses and placebo at 19 $^\circ\text{C}$  and Table 2 presents jnd's obtained at 22 $^\circ\text{C}$ . Temperature was found to be of no significance since the mean Weber ratio at 19 $^\circ\text{C}$  of  $33.03 \pm 2.6$  per cent (distilled water) and  $33.23 \pm 7.52$  per cent (tap-water), com-

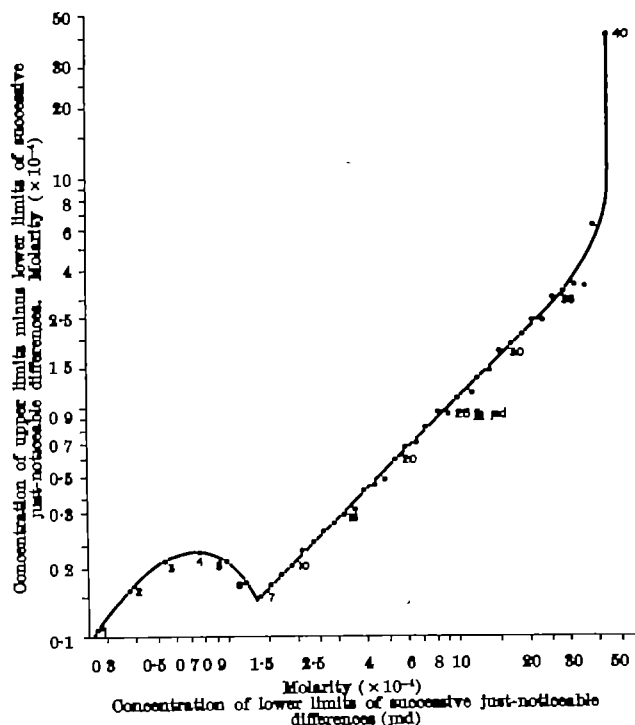


Fig. 2. Difference thresholds for sodium saccharinate. Logarithmic relation between the magnitude (in molarity) of successive just-noticeable differences on the ordinate and the concentration of their corresponding lower limits on the abscissa. Plot based on data of Lemberger (ref. 5).

Table 1. UPPER AND LOWER LIMITS AND THEIR CONCENTRATION DIFFERENCES OF THE FIRST 11 SUCCESSIVE JND'S EXPRESSED IN MOLARITY WITH CORRESPONDING WEBER RATIOS AS A PERCENTAGE FOR SODIUM SACCHARINATE AT 19° C USING DISTILLED WATER OR TAP-WATER RINSES

Successive jnds	Distilled water rinse				Tap-water rinse			
	Conc. of lower limit ( $M \times 10^4$ ) (S)	Conc. of upper limit ( $M \times 10^4$ )	Conc. diff. ( $M \times 10^4$ ) ( $\Delta S$ )	Weber ratio (per cent) ( $\Delta S/S \times 100$ )	Conc. of lower limit ( $M \times 10^4$ ) (S)	Conc. of upper limit ( $M \times 10^4$ )	Conc. diff. ( $M \times 10^4$ ) ( $\Delta S$ )	Weber ratio (per cent) ( $\Delta S/S \times 100$ )
1	2.53125	8.5525	1.03125	40.74	2.4844	8.5156	1.8012	41.51
2	3.5525	4.5000	0.9375	26.33	3.5156	4.4581	0.9375	26.67
3	4.5000	6.1875	1.6875	37.50	4.4581	6.0000	1.5419	34.74
4	6.1875	8.0625	1.8750	30.30	6.0000	7.9688	1.9688	32.81
5	8.0625	9.3750	1.3125	16.28	7.9688	10.3125	2.3437	29.41
6	9.3750	14.0625	4.6875	50.00	10.3125	14.0625	3.7500	36.36
7	14.0625	17.8125	3.7500	26.67	14.0625	17.6250	3.5625	25.33
8	17.8125	24.3750	6.5625	36.84	17.6250	24.3750	6.7500	38.30
9	24.3750	31.8750	7.5000	31.18	24.3750	31.8750	7.5000	30.77
10	31.8750	43.0000	10.1250	31.76	31.8750	37.5000	5.6250	17.65
11	43.0000	57.0000	15.0000	35.71	37.5000	57.0000	19.5000	52.00
Mean Weber ratio: 33.03 $\pm$ 2.64 per cent (S.E.)					Mean Weber ratio: 33.23 $\pm$ 7.52 per cent (S.E.)			

Table 2. UPPER AND LOWER LIMITS AND THEIR CONCENTRATION DIFFERENCES OF THE FIRST 11 SUCCESSIVE JND'S EXPRESSED IN MOLARITY WITH CORRESPONDING WEBER RATIOS AS A PERCENTAGE FOR SODIUM SACCHARINATE AT 22° C USING DISTILLED WATER OR TAP-WATER RINSES

Successive jnds	Distilled water rinse				Tap-water rinse			
	Conc. of lower limit ( $M \times 10^4$ ) (S)	Conc. of upper limit ( $M \times 10^4$ )	Conc. diff. ( $M \times 10^4$ ) ( $\Delta S$ )	Weber ratio (per cent) ( $\Delta S/S \times 100$ )	Conc. of lower limit ( $M \times 10^4$ ) (S)	Conc. of upper limit ( $M \times 10^4$ )	Conc. diff. ( $M \times 10^4$ ) ( $\Delta S$ )	Weber ratio (per cent) ( $\Delta S/S \times 100$ )
1	2.3906	3.28125	0.89065	37.23	2.3906	3.28125	0.89065	37.23
2	3.28125	4.2656	0.98435	29.99	3.28125	4.21875	0.9375	28.57
3	4.2656	5.53125	1.26565	29.67	4.21875	5.53125	1.3125	31.11
4	5.53125	7.40625	1.8750	33.90	5.53125	7.5000	1.96875	35.59
5	7.40625	9.3750	1.96875	26.58	7.5000	9.3750	1.8750	25.00
6	9.3750	13.1250	3.7500	40.00	9.3750	13.1250	3.7500	40.00
7	13.1250	16.8750	3.7500	28.57	13.1250	16.6875	3.5625	27.14
8	16.8750	22.1250	5.2500	31.11	16.6875	23.5000	6.8125	34.63
9	22.1250	30.0000	7.8750	35.59	23.5000	30.0000	6.5000	27.66
10	30.0000	37.1250	7.1250	23.75	30.0000	37.5000	7.5000	25.00
11	37.1250	53.2500	16.1250	43.43	37.5000	53.2500	15.7500	42.00
Mean Weber ratio: 32.71 $\pm$ 1.79 per cent (S.E.)					Mean Weber ratio: 32.71 $\pm$ 5.58 per cent (S.E.)			

pared with the mean Weber ratio at 22° C of 32.71 = 1.79 per cent (distilled water) and 32.71  $\pm$  5.58 per cent (tap-water), are clearly of the same order of magnitude.

Secondly, the influence of the type of rinse water was examined. Both sodium saccharinate and sucrose jnd's were determined with tap-water and distilled water rinses (Tables 1, 2 and 3). No significant differences were found in mean Weber ratios for sodium saccharinate at 19° C, 33.23  $\pm$  2.6 per cent (distilled water) and 33.23  $\pm$  7.52 per cent (tap-water); for sodium saccharinate at 22° C, 32.71  $\pm$  1.79 per cent (distilled water) and 32.71  $\pm$  5.58 per cent (tap-water); for sucrose at 22° C, 41.06  $\pm$  1.02 per cent (distilled water) and 40.48  $\pm$  1.14 per cent (tap-water).

Our investigation of the third variable, the possible influence of the chemical structure of a compound on the mean Weber ratio, was a comparison of the size of mean Weber ratios for sucrose (an un-ionized compound) with the data for sodium saccharinate (an ionized compound) at 22° C for the two-rinse water conditions (Tables 2 and 3).

A survey of our experimental data allows us to state the following: (1) A 3° C difference in temperature has no effect on the mean Weber ratios for sodium saccharinate other than to increase slightly the standard error at the lower temperature. (2) The type of rinse water has no influence on the mean Weber ratios for sodium saccharinate; however, the effect of a tap-water rinse is to increase the standard error (see Tables 1 and 2); (3) the tap-water rinse has no effect on the mean Weber ratios for either of the two compounds at 22° C; however, the standard error with tap-water rinse for the ionized compound (sodium saccharinate) is increased more than for sucrose, the un-ionized compound (see Tables 2 and 3).

In spite of controlling temperature and type of rinse water, we are unable to reproduce the initial curvilinear portion of Lemberger's sodium saccharinate curve ('half-moon') nor to establish a single factor to account for the lack of this initial curvilinear portion (from the first to the sixth jnd) in our data. Nor can we find any explanation for the 2.5–3 times smaller size of Lemberger's

mean Weber ratios for sucrose and sodium saccharinate, respectively, within the linear range of her data (from the 7th to the 36th jnd) compared with our mean Weber ratios.

Further differences between Lemberger's and our methodologies, however, may account for our inability to reproduce the curvilinear 'half-moon' (the range between the first and sixth jnd) and for the fact that her Weber ratios were 2.5–3 times smaller than ours:

(1) Our average daily performance consisted of determining up to 20 jnd's; whereas Lemberger, according to her description, can be assumed to have taken 2–3 days for the determination of one jnd.

(2) Our subject, as already mentioned, was 'learned', that is, his threshold for quinine due to conditions of well-defined practice suddenly decreased three thresholds in solution numbers, that is, eight-fold from the point of view of concentration<sup>18</sup> before this experimentation (each successive solution number represents a doubling concentration). We can define his taste sensitivity prior to learning: taste threshold for quinine in solution numbers equals 3, whereas that for 6- $\pi$ -propylthiouracil (PROP) equals solution number 6. The sucrose threshold of our subject was solution number 15, quite similar to that of Lemberger, whose threshold 16, expressed in our solution numbers, corresponds to a concentration of  $2.4 \times 10^{-4}$  M. Lemberger could not have been a 'learned' subject before her experimentation since, according to our experience, learning is apparently restricted to taste practice with quinine under defined conditions and it is not known whether learning can be achieved by tasting sodium saccharinate under Lemberger's experimental conditions. We also attempted to reproduce Lemberger's data with an 'unlearned' subject also slow in taste testing, who, under our experimental conditions, displayed from 25 to 100 per cent variation in the size of his first jnd for sodium saccharinate. We do not know whether the extent of variability is a characteristic of unlearned subjects or an individual characteristic of this 'slow' subject.

Although Lemberger repeated the determination of each jnd until the standard error was minimal for it, she

Table 3. UPPER AND LOWER LIMITS AND THEIR CONCENTRATION DIFFERENCES OF THE FIRST 11 SUCCESSIVE JND'S EXPRESSED IN MOLARITY WITH CORRESPONDING WEBER RATIOS AS A PERCENTAGE FOR SUCROSE AT 22° C USING DISTILLED WATER OR TAP-WATER RINSES

Successive jnds	Distilled water rinses				Tap-water rinses			
	Conc. of lower limit ( $M \times 10^{-4}$ ) ( <i>S</i> )	Conc. of upper limit ( $M \times 10^{-4}$ ) ( <i>S</i> )	Conc. diff. ( $M \times 10^{-4}$ ) ( $\Delta S$ )	Weber ratio (per cent) ( $\Delta S/S \times 100$ )	Conc. of lower limit ( $M \times 10^{-4}$ ) ( <i>S</i> )	Conc. of upper limit ( $M \times 10^{-4}$ ) ( <i>S</i> )	Conc. diff. ( $M \times 10^{-4}$ ) ( $\Delta S$ )	Weber ratio (per cent) ( $\Delta S/S \times 100$ )
1	0.780	1.080	0.300	38.46	0.780	1.080	0.300	38.46
2	1.080	1.560	0.480	44.44	1.080	1.560	0.480	44.44
3	1.560	2.160	0.600	38.46	1.560	2.160	0.600	38.46
4	2.160	3.120	0.960	44.44	2.160	3.120	0.960	44.44
5	3.120	4.320	1.200	38.46	3.120	4.320	1.200	38.46
6	4.320	6.144	1.824	42.22	4.320	6.144	1.824	42.22
7	6.144	8.544	2.400	39.06	6.144	8.544	2.400	40.535
8	8.544	12.480	3.936	46.07	8.544	12.480	3.936	46.07
9	12.480	17.280	4.800	38.46	12.480	17.280	4.800	38.46
10	17.280	24.960	7.680	44.44	17.280	24.960	7.680	44.44
11	24.960	34.176	9.216	36.92	24.960	34.176	9.216	36.92
Mean Weber ratio, 41.06 $\pm$ 1.02 per cent ( <i>S.E.</i> )					Mean Weber ratio, 40.48 $\pm$ 1.14 per cent ( <i>S.E.</i> )			

did not repeat the whole procedure of successive jnd determinations. We are unable, therefore, to calculate her standard error for reproducing the whole curve or to compare her data with that of our 'unlearned' subject.

The relation between the magnitude of stimulation and sensory response is tabulated for the six sets of data in Tables 1, 2 and 3, of which the latter deserves special attention. With arithmetically increasing concentration limits there is a corresponding geometrical increase in magnitude of jnd. This increase, evident in Table 3, is progressing in a quantum fashion, whereby each quantal unit is exactly the double of the previous one. The sinusoidal fluctuation in the magnitude of the first eleven Weber ratios (see Table 3), alternating with troughs of 38.46 per cent and peaks of 44.44 per cent, correspond to a period of 30 min.

In summary, a graphic and experimental re-examination of Lemberger's data led us to the elucidation of methodological factors in jnd determination influencing the size of a dimensionless ratio, the Weber ratio. For both sodium saccharinate and sucrose, we found a straight line plot for the Weber ratios with rhythmical doubling of concentration limits and sinusoidal fluctuations in the Weber ratios.

#### Size of a Weber Ratio as an Indicator of Systemic Reactivity

Recently it was found that a statistically significant relationship exists (within a group of 48 acutely ill female schizophrenic patients) between extrapyramidal side effects induced by a low cumulative dose of trifluoperazine and taste sensitivity. Side effects displayed in response to higher trifluoperazine dosage were more likely to occur in taste-insensitive patients<sup>12</sup>.

We wondered whether the size of a Weber ratio, instead of taste threshold, would also be related to systemic (drug) reactivity. Our pilot experiments indicate that trifluoperazine-induced tranquillization increases, whereas 'Piloocybin'-induced (central) sympathetic stimulation decreases, the size of an individual's Weber ratio for sodium saccharinate (see Table 4).

Our sample consisted of 12 acutely ill mental patients, 7 of whom were on high and 5 on low trifluoperazine medication with a male to female ratio 1:1; 7 healthy volunteers of whom 5 were under the influence of 'Piloocybin' and a week later without any drug. 'Piloocybin' was chosen because it produces a transient state of central sympathetic stimulation<sup>14</sup>, thus contrasting the tranquillizing effect of trifluoperazine and enabling us to compare the size of Weber ratios under these two extreme systemic conditions.

We used sodium saccharinate at 22° C with distilled water rinses when determining a jnd approximately two thresholds above the sodium saccharinate threshold of a patient but still well within the linear portion of the Weber ratio plot.

Even individual variations in reactivity to trifluoperazine or to 'Piloocybin' seem to be mirrored in the size

of the Weber ratio. To give a specific example: a taste-sensitive volunteer reacted to 12.5 mg 'Piloocybin' more intensely than another less taste-sensitive subject to 15 mg of the same drug; their Weber ratios at the peak of drug experience were 29 and 39 per cent respectively.

One set of data requires comment, namely, the approximately 20 per cent higher mean Weber ratio of the 7 healthy volunteers in Table 4 (48.9  $\pm$  1.8 per cent) as compared with the size of the mean Weber ratio for sodium saccharinate in Table 2 (32.71  $\pm$  1.79 per cent). The difference may be accounted for by our observation that continuous taste practice diminished the size of the Weber ratio of our subject in Table 2, whereas the 7 volunteers in Table 4 were not previously exposed to such practice. The nearly identical size of the Weber ratio (48.9  $\pm$  1.8 per cent) in our 7 volunteers may be accounted for by one factor: the sodium saccharinate baseline used in all 7 subjects was identical, namely,  $9.38 \times 10^{-3}$  M.

Table 4. RELATION BETWEEN DEGREE OF TRANQUILLIZATION OR SYMPATHETIC STIMULATION, THAT IS, SYSTEMIC (DRUG) REACTIVITY AND THE SIZE OF THE WEBER RATIO FOR SODIUM SACCHARINATE AT 22° C WITH DISTILLED WATER RINSES

Subjects	Mean Weber ratio for sodium saccharinate at 22° C
(1) Patients on a high daily dose of trifluoperazine (30-60 mg). <i>N</i> = 5, 25-40 years of age	21.7 $\pm$ 9 per cent ( <i>S.E.</i> )
(2) Patients on a low daily dose of trifluoperazine (5-20 mg). <i>N</i> = 7, 25-40 years of age	18.2 $\pm$ 13 per cent ( <i>S.E.</i> )
(3) Healthy volunteers without any drug. <i>N</i> = 7, 21-28 years of age	48.9 $\pm$ 1.8 per cent ( <i>S.E.</i> )
(4) Healthy volunteers, 100 min after the ingestion of 10-15 mg of 'Piloocybin', same subjects as (3). <i>N</i> = 5, 21-28 years of age	34 $\pm$ 2.7 per cent ( <i>S.E.</i> )

\* Only subjects previously exposed to 'Piloocybin' were included to eliminate effects related to 'novelty of experience'.

There are two other factors which may influence the size of a Weber ratio, namely, taste sensitivity and sex. Sensitive tasters have smaller Weber ratios than insensitive tasters; women are, under otherwise identical conditions, more sensitive tasters than men<sup>15</sup>. That excitation diminishes the size of the Weber ratio should not be surprising in view of the data of Kimura<sup>16</sup> and Chernotaki<sup>17</sup>. The latter author found that systemic sympathetic stimulation enhances afferent activity recorded from the glossopharyngeal nerve during application of gustatory stimuli to a frog's tongue. This article also offers an explanation for the sympathetic enhancement of peripheral gustatory activity noted by Kimura in the rat.

We have dealt with methodological and systemic factors related to the Weber ratio in gustatory chemoreception. The constancy within limits of this dimensionless ratio reminds us that we live in a world in which the absolute magnitudes of stimuli go unperceived, a perceptually compressed but "the best of all possible worlds".

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## IMPORTANCE OF MEMBRANES IN PROTEIN BIOSYNTHESIS

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THREE years ago, on the basis of a wide variety of observations reported in the literature from this and other laboratories, a general model for protein synthesis was proposed which suggested that ribosomes are membrane-associated when they carry out their synthetic activities in the living cell<sup>1</sup>. Since that time many new investigations bearing on the validity of such a functional cytological relationship have appeared. Since, at the moment, these important and related papers have not been considered as a unit, it seemed of distinct value to assemble the information so that a clear picture could be drawn of the existing evidence. Although many earlier reports demonstrated that membrane preparations, obtained principally from bacteria, were capable of efficient amino-acid incorporation into protein, the question of whether or not this represented a different mode of incorporation than the more usual free ribosome mediated systems was not amply considered. Furthermore, some systems actively studied, notably *E. coli*, were capable of efficient amino-acid incorporation even though an intracytoplasmic membrane system does not appear to be present and the ribosomes are considered to exist free in the cytoplasm. For the generality of the proposed ribosome-membrane model to extend to such systems as *E. coli*, the possibility was considered that a certain proportion of the ribosomes of *E. coli* were attached to the plasma membrane at any time. These attached ribosomes would be responsible for protein synthesis in the intact cell and due to a lability of their linkage to the membrane would be dissociated by the forces used to break the cell and afterwards would appear as free ribosomes.

In a series of papers, Hendler and Tani presented evidence that the foregoing description does apply to *E. coli* and that the generality of the membrane-ribosome structure for protein synthesis is not compromised by the finding of high amounts of incorporated amino-acids in free ribosome fractions<sup>2-4</sup>.

In several investigations dating from 1958, the group at the Chester Beatty Institute, including Drs. Butler, Hunter, Godson, Crathorn, and Goodall, examined the site of amino-acid incorporation in *B. megaterium*<sup>5</sup>. They showed that the membrane represented the major site of incorporation, that an appreciable fraction of the cellular RNA sedimented with and was held by the membrane, and that ribosomal particles could be released from the membrane by washing with buffers of low ionic strength. Schlessinger recently re-examined this system and confirmed that an appreciable fraction of the total RNA was bound to membranes (more than 50 per cent<sup>6</sup>). Most of this RNA was released from the membranes by lowering

the magnesium concentration from  $10^{-3}$  M to 0. Similarly, 0.2 per cent DOC or subsequent incubation alone was capable of releasing the bound RNA. The RNA released by DOC treatment sedimented in a sucrose gradient principally as polysomes of 4 or more ribosomes. The isolated membranes were capable of incorporating up to 6 times more amino-acid into protein per unit weight of RNA than the ribosome-polysome mixture obtained from a crude extract. Schlessinger pointed out that there was no direct evidence that the polysomes found in the DOC extracts of membrane were present in the membranes as polysomes. In yeast, a membrane-ribosome fraction was described which could incorporate sulphur-35 into stable peptide linkage 5-7½ times faster than free ribosomal particles on a per unit RNA basis<sup>7</sup>.

Moore and Umbreit, in a preliminary communication<sup>8</sup>, reported that in *S. fecalis*, when a washed membrane fraction and a ribosomal fraction were compared, it was found that the membrane-bound ribosomes were about 5-fold more effective than cytoplasmic ribosomes for the incorporation of radioactive amino-acids. This work has continued and Moore and Umbreit have demonstrated that the incorporation of amino-acids in the free ribosome fraction is proportional to their phospholipid concentration. Phospholipid is found only in membranes of *S. fecalis* and is absent from cell walls and cytoplasm. Consequently, these authors conclude that the results indicate a membrane constituent to be the limiting factor in protein synthesis in this system even for the 'free' ribosome fraction<sup>9</sup>.

The localization of ribosomes at membrane sites is further demonstrated by studies involving the incorporation of labelled precursors into RNA in *E. coli*<sup>10</sup> and *Streptococcus fecalis*<sup>11</sup>. In HeLa cells infected with polio virus, it has been shown that the polio-specific polysomes are also membrane-attached<sup>12</sup>. Another study having a possible bearing on this question is that of Neu and Heppel<sup>13</sup>. They found that during the formation of EDTA-lysozyme spheroplasts of *E. coli*, all the latent ribonuclease activity was released from the unlysed cells.  $\beta$ -Galactosidase was not so released, but could be obtained after lysis. If, as is commonly thought, the latent RNase is a part of the *E. coli* ribosome, then this investigation would be consistent with a membrane localization of the ribosomes. Another alternative considered by these authors is that the ribonuclease may actually not be part of the ribosome, but could exist free in a pericytoplasmic compartment.

The sensitivity of micro-organisms to antibiotics also points to a functional association of membranes and

ribosomes. In an investigation of the early sequence of events following the addition of streptomycin to growing cultures of *E. coli*, Dubin *et al.* have demonstrated that loss of membrane integrity and impairment of protein synthesis appear to be related events<sup>13</sup>. More recent work has fixed the site of ribosome damage at the 30S component of the ribosomes<sup>14,15</sup>. Nomura, in a recent investigation of the mechanism of killing action of antibiotic colicines, has also presented evidence in favour of a functional co-ordination of macromolecular synthesis and cell membranes<sup>16</sup>. Thus, several of the colicines inhibit either together or separately the processes of RNA and protein synthesis, oxidative phosphorylation, and maintenance of DNA. The addition of trypsin reverses the action of the colicines. Since the trypsin does not penetrate the bacteria, the colicines presumably act at the cell surface. Nomura discussed the possibility that reactions involved in macromolecular synthesis are integrated in the cell membrane. In a different but related study<sup>17</sup>, it was found that suspensions of *E. coli* cells, after freezing or incubation with EDTA or polymyxin B, participated in amino-acid catalysed ATP-pyrophosphate exchange. It was shown that the reaction took place at the cell surface of the intact cells. The reaction products were found extracellularly. The authors considered these results in terms of the possible localization of conventional amino-acid activating reactions at the cell membrane.

Stafford *et al.* found active polysomes bound to membranes in fertilized sea-urchin eggs<sup>18</sup>. The major fraction of incorporated radioactivity was into a heavy, easily sedimentable fraction. Ribonuclease partially released ribosomes from this material, whereas 0.3 per cent DOC totally released radioactive ribosomes. Hultin found that the major fraction of amino-acid incorporation in fertilized sea-urchin eggs was accounted for by structures which reached the bottom of a sucrose density gradient (5-22.5 per cent concentration) in 60 min at 53,000g (ref. 19).

There are several recent investigations carried out with liver preparations which underline the importance of membrane-bound ribosomes in protein synthesis. Henshaw *et al.* showed both *in vivo* and *in vitro* that membrane-bound ribosomes accounted for virtually all the incorporation of amino-acids<sup>20</sup>. Although the authors suggested that this situation may be contrasted with that existing in cells lacking an endoplasmic reticulum, the work by Handler and Tani<sup>2-4</sup> in *E. coli*, already mentioned here, suggests that, even in the latter case, membrane-bound ribosome may be functioning.

Campbell *et al.* examined the amino-acid incorporating ability of microsomes, RNP particles released from the microsomes by DOC, and free ribosomes in the presence and absence of poly-U, and as obtained from normal and regenerating liver<sup>21</sup>. They concluded that the major synthesizing activity of the liver cell was associated with membrane-attached ribosomes, that the membrane plays a part in the control of the activity of the attached particles and that free ribosomes are comparatively inactive, but may be stimulated by adding RNA such as poly-U. Similarly, Howell *et al.*, as a result of incorporation studies with liver both *in vivo* and *in vitro*, have concluded that membrane-bound ribosomes are responsible for the bulk of the protein synthesis<sup>22</sup>. Von Der Decken concluded similarly that microsomal membranes play an important part in the synthesis of serum albumin<sup>23</sup>. This conclusion stemmed from the observation that although microsomes appeared to be capable of synthesizing serum albumin, and RNP particles released from microsomes could incorporate amino-acids, the released particles could not make serum albumin.

Several groups have investigated protein synthesis in mitochondrial preparations. Roodyn used rat liver mitochondria for the *in vitro* incorporation of amino-acids<sup>24</sup>. Afterwards he step-wise fractionated the labelled mitochondria by titration with detergent. He found

that the bulk of radioactive protein was in a fraction rich in phospholipid and RNA. Further fractionation showed the radioactivity to be closely associated with the lipoprotein components which Roodyn suggested were derived from the mitochondrial membrane. From *in vivo* labelling studies with rats, Truman also concluded that the site of incorporation of amino-acids in liver mitochondria is associated with the mitochondrial membrane<sup>25</sup>. In a separate *in vitro* study with ox heart mitochondria, Truman showed that the most highly labelled proteins were soluble in 50 per cent ethanol, and were of low density and low polarity<sup>26</sup>.

The cytological validity of an intimate association of ribosomal and membrane components receives support from recent electron microscopic studies of mouse pancreas by Sjöstrand and Elvin<sup>27</sup>. These workers confirm and extend earlier observations by Sjöstrand and Baker<sup>28</sup> and Hanson and Hermodsson<sup>29</sup> concerning the use of osmium-fixation as opposed to controlled freeze-drying in preparation for electron microscopy. They present evidence that the ribosomal material forms an almost complete carpet covering the membrane elements. The subdivision into particles that is more commonly observed they attribute to artefacts of fixation. Recent electron microscope investigations with *B. cereus*<sup>30</sup>, *B. megaterium*<sup>31</sup>, and *E. coli*<sup>32</sup> clearly show ribosomes and ribosomal clusters attached to membranes.

Investigations on the existence and turnover of lipoprotein and lipopeptides from a wide variety of sources continue to appear. With the clearer establishment of active ribosomes at lipoprotein membrane surfaces during their participation in protein synthesis, the potential significance of these findings is considerably strengthened<sup>33-35</sup>.

During the past several years it has become quite clear that the reactions of electron transport are structurally integrated into the fabric of the membrane of the mitochondria or bacterial cytoplasmic membrane. In such a structure, energy released by oxidation is converted to ATP via high-energy intermediates. The ATP, being water-soluble, is then available throughout the cytoplasm for energy-requiring reactions. Because protein synthetic reactions may be similarly integrated by a membrane, the suggestion was offered<sup>36</sup> that high-energy intermediates produced in a membrane may be directly used for protein synthesis without the necessity of being converted first to ATP. Bronk added considerable support to this idea in a study of energy-dependent amino-acid incorporation in rat liver mitochondria<sup>37</sup>. He found that, at concentrations of oligomycin which blocked the terminal phosphorylation steps leading to ATP formation, the incorporation of <sup>14</sup>C-leucine was not inhibited. Thyroxine and tetraiodo-thyroacetic acid both blocked phosphorylation of ADP while causing a stimulation of amino-acid incorporation, presumably by diverting energy in the direction of the protein synthetic reactions. The same question is the subject of a careful study by A. M. Kroon, with mitochondria and microsomes isolated from rat liver<sup>41</sup>. Although some of the evidence points to the direct utilization of high-energy intermediates, Kroon gives reasons why it is not conclusive at the present time. Handler and Jarett have attacked the problem in a different way<sup>42,43</sup>. Yeasts were grown under strict anaerobiosis with energy supplied by fermentation of glucose. In confirmation of earlier reports<sup>44-46</sup>, it was found that both DNP and azide, in concentrations which were capable of inhibiting oxidative phosphorylation, could block protein synthesis. It was determined that fermentation was not affected and that ATP-levels in the inhibited cells were equal to or greater than those in the uninhibited controls. DNP and azide had no inhibitory effect on any of the reactions involved in *in vitro* amino-acid incorporation when ATP was present. This was also true for reactions that were stimulated by the addition of poly-U. Since these two

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## BRITISH ASSOCIATION MEETING IN CAMBRIDGE

### SCIENCE AND SCIENTISTS

By SIR CYRIL HINSHELWOOD, O.M., F.R.S.

President of the British Association

THE annual gathering of this Association is traditionally the occasion when men of science and laymen come as it were into conference, when the scientists try to explain themselves and expound what they are doing, and the lay world listens with varying proportions of excitement, sympathy, criticism, scepticism or amusement. Attitudes have varied. The early comments of august sections of the Press on the proceedings seem to have been inspired by the spirit of Dean Swift at his fiercest. Bishop Wilberforce acted the buffoon though his intentions can scarcely have been playful, and the well-known abbreviation of our title, while indicating that element of popularity which most nicknames connote, summed up what a large part of Britain in its heart of hearts long felt about science and all connected with it.

To many, however, the story has been a very great one. I remember in my youth being thrilled to the core at presidential addresses by Oliver Lodge and by J. J. Thomson. We were at the heyday of the Wellsian enthusiasm for scientific progress. Utopia and revelation seemed just around the corner. Let the scoffers scoff: let the die-hard upholders of the classics denounce science if they wished, or suggest that it was not quite the thing, it was nevertheless a wonderful and moving adventure. It had long been the affair of a small minority of people. The originators of the Royal Society had been exceptional spirits, though we should never forget how much more exceptional must have been those unknown men of genius who in the course of the long ages of pre-history harnessed fire, drew forth metals from their ores, tamed animals and cultivated crops. One would give much to know their unrecorded saga.

But science, which was once a private affair, has now become a public concern. Several things have contributed to this. Science in its own right made at length progress in the battle for a place in education, though vestiges of the old prejudices long survived. More important, everyone has become increasingly aware of the power of applied science to affect our lives. Sad to say, two great wars played a major part in this enlightenment, and, perhaps in consequence, some people fear the destructive powers of science more than they appreciate its beneficent gifts. But most will recognize that our own choice decides what use we make of our control over Nature, and what any one of us thinks the balance will be depends chiefly on whether he expects good or evil to prevail in the world generally. In the last resort I believe the majority

of people expect, on the whole, good to flow from the use of knowledge.

Apart, however, from a desire for practical benefit, something of the romance of science has spread widely, and here, not only inexpensive literature, but also radio and television, themselves the children of applied science, have greatly contributed to what can very nearly be called a revolution.

There is, nevertheless, not much doubt that more general interest centres on applied science than on pure science. Governments are anxious for economic advantages from technology, so too is industry, and so, on the basis of what he is told, is the ordinary man. Everybody agrees that we now need many more scientists, and that we have an even greater need of technologists or applied scientists. Rather ambitious plans, which it is to be hoped are all quite sincere and genuine, are laid for unprecedented expansion, and certainly the desire for new things and for the means to improve the common lot is widely felt.

All in all, at the present time, it seems not inappropriate to say something about science and scientists in general.

There is a certain lack of clarity in many quarters about the relation of pure and applied science, and that is the first matter about which I would like to say something. Scientists themselves were at one time commonly believed to be mainly concerned with practical ends, and indeed there are still people who think that scientists care only about gadgets and gimmicks. This is by no means true, and as a matter of fact, to-day, scientists who were once regarded as 'rude mechanicals' are now quite commonly criticized for neglecting practical ends. They are now reproached for not doing quickly enough things which they were once laughed at for doing at all. But it is no use being amused or resentful at this odd example of time's revenge. We must try to see more clearly what the situation is and what might be done about it.

The relation of pure science to applied science is exceedingly subtle and complex. The urge to explore, understand and depict Nature is by no means the same thing as the desire to master and control it, nor are the relevant talents and abilities the same. Some men of science have had a keen desire to see their discoveries turned to practical account: others have been indifferent. Some have even affected to despise utility; but this was, I fancy, rather a gesture of defiance to their nineteenth-century detractors.

History shows a long record of discoveries made by one man and turned to account by others far removed in space and time. This state of affairs was well enough when all these things rested with amateurs. But the problem of application has now to be much more squarely faced, the more so in that the great majority of scientists to-day are people to whom society has explicitly provided opportunities in the expectation of tangible and useful results. What then is the right policy to follow?

What would be quite useless would be any kind of Draconian edict that all scientific work must henceforth be devoted to demonstrably practical ends. There could be no surer way of rendering the future completely barren, since nearly all great technological advances depend on discoveries so unexpected as to be unplannable. Nature in her own time reveals her secrets to the patient questioner, and the plain fact is that Nature is infinitely cleverer than man. Pure science, therefore, cannot be dismissed as a polite amusement and it would be a great tragedy if people with a real vocation for it were seriously diverted in the supposed interest of utility.

Nevertheless, it is deplorably true to-day that of the very large numbers now joining the ranks of science far too few seem to be evincing the technological interests which the future calls for. Reflexion on the native genius of our country suggests that the mechanically minded, the adventurously practical kinds of people should be by no means rare. Why then do we hear complaints about the lack of recruits for applied science? This is a very complex problem with no short and simple answer; but I think that two influences have been important. The traditional second-class status of science in the Establishment tended to deter all save the minority with a real vocation for it. The efforts of this dedicated minority not unnaturally won for British science no little prestige in the world, enough in fact to make this same field seem the most attractive to the rapidly increasing number of uncommitted entrants. Some young people are reproached for what is now alleged to be snobbery in their preference for pure science. On most of them this is a quite unjust judgment. Some are led there by real vocation, some by example, some because nobody has offered them an alternative, and some because large sections of opinion still draw no distinction between pure and applied science.

If some fault in the system of values is responsible, then the blame lies rather with the climate of society which has withheld its due esteem from practical skills, and until recently from science itself, the real nature of which it misunderstood. There is also another very significant facet of the situation. Great technological advances are made, not necessarily by those with profound insight into the secrets of Nature but by those with alert, enterprising, ingenious but quite unacademic minds, in some ways at least as much men of action as men of thought, who ask the question not so much 'Why does this happen?' as 'How could this be done?' or 'What use could be made of this?'. In the past our educational system would largely have diverted young people of this sort from any kind of science, pure or applied, and their talents would have flowered in other fields, where the rewards were greater. The damage is then done, since clearly nobody can apply scientific facts if he does not know any of them to apply.

Another trouble with our traditional methods of education, selection and promotion is the unjustified primacy which we have accorded to skill in purely verbal expres-

sion. It is quite wrong to suppose that all thought is through the use of words. Non-verbal images can be just as important. Some people think with their hands, and we need to give much greater credit to manual skill than we do (oddly enough with the exception of athletics).

It may indeed well be, as things are, that some appreciable diversion from the pure sciences to the applied sciences is called for. If it is really true that there is any impression abroad among young people that applied science is in itself a sort of second best to pure science in the scheme of things, then it ought to be dispelled. The adventure of controlling Nature is no less great than that of understanding its deep secrets, and the building of bridges as much artistic creation as the building of abstract systems of thought. We should be foolish indeed if we rejected the creations of Michelangelo because they are hewn in stone and accepted those of musicians or writers because they exist only in the mind.

But by no means all the recruits for technology need come from pure science. Many indeed could and should come from quarters which society has hitherto discouraged from developing any interest in science at all. It appears that lately there has been a turning away from pure science too. But can we blame young people with uncommitted minds for not choosing a course which means harder work and smaller prospects of esteem. They still feel, no doubt, that their ultimate success and influence will be limited by the old slogan of the administrator who proclaimed that the scientists must always be on tap and never on top. The formula which should be followed is simple enough, however difficult its application may prove to the complex situations of real life. Since men with the talent and the temperament for turning knowledge to practical use may turn up anywhere, in any social stratum, in any academic discipline and with any general intellectual background, we should see that far, far fewer of them any longer remain ignorant of science and perhaps even proud of their condition, and that those who respond should not find themselves relegated to a subaltern status.

Another important question of the day, and one not unrelated to what I have said, is that of the organization of research. In the grand perspective of world history the interplay of discovery and application has been largely an affair of chance. Now we wish to guide and plan the process, to rationalize and accelerate it.

A popular idea sees the planning and direction of research as a logical process proceeding by obvious stages from point to point to a predetermined goal after an expenditure of time and money calculable in advance by accountants. It is unfortunately nothing of the kind. Anybody can state a goal, whether the cure of the common cold or the control of the weather, and the proper organization may provide money for the effort. But inspiration comes only to rare individuals: it floats up intuitively from the subconscious. It is communicable from a leader to a team only if the members have confidence in him, both as a man and as a scientist. In some respects the situation in scientific research is rather like that in a football match. The players, the best available, must know the rules and will with advantage have concerted some general lines of strategy. The overall direction of play is predetermined. But after the start each new development grows out of what immediately precedes it, and depends on the inspiration of individuals as much as on the understood policy. Any captain who tried to plan too much in too great detail would lead the team only to

defeat. The captain himself must be at least a competent player in his own right.

There is little place in scientific research for philosopher kings, prescribing broad human objectives to teams of docile scientists kept 'on tap but not on top'. Nor, however, is it expedient to leave the romantics, the artists, the creators and the dreamers in ivory towers (whether in affluence or poverty). Nor again is it wise to try to transform them, by persuasion or otherwise, into something radically different from what they are. The world would probably have been poorer had Beethoven been forced to carve statues. Nor is it any advantage to transfer all confidence to the practically minded inventor, who would eventually be left without any new knowledge to apply. Again and again the key to a great discovery has been an unexpected observation, but generally speaking if it is to be quickly fruitful it must have been made in the context of a well-defined pattern of thought and effort. The famous Fleming mould would not have meant much to an administrator or even a nuclear physicist. But quite apart from these anecdotal accidents, and more important, is the fact that as the chart of the unknown becomes filled in, judgment of the most profitable course to follow itself changes. Mysterious inlets may prove dead ends or may open into vast seas. Research on the distribution of energy in the spectrum of a hot body might have proved a mere student exercise in physics; in fact, it revolutionized the whole structure of scientific thought.

If we wish to plan research we can only do so by assembling a community of people with varied and mutually complementary talents, operating with strategic flexibility, and in an atmosphere of curiosity in which the members know and understand what the others are talking about and respect their leaders. We shall forget at our cost that an honoured place in such a community must be found for the dedicated thinker. We must remember the words of Newton who, when once asked how he made his discoveries, said: "By always thinking unto them. I keep the subject constantly before me and wait till the first dawnings open little by little into the full light".\*

No committee structure, however logical and tidy, will replace the devotion of which Newton speaks.

Let no one think that a scientific community can be led either on one hand by people uninterested in human relations, or on the other hand by professional humanists without real personal understanding of what science involves.

A great deal of nonsense has at one time and another been talked around the allegation that scientists are interested in things rather than people. I have only to look at my own friends to know that this is utterly untrue; but if it had any truth the fault would rest with the educational system of selection which has indeed quite often attempted to segregate impressionable youth in accordance with this false philosophy, and I am afraid may still occasionally try to do so.

The prescription for future success in applied science is, after all, humane enough: a well-assorted community of human types, the analytical, the creative, the enterprising, the manually dexterous, all enjoying equal status, so that each part of the task may be taken up by some when others have made their contribution. True, all concerned must know the rudiments of the same language,

the general idiom of science. Is this an impracticable demand? Is it an aim lacking in humanity or social conscience?

We hear a good deal of talk about speeding up the advance of discovery and application. We all hope this can be done. But let nobody suppose it can be done by mere administrative elaboration or by the imposition of political doctrines. Let us imagine that somehow we can find the right balance between democratic equality on one hand and incentive and leadership on the other, and that by some miracle we achieve an administration capable of fostering effort while curbing extravagance and waste: the fact will still remain that scientific research of a creative kind is a very difficult business, and that its application is both adventurous and laborious, calling not only for courage and skill but also for that old-fashioned commodity known as faith. If the spirit is to spread more widely from the relatively few individuals of the past the whole climate of society must be favourable.

I have spoken so far of the application of science, because at certain seasons the most urgent task may be to gather the fruit from the tree. But woe betide those who kill the roots of the tree in this process: and this thought leads to deeper matters. I have the honour to be addressing the British Association for the Advancement of Science. Let there be no doubt about it, science is one of the great activities of the human mind and soul, and in the last resort let us remember that society was made for men and not men for society. As Dante's Ulysses says to his companions: "You were not born to live like the beasts, but to strive after virtue and knowledge". The question why we pursue knowledge is, I suppose, only part of that wider and nearly unanswerable one, "What is the object of life?". Even if they cannot give a satisfactory answer to the riddle, many men will undoubtedly continue the pursuit. Some may answer that we pursue knowledge to gain affluence and leisure. What affluence and leisure are to be used for is a personal question. To pursue knowledge will probably be the reply of the true scientist.

How then do scientists work? Does their work change their attitude to existence? Most of the conventional generalizations about them are quite crudely wrong, as becomes obvious if one reads the biographies of a few.

The old-fashioned view, the infection of which still tends to be inherited, saw them as materialistic, ignorant or contemptuous of beauty and ethics, respecters of the narrow truth they can comprehend, and uninterested in human relations to the point of ruthlessness. Sometimes they have been seen as supermen who could open quick and easy roads to affluence, though, it must be said, latterly sometimes also as supermen who can be mass-produced by administrative strokes of the pen.

To see what kinds of generalization are possible at all let us consider briefly the case-histories of a few outstanding scientists of the last generation. We will begin with three great physiologists, all Nobel Laureates, who worked in related fields: Pavlov, who created the science of conditioned reflexes; Ramón y Cajal, who was described by Sherrington as the supreme analyst of the cellular architecture of the nervous system; and Sherrington himself, who charted the functioning of the nervous system as a whole.

The work of the Russian, Pavlov, on reflex responses has been much quoted in anti-religious propaganda and to a superficial view it might well seem to assimilate living creatures more closely than before to automata. Pavlov

\* This saying was first made known to me by my friend Prof. R. N. da C. Andrade. If he had not been a great scholar he would not have known it, and if he had not been a fine physicist I do not suppose he would have realized its force.

himself is said to have spoken of religion as a social conditioned reflex. He was reputed to have been tenacious, hardworking, exacting and occasionally intolerant, averse to mathematics, chemistry and to too much theory, and to have preferred muscular to mental satisfaction. He was, however, not the product of a scientific environment: his father was a provincial clergyman.

The Spaniard, Ramón y Cajal, on the other hand, was the son of a surgeon of sorts, who taught his son anatomy, but the boy spent most of his time reading romances, was ill-treated at school, apparently for objecting to Latin, and presently expelled to become the leader of a rebellious gang. His passions were sketching, verse, fiction and philosophy. Wonder was aroused in him when he first saw the blood circulate in the web of a frog under the microscope: he found it what he described as "an astonishing spectacle, an unforgettable event in my life", and surely it was an artist who had no sleep the night after he had first seen a finely stained preparation of cerebral cortex in which, as he said, "all was sharp as a sketch with Chinese ink on transparent Japan-paper".

Family influences interested Sherrington in medicine and he passed by more or less conventional channels into research. He grew up among pictures of the Norwich school, wrote poetry and it has been said of him that the poet was never deep down. Though he, more than any other man, elucidated the nature of nervous reflexes, he was strongly opposed to any mechanistic view of the world. "Mind," he wrote, "knows itself and knows the world: chemistry and physics, explaining so much, cannot undertake to explain Mind itself."

The work of these three men, revealing the marvels and subtleties of nervous mechanisms, could no doubt be cited to support the views of behaviourists and materialists. Pavlov may have been one: the other two were not, and Sherrington never ceased to protest against attempts to explain away the phenomena of consciousness. The trio differed in background, temperament and tastes. What they had in common was the passion for knowledge, for the discovery of things previously unknown, and for the unravelling of mysteries—a characteristic which reflects a strong vein of romance. Another major fact, and one which is true of most scientists, is the essentially aesthetic nature of the appeal which their work made to them. Ramón's wonder at the spectacle of the circulating blood and Sherrington's poetry are indications of it and it appears indirectly in Pavlov's love of collecting, which ranged from butterflies to paintings. But romantic curiosity and the enjoyment of wonder and beauty do not make the man of science unless they are allied to a deep wish not only to know but also to organize knowledge into a coherent picture of the world. Science, whatever else it may be, is a form of creative art.

Let us now see how these themes appear in the lives of some great physicists of roughly the same generation. Max Planck at the turn of the century revolutionized thought by revealing an essential discontinuity in the working of Nature. But he came from the most respectable stratum of the German Establishment of his time. If we seek to pluck out the heart of his mystery we may listen to his own words: "the outer world," he says, "represents something independent of us and absolute with which we are confronted, and the search for the laws which govern this absolute has appeared to me as the most fascinating work of a lifetime." Rivalling the passionate concern with the scheme of Nature was the love of music which was a major element in his life, and which he shared

with Einstein and with a surprisingly high proportion of physicists.

Another man who sought with adventurous boldness to depict the system of Nature was Eddington, who had a basically mathematical approach in his interpretation of the world, and spent the latter part of his life in a titanic attempt to find the inner relations between the microcosm within the atom and the macrocosm of the stellar universe.

Very different in origin and in his exuberant, extrovert personality was Rutherford, the New Zealander. Like Newton and like Wren, Rutherford had as a boy a taste for mechanical construction—though he was also no mean scholar and he also believed in keeping mathematics in its place. One of his reported *obiter dicta* is to the effect that no physical theory is worth much if it cannot be explained to a barmaid (a class of lady who still existed in Rutherford's day). He, too, had the intense energy and the concentrated urge to discover, and he, too, created his own pictures of the world of Nature, but ones very different from the abstract patterns of Planck and Eddington and based on conceptions of atomic structure vivid and powerful enough to appeal to the most simple-minded.

Just as these men differ, so does the actual idiom of scientific representation, which varies from the most concrete to the most intangible. Rutherford liked the most down-to-earth imagery. Yet he was far from simple-minded himself, and his own symbolization moved chemistry and physics forward by a generation. Einstein, on the other hand, brought facts and ideas about the speed of bodies and of light and about gravity into formulations, which some philosophers declared contrary to common sense and which certainly are of an extremely abstract character. Oliver Lodge, who presided over this Association just half a century ago, had a taste for more shadowy prescriptions. He wrote, "my instinct seemed to be more abstract, rejoicing in hidden forces... ultimately becoming more absorbed in the ether as a non-sensuous reality—than in the material objects themselves". But the primitive models of Rutherford, the elusive ether of Lodge and the Lewis Carroll-like mathematics of Einstein were no idle playthings. They were the working tools with which radio was developed, the existence of nuclear energy predicted, and the method for its release discovered.

The creations of various men of science may be likened to pictures by artists of different schools, all conveying essential truth but in varied forms. The individual nature of its symbolism lays science open to the attack of those philosophers who like to question minutely the use of language in every statement. Men of science, although some of them play at this game themselves, tend on the whole to be impatient of purely semantic discussions and indeed most of them would probably feel that present-day linguistic philosophy has been not unjustly described as an affair of limited risks and small returns. In many ways creative science is just the converse of this. A philosopher once told me that the now notorious second law of thermodynamics was not so much false as meaningless. So be it. Force and energy themselves as concepts can be subjected to destructive analysis yet these elements are combined by scientists into patterns which not only give artistic satisfaction but also lead to world-transforming practical discoveries. Newton's treatment of force and mass can easily be represented as a circular argument and Einstein's spatio-temporal schemes dismissed as illogical. Yet these things are part of the

foundation of nuclear physics, and nuclear energy and the hydrogen bomb, whatever else they may be, can scarcely be written off as meaningless.

The creative scientist is in fact usually more concerned with the relations of things to one another than with precise verbal analysis of what these things are. He seeks a representation of the world which continually grows by an extension or transformation of what is there already. Thus what many scientists are really after is the adventure of discovery itself.

Rutherford used to talk about science as though he believed—and he did believe—that wonderful new revelations and illuminations were just about to occur. We are at a most exciting stage, he would say. There is ample ground for such a feeling at the present time. The last section of this address will therefore be devoted to a brief survey of some of the great fields of present activity, and I shall try to say something of the fundamental problems of science which are of the deepest significance in their own right. By a strange uncovenanted mercy it is indeed the study of these, if history has any lessons to impart, which in the long run will have the greatest effect on human life. But I hope that what I have already said will have shown that this observation implies no reserve about the urgent necessity of other work of shorter range.

The questions which still remain the most absorbing are the oldest of all: the nature of the cosmos, the nature of matter, the nature of life.

People have, I suppose, always wondered about existence. The most primitive thinking scarcely distinguished the inner world of consciousness from the external world of space and matter, and many strange phantasmagorias of magic and myth have been conceived. Chinese philosophers saw in the world a conflict of positive and negative principles, and Pythagoras thought that geometry and number ruled the order of Nature. These systems are much more abstract than the atomic theory which even as expounded by the Greeks and by Lucretius gave a vivid idea of the hidden happenings underlying visible phenomena, and as developed in modern times served as a basis for most of the great triumphs of chemistry. But still more recently a great wave of abstraction has again swept over science.

What Jeans called the "Mysterious Universe" has lost nothing of the mystery which surrounds its structure, its origin and its history, and which extends to the stuff of which it is made and to the forces which act between its parts. But, in all these respects, lively imagination is continually playing on a stream of new discovery.

What is already known about the structure of the universe is surprising enough: thousands of millions of stars forming galaxies; countless galaxies rushing apart from one another with velocities which appear to increase with the distance from some assumed centre. This last fact has suggested, as everyone knows, that at some cosmic zero hour a vast central agglomeration might have exploded, the parts which then received the greatest velocity having by now travelled farthest. I must remark in passing that to identify this zero with a moment of creation seems to me neither sound science nor sound theology but a sentimental pastiche of both. To speak, moreover, of a beginning of time itself marked by this spectacular event seems meaningless. If this singular moment actually occurred it must have represented some sort of nodal point in a process on a still vaster scale, and of this we know absolutely nothing. But the words of

Hamlet to Horatio still remain one of the wisest warnings ever uttered.

The whole idea of a unique origin of things has its rival in that of a continuous creation of matter fed into the known universe steadily in the form of hydrogen, so that what we observe now may have persisted for all time (whatever the phrase 'for all time' may mean).

Experimental discrimination between the two theories is hoped for from observations on the remoter parts of space, but so far a definite or at any rate an agreed decision has not been achieved. In any event what is meant by continuous creation of matter raises questions as difficult and at present as unanswerable as those connected with the grand inaugural explosion. Against this bizarrely romantic background research continues with radio and optical telescopes, soon no doubt to be mounted in extra-terrestrial observation posts. Meanwhile Nature whispers warnings against over-simplification by revealing in very remote places the possibility of agglomerations of matter quite different from anything hitherto known, including what have been called 'quasi-stellar radio sources'. According to previous ideas these are much too far away for their brightness and are fantastically more energetic than they should be in the emission of radiation. In astronomy it would seem to be one of Rutherford's most exciting moments.

The gravitational forces acting throughout the universe present one of the most epic of all problems. Nothing has diminished the grandeur of Newton's conception of a force governing all the movements of the cosmos which he revealed for the first time as an understandable system. In the thought of Einstein this force, though its manifestations were very nearly in accordance with Newton's law, was not a real force but something simulated by the inherent geometry of space and time. Einstein's equations, however, lacked the fine simplicity of Newton's, and his thought, for all its power, had no finality. There has been a burst of renewed interest in relativity, and the theory of gravitation. If the geometry of space and time is a function of the masses present, what is mass? Mach had once suggested that the mass of any body arose somehow from its interaction with all the rest of the universe. Hoyle and Narlikar think they have overcome certain difficulties about this idea, and by combining it with Einstein's kind of space-time geometry have constructed a more unified theory. According to the equation which they develop there can be no description of 'empty space'. Space itself depends on what it contains and an empty world is a non-existent world.

If gravitation is the major force, or apparent force acting across the stellar universe, it has little to say about the structure and properties of matter itself, which has its own special mysteries in plenty. The intuition of the ancients that matter is particulate has stood the test of time, but the twentieth century has shown that the particles are particles with a difference. They behave in ways which are not easily describable in everyday language, though to a mathematician they resemble waves. Nevertheless the quality of discreteness remains, and between the particles a whole hierarchy of forces act.

Ordinary terrestrial life is very much an affair of chemistry, the whole panorama of which, including the variegated array of the elements and their properties, can be interpreted, on the whole very satisfactorily, in terms of the simple set of particles, proton, neutron, electron. But ordinary terrestrial life is an affair of low energies. In the stars and nebulae, Nature provides temperatures of

fantastic sounding magnitudes, and the great accelerating machines of modern laboratories provide, at fantastic sounding cost, energies which transcend many millions of times those met in ordinary chemistry. In this strange world of energy affluence and violence a bewildering collection of new particles appear. Some are heavy, some light, some are stable, some transient. They appear and dissolve: one type generates others. As discovery followed discovery it seemed that chaos and complexity were the rule of the subatomic microcosm. But at length some degree of order is beginning to emerge, though it is order of a rather peculiar kind.

The particles differ in mass and in charge: they differ in life-time and in the intensity with which they appear to act on one another. Powerful forces exist which are neither electrical nor gravitational. The bizarre appearances and disappearances of these entities prove to be governed by rules according to which various measurable quantities must maintain fixed relations, the so-called conservation laws. A system of numerical labelling of the possible modes of this particulate existence proves feasible, and it leads to the definition of abstract quantities known as 'isotopic spin' and 'hypercharge'. Observed values of these can be plotted one against the other on charts, and it now appears that the known particles (or particulate states) yield diagrams with striking elements of geometrical symmetry. This discovery indicated clearly an underlying law, and in 1964 a gap, a missing point, in one of these diagrams led to the finding of a hitherto unknown particle with even odder properties than usual (the 'omega minus' particle). Within the past few months there have been reported indications not merely of a new particle but of a new group of particles. What it all means is far from clear, but if we are here glimpsing a fundamental law of Nature it is certainly one which would have appealed to Pythagoras, for geometry and number are indeed coming into their own. The theories about these matters are now being made still more powerful by the introduction of considerations based on relativity, so in nuclear physics too it is one of Rutherford's exciting moments\*.

The non-electrical forces between sub-atomic particles are profoundly puzzling. Some are immensely powerful; some, acting in the same sub-atomic microcosm, relatively feeble. There are mathematical theories about them, which while far from comprehensive are in some respects of great power, as was witnessed long ago by Yukawa's feat in predicting one of the meson-type particles before it was ever observed. Here again we move in a strange world of mathematical abstraction.

The world of chemistry, many orders of magnitude larger, in which the drama, not of the weird sub-atomic entities but of the more homely, entire atoms and molecules, is enacted is in most respects a more comfortable one of those who like relatively concrete images of reality. Much is now understood, though plenty, both for enlightenment and for profit, remains to be discovered about the nature of metals, minerals, dyes, drugs, natural products of all kinds and artificial polymers, in respect of their structure, their transformations and their properties.

One of the greatest concentrations of recent effort has been on the physics and chemistry of living matter. There is now an essential understanding of the complex molecular patterns which, repeating themselves in endless permuta-

tions, can store information in the sequence of their units and in this way constitute a genetic code. A particular kind of chemical fitting together of complementary molecular structures is seen to play a major part in the replication of these codes during the multiplication of cells and the handing on of life. All this is a triumph of structural chemistry and of X-ray analysis.

It would be fallacious, however, to suppose that the major code-bearing molecules, the nucleic acids, can as a matter of pure structural chemistry replicate themselves in isolation. They do so only in the integrated organization of a living cell. The manner of this organization and the way in which it determines the characteristics of life are gradually being pieced together.

From the organization of the cell and the specialized structure of its surface the next step is to ask how cells can group themselves to form tissues and organisms. How the morphology of organisms is established and maintained is also a matter of chemistry and physics which is gradually yielding to patient search and thought. Great advances have been made in understanding the working of the nervous system, and we have a rough idea how that most subtle and elaborate of all computers, the human brain, performs its functions. The conceptions of molecular codes and the chemical storage of information which have arisen from work on reproduction have stimulated fascinating speculations about the mechanism of memory and even about the ancient mystery of dreams.

But what remains utterly incomprehensible is how and why the brain becomes the vehicle of consciousness. Great heat has sometimes been generated by debates about whether scientists will ever, as it has been crudely expressed, be able to make life in a test-tube. The heat at least is wasted. It need change little in our conception of things if they did. Suppose that in the course of centuries the transcendent technical difficulties are overcome, and that the appropriate enzymes, synthetic nucleic acids and so on are assembled together cunningly enough and that a cell is constituted. Suppose even that two suitable cells are made and unite and, in an appropriately controlled environment, develop into a man. The inscrutable mystery of the relation between this piece of chemistry and physics and the conscious mind remains precisely the same as if the cells were formed by the biochemical processes of human bodies. Some philosophers have wanted to talk away the mind-matter problem as a mere verbal confusion. I suspect that at bottom they simply attach no importance to the scientific description of things and are therefore indifferent to any divorce between it and the language which describes the world of conscious experience. If so, they are of course entitled to remain indifferent; but men of science presumably do not.

At all the boundaries of science we come up against what are probably the inherent limitations of human understanding. At the edge of biology we meet the chasm between what science describes and what the mind experiences. In the physical sciences, too, we encounter insoluble contradictions if we try to contemplate the limits of space, or the beginning of time. Why, moreover, should numerical and geometrical laws govern the transformations of the transient entities of the sub-atomic world? If there is continuous creation in the cosmos, what can we really mean by this process? If reality is describable by a four-dimensional space-time continuum why does the time dimension present itself differently to our consciousness? If some universe of anti-matter, where our protons and electrons are replaced by their

\* In passing, I might remark that one sometimes wonders whether 'manifestations' would not be a more appropriate word than 'particles'.

electrical opposites, were to drift into ours a nearly inconceivable thing would happen. The two universes would annihilate one another and leave not a wrack behind. If Shakespeare has helped us to feel this event emotionally, the transformation of the whole world into energy is something which the intellect can grasp only in the form of a mathematical equation and, like that of so many other ultimate things, its essence seems to pass outside the framework of our comprehension.

There are, no doubt, some who on the basis of reflexions like this would depreciate the whole of science. But are they themselves sure that their own systems do not also contain ultimate incomprehensibilities and even inner

contradictions and that we are not all equal in the finitude of our vision?

If men of science are at all as I attempted to depict them earlier in this address, they are not perturbed by the limitation of their possible understanding. There seems at any rate to be no foreseeable terminus to their own adventure. If the canvas on which they represent the world is bounded it still has plenty of room on it to paint magnificent pictures which inspire the enquiring mind, delight those who have the sense of wonder, and, if the natural perversity of man does not frustrate the effort, show the way to benefit humanity for many centuries to come.

## SUMMARIES OF ADDRESSES OF PRESIDENTS OF SECTIONS\*

### PHYSICS OF RAINDROPS AND HAILSTONES

**I**N his presidential address to Section A (Physics and Mathematics), Prof. B. J. Mason outlines recent advances in the physics of rain and hail formation and demonstrates some of the experimental techniques used at the Imperial College of Science and Technology for investigating these processes.

*Growth of raindrops.* Raindrops are formed either by the melting of snowflakes and hailstones, or by cloud droplets of differing sizes and fall-speeds colliding and coalescing to form larger drops. In the latter case the growth rate of a drop depends on its collision cross-section for smaller drops and whether, following collision, the drops coalesce or bounce apart. Theoretically this problem is difficult and, though some progress has been made with the help of large computers, definitive answers are sought in the laboratory.

Experimental work has recently been greatly facilitated by the invention of a vibrating-needle device capable of producing droplets of very uniform and controllable size. Collection efficiencies have been measured for drops of radius 20–60  $\mu$  colliding with droplets of radius 1–12  $\mu$ , and accord well with theory. An important fact, predicted by theory and confirmed by experiment, is that drops of radius < 18  $\mu$  fail to collect droplets of any smaller size. These experiments, supported by high-speed photography of colliding droplets, show that collisions produced by differences in terminal velocity are almost always followed by coalescence, and separation is rare. However, similar droplets impacting at relative velocities of metres/sec rather than cm/sec oscillate violently after coalescence and break up again.

Collisions may occur between equal-size drops falling at near-terminal velocity if one enters the wake of the other. Photographs of such events show that collisions occur only if the drop radius exceeds 85  $\mu$  and the Reynolds number exceeds unity. When two drops collide, the barrier to coalescence is presented by the air film trapped between the drop surfaces, and this has to be expelled by the action of dynamical, gravitational, or electrical forces working against the viscous resistance of the air. The basic physics has been investigated by studying the impaction, bouncing and coalescence of small water drops at a plane

air-water interface. Whether the drops bounce or coalesce depends on their size, impact velocity and angle of incidence, and drops striking the surface normally behave quite differently from those which strike nearly tangentially. Deformation of the droplet surface on impact assists in the draining and rupture of the air film and raises the probability of coalescence. Drops carrying a net electric charge and drops polarized in an applied electric field coalesce with the water surface more readily than uncharged drops of the same size and impact velocity.

Summarizing, then, we now have fairly reliable data on collision efficiencies for water drops in air, with experiment and theory in reasonable accord, and evidence that gravitationally induced collisions are followed by coalescence. Applying these data to the growth of raindrops in model clouds with simplified patterns of the air motion, we find that raindrops may form by coalescence in summer cumulus clouds provided that they are sustained by upcurrents of at least 1 m/sec, attain depths of at least 1 km, and persist for at least 1 h. The extent to which these conditions may be relaxed by the action of electric fields and turbulence in the cloud is not yet certain, but it seems that these effects are unlikely to be very important.

*Structure and growth of hailstones.* Hailstorms occur most frequently in the continental interiors of middle latitudes where they cause extensive damage to crops, buildings and livestock, and present a serious hazard to aircraft. Hail damage in the United States is estimated at 100 million dollars per annum.

Hailstones vary widely in shape: spherical, ellipsoidal, conical, discoidal and irregular forms sometimes occur in one storm. The surfaces of the stones may be smoothly curved, scalloped, or irregular with protuberances and spikes. In size they range from currants to grapefruit. The largest stone ever reported, from Nebraska, was 5.4 in. in diameter and weighed 1.5 lb.

A hailstone may originate either on an ice crystal or a frozen raindrop and grow by the accretion and freezing of supercooled cloud droplets. The structure and mode of growth have been investigated by studying natural stones and by growing artificial stones in the laboratory. The internal structure is studied by viewing 0.5-mm slices under the microscope in both transmitted and polarized light. The crystalline structure, as revealed by the size, shape, spacing and orientation of the component crystals,

\* At Sir Peter Medawar's request, an abstract of his presidential address to Section D (Zoology) is not published in this issue of *Nature*. It is hoped, however, to publish the full address in a later issue.



is determined by many factors, such as the number concentration, size and impact velocity of the supercooled droplets, the temperatures of the air and hailstone surface, and the detailed mechanisms of freezing.

If the surface temperature of the ice pellet is well below  $0^{\circ}\text{C}$ , the impacting droplets freeze individually to produce a rimed structure of low density and composed of many small crystals. During rapid freezing at these low temperatures, numerous tiny air bubbles are released, remain trapped in the ice, and scatter light to give the ice an opaque, white appearance. On the other hand, if the transfer of heat between the pellet and the environment is just sufficient to allow all the deposited water to freeze and maintain the surface temperature at  $0^{\circ}\text{C}$ , freezing proceeds slowly, the droplets have time to spread over the surface before freezing and then produce a compact, glaze ice of high density, composed of rather large crystals with few air bubbles, which is transparent.

If the hailstone grows larger than about 1 cm in diameter, it is likely to collect supercooled water at such a rate that it cannot all freeze immediately because the latent heat of fusion cannot be conducted sufficiently rapidly to the air. Only a fraction of the water freezes to produce a skeletal framework of ice that retains the remainder of the unfrozen water, the whole mixture being at  $0^{\circ}\text{C}$ . The hailstone now builds up a coating of wet, spongy ice which is often transparent, but sometimes contains small air bubbles which give it a milky appearance. The airflow past such a large hailstone falling at, perhaps, 30 m/sec may deform the spongy ice and produce an ellipsoidal or discoidal shape. Large stones are often composed of several alternate layers of opaque and clear ice which reflect changes of crystal size and air-bubble content of the ice, these being induced by variations of temperature and water content inside the cloud.

But why are giant hailstones so rare, and how is their manufacture in the cloud controlled? Large stones of 5 cm diameter have terminal velocities of about 30 m/sec

and any theory of their production requires that the cloud shall be many kilometres deep and contain updraughts of comparable magnitude to keep the stones suspended for growth periods of about 20 min. But, because a strong, steady updraught will carry a nascent hailstone through the supercooled region of the cloud before it can attain a large size, it seems necessary to allow it to re-enter this zone and continue its growth on a second journey. It has been suggested that if the updraught were intermittent, the hailstone might repeatedly fall from a high level and then be carried up again, but there is no evidence that the strong updraughts are intermittent.

However, these difficulties largely disappear when we recognize that, because in middle latitudes the horizontal wind usually increases with height, the updraught is not usually upright but strongly tilted. Particles which achieve insufficient size on their first journey in the updraught may be thrown out of it as they reach the tops of their trajectories, fall down outside it, and re-enter the updraught at a lower level. If the updraught speed increases with increasing height, then a small proportion of the re-entering particles having just the right size may be lifted slowly by the updraught, growing at such a rate that the increase in their fall-speed closely matches the increasing speed of the updraught, and acquire a fall-speed equal to that of the updraught where it is strongest. Finally, they will fall down through the updraught again and reach the ground as very large hailstones.

In other words, the storm acts as a sorting machine, winnowing out most of the stones and selecting only a few for growth to the largest sizes.

Although our understanding of the hail problem has greatly increased in recent years, we still have a great deal to learn about the organization and development of these damaging storms, in which the strong interactions between the dynamics of the air motion and the microphysical processes of droplet-freezing and ice-particle growth are of peculiar complexity.

## THE CHANGING FACE OF ORGANIC CHEMISTRY

THE Changing Face of Organic Chemistry" is the subject of Prof. D. H. Hey's presidential address to Section B (Chemistry). Organic chemistry is to-day passing through a period of change greater in scope and more fundamental in character than ever before. This is largely, but not wholly, due to the rapid advances which have been made in the development of physical techniques. Side-by-side with these advances must be placed what has been described as a "veritable hurricane of new theoretical concepts". Foremost among the physical techniques must be mentioned the various branches of spectroscopy, mass spectrometry, and X-ray crystallography. The full exploitation of these developments has not only accelerated the process of structure determination but it has also to a large extent taken away from the organic chemist much of the interest and significance of the part which he has traditionally played in the analytical degradation and synthesis of a complex natural product.

If the total synthesis of a complex natural product has no longer an essential part to play in the elucidation of a molecular structure, there is nevertheless still wide scope for the study of reactions and transformations, which reveal much more than merely the relative positions of the atoms in a molecule. The changing pattern in organic synthesis is illustrated by a comparison between the synthesis of equilenin by Bachmann, Cole and Wilds in 1939 with the achievements of Cole, Johnson, Robins and Walker (1958), Velluz and his colleagues (1960), and Crispin and Whitehurst (1963), in the more difficult

synthesis of oestrone in which stereochemical control plays a dominant part.

Recent developments in X-ray crystallography have made an enormous impact on organic chemistry. The year 1955, when Dorothy Hodgkin announced her contribution to the elucidation of the structure of vitamin B<sub>12</sub>, marked the beginning of a new epoch in the relationship between the organic chemist and the crystallographer, the consequences of which have yet to be fully realized. More recent work on the antibiotic cephalosporin C is cited as an example of the organic chemist and the crystallographer working as complementary partners.

Another characteristic feature of contemporary organic chemistry is the wider recognition of the participation of relatively unstable intermediates, such as the carbenes, nitrenes, benzyne and free radicals, in many organic reactions. Reactions of organic-free radicals are now widely recognized and examples are cited which show their relevance to a broad range of topics. These include the production of phenol and acetone from cumene, the reaction between acetic acid and ethylene to give butyric acid, the exploitation of fluorocarbon chemistry, the manufacture of nylon-7, and the mode of action of the new herbicides diquat and paraquat.

Recent advances in the scope, control and ingenuity of synthetic processes are well illustrated by the preparation for the first time of such structures as those of Dewar and Ladenburg for benzene, first put forward almost a hundred years ago but long since rejected. Even more remarkable are the syntheses of the hydrocarbons cubane

and conglomerates and of the interlocking ring structures known as the catenanes.

The power now placed in the hands of the organic chemist is changing the pattern on which for so long he has worked, but in doing so new and exciting possibilities

are opened up. The new organic chemist has been aptly described as "one who is versed in all the disciplines of chemistry from chemical physics to chemical biology and who may be called an organic chemist solely because he happens to work on the chemistry of carbon compounds".

## ROLE OF THE FIELD GEOLOGIST

WE are reminded by Dr. J. V. Harrison in his presidential address to Section C (Geology) that during the time of the French Revolution and also the Napoleonic Wars geology was evolving rapidly as field-work became popular and furnished abundant facts. Search for valuable minerals to keep established trades in existence had been going on for many centuries and had made rocks and ores familiar objects in some societies with special interests. Later, bedded rocks came to be recognized as having 'ages'. In most exposures the lower bed was the older and the higher one the younger. It was not until a century later that it was realized that ages of rocks could be given in terms of years. Fossils were discovered in many formations and their classification undertaken. Their distribution was found to be compatible with an age concept.

Superposition of strata and their fossil sequences were, and still are, the most reliable criteria on which to establish relative age relations. By the beginning of the nineteenth century William Smith had acquired an exceptional knowledge of the rock series in parts of central England and had developed a method for recording his observations by colouring and annotating topographical maps to show the distribution and structure of the rocks. This led him, after years of careful field work, to produce and publish in 1815 a geological map of England. Government-sponsored geological maps of Scotland and Ireland soon followed as well as those of certain countries overseas, for example France. At about the same time, groups of strata in England and Wales were being studied, compared, sub-divided and mapped. The description and recognition of the fossils which many of them contained helped to establish the series of 'systems' familiar to all present-day geologists. Rocks of all ages, from the oldest Pre-Cambrian to the youngest Pleistocene, were included in an acceptable working plan, a world-wide classification.

Geologists were thus able to compare observations made in all parts of the world. Whatever the quest, the geologist's instructions were much the same: observe, record and interpret. Each expedition required a map. The course of geological mapping in Britain was determined

by the availability of topographical maps published by the Ordnance Survey from 1801 onwards. These provided splendid base maps for the geologist; but in most parts of the world such base maps were not available. Even nowadays much of the time spent in geological exploration has to be devoted to the production of a base map. Sometimes this is done by using very approximate methods, as in Central America and Borneo, and sometimes by modifying more accurate ones, such as in Persia and Peru, to fit with the requirements of the time-table, but nowadays vertical photographs taken from high-flying aeroplanes help to establish accurate base maps. Experience has shown that the value of a geological map depends largely on the clearness of the exposures. Only the maps made in countries where exposures are good are easy to interpret, and where they are not, help has to be sought from physical and chemical techniques—this has only been possible since the end of the 'twenties when many useful techniques were refined. Gravity maps, seismic data, electric logs and concentrations of selected elements may help in some aspect of a particular enquiry.

If such geophysical and geochemical help is forthcoming, why, it may be asked, should geologists be retained? The answer lies in the fact that each of these methods yields evidence about only one feature of the structure beneath. Their value depends largely on the interpretation of the immediate results; the geologist has been trained by seeing and examining many rock structures. Someone must have knowledge of the rocks as they are, that is, as they are exposed on the surface of the Earth. In each generation they must be studied, and each student must start with something of the heritage handed down; by this means the new geologist can set out with freshly tutored eyes expecting to see something which has previously been overlooked and which may be a key to future revelations. Through discussion and contact with his colleagues in the field, the geologist can tackle the problems of present geology with enthusiasm yet retain a good sense of proportion. The field geologist is as essential to-day as he was when field geology first became fashionable 150 years ago.

## THE CITY REGION

ONE hundred years ago, the British Association, then meeting in Birmingham, concerned itself with the problem of the growth of the industrial town, drawing illustrations from the social conditions which were accompanying the industrialization of the South Staffordshire coalfield. To-day the problems of urban growth are on a regional, rather than a local, scale and, despite the progress made in town and country planning and in 'environmental control', they present problems of at least equal difficulty and challenge. One of the most difficult of these is the problem of the 'city region', and it is pertinent that Prof. M. J. Wise should choose this as the subject of his presidential address to Section E (Geography).

While much work has been achieved, or is in progress, the scale of the research effort is not yet adequate. In

Britain greatest attention has been focused on the city region of London. A. G. Powell's paper to Section E in 1960, and other studies of this and other city regions, have demonstrated the principal features of the changing urban form. These include the growth in scale of the operations of the city and the increasing intensity of their influence over an area of regional scale. The outward movement of population from the inner areas, the result of both planned and independent removals, has assisted the creation of an outer fringe of rapid population growth. The dispersal of light industry and some types of servicing activity are associated with this movement. By contrast, increasing economic activity in the central areas of the city and in some other parts of the inner area, coupled with improvements in transport opportunities, is linked

with a further growth of long-distance journeys to work. Changes in the relationships between existing and new service centres are noted, and increasing specialization of area within the city region is seen to be an emerging trend. Individual localities within the region change their functions and their forms. Relationships between towns and countryside within the region become closer and provide new problems.

Similar, if not identical, trends are to be seen in many lands and insufficient attention has been directed in Britain to the possibilities of comparative city region study. The growth of city regions is a phenomenon of almost world-wide distribution. Despite understandable differences, there are striking resemblances in the city region problem in countries of diverse character, for example, in Japan, Hong Kong, the U.S.S.R., Australia, India, as well as in Western Europe and in the United States. Comparative studies may throw new light on the forces promoting growth of the city region; on the processes of change; on the results of actions taken to contain rates of growth and to modify the newly emerging urban forms; on the relations between transport changes and urban growth; and on the new relationships between the city region and the national economies and between the constituent parts of the city region itself.

All these topics merit more attention from geographers. The excellence of past achievements in, for example, central place problems, town-country relationships and urban morphology should encourage the extension of research work into the problems of identifying the forces and processes of change. The detailed characteristics of individual city regions need to be established and compared. While there are recent examples of encouraging work, some major contributions are handicapped by lack of resources. There is a strong need for more interdisciplinary work both among social scientists and between them and architects, planners and engineers. The case for the formation of a national institute for urban research is a strong one.

While the concept of the city region has gained widespread acceptance recently, it is not in itself new, perhaps at least half a century old. While many British geographers have contributed to its development, the work of R. E. Dickinson is particularly noted. Further refine-

ment of the concept is needed. However, town and country planners should not expect to find ready-made solutions to such problems as the practical boundaries of appropriate planning regions emerging from academic work. Too little attention has yet been directed to the measurement of changes in the region. The concept is not a static one, as is sometimes assumed. City regions are in the course of continuous change and adaptation. New tools for analysis are required.

A dynamic view of the city region is especially necessary when planning policies are being devised. For example, new appreciations appear to be needed of the roles of central business districts. The problem of devising clear but adaptable regional land-use plans is especially difficult. The fact of city region growth must be accepted and plans laid to maximise its advantages and lessen its disadvantages. There are encouraging signs of new work in land classification which may draw back interest to the problem of agriculture in the city region. Some changes in the countryside are inevitable: urgent studies are needed if they are not to be disastrous. The problem of changes in transport facilities in their effects on regional land-use patterns is especially evident and points again to the need for more effective links between economic planning and physical planning.

Linked trends in planning thought, related to the use of 'growth points', 'counter-magnets', or development 'poles', are of relevance to the problem of the city region. In what circumstances can positive planning for such 'points' be of value either within or outside the city region? Again, further progress is necessary with theoretical studies.

The new forms of city growth override existing local government arrangements. How far does the regional nature of city growth provide possibilities for the creation of new forms of government and administration?

The scale of the city region problem is immense. While it presents vital problems for immediate decision, the long-term implications demand the establishment of research effort on a much larger scale than can at present be attempted. To this end both universities and planning authorities should contribute. Countries in many parts of the world look to Britain for guidance in the theory and practice of urban growth. There is a special task for geographers.

## IS INFLATION INEVITABLE?

FOR the purpose of his presidential address to Section F (Economics), Prof. E. V. Morgan defines 'inflation' as, "a situation in which either the national money income is growing faster than the national real income or there is a growing backlog of unfilled orders".

In many sectors of modern industry, neither prices nor wages adjust themselves to changes in supply and demand promptly and in a manner which will clear the market. The study of inflation, therefore, requires a theory which will explain how prices and wages are actually fixed.

Economists have sought to explain the movement of wages in terms of the demand for labour, the cost of living, the rate of profit, productivity, the 'pushfulness' of Trade Unions and considerations of 'fairness' as between different industries and occupations. A review of work in this field suggests that: (1) The demand for labour is an important influence, though it is somewhat less important than it used to be, and it may now operate largely through employers' expectations of how far they can pass on higher costs in higher prices. (2) Recent changes in the cost of living are a very important influence on present wage negotiations. (3) The growth of productivity is less important than considerations of fairness, so that indus-

tries with widely differing rates of productivity growth often have much the same increase in wages. (4) There is little evidence of a close connexion between the growth of wages and that of profits in Britain, though this connexion appears to be much stronger in the United States. (5) The relative 'toughness' in bargaining of employers and Unions has some independent influence, though it is largely a reflexion of Unions' fear of unemployment and employers' expectation of being able to pass on increases in costs.

Studies of methods of price-fixing are inconclusive, but it is agreed that many prices are fixed at a level below that which would maximize short-run profits; that they are changed at fairly infrequent intervals; that they tend to reflect changes in cost; and that they are not very sensitive to changes in demand unless these are large.

Prof. Morgan examines the genesis of inflation when some prices are adjusted so as to clear the market, but some prices and most wages are fixed in the way described here. It is concluded that: (1) Neither rising import prices nor rising profit margins can create a self-sustaining inflationary tendency, but rising wages may do so, unless restrained, at the risk of creating unemployment, by

monetary or fiscal measures. (2) The changes in supply and demand that are part of a progressive economy may impart an upward bias to the price-level. (3) Differences in the rate of growth of productivity between industries may also impart an upward bias.

It will be impossible to escape these tendencies while there is excess demand for goods or for labour, and the prospect of price stability and of long-run growth would be improved by running the economy a little less near to the absolute limit of capacity than it has been in recent

years. Other necessary conditions for the avoidance of inflation are the breaking down of expectations that higher wages costs can be automatically passed on in higher prices; and the use of a greater part of the gains from increased productivity to reduce prices rather than to raise wages or profits. Discussions between Government, employers' organizations and Unions can help in establishing these conditions, though it is necessary to guard against dangers of inflexibility which may be implied in an incomes policy.

## MAN'S USE OF NATURAL RESOURCES

PROF. E. M. JOPE, in his presidential address to Section H (Anthropology), points out that some natural resources have, in human hands, held high potential in shaping (or hindering) cultural development. It is man's awareness of his just place in a finely balanced ecology at both subsistence and cultural levels which leads to civilized fulfilment.

Some stages seem to have been particularly crucial in rising humanism. There is the long upsurge of controlled food-production through Asia into Europe and in the New World; there is the rise of metallurgy, particularly of steel, and the steepening rise of power-technology in the West from the Middle Ages onwards; and there is the increasing command over materials which has led to the creative molecular architecture of specifically designed synthetic substances.

The evolution of effective food-production was a long-protracted process, first in western Asia from about 9000 to 5000 B.C., there and in south-east Europe adapted to a particular range of terrains. We should not too readily assume that this pattern of farming was implanted ready-formed into the very different conditions of temperate Europe, to yield the viable food-producing way of life found extending across European temperate forests from the west Balkans to the North Sea coastlands during the fifth millennium B.C.

Early domestication of plants is perhaps a little better understood than that of animals which, in the initial stages, must be seen largely through the archaeological contexts of faunal remains differing little, if at all, from the wild. Biological data can, however, be most revealing of lines of ancestry in domestic stocks, particularly in the ancestral relations to be traced in their multiple protein systems, or even in polysaccharides. Such molecular genetics is of great potential, especially if it could be extended to the insoluble proteins such as keratins or those of bone structure, a residuum of which survives intact in ancient bone; it might also be applied to soft tissues preserved by special conditions, as with the Altai horses. With these insoluble proteins, two-dimensional mapping of the constituent peptides ('finger-printing') is probably necessary.

The use of traction animals, and hence of wheeled transport, is another aspect of controlled animal breeding, and properly designed harnessing of their effort is vital. Here the Chinese, as in many other matters, initiated crucial advances long before they appeared in the West, as with the padded horse-collar, or effective harnessing

of wind-power in sailing ships controlled through the stern-post rudder. But it was only through the social urge of the medieval West that these germs were exploited to lead into modern power technology. That England, even in later Saxon times, was well to the forefront of these medieval advances is well shown by recent large-scale excavations, as of the tenth-century water-mill system at Old Windsor.

Two inventions, the fire-piston and the crank, again initiated early in the Far East, were not exploited in combination as a source of powered continuous rotary motion until the great Western developments of steam engines in the nineteenth century and the internal-combustion engines of the twentieth century. In these, especially the former, Britain again led the field. Demand on her excellent coal resources increased all this (the rising province of industrial archaeology), yielding a large chemical industry from its by-product outfall.

The archaeology and social anthropology of food and power resources are of the greatest importance in planning the viable use of the Earth, and the avoidance of man-made desolation, again as much at the level of cultural satisfaction as of subsistence.

Unlike the more dynamic resources, static resources are restricted and not continually regenerated from solar energy or as part of a natural biological cycle. But by expanding the use of varied materials into a profusion of man-made and man-designed substances, man has deployed the resources of Nature into an advantageous ecological pattern. Most important of all was the rise of alloy metallurgy, the interim stage of bronzes, then the carbon- and, from the nineteenth century, the alloy-steels. The refined use of materials and of the subtle nuances of their texture for artistic expression must not be overlooked. The ultimate elegance is seen in our full understanding of structure in relation to behaviour in even the most complex molecules of Nature, and the recent development of artificial substances designed with increasing precision for specific purposes (molecular architecture or molecular structural engineering) is one of man's greatest ecological achievements, for he has thereby created renewable cycles from the simplest of raw materials, the atoms themselves. The implications of extending this molecular architecture to full genetic control are serious and must not be ignored. It remains to be seen how these and the further extension into nuclear architecture can influence the nature of human society.

## THE ENERGETICS OF RUNNING

FORTY years ago the subject of Prof. A. V. Hill's presidential address to Section I (Physiology and Biochemistry) was "The Physiological Basis of Athletic Records", and Mr. B. B. Lloyd reverts to Hill's theme in his

presidential address this year, applying Hill's arguments and ideas to present-day records in the light of modern views on energy stores and supply rates and on the relation between the power output of a runner and his velocity.

As Hill pointed out, the energetic economics of running depend on two fundamental relations. First, the total energy,  $E$ , available to a runner in a time  $t$  is a store of energy,  $S$ , added to the product,  $Bt$ , of a rate of energy supply,  $B$ , and the time. Secondly, according to the most recent work of Margaria and his school, the energy,  $E$ , needed for running a given distance  $y$  is proportional to the distance, independently of the velocity; that is,  $By$ , where  $B$  is about 65 calories per metre. Thus the total energy used in a race lasting  $t$  sec will be  $By + At$ , where  $A$  represents the non-running metabolism. Thus  $By + At = S + Bt$ , so that  $y = S/B + t(B - A)/B$ ; that is, distance run is a linear function of time taken.

This is well borne out by plotting distance against time for the world running records, ranging from 50 yards to 600 miles, which give six distinct straight lines. The first covers the sprints up to 20 sec, but it is a straight line only in so far as the effects of acceleration and the increase in oxygen debt with time cancel each other out. The second line runs between the half-mile and the mile, the energy store  $S$  being Hill's oxygen debt, and the rate  $B$  being determined by the maximum oxygen usage of which the runner is capable, which itself depends largely on the cardiac output. If the energy store corresponding with the oxygen debt runs down exponentially, there must be a linear relation between time and the logarithm of the vertical distance on the time-distance plot between the points for the sprints and the extension of the second line from the mile through the 1,500 m, 1,000 m and half-mile. This is indeed found to be so, the half-life of the energy store being about 18 sec; this means that all the records

from the sprints to the mile can be regarded as governed by a single exponentially declining store, of about 11 Cal, and a single rate of oxygen consumption, of about 5 l./min, yielding some 25 Cal/min.

The third line runs from the mile to 10,000 m (28 min), showing a slightly larger store and a rate reduced by a few per cent. The fourth line runs from 10,000 m to the marathon (2 h 12 min), a range over which not oxygen usage but fuel supply appears to be limiting. The fifth line applies to distances greater than the marathon up to more than a hundred miles. Most of the fuel used must come from outside the muscles, and the slope of the line is about half that of the second line. Finally, the sixth line covers intermittent running, at an approximate rate of 100 miles a day. The energy expenditure is about 10,000 Cal a day, twice that of the most energetic lumberjack.

This analysis has also been applied to the records of the past fifty years. There seems to have been a steady enlargement in the rate at which oxygen can be used, though not in the oxygen debt, and on present trends the mile, for example, will be run in 3 min 40 sec by the turn of the century. Sprints will show proportionately less improvement, distances beyond 10,000 m proportionately more.

The scantier records of women runners fit the foregoing analysis well. Women have shown a bigger rate of improvement with time than men. They also seem to be able to use up their oxygen-debt store of energy faster, so that it is possible that one day the momentary maximum speed of the champion woman sprinter will exceed that of her male contemporary.

## THE HEYWORTH REPORT, PSYCHOLOGY AND THE SOCIAL SCIENCES

IN view of the recent publication of the *Report of the Committee on Social Studies* (H.M.S.O., Cmd. 2660), it is apt that Prof. George Westby in his presidential address to Section J (Psychology) has chosen to treat, as a contribution to the inter-section discussion which has been planned for the Cambridge Meeting, some of the problems of psychology and the social sciences affected by its argument and recommendations.

The Committee on Social Studies, under the chairmanship of Lord Heyworth, had terms of reference from the Ministry of Education and Science "to review research at present being done in Government Departments, Universities and other institutions and to advise whether changes are needed in the arrangements for supporting and co-ordinating this research". Prof. Westby begins by laying special emphasis on the important, but recently misrepresented, fact that even in those university departments which have traditionally had close links with biological departments, psychologists have always been aware that their science had potentially high contributions to make to society at large. Far from being tied to laboratory benches the 'founding fathers' of the subject in Britain undertook considerable obligations in the applied psychology of industry, education, health and other government services.

It is suggested that attention should be directed to a radical recommendation which seems to be implicit in the Heyworth Report. This speaks of psychology departments as having had their "main interests" in the biological aspects of the subject. The proposal seems to be that 'social psychology', which has by some American universities been developed mistakenly in recent years out of close relationship with the biological experimental aspects of psychology, should be considered either as a 'social science' separate, in some critical sense, from experimental psychology and biology, or possibly (the

Report is not clear) that psychology as a whole is 'really' a 'social science' and should be the special concern of the proposed Social Science Research Council. In any event, it would appear that a rapid expansion of social psychology is proposed, marching along with the expansion proposed for the other social sciences.

A number of arguments are adduced by Prof. Westby which suggest a pause for reconsidering such proposals. The present shortage of scientific man-power in this field is acute and the first requirement, therefore, is adequate finance, earmarked through the University Grants Committee, for expansion at first-degree level. The increased output and improved quality at this level which would occur as a consequence would then support the proposed, and necessary, expansion of postgraduate training courses and research studentships. An advance in applied research at the present stage which was too rapid would result in disappointment and disillusion for the many friends that the social sciences have gained in industry and government. Furthermore, outstanding methodological and theoretical problems remain half-solved at the most; there is always a danger of distraction from these by the need (too often pressing in the applied fields) "to show something practical for the money". There are signs, too, that the present atmosphere of 'social science euphoria' is endangering university teaching standards; in psychology and sociology there must be no premature dogmatism. A rush of what can only be called 'social science fiction' in the guise of sociology tempts even practical and informed administrators into premature policy judgments on the assumption that there is firm 'scientific evidence'.

In these circumstances it seems unwise to uproot psychology or its off-shoot, social psychology, from the seed-bed of the natural sciences, either theoretically by denying any common methodology, or practically by making exclusive allies with the sciences of economics,

politics and sociology. This is not to suggest that there can be a 'pure' psychology. Psychologists realize, like sociologists, that 'man is a social animal', but the biological aspects of social problems become increasingly important with advance in the biological sciences.

For these reasons the organization of psychology within the degree-structures of some of the universities of traditional pattern still offers the best path of progress. Where psychology has membership of the three faculties of arts, science and social sciences, and has appropriate joint honours options in each, all the essential links of the

discipline, in conformity with the principle of the 'unity of knowledge', will be safeguarded.

It is suggested finally that the implementation of the 1946 Clapham Report, though perhaps more cautious and sceptical than would meet the changed situation of to-day, put the stress of any expansion in the right place—in the proper staffing of disciplines which are faced with especially time-consuming problems of applied research, in a period of explosive interest in the social sciences among sixth formers and a Robbins expansion which will probably, in these subjects, have been underestimated.

## PHYSICAL APPROACHES TO SOME BOTANICAL PROBLEMS

IN his presidential address to Section K (Botany), Prof. R. D. Preston considers the fine structure of plant cell walls as an example of the powers of physical approaches to some botanical problems. In common with all other metabolic products, the polysaccharides of plant cell walls are the consequence of a chain of enzyme-controlled reactions specified by the appropriate DNA, and he takes the opportunity both of examining the bearing of wall structure on cell growth and shape and of tracing back some steps in wall biosynthesis as they appear to be demanded by wall structure, as part of the chain of events leading from DNA to the expression of plant form.

The walls of almost all plant cells are based on a common plan involving, namely, an array of long thin rods (microfibrils) some 100 Å–300 Å in diameter and of indefinite length, embedded in a matrix. The rods are in part crystalline; so far as is known, they consist in all higher plants of cellulose, a  $\beta$ -1,4-linked glucan, though in some lower plants, all seaweeds, they contain instead a  $\beta$ -1,3-linked xylan with distinctly different physical structure and properties which are discussed. Whether glucan or xylan, the rods are strong in longitudinal tension; laterally they are bonded together through other polysaccharides in the matrix, giving a structure which is less resistant to tensions applied at right angles to microfibril direction. The (in this sense) anomalous condition of some other seaweeds, in which the crystalline component is  $\beta$ -1,4-linked mannan not organized into visible microfibrils, is not necessarily a departure from the common plan.

The mechanical properties of an array of parallel microfibrils are, therefore, anisotropic; in particular, such an array, when subjected to stress, creeps much faster when the direction of stress lies at right angles to the microfibril direction than it does when stressed parallel to this.

Since plant cells grow, at least in large part, through a yielding of the wall due to stresses invoked by turgor pressure, it follows that the rate of cell growth, and the direction of yielding of the wall and, therefore, the ultimate shape of the cell—and indirectly of the plant—should be a function of wall structure. Examples are given to show that, in those instances examined, these expectations are fulfilled. There is no doubt but that control of cell growth and cell shape is achieved in large part through wall structure. The control mechanism is, therefore, in principle on call from the first moment of deposition of the wall.

The details of wall architecture vary widely over the plant kingdom, but it is striking how many plants possess lamellated walls in which the constituent microfibrils of each lamella lie rather beautifully parallel to each other and in how many of these a single cell wall shows two or three different microfibril directions and no other. Detailed physical examination of these walls allows the main features of the microfibril-synthesizing and -orienting mechanism to be defined in principle. Each microfibril must be produced by end-synthesis through an enzyme complex which may be envisaged as particles larger in diameter than the microfibrils and attached to at least one end of each. These particles must be ordered at the surface of the cytoplasm, probably in cubic close packing. Such arrays of particles have been seen in the electron micrographs of formalin-fixed material, but since then, and much more convincingly, in freeze-splintered material in Frey-Wyssling's School at Zurich. It remains to be seen whether these can be identified with the particles shown by Hassid to build the glucose moiety of guanidine diphosphate glucose into cellulose, and to assess the significance of the microtubules now known in many cells to lie near to the wall-synthesizing cell surface.

## THE CHANGING AIMS OF FORESTRY

MR. C. W. SCOTT has chosen as the subject of his chairman's address to Sub-Section K\* (Forestry) "The Changing Aims of Forestry". The aims of forestry are to supply what is wanted from the forests, and as the demands change, so also must the aims. The trend of present demand is for woods of different type and size from those required in other centuries—or even recent decades. Growing wood is the primary function of forestry, though forests have other important functions.

Great changes are in hand to meet the swift rise of human populations and standards of living, and the rising demands for wood on a great scale in the form of plywood, man-made boards, and pulp and paper. Other causes of

change in forestry are new machines and the cost and limited supplies of labour. Work and life in or near forests are usually less attractive to labour than on farms or in towns.

*The demand for wood in Europe.* In 1964 the United Nations published a valuable study in detail of the demand for wood in Europe, excluding the U.S.S.R., during the period 1900–2000, and specially the period 1950–75. During 1900–50 the growth of demand was modest; but during 1950–75 this demand is likely to double, with larger imports. By the year 2000 the gap between Europe's annual growth of wood and her annual use may be 100–160 million m<sup>3</sup>, or some 3,500–5,000

million ft.<sup>3</sup>. A.D. 2000 is only thirty-five years ahead, or about half the time that it takes to grow a fair Scots pine saw-log in the north of Britain. The magnitude of the foregoing estimate of future demand may be compared with the planned initial needs of the new pulp and paper mill at Fort William, Scotland, which are for some 15 million ft.<sup>3</sup> (true) per year. It will strain not only the present Scottish forests (predominantly young Sitka spruce in that area) but also the present roads to supply 10 million ft.<sup>3</sup>. The balance must be imported.

Sawn wood is losing ground to man-made panels of wood pulp and other materials, but the demand for sawn wood is still immense and likely to remain so if it is cheap enough. But the demand for wood-pulp is growing much faster. By 1975 half the total demand for wood in Europe is likely to be for pulp and panels, with obvious effects on silviculture.

*The growing of wood in Europe.* There is a change of emphasis from slowly growing trees, like oak, to faster trees, such as poplar, eucalypts and conifers, and especially the faster conifers such as certain pines and Sitka spruce. Poplar gives four times the annual volume of wood per acre than does oak; and in France a quarter of the total annual cut of broad-leaved trees is now poplar. Prime oak has special value, but the day of oak coppice for fuel is over, or at least passing. The climatic, soil and water demands of various trees govern what can be grown well on any given site, but where there is a choice to-day it is rightly for the faster and cheaper production of what the markets want.

How much wood Europe is to grow for itself and how much to import is a problem in land use and economics. So also is the matter of helping poorer countries by buying their surplus wood, if any, if only to help them buy European machinery with which to help themselves.

In northern Europe the annual growth of wood averages about 1–3 m<sup>3</sup> per hectare, with a tree-life or rotation of about 100 years. In the warmer south and south-west of Europe the faster species grow at 15–20 m<sup>3</sup> per hectare per year, on suitable sites, with a rotation as low as twenty-five years, or lower for coppice. Slowly grown coniferous wood has special physical values, and both slow and fast wood are needed; but slowly grown trees alone cannot meet the future demand in Europe, and still

less so in Asia and Africa. The rising cost of forest labour and the need for mechanization with simplified and cheaper production favour even aged plantations of one species in each block, and blocks of considerable size. Monoculture is no more to be deplored in production forestry than in agriculture, with proper soil maintenance and steps against pests.

*The defects of some natural forests.* The late T. R. Peace warned the British Association in 1960 how wrong it is to worship blindly the "natural forest" as a desirable aim. The virgin rain forests of the Amazon are the greatest in the world but they export very little timber because the species are in general too hard and heavy, and often contain silica and extractives. The rain forests of South-east Asia and West Africa are at present much more valuable to man because they provide exports of moderate- or light-weight timbers. One reason for this difference may be that the Amazon has been less disturbed; and human disturbance tends to bring in the lighter wooded trees, provided of course that the disturbance is not too severe.

The wild rubber (*Hevea*) trade of the Amazon flourished until about 1914 but was soon eclipsed by plantations of the same tree in South-east Asia. Even now the latter survive against synthetics only by cheap production, helped by abundant labour and tree breeding which has raised the annual output per tree five-fold.

*The future.* How much land and what land should be used for farming, forestry, recreation and nature conservation is a problem in land-use in Britain as elsewhere. Western Europe and America north of the Rio Grande are not typical of the world as a whole, in which many millions lack adequate food, fuel, housing and education. For the last of these four, paper seems likely to be wanted on an immense scale. By the end of 1964 about 11 million acres of fast-growing tree plantations had been made in the poorer countries alone, with eucalypts, true teak (*Tectona*) and fast pines such as *P. radiata* and *patula* prominent. Even if pulp and paper are eventually made on a grand scale from materials other than wood, it is likely that cheap wood will long be wanted by the world as a whole: but, as the late W. E. Hiley used to emphasize, the cost of production is vital, and the consumer has the last word.

## TECHNOLOGY IN A LIBERAL EDUCATION

IT is commonly thought that the antithesis between 'liberal' and 'technical' education goes back to the ancient Greeks. This is at most only partially true, as J. C. Dancy points out in his presidential address to Section L (Education). The Greeks of the Golden Age respected the crafts along with the arts. It was Plato who invented the caste-system which set ideas above matter, theory above experiment, pure above applied learning, and which linked epistemological with social distinctions.

Plato's influence has distorted the British theory and practice of education. That influence has been exercised in part directly, but in part also through the great thinkers of the early nineteenth century, especially Coleridge. Plato was one of the ancestors of the Romantic Revival, whose hatred of the Industrial Revolution and emphasis on literary values have for years dominated our educational thinking. Add these ideas to the traditional English social caste-structure, and you are in for trouble.

In the past hundred years 'pure' science has broken through the barrier of socio-academic respectability, but technology has still to do so. Yet some aspects of technology deserve a place as an integral part of a truly liberal education. No school would call its curriculum liberal

unless it offered its pupils the creative experience of art and music. So all pupils should be given the opportunity of making things which work—the opportunity provided in such places as Mr. Gerd Sommerhoff's Technical Activities Centre at Sevenoaks School, Kent. Such work is creative in three separable senses: it requires and develops the creative activity of mind, hand and eye; the activities of the scientist, the artist and the craftsman.

Intellectually, technical project work can restore to science in schools the challenge, the excitement, the uncertainty, the open-endedness which traditional school syllabuses and approaches (not excluding practical work) have gone so near to killing. Aesthetically, such work contains an element of design which is distinct on one hand from styling and on the other from efficiency, and is analogous to elegance in mathematics. Nor, thirdly, can we afford to scorn the skills of the craftsman: an activity in which hand and brain work together is a supremely human activity.

Technology is indeed in many respects a bridge between science and the humanities. It may even be that the psychological make-up of the engineer is nearer to that of the artist than to that of the scientist, and that the country's shortfall of engineers should be made up from



the ranks not of our science sixth forms, but of our arts sixth forms.

Over and above such theoretical considerations, the art of making things which work is one which gives great

satisfaction to the individual—a matter the importance of which increases *pari passu* with the increase of leisure—and which obviously stands to benefit society, both in the advanced and in the underdeveloped nations.

## ASPECTS OF SOIL, PLANT AND ANIMAL RELATIONSHIPS

**D**URING the past fifty years much has been learned about soil conditions in relation to both plant growth and animal health. In particular, it has been recognized that, in addition to the accepted major nutrients, trace amounts of a number of elements are essential to both plants and animals. It is this aspect of soil-plant-animal relationships which forms the main theme of Dr. A. B. Stewart's presidential address to Section M (Agriculture).

Because of variations in environmental and soil conditions as well as in crop and animal requirements, soil-plant-animal relationships are necessarily of considerable complexity. Unlike the plant, the animal may derive much of its food from sources unrelated to its immediate environment. Direct relationships between soil conditions and animal health are in consequence more difficult to establish than are corresponding relationships between soil conditions and plant growth. On the other hand, intensification of agriculture and restriction of livestock to narrower ranges of soils has undoubtedly done much to direct attention to specific mineral deficiencies. Refinements in analytical procedures, the availability for experimental purposes of radioisotopes of most of the biologically important elements, and developments in the field of enzymology have all contributed substantially to the striking advances which have been made in knowledge of the mineral, including trace-element, requirements of plants and animals. With continuing advances in these and allied fields of research, it is probable that certain of the elements at present regarded as non-essential may be found to play vital parts in plant and animal nutrition.

Following discussion of soil conditions in relation to plant growth, reference is made to the various macro- and micro-nutrient elements now known to be essential for either plant growth or animal health or both. These include carbon, hydrogen, oxygen, nitrogen, phosphorus,

potassium, calcium, magnesium, sulphur, sodium, iron, manganese, copper, zinc, boron, molybdenum, cobalt, selenium, chlorine, iodine, and possibly fluorine, bromine, barium and strontium. Others such as aluminium and silicon are beneficial to some plants under some conditions, but the possible significance in agriculture of many elements which are taken up adventitiously by plants is still uncertain.

The principal factors determining the trace-element status of soils in Britain are soil parent material, drainage conditions and degree of acidity or alkalinity. The trace-element composition of plants depends, however, not only on soil conditions but also on such other factors as genus, species or strain of plant, environmental conditions during growth and, because of seasonal variations, the stage of maturity at which the plant is gathered or consumed. In many instances the incorporation into fertilizers of an appropriate quantity of a particular element may be a simple means of remedying trace-element deficiency in soil. It is of the greatest importance, however, to remember that with trace elements the range between deficiency and excess is a narrow one, and that unnecessary application of trace elements to a soil increases the risks of possible toxicity associated with the build-up of trace elements in available form. Toxicity conditions associated with the natural occurrence in soils of excessive amounts of certain elements such as nickel, leading to crop failure, and molybdenum and selenium, affecting adversely the health of livestock, are not uncommon. In Britain, geological complexity and the transport of soil parent materials during periods of glaciation have led not only to regional but also field-to-field variations in soil contents of biologically important trace elements. Under such conditions the indiscriminate application of trace-element supplement as a general insurance measure against possible deficiency is inadvisable.

## THE SOCIOLOGY OF SECULAR RELIGIONS

**T**HE purpose of Prof. D. G. MacRae's presidential address to Section N (Sociology) is to attempt to correct a widely spread set of beliefs about modern industrial societies—beliefs which are held both by the lay public and professional sociologists. Its second intention, which is a consequence of the first, is to correct certain largely unconscious assumptions of theoretical sociology.

It is widely accepted to-day that we are living in a period of intense secularization and that modern societies, particularly industrial societies, are the furthest advanced along this road. Churches and church attendance are believed to be in decline; material and scientific values are believed to be replacing non-natural and religious values, and patterns of behaviour based either on scientific procedures or economic calculation are believed to be becoming dominant in our societies. In a word, our societies are supposed to exemplify in an extreme form the process characterized by the German sociologist Max Weber as "increasing rationalization".

It is, of course, not the intention of this address to deny the importance of scientific procedures or their increasing role in our societies. No more is it the intention to deny that organized religion in Britain has probably less grip

on the general public and less affects our social structure than was true in the past or than is true in almost any other non-Communist society (equally, as Prof. MacRae states, it is not intended that the very high rate of religious observation in the United States should be explained away). Rather, what is claimed is that we are in a period in which there is a marked rise in secular religions.

By secular religion is meant: (1) The spread and elaboration of belief-systems based on non-natural authority, usually accompanied by specific ritual systems, and usually offering individual consolations based either on ordinary technical factors or on day-to-day social relationships. (2) In the strict sense these non-secular religions usually involve the idolatry of abstract idols: nationalism and communism will both be examined from this point of view. Not all secular religions, however, carry such heavy political weight, and various forms of scientism (including sociologism) are examined—by scientism is meant the idolatry of the appearance and the mimicry of the forms of natural science and specific technologies.

In addition, it is argued that the contemporary world is pervaded by various forms of mythical thought. This

latter term is understood in the sense used by Cassirer in his *Philosophy of Symbolic Forms* and used also by such prehistorians as Frankfort. The connexion of the mass media advertising and new culture heroes and heroines with contemporary myth is outlined.

Consideration of these social realities of our time must involve a criticism of one of the fundamental aspects of sociological theory: this is the unstated idea that some sort of equilibrium system or balance sheet formulation can be used in sociological study of religion and ideology.

Rather is it suggested that the logical incompatibility of ideological elements is no barrier to their simultaneous growth and development in social structures, although in certain specific circumstances such incompatibilities may emerge and be of real importance in precipitating radical social change. Finally, an attempt is made to relate the account of contemporary secular religion and the criticism of sociological theory that this account entails to the general sociology of religion current among contemporary sociologists.

## SCIENTIFIC UNDERSTANDING AND THE CHIEF END OF MAN

SINCE the collision between T. H. Huxley and Bishop Wilberforce at the British Association meeting in Oxford in 1860, further radical advances in scientific understanding have taken place. The enormous industrial productivity of a modern State is based on Faraday's studies of electromagnetism, the work of Joseph Black on latent heat underlying the perfection of the steam engine, chemical researches into large molecules leading to the present plastics industry and, more recently, on a combination of investigations of solid-state physics culminating in the development of transistors combined with Babbage's conception of a general-purpose digital computer. Dr. Magnus Pyke, in his presidential address to Section X (General), considers that all this and more is bringing about a state of affairs when earning a living can no longer be considered a major goal of human endeavour.

In the past hundred years, advances in chemistry and biology have changed men's ideas of the nature of famine and pestilence. The yields of arable crops have been consistently increased by the use of chemical fertilizers and, above all, by the breeding of improved strains of both plants and animals. Statistical evidence has been presented by Mayer<sup>1</sup> suggesting that the proportional increase thus achieved in world food supplies during the past two decades has been greater than the parallel increase in population and that this is part of a general trend that has been proceeding since the 1850s. The effect of scientific advance on medicine has been even more profound. Understanding of the nature of infectious diseases originated with Pasteur; aseptic surgery, anaes-

thetics and immunology are further examples of new knowledge; and chemotherapeutic agents and antibiotics were only developed by scientists of the present generation.

Great though the effect of scientific understanding has been on man's material condition, its influence on his thought and belief has been more profound still. Earthquakes and epidemics can no longer be feared irrationally as capricious retribution for public sins, nor need individual illnesses any longer be attributed to the evil eye. The universe has become a rational and, in many respects, a controllable place. The nature of the intellectual process which constitutes science which has brought this about can be seen to be an amalgam of observation, reason and imagination constantly refined by repeated reference back to Nature. Science can thus in many respects be compared with poetry as a way of finding truth. The present intellectual schism is between those who base their behaviour on dogma and those who put their faith in the belief that the exercise of reason and imagination in search of truth is the basic human goal.

MacKay<sup>2</sup> has already pointed out that it was concern for truth which begot science, not science which begot concern for the truth. It is argued that to continue to pursue the truth, which is, in its essentials, the pursuit of a moral goal, by means which have already successfully brought material wealth, food, healing and understanding of Nature and of man himself, is a worthy end for human endeavour.

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## PRESIDENT FOR 1966 OF THE BRITISH ASSOCIATION

SIR JOSEPH HUTCHINSON, C.M.G., F.R.S.

SIR JOSEPH HUTCHINSON, Draper's professor in the University of Cambridge, has been elected president for 1966 of the British Association for the Advancement of Science. Sir Joseph will deliver his presidential address at the next annual meeting in Nottingham.

Sir Joseph succeeded Sir Frank Engledow in the chair at Cambridge in 1957, and to repeat that which appeared in *Nature* on that occasion should indicate sufficiently that the British Association has made an excellent choice in the successor to Sir Cyril Hinshelwood as president.

Sir Joseph was born in 1902 and was educated at Ackworth and Bootham Schools, and at St. John's College, Cambridge. After graduation, he took a postgraduate course at the Imperial College of Tropical Agriculture, Trinidad, and then joined the Empire Cotton Growing Corporation's Cotton Research Station as assistant geneticist in 1926, the year in which the Station was established. He soon proved himself a worker of notable quality, and began a long series of researches on the genetics and taxonomy of the cotton plant which ultimately established him as one of the world's leading

authorities. After seven years in Trinidad he gained further valuable experience in India, where he was from 1933 until 1937 geneticist and botanist at the Indore Institute of Plant Industry. Returning to Trinidad as chief geneticist at the Cotton Research Station, he continued, with the aid of his colleagues, Dr. R. A. Silow and Dr. (now Prof.) S. G. Stephens, the elaborate series of genetical and evolutionary studies on the genus *Gossypium* which culminated in a joint work entitled *The Evolution of Gossypium and the Differentiation of the Cultivated Cottons*. This book was highly praised and takes its place among the classics of biology as perhaps one of the most comprehensive studies of the evolutionary history of a single genus ever carried out.

With the shutting down of the Trinidad Station in 1949, Sir Joseph went to Uganda as the first director of the Empire Cotton Growing Corporation's Cotton Research Station at Namulonge. Elected to the Royal Society in 1951, he was knighted in 1956. Since 1953 he has served as chairman of the Council of Makerere College, the University College of East Africa.

(Continued from page 1054)

different poisons are known to be related by their effects on the reactions involved in converting high-energy intermediates to ATP, the findings are interpreted in terms of the necessity for ATP to communicate with the high-energy intermediates of the terminal stages of oxidative phosphorylation in order to energize the *in vivo* synthesis of proteins.

Thus it is seen, from a wide variety of different approaches, that the evidence pointing to the importance of structural organization for protein synthesis is accumulating. Biochemistry has made remarkable advances by dissecting the cell into various independent metabolic steps. The cell is a structure which is composed of structures. Protein synthesis represents the combined activity of many integrated reactions which deal with precursors, flow of information, energy supply, interlinked enzyme reactions and controls. It is not surprising that the search should now be turned back into the cell to find places for the various parts that have been studied in isolated systems.

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## LOCALIZATION OF GAMMA-GLOBULIN WITHIN INCLUSION BODIES OF L. E. (LUPUS ERYTHEMATOSUS) CELLS

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**I**NCLUSION bodies of L. E. cells are derived from nuclei of leukocytes as a result of changes induced by the L. E. factor present in the serum of patients with systemic lupus erythematosus. The mechanism of action of this factor and the nature of the changes responsible for the transformation of nuclei into homogeneous inclusion bodies are not yet fully understood. Histochemical investigations have shown that the altered staining properties of the DNA in inclusion bodies with basic dyes is due to the presence of increased amounts of proteins foreign to normal nuclei<sup>1</sup>. More specifically, investigations with immunofluorescence techniques have demonstrated that inclusion bodies contain gamma-globulins not present in normal nuclei<sup>2</sup>. However, to our knowledge, the histological localization of gamma globulins with fluorescence-labelled antibodies has been carried out only in smears and not in sections of L. E. cells. As a consequence, it is not clear whether the demonstrated gamma-globulins diffusely permeated the inclusion bodies or simply coated them. For the same reason, it is difficult directly to correlate the results obtained with histochemical and immunological methods. In our investigations on the composition of inclusion bodies, it seemed appropriate, therefore, to attempt a more critical localization of gamma-globulin on sections rather than on smears of L. E. cells.

The capillary test developed in our laboratory<sup>3</sup> has permitted us to obtain cell blocks of buffy coat which contained large numbers of L. E. cells and could be readily sectioned. The localization of gamma-globulin in the

sections was accomplished with the help of labelled sera by means of autoradiography, fluorescence microscopy and electron microscopy.

**Autoradiography.** The globulins of L. E. sera were labelled with iodine-125 as recommended by Fitch, Winebright and Harper<sup>4</sup> and according to the technique of Bale *et al.*<sup>5</sup> with the modification that all rinses were carried out with normal human serum rather than with buffered saline. This modification was introduced in order to minimize the use of buffered saline, since the latter was found to interfere with the production of satisfactory L. E. cell preparations. The specific activity of the labelled globulins was 5  $\mu\text{Ci}/\text{mg}$  of protein contained in a 0.3 ml. volume. The blood of a healthy donor was collected in heparinized capillary tubes. After centrifugation, the plasma was replaced carefully with the labelled globulins. A syringe was inserted to mix the white cells and the labelled globulins thoroughly. The capillary tubes were then incubated at 37° C for 30 min and centrifuged again. The resulting white cell pellets were removed, washed, and frozen in a cryostat after infiltration with an embedding compound (O.C.T. for temperature zone 0° C to -15° C, Lab-Tek, Westmont, Illinois, U.S.A.). Frozen sections about 4  $\mu$  thick were mounted on microscopic slides, allowed to dry at room temperature and fixed in 95 per cent ethanol for 5 min. Sections were then coated in the dark room with Ilford K-5 nuclear research emulsion in gel form diluted with an equal volume of water. The slides were sealed in a dry, light-tight box and



Fig. 1. Autoradiograph of sectioned L. E. cells produced with  $^{125}\text{I}$ -labelled globulin of L. E. serum. Note the concentration of grains over the inclusion bodies. Sixteen days' exposure. ( $\times 8,700$ )

kept at  $4^\circ\text{C}$  for one to three weeks. Sensitized silver grains were developed with Kodak D-19 and sections were stained with Wright's stain<sup>4</sup>.

After 7 days' exposure, the number of grains produced by  $^{125}\text{I}$ -labelled globulins was found to be greater over the inclusion bodies of L. E. cells than in the background. The proportion of inclusion bodies with high grain counts increased with the exposure time. After 3 weeks' exposure, more than 80 per cent of the inclusion bodies showed higher numbers of grains than the background. At this time, some inclusion bodies were overlaid by more than 40 grains, while only scattered grains were found in the background. A representative autoradiograph of sectioned L. E. cells after 16 days' exposure is shown in Fig. 1. Increased numbers of grains were also seen over free haematoxylin bodies but rarely over unaltered leukocytes.

**Immunofluorescence.** The direct method using fluorescein-labelled globulins of L. E. sera was found unsatisfactory because only a few L. E. cells were produced by such globulins. The indirect method was therefore applied, by which L. E. cells were produced with unlabelled L. E. sera and afterwards exposed to labelled anti-human gamma-globulin globulins. The labelled globulins had been absorbed with dry mouse liver powder before use. Pellets of leukocytes containing L. E. cells were washed repeatedly with normal rabbit serum to remove unbound L. E. serum. Frozen sections of the pellets were fixed in 95 per cent ethanol for 5 min and incubated with normal rabbit serum to block non-

specific binding sites and then with fluorescein-labelled rabbit anti-human gamma-globulin globulins. Each incubation was carried out at room temperature for 30 min. After washing with buffered saline, the sections were covered with buffered glycerol, and examined under a fluorescence microscope. In order to examine the morphological details of the structures exhibiting specific fluorescence, the same preparations were afterwards stained with Wright's stain.

Inclusion bodies of L. E. cells and free haematoxylin bodies showed specific green fluorescence. In contrast, the leukocytic nuclei of the same L. E. cells and of other leukocytes were devoid of any fluorescence. The specific fluorescence of the inclusion bodies had a homogeneous appearance (Fig. 2, A and B) which correlated well with the homogeneous character of the same bodies as seen with Wright's stain (Fig. 2 C). Pre-treatment of control preparations with unlabelled rabbit anti-human globulin serum blocked the specific fluorescence completely.

**Immuno-electron microscopy.** Globulins of L. E. sera and rabbit anti-human gamma-globulin globulins were labelled with ferritin by means of *m*-xylylene diisocyanate according to the method of Singer and Schick<sup>5</sup>. Labelled globulins of L. E. sera gave unsatisfactory L. E. preparations and could not be used for the purposes of this investigation. On the other hand, recovery of antibody activity in rabbit anti-human gamma-globulin globulins was excellent following their conjugation with ferritin by the same technique. An indirect method was therefore applied as in the case of the immunofluorescence. Pellets of L. E. cells were first prepared with unlabelled sera by the capillary method, washed thoroughly with normal rabbit serum, and fixed for 15 min in 3 per cent buffered formalin, pH 7.4. After rinsing with the same buffer, they were incubated at room temperature with ferritin-conjugated rabbit anti-human gamma-globulin globulins for 60 min. After repeated washings, they were fixed in osmium tetroxide and embedded in 'Epon-812'. Ultrathin sections were examined both before and after staining with uranyl acetate or lead hydroxide in order not to confuse ferritin particles with precipitates of the metallic stains.

A survey view of an L. E. cell from a stained section is illustrated in Fig. 3A. Inclusion bodies appeared homogeneous at low magnification and were always surrounded by a single membrane. The latter could be demonstrated to consist of the invaginated plasma membrane of phagocytizing cells and not of a nuclear membrane of the altered phagocytized nuclei. These and other fine structural characteristics of L. E. cells will be reported in detail elsewhere. Fig. 3B illustrates a field from the same inclusion shown in Fig. 3A before staining. Electron-dense ferritin particles appear irregularly scattered throughout the inclusion body. A detailed count of

ferritin particles was made in several inclusion bodies. Twenty to fifty particles per square micron of section surface were found in sections of an estimated thickness of 800–1000 Å. There were no ferritin particles in the intact nuclei of phagocytizing cells or in the platelets and intact lymphocytes usually present in the same preparations. Occasional particles, however, were attached to the plasma membranes of phagocytizing cells.

The three techniques used in this investigation are complementary, as they permit, by different means, the detection and precise localization of gamma globulin in cells and tissues. They gave uniform and consistent results which are believed reliably to demonstrate the presence of gamma globulin within the inclusion bodies of L. E. cells. While the indirect methods using anti-globulin

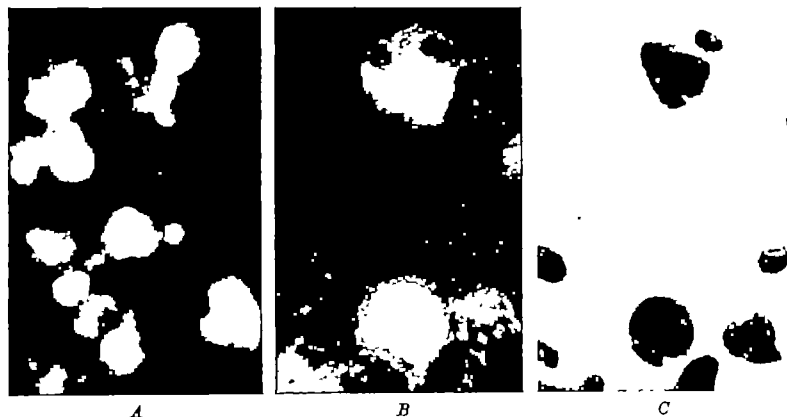


Fig. 2. A, Section of L. E. cells treated with fluorescein-labelled antihuman gamma-globulin globulins. Note the specific fluorescence of the inclusion bodies. ( $\times 600$ ). B, Detail of two sectioned L. E. cells showing specific fluorescence of inclusion bodies. ( $\times 1,065$ ). C, Same field as in B after staining with Wright's stain. ( $\times 1,065$ )

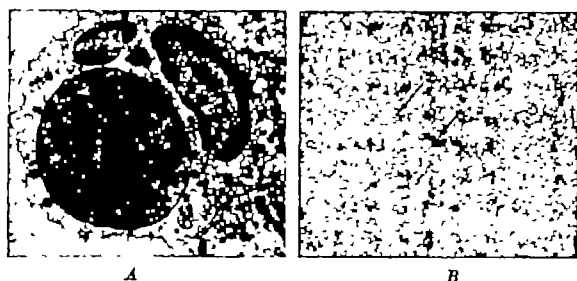


Fig. 3. A, Survey electron micrograph of an L. E. cell treated with ferritin-labelled anti-human gamma globulin globulins. Uranyl acetate stain. ( $\times 5,000$ .) B, Detail of the inclusion body shown in A, before staining. Note the electron-dense ferritin particles (arrows). ( $\times 45,000$ )

antibodies in conjunction with immunofluorescence and immuno-electron microscopy gave strong indication to this effect, more direct evidence was provided by autoradiography in which the labelled globulin of L. E. serum

itself could be used. Since physicochemical differences between L. E. factor and 7-S gamma globulin have not yet been demonstrated, it would seem reasonable to assume that gamma globulin within inclusion bodies consists, at least in part, of the L. E. factor.

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## CHEMI-LUMINESCENCE AND CHEMI-ELECTRON EMISSION FROM ALKALI AZIDES

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At elevated temperatures, Audubert *et al.*<sup>1-3</sup> observed a faint emission of ultra-violet light from various azides by means of a photosensitive Geiger-Müller counter. They ascribed the emission to the thermal decomposition of the specimens and assumed that the intensity of the radiation was a direct measure of the rate of decomposition. Thus they were able to calculate activation energies for the decomposition process from an Arrhenius plot. For the different azides similar activation energies were found and the spectral characteristics of the emissions were practically the same; this was taken as an indication that the same excited species was involved in all cases. Audubert *et al.* concluded, in particular, that the luminescence was due to excited nitrogen and made tentative designations of observed bands to electronic states of molecular nitrogen<sup>4,5</sup>. Recently, a somewhat different designation of these bands has been given by R. W. Nicholls<sup>6</sup>.

It was suggested by Audubert and Mattler<sup>4</sup> that the influence of metal nuclei formed during decomposition is to decrease the intensity of luminescence, the energy of the activated molecule being used to detach an electron rather than to produce photons. Thus, chemi-electron emission might be expected as a result of the reaction. In a recent investigation of the thermo-luminescence of ultra-violet-irradiated alkali azides<sup>7</sup> we observed the simultaneous occurrence of a stimulated electron emission which we associated with the decomposition of the azide. The present investigation was undertaken in order to check this assumption as well as to investigate the feasibility of examining quantitatively the decomposition kinetics of azides by means of chemi-electron emission and chemi-luminescence.

In our experiments the chemi-luminescence of the azides was measured by a photomultiplier with maximum sensitivity in the near ultra-violet. The spectral response of the system was determined by the cut-off of the quartz window of the sample chamber. The limit of detection was of the order of  $10^3$  photons/sec. Electron emission was detected by replacing the photomultiplier arrangement by an open Geiger counter which was insensitive to light, including the near ultra-violet. The sample was shielded from the electric field of the counter by a wire grid which was kept at the potential of the counter

cathode. With this arrangement we could distinguish a few electrons/sec. Some error was introduced by poisoning the counter with decomposition products as the experiment progressed. The sample was mounted on a flat slide of 'Pyrex' glass which could be heated by means of an electric heater.

In accordance with the work of Audubert *et al.*, an emission of ultra-violet light was observed that increased exponentially with temperature. It was found that the presence of a gas atmosphere enhanced the luminescence, although the nature of the gas used (argon, air, carbon dioxide, methane) had little apparent effect. We observed, however, that there was always an optimum pressure (varying from 10 to 100 torr) at which to obtain maximum light emission; in vacuum or under atmospheric pressure the luminescence approached the lower limit of detection. Slowly flowing argon at a pressure of 40 torr was used in most of our measurements in order to achieve a good luminescence yield.

In the experiments the temperature of the sample was raised or lowered at a rate of about 10 deg/min, while the intensity of the light or electron emission was measured as a function of temperature. Assuming that the intensity  $I$  of the emission is proportional to the rate of decomposition  $dc/dt$  and that the amount of the specimen decomposed during the experiment is negligible, the differential equation for the kinetics of the reaction may be written as:

$$I = -\frac{dc}{dt} \approx \text{constant} \cdot \exp(-E/RT)$$

and the energy of activation  $E$  can be readily computed from the slope of an Arrhenius plot. Fig. 1 shows such a plot for the luminescence of  $\text{KN}_3$ ; in Table 1 the average values of  $E$  calculated from our measurements are given for various azides. The spectral distribution of the luminescence was qualitatively determined by means of sharp cut-off filters since the low intensity of the light emission did not permit the use of a spectrometer. More than 90 per cent of the ultra-violet emission was found to lie between 240 and 260 m $\mu$ . There was also luminescence in the visible range, mainly around 560 m $\mu$ , as well as some emission in the infra-red.

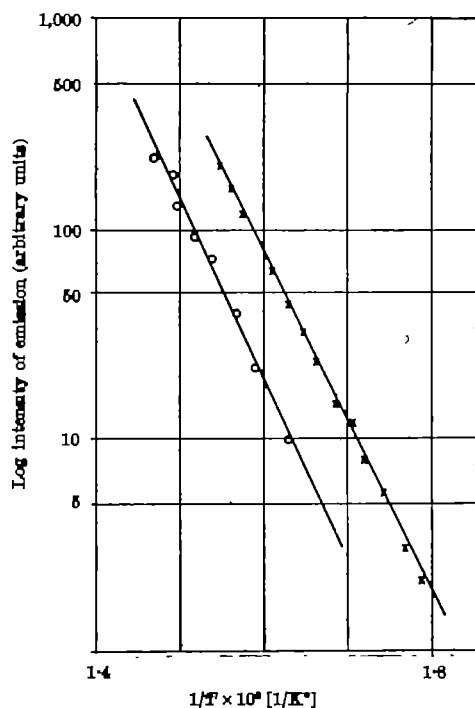


Fig. 1. Arrhenius plot for  $\text{KN}_3$ . O, Ultra-violet emission; x, electron emission

At elevated temperatures, electron emission was found for all the azides both in the solid and liquid state. That the emission is entirely made up of negative particles was verified by an electric potential between the sample holder and the Geiger counter; when the sample holder was made negative a small voltage caused an increase in counting rate, while a reverse voltage reduced the counting rate to zero. Fig. 2 shows the electron emission of  $\text{KN}_3$  as a function of decreasing temperature. Although the temperature ranged both below and above the melting-point of the specimen, there was within the experimental errors no change in the exponential slope of the curves when passing from the liquid to the solid phase. At the fusion temperature, however, we frequently noted a peak of the electron emission as seen in Fig. 2. This increase in electron emission that accompanies the solidification of the molten azide is due to the under-cooling of the melt and subsequent sudden release of the heat of crystallization. The tendency of alkali azide melts for under-cooling is also known from calorimetric measurements<sup>8</sup>.

Although the individual luminescence or electron emission curves could be fitted very well to Arrhenius plots, the energies of activation calculated from such plots showed considerable variations. Average activation energies of about 35–42 kcal/mole were found for most of the processes. They are in the same range as the energies observed by Audubert<sup>8</sup> and agree with values of 36 kcal and 41.5 kcal found by others<sup>9,10</sup> for the decomposition of  $\text{KN}_3$  in the presence of potassium vapour.

The ultra-violet emission from  $\text{NaN}_3$  was dependent on the pre-treatment of the sample; initial runs on  $\text{NaN}_3$  sometimes produced activation energies as high as 75 kcal/mole. Energies of this magnitude were also found for the other azides when the runs were carried out in vacuum, or for initial electron emission runs of  $\text{RbN}_3$  and  $\text{CaN}_3$ . In all these cases, however, the energy values showed such a large spread that computation of an average value was not considered to be meaningful, and therefore such values were not included in Table 1. In contrast to the work of Audubert, we did not observe different energies of activation for  $\text{NaN}_3$  above and below 365° C.

From isothermal experiments the order of the decomposition reaction was found to be zero for both lumin-

Table 1. ACTIVATION ENERGIES FOR THE DECOMPOSITION OF ALKALI AZIDES

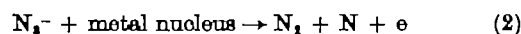
	(kcal/mole)		
	From luminescence	From electron emission Solid	Melt
$\text{KN}_3$	$41.7 \pm 5.2$	$35.1 \pm 6.3$	$41.5 \pm 6.3$
$\text{RbN}_3$	$39.8 \pm 3.7$	$43.5 \pm 6.1$	$40.5 \pm 3.9$
$\text{CaN}_3$	$41.0 \pm 3.6$	$43.5 \pm 6.5$	$43.6 \pm 7.9$
$\text{NaN}_3$	$35.2 \pm 4.8$	$36.8 \pm 7.1$	

escence and electron emission. This result is in accordance with recent measurements of the nitrogen evolution during the isothermal decomposition of various alkali azides<sup>11</sup>.

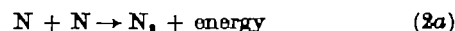
The thermal decomposition of metal azides is known to proceed according to the overall equation:  $2\text{MAN}_3 \rightarrow 3\text{N}_2 + 2\text{Me}$ . The details of the reaction are not fully understood yet. The initial step, however, must be the detachment of an electron from the  $\text{N}_3^-$ , either in form of an exciton or by complete ionization. The fact that, so far, it has not been possible to find free  $\text{N}_3$  radicals by spin-resonance techniques during the decomposition of inorganic azides suggests the following two reactions in which free azide radicals are not involved:



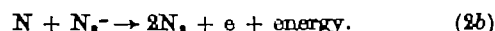
the excess electrons usually being captured by metal ions or specks; or in the presence of metal nuclei:



followed by:



or more likely:



From theoretical considerations one should expect for process (1) an energy of activation of about 70–80 kcal/mole<sup>12</sup>. The experimentally observed activation energies with values as high as 75 kcal/mole indicate that reaction

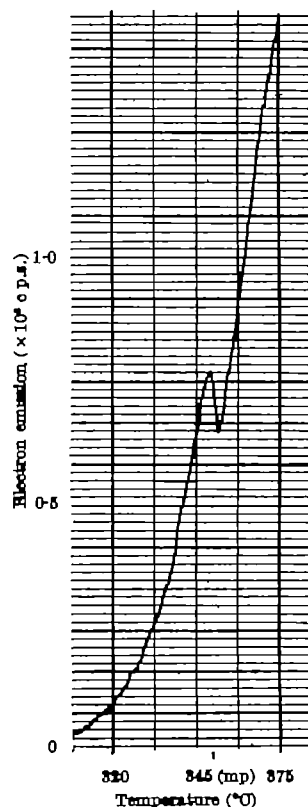


Fig. 2. Electron emission of  $\text{KN}_3$  as a function of decreasing temperature

(1) dominates in the early stage of decomposition, or in vacuum where the metal formed during the decomposition may escape by evaporation. With progressing decomposition the metal produced by the reaction begins to nucleate and the interface reaction (2) with an activation energy of about 35–42 kcal/mole becomes predominant. We consider the simultaneous occurrence of two decomposition reactions which proceed with different  $E$  to be the main reason for the comparatively large fluctuations of the  $E$  values computed from the Arrhenius plots. Although usually one of the competing reactions is dominant and, therefore, the intensity versus temperature curves can be quite satisfactorily fitted to Arrhenius plots, their slopes may differ in identical experiments depending on the contribution of each reaction. Thus, the experimentally determined energies of activation are essentially composite values partly depending on parameters such as initial concentration of metal nuclei, size of interface area, presence of surface impurities, etc. These energies are meaningful, however, in that they represent approximations of the true energies of activation. The limited reproducibility of decomposition experiments with azides under identical experimental conditions is by no means typical for luminescence or electron emission measurements, but has also been experienced when using the nitrogen evolution technique. It probably accounts for the different  $E$  values frequently reported in the literature for the decomposition of azides.

From the thermochemical values given in the literature<sup>13</sup> and from the known energies of activation, the maximum energies made available for excitation by processes (1), (2a) and (2b) were estimated to be 139, 225 and 147 kcal/mole, respectively. If given to a single  $N_2$ , these energies are sufficient to lead to the emission of the observed ultra-violet chemi-luminescence. Nicholls<sup>4</sup> has suggested that the emission in the ultra-violet is identical with the Vegard–Kaplan bands of  $N_2$ , which may be considered to arise from the recombination of atomic N in the  $4S$  state to  $N_2$  in the  $1\Sigma$  state<sup>14</sup>. Process (2) could lead to a single N atom excited with the activation energy of the reaction. This energy, however, is not sufficient to account for the emission observed at 560 m $\mu$ , which may be chiefly due to traces of excited oxygen<sup>15</sup>.

The fact that the energy of activation computed from the electron emission is practically the same as that obtained from luminescence measurements or by observing the nitrogen evolution suggests that the free electrons are an immediate product of the decomposition process, rather than exoelectrons or photoelectrons. The decomposition was found to be a reaction of zero order, indicating a process proceeding at an interface, such as the surface proper of the sample or the interface between the azide and the metal nuclei. This view is supported by the consideration that, in contrast to luminescence, free electrons can be detected only if they are released in the

surface region, but the same course of reaction was observed regardless of whether electron emission or light emission was monitored. It may be assumed, therefore, that reaction (1) occurs only on the surface. This is in accordance with the opinion held by other investigators that the decomposition in the azide matrix is unlikely to occur unless there are metal nuclei present. As to reaction (2), it cannot be decided on the basis of our experiments whether the catalytically active nuclei are homogeneously distributed throughout the volume of the sample or concentrated at the surface only. In both cases a zero-order reaction is to be expected. The observation, however, that the Arrhenius plot shows no substantial change in the slope when passing from the liquid to the solid phase indicates that there is no difference in the catalytic reaction in the two phases. It also shows that reaction (2) is largely independent of specific solid-state defects as well as of the crystalline structure of the solid.

Audubert and Mattler proposed a mechanism, according to which the luminescence is quenched by resonance induction involving metal nuclei, to explain their observation that the luminescence was enhanced by a moving gas. Our observation was that the pressure of the gas rather than the circulation was of primary importance, and we propose the following explanation for the existence of an optimum pressure. The increase of luminescence with increasing pressure can be understood as a pressure dependency of the decomposition reaction itself. The formation of metal nuclei is governed by two competing processes, that is, formation by the decomposition and simultaneous evaporation of the nuclei. An external pressure will favour the growth of nuclei by reducing the evaporation rate and thus eventually cause an increase of the reaction rate. However, with increasing pressure the probability for quenching of the luminescence of the excited  $N_2^*$  and  $N^*$  by collisions of the second kind, either with metal atoms or molecules of the surrounding gas, will also increase, causing a decrease of the luminescence yield. Superposition of both effects results in the occurrence of an optimum pressure for the luminescence.

We thank Dr. Z. V. Harvalik, director of the Basic Research Laboratory, and Mr. R. C. McMillan for their advice

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## RADAR INVESTIGATIONS OF WORLD-WIDE IONOSPHERIC DISTURBANCES ASSOCIATED WITH SATELLITES

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**R**ESULTS of high-frequency and very-high-frequency radar investigations are described, which seem to indicate that ionospheric disturbances associated with a large satellite can be detected by a north-looking radar, situated close to the northern auroral zone, when the satellite is approaching the magnetic latitude of the radar either in the northern or in the southern hemisphere. The reflexions from the disturbances have no Doppler-shift and

their occurrence is dependent on the local time and the heading of the satellite. These phenomena are detected during magnetic disturbances only and are thought to be correlated with magnetic shell enhancements of the electron density in the upper ionosphere. When the satellite passes such an enhancement it may trigger a disturbance which can be detected at places close to the corresponding magnetic shell.



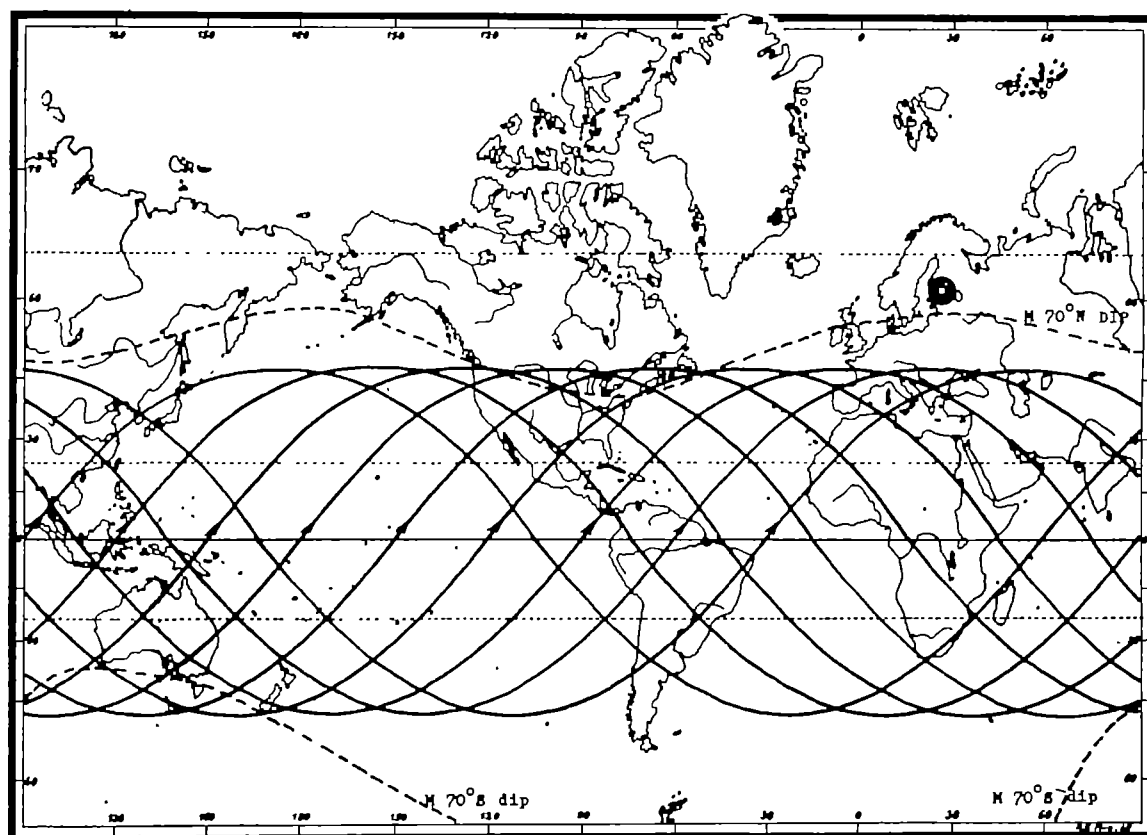


Fig. 1. Orbit of Echo 1

Satellite associated ionospheric disturbances occurring close to the satellites have been searched with positive<sup>1,2</sup> and negative results<sup>3</sup>. In the pulse radar investigation in Ohio in winter 1961-62 (ref. 2) the large radiotelescope antenna was used to achieve high sensitivity. Only relatively strong echo signals were taken into account in the analysis. It was found that these signals, which did not have any Doppler shift, tended to occur at times when the largest existing satellite *Echo 1* was close to the north-looking antenna beam. Disturbances seemed to occur in the antenna beam especially at the time when *Echo 1* was near to the most northerly point of its orbit about 100-500 km westward from the longitude of the radar. This seems to indicate that satellite-induced disturbances can travel rapidly eastward along the line of magnetic latitude.

**Investigations using 17 Mc/s CW radar.** In spring 1964 an investigation was started in Finland to find out how far away from the satellite it would be possible to detect satellite-correlated ionospheric disturbances. Assuming that disturbances travel to the east along the lines of magnetic latitude, disturbances associated with *Echo 1* can be detected in Finland only when *Echo 1* is near to the most northerly point of its orbit over the United States. The corresponding equator-crossing longitudes of the orbits are about 120°-230° W (Fig. 1). Elsewhere the orbit is to the south from the magnetic latitude of Finland. The orbit of the largest satellite *Echo 2* reaches the latitude 81.5° N and hence crosses the magnetic latitude of Finland twice during each revolution.

The transmitter power of the CW radar was 1 kW at 17 Mc/s. The antenna was a quarter-wave vertical mast with director pointing to the north. The receiving antenna consisted of two horizontal north-looking three-element yagis in parallel. The receiver bandwidth was about 1 kc/s. Owing to interference observations were

possible only during night time, and were performed during two months beginning February 22, 1964. In the analysis all signals lasting over 25 sec and less than 2.5 min and having a receiving power over -115 dBm were taken into account. Almost two hundred such signals were obtained, that is, on the average three signals a night. The lower limit of 25 sec was chosen to leave out of the analysis most of the meteor signals.

**Echo 1 results.** Fig. 2 shows the histogram of 65 signals obtained during the time *Echo 1* was in the orbits 120°-230° W. The number of signals per 10-min intervals is

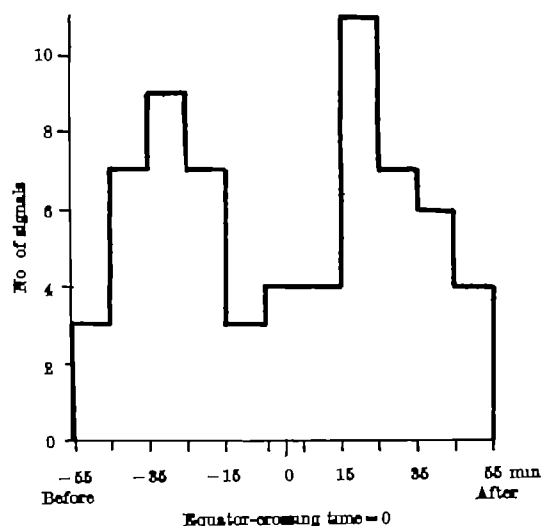


Fig. 2. Histogram of the signals received during the orbits 120°-230° W of *Echo 1*, February-April, 1964. 17-Mc/s CW radar in Finland

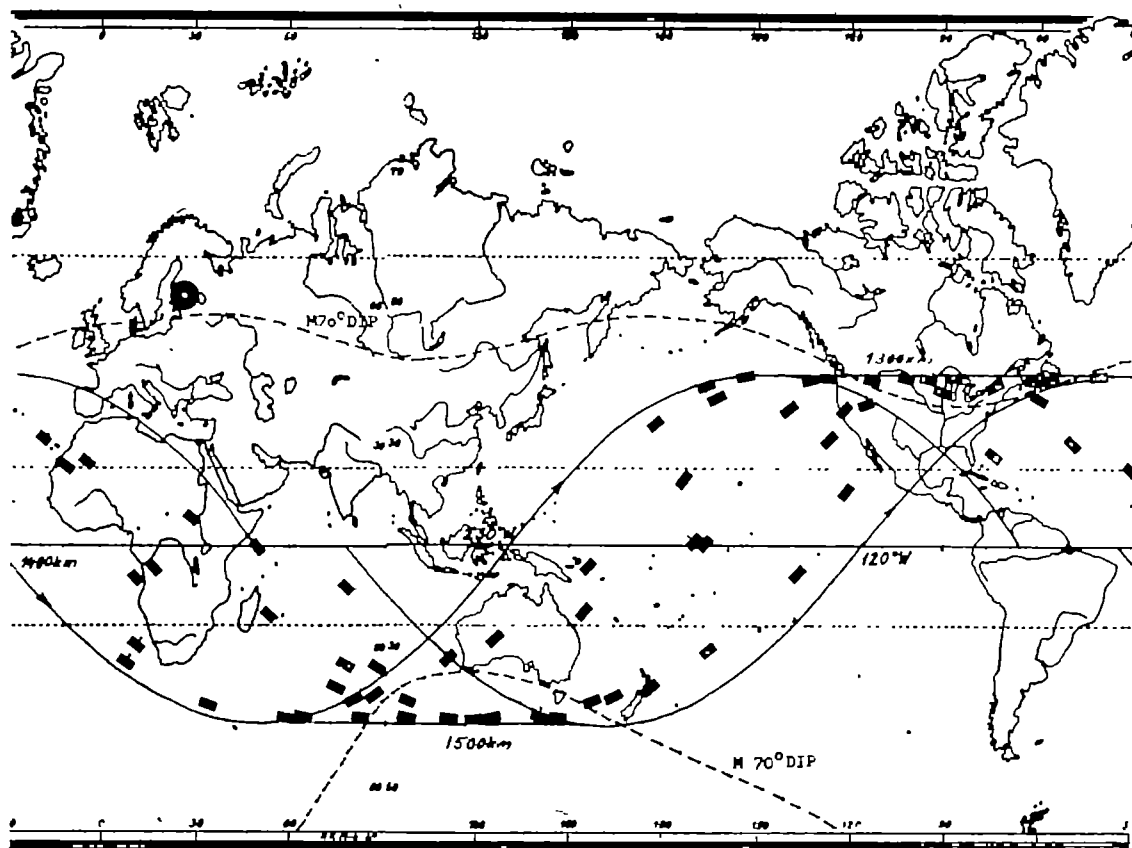


Fig. 3. Positions of *Echo 1* when radar reflexions were received in Finland, February–April, 1964, 17 Mc/s

shown with respect to the south–north equator-crossing time (ECT) of *Echo 1*. Fig. 3 shows the map of the positions of *Echo 1* at the times when signals were received. Lines of constant magnetic dip angle of  $70^\circ$  are also shown. They correspond approximately magnetic co-ordinate value  $L=3$ . The height of the satellite varied from 1,200 to 1,600 km. The histogram has two peaks about 30 min before ECT and about 25 min after it. The positions of *Echo 1* tend to concentrate close to the most northern and most southern points of the orbit. The most southern points happen also to be close to the southern magnetic latitude corresponding to that of the radar station. This unexpected result seems to indicate that satellite-associated disturbances are able to travel from the southern to the northern hemisphere with the same magnetic dip angle. The velocity of travel must be high in comparison with

the velocity of the satellite. In Fig. 4 only signals lasting more than 40 sec are shown. The peak at  $-30$  min has completely disappeared. This indicates that disturbances from the southern hemisphere are weaker. Satellite-related ionization effects between hemispheres have also been suggested by Kraus<sup>4</sup>.

Eighty-three signals were received during the time *Echo 1* was on the orbits  $230^\circ$ – $350^\circ$  W. Fig. 5 shows the histogram of these signals with respect to the ECT of *Echo 1*. As expected it resembles a more uniform distribution.

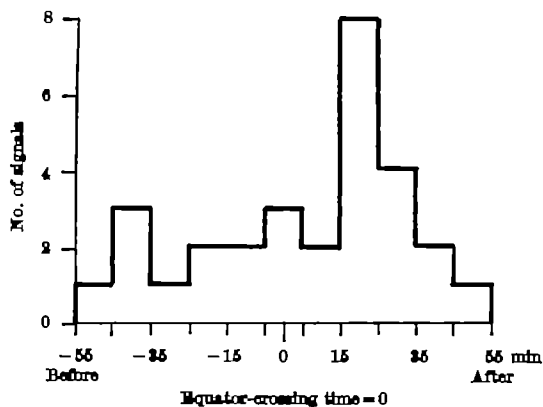


Fig. 4. Histogram of signals received during the orbits  $120^\circ$ – $150^\circ$  W of *Echo 1* and lasting more than 40 sec. 17-Mc/s OW radar in Finland

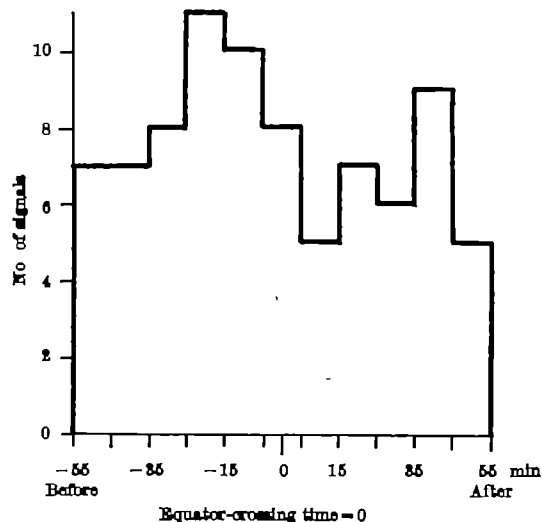


Fig. 5. Histogram of the signals received during the orbits  $230^\circ$ – $350^\circ$  W of *Echo 1*. February–April, 1964. 17 Mc/s OW radar in Finland

**Echo 2 results.** *Echo 2* traversed approximately in orbits  $100^{\circ}$ – $200^{\circ}$  W when the signals in Fig. 2 and 3 were obtained. The histogram of all the signals during the orbits  $100^{\circ}$ – $100^{\circ}$  W with respect to the ECT of *Echo 2* is shown in Fig. 6. No prominent peaks are present. *Echo 2* passed the radar station due south at midnight and just after midnight.

The orbit of *Echo 2* is approximately tangential to the southern magnetic latitude corresponding to that of Finland when the satellite moves in the south from Africa. The number of signals received during these orbits (equator-crossing longitudes  $200^{\circ}$ – $280^{\circ}$  W) was 106 including most of the signals shown in Fig. 5 and only a few from Fig. 2. Fig. 7 represents the histogram of these signals with respect to ECT of *Echo 2* and Fig. 8 shows a part of the map of the positions of *Echo 2* when signals were received. The height of *Echo 2* varied from 1,000 to 1,300 km. There is a distinct peak at 25–30 min before ECT when *Echo 2* has been directly to the south from Finland. This

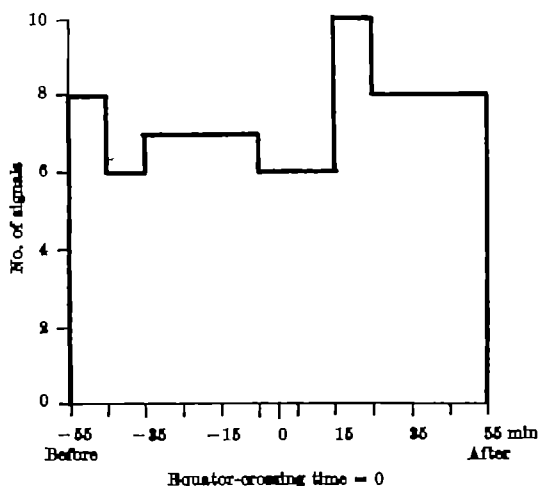


Fig. 6. Histogram of signals received during the orbits  $100^{\circ}$ – $200^{\circ}$  W of *Echo 2*. February–April, 1964. 17-Mc/s OW radar in Finland

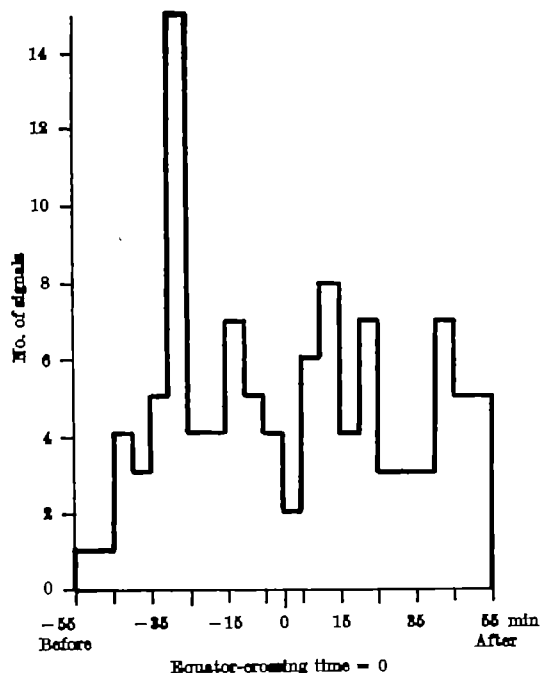


Fig. 7. Histogram of signals received during the orbits  $200^{\circ}$ – $280^{\circ}$  W of *Echo 2*. 17-Mc/s OW radar in Finland

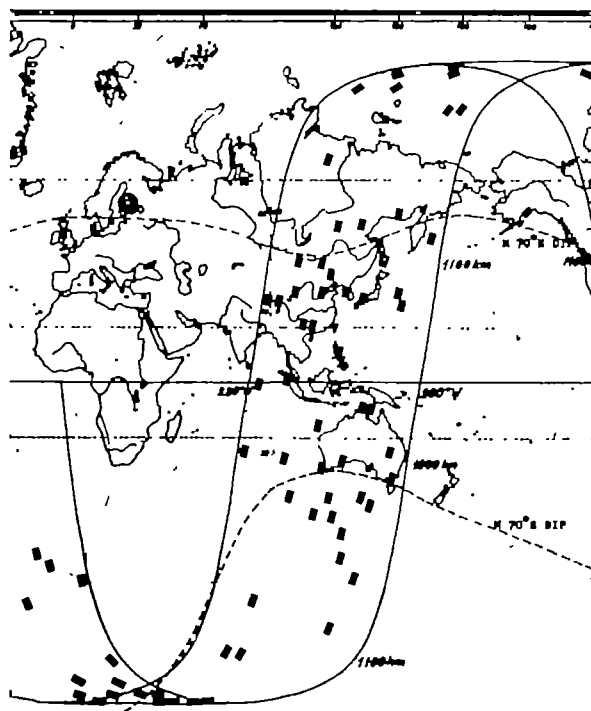


Fig. 8. Positions of *Echo 2* when radar reflections were received in Finland February–April, 1964. 17-Mc/s OW radar

seems to confirm the assumption that satellite-associated disturbances can travel from the south to the corresponding northern magnetic latitude.

**Sputnik 3 results.** During the winter 1959–60 *Sputnik 3* was the only large satellite. 100-Mc/s CW radar observations of satellite-associated ionospheric disturbances were made in Finland\*. The geomagnetic geometry of orbits  $30^{\circ}$ – $90^{\circ}$  W of *Sputnik 3* was similar to the orbits  $120^{\circ}$ – $230^{\circ}$  W of *Echo 1* except that *Sputnik 3* passed the radar station almost at the most northerly point of the orbit. The analysis of observations shows characteristics similar to Fig. 2–4 of *Echo 1*.

**Conclusions: related phenomena.** The results obtained suggest that large satellites when orbiting at heights of 300–1,500 km close to the auroral zones ( $L$  co-ordinate values between 3 and 6) are able to induce ionospheric disturbances which can be detected at the corresponding magnetic latitudes on the northern and on the southern hemisphere. These phenomena imply a moderate magnetic storm and are also dependent on the local time. Favourable times for receiving signals are from about 4 to 10 a.m. and from 4 to 10 p.m. The heading of the satellite is also important.

The satellite *Ariel* has measured electron density variations in the heights of 400–1,200 km (ref. 6). During magnetically disturbed periods sharp electron density enhancements were observed with a structure resembling magnetic shells ( $L$  values 3–8 for moderate storms). It is supposed that a large satellite, when grazing or crossing such a shell, can trigger a disturbance detectable by high-frequency radar in the vicinity of the corresponding northern and southern magnetic latitudes.

Very-low-frequency noise bands observed by the *Alouette 1* satellite between  $L$  values 2.5 and 4 (ref. 7) can be related phenomena. There are also similarities between satellite-related ionization effects and the way the satellite *Io* controls the decametric radiation from Jupiter\*. Decametric radiation from Jupiter is obtained when *Io* is near local sunrise to sunset and at the same time near magnetospheric shells with the highest  $L$ -values (about

6.5). These similar circumstances suggest that the satellite ionization phenomenon and the decametric radiation from Jupiter may be different manifestations of the same mechanism in which a satellite interacts with an electron shell in the magnetosphere<sup>5</sup>.

This work was supported by the Foundation for Promoting Technical Research in Finland.

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## MATTER CREATION BY GRAVITATIONAL WAVES

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THE idea of a steady-state universe has an immediate aesthetic appeal which is marred by the reflexion that the spontaneous creation of matter appears to violate the most fundamental conservation law known to science. It is true that the quantities involved are so minute as to be undetectable by direct observation, but this does not lessen the surprise. For the conservation of energy and momentum is equivalent to the invariance of time and space under translation, and a minute violation of a symmetry principle is just as surprising as a large violation.

If a particle is created in our locality, we should look for some asymmetry in the neighbouring space time to account for the apparent non-conservation of energy and momentum. I suggest that we might find such an asymmetry in the form of a rapid fluctuation of the curvature of space time in the neighbourhood of the created particle. We should identify this rapid fluctuation with gravitational radiation, and we should say that the absorption of gravitational radiation had resulted in the emission (or creation) of matter.

While there can at present be no direct experimental evidence for the creation of matter by the action of gravitational radiation, there is at least some indication from orthodox relativity theory that such a mechanism should be possible. Wheeler<sup>1</sup>, in discussing the superdense star, comes to the conclusion that pressures enormously greater than those in nuclear matter must be able to convert nucleons into free radiation. Now, if this is so, the reverse transformation should be possible—free gravitational radiation should be convertible into nucleons. McCree<sup>2</sup> has discussed the fall of a particle from infinity on to the Schwarzschild singularity of a large body. If the particle is brought to rest, and the kinetic energy transformed into radiation, then there is no increase in the proper mass of the body, and the energy of the radiation (observed at infinity) is just equal to the rest mass of the particle. Again, the reverse transformation should be possible: gravitational radiation should be able to detach a particle from the Schwarzschild singularity of a body, and impart to the particle sufficient kinetic energy to escape. The proper mass of the body will be unchanged, and the energy supplied by the radiation will be equal to the rest mass of the particle.

The properties of gravitational radiation are difficult to observe experimentally and are theoretically obscure. Let us assume that gravitational radiation is quantized into gravitons, each of which has energy given by the Planck relation  $E = h\nu$ . Now, most known gravitational processes take place at very low frequencies, whereas the production of nucleons would require high-energy gravitons with frequencies of the order of  $10^{24}$  c/s. Where can such high frequencies be found? I suggest that the answer lies in the advanced wave from distant sources.

The Wheeler-Feynman<sup>3</sup> formulation of electrodynamics postulates a time-symmetric solution, with an advanced field as well as a retarded field. The advanced field is converted into a retarded field plus radiation reaction by the action of a distant absorber. Presumably, a similar formulation may be made for gravitation, with this difference—that the absorption of gravitational radiation is extremely low. The advanced wave, instead of being converted into radiation reaction, will be propagated to the remotest parts of the universe with negligible attenuation.

Gravitational waves, like light waves, will be affected by the Doppler shift due to the galactic recession. Whereas the retarded wave is shifted to lower frequencies, the advanced wave is shifted to higher frequencies and to higher energies.

We can derive the Doppler shift most easily by subjecting the momentum 4-vector of the graviton to a Lorentz transformation. Considering for simplicity the case of one space dimension, we can write the momentum of the retarded graviton as  $\{p, +ip\}$ , and that of the advanced graviton, which travels backwards in time (so that  $dt/ds$  is negative), as  $\{p, -ip\}$ . If the observer is receding from the source with velocity  $v = \beta c$ , then the observed momentum of the retarded graviton will be

$$\begin{bmatrix} \gamma & +i\beta\gamma \\ -i\beta\gamma & \gamma \end{bmatrix} \begin{bmatrix} p \\ +ip \end{bmatrix} = \gamma(1-\beta) \begin{bmatrix} p \\ +ip \end{bmatrix}$$

while that of the advanced graviton will be :

$$\begin{bmatrix} \gamma & +i\beta\gamma \\ -i\beta\gamma & \gamma \end{bmatrix} \begin{bmatrix} p \\ -ip \end{bmatrix} = \gamma(1+\beta) \begin{bmatrix} p \\ -ip \end{bmatrix}$$

Here  $\gamma = (1-\beta^2)^{-1/2}$  and so the momentum, and hence the frequency, of the advanced graviton is increased by a factor of:

$$\gamma(1+\beta) = \{(1+\beta)/(1-\beta)\}^{1/2}$$

The overall effect can be seen from a space time diagram, as in Fig. 1. Pulses emitted by the source are received at longer intervals at the retarded wave, and at shorter intervals at the advanced wave.

As the advanced wave approaches the horizon of the universe, the velocity of the source relative to the local galaxies approaches the speed of light, and the frequency of the advanced wave increases without limit. Thus, even though the source frequency may be very low, the observed frequency will eventually be shifted above the critical frequency necessary for matter creation.

Since a graviton has zero rest mass, momentum can be conserved during matter creation only by collision

with another particle which can absorb the excess momentum of the graviton. Now McCrea<sup>2</sup>, in an interesting paper, has suggested this postulate: that new matter is created only in the presence of old matter. If matter creation does take place by the action of gravitational radiation, then the conservation of momentum, which we have been at such pains to retain, automatically requires that McCrea's postulate be satisfied.

The most likely spin for a graviton is 2, and so the annihilation of a graviton should result in the creation of four particles of spin  $\frac{1}{2}$ : for example, two protons and two electrons, or perhaps four neutrons. The reverse reaction, in which four particles are annihilated in a collision with a fifth particle to form a graviton, should also be possible. Since this involves a five-body collision, we may expect that this reverse reaction will only take place at extremely high densities, such as are produced by gravitational collapse. In moderately dense matter, we may expect creation to predominate over annihilation.

If, as I have suggested, it is the advanced gravitational wave that is responsible for matter creation, then the matter created will be in the form of anti-particles. This follows from the conservation of momentum.

It is easiest to work in the rest frame of the incoming scattered particle, which then has momentum  $\{0, +im\}$ . Suppose the incoming graviton and the outgoing scattered particle have momenta  $\{p, -ip\}$  and  $\{\beta\gamma m, +i\gamma m\}$ . Then the total momentum of the outgoing created particles will be

$$\begin{bmatrix} p \\ -ip \end{bmatrix} + \begin{bmatrix} 0 \\ +im \end{bmatrix} - \begin{bmatrix} \gamma\beta m \\ +i\gamma m \end{bmatrix} = \begin{bmatrix} p - \gamma\beta m \\ -i(p + (\gamma - 1)m) \end{bmatrix}$$

The created particles thus have negative energy, and so are anti-particles. This at first sight contradicts the elementary observation that the universe consists of ordinary matter, and not anti-matter.

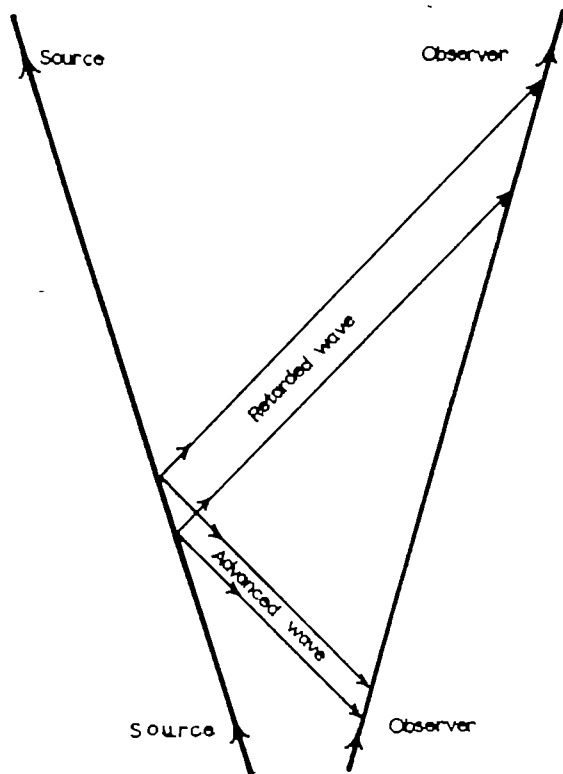


Fig. 1. Doppler shift of advanced and retarded waves

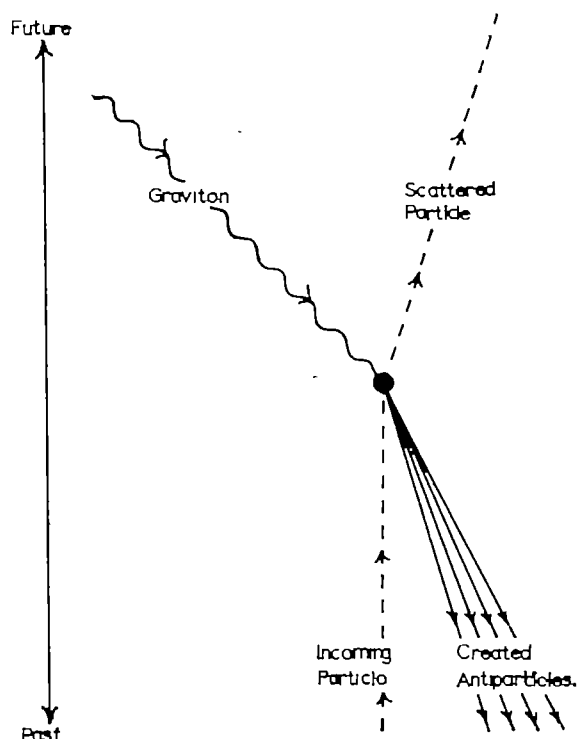


Fig. 2. Emission of anti-particles into the past

However, as Fig. 2 indicates, the created antiparticles are emitted into the past, which is equivalent to the emission of ordinary particles into the future. Presumably the antiparticles will at some point on their world lines be scattered into the future as ordinary particles. In any event, the result is an increase in the net number of ordinary particles towards the future, since antiparticles may be counted as the negative of ordinary particles.

I have suggested that matter may be created by the conversion of gravitational energy into mass energy, without violating any conservation laws other than that of baryon number. However, although on this model energy is conserved locally, it is not necessarily conserved on a cosmic scale. Indeed, on a cosmic scale there is probably no consistent way of attributing energy to a gravitational field. For the energy of a gravitational field is defined by the integral of the energy momentum pseudo tensor over a three-dimensional region which is chosen to be so large that the gravitational field vanishes outside this region. Such a region does not exist if we are considering the gravitational field of the entire universe. There seems to be no reason, therefore, why the total amount in the universe of mass, and of such energy as may be definable, should remain constant—instead, it may fluctuate from epoch to epoch.

Even though this model may perhaps violate the perfect cosmological principle, it still possesses some of the satisfying features of a steady-state model. In particular, it brings the phenomenon of creation within the scope of physical enquiry. Moreover, it does this without introducing any special modifications to terrestrial physical laws. We need not assume that the present laws of physics are inviolate, of course; but in view of its speculative nature, cosmology does not offer a very firm basis for suggesting which of the many possible variations are most likely to be correct.

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## LETTERS TO THE EDITOR

## GEOPHYSICS

## Continuous Observation of the Auroral Belt by Means of Radio

The purpose of this communication is to show that the southern edge of the auroral belt (the latitudinal range over which aurorae occur at a given time and longitude) can be reliably tracked by radio means. With the multi-frequency high-frequency system used at College, Alaska, the auroral belt can be tracked from overhead at  $65^\circ$  to above  $80^\circ$  north latitude. This wide coverage is possible because the high-frequency system utilizes ordinary ionospheric refraction to extend the northern limit of detection, and normal *H* and *F* layer refraction to produce perpendicularity between the wave and the geomagnetic field. The very-high-frequency auroral radar, on the other hand, can detect only those aurorally associated scatterers located in the *H* layer near the relatively limited region where the line of sight is normal to the geomagnetic field.

sky as the received ultra-high-frequency scatter echoes, and that, furthermore, the amplitudes were proportional. Unfortunately, neither ranges nor heights of the visual aurorae were measured, so these results do not conclusively show that the radio and visual aurorae coincided in space.

Results from the all-sky-camera network north of College, the College and Fort Yukon meridian scanning photometers ( $5577 \text{ \AA}$ ), and the 4-64 Mc/s College step sounder were utilized in the work recorded here. The all-sky-camera photographs and the photometric records were used to determine the position of the auroral belt. From the backscatter records the ranges of the echoes were scaled and converted to latitude by assuming that the slant range of any echo trace equalled the ground distance to the corresponding scattering irregularity. The auroral belt position and the backscatter range results were then plotted versus local time for the 12 days in December 1964, when simultaneous radio and optical data were available; the diagrams for December 7 and 31 are shown in Fig. 1. Cross-hatched areas represent the optically determined

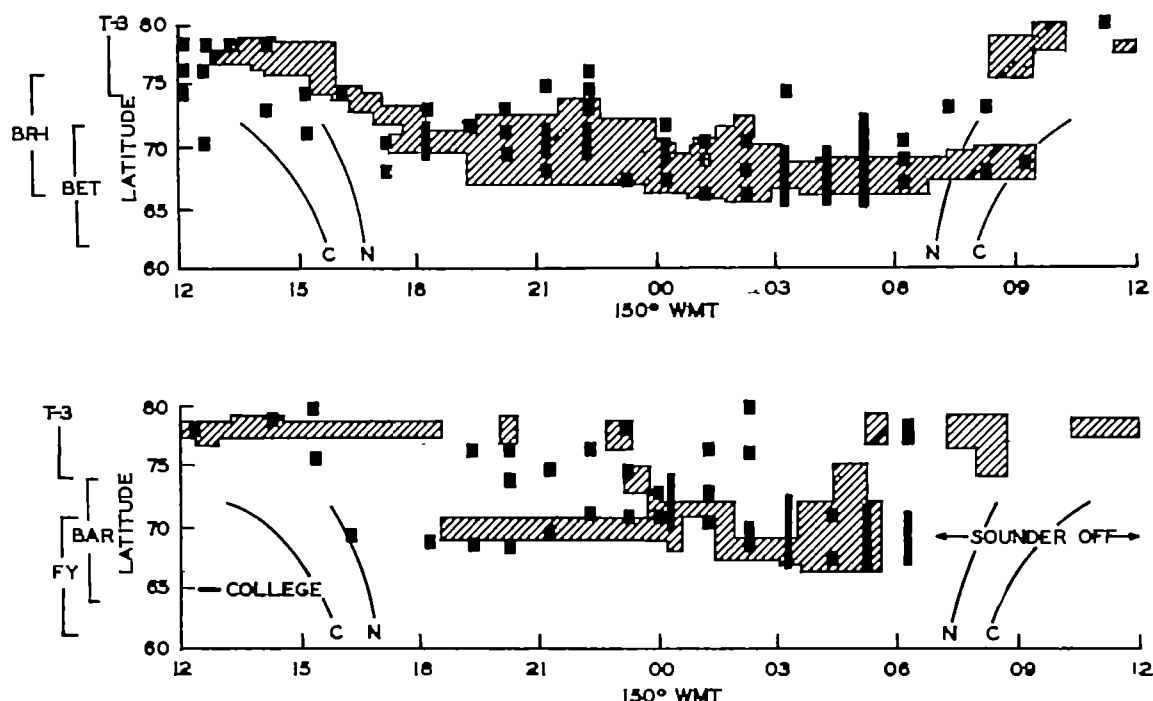


Fig. 1. Optical and radio auroral regions are shown for December 7-8, and December 31-January 1, respectively, in the upper and lower plots. Aurorae are shown as cross-hatched areas, and backscatter irregularities as blocks. Coverage by the optical sites is indicated. Civil (O) and nautical (N) twilight lines are shown.

The precise connexion between radio and visual aurorae is still relatively unknown. The phenomena appear to be closely connected, but, as Chamberlain<sup>1</sup> pointed out in his review of radio auroral work, the relationship is somewhat vague. Leonard, Romick and Belon<sup>2</sup> found that, although the region producing the very-high-frequency radio echoes was grossly associated with that containing the visual aurora, there was no detailed spatial correlation between individual echoes and auroral forms. Using an ultra-high-frequency antenna and a  $5577\text{-\AA}$  photometer with similar beam-widths, Kelly<sup>3</sup> found that auroral light was observed from the same angular portion of the

auroral belt, and solid blocks the backscattering irregularities. The agreement between the optical and the radio data illustrated in Fig. 1 is quite good considering the roughness of the method of scaling the radio data.

The operating optical observing sites and their regions of coverage are shown in Fig. 1 along with the civil (O) and nautical (N) twilight lines up to  $72^\circ$  for the days shown. The December 7 record shows the best agreement of the group while the December 31 record shows one of the poorer examples.

Backscatter echoes frequently arise from discrete sheets located to the north of College and aligned along the

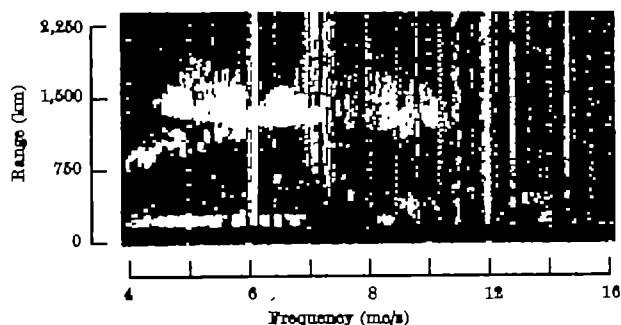


Fig. 2. A typical backscatter ionogram from a discrete E-F sheet. The upward sloping trace was produced by F supported groundscatter

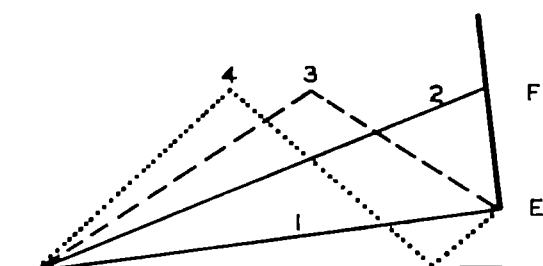


Fig. 3. Four common ionospheric scatter modes observed at College. Modes 1 and 2 are direct E and F scatter, modes 3 and 4 are E scatter which propagate via the F region

geomagnetic field through the E and the F regions<sup>4</sup>. Such a backscatter record is illustrated in Fig. 2. The four most frequently observed high-frequency scatter modes are illustrated schematically in Fig. 3. The method of determining the mode of propagation of a particular echo has been discussed in detail by Bates<sup>5</sup>. Every recorded echo trace other than those from vertical incidence, meteor, groundscatter, and slant F (polar spur) echoes was included in making these plots.

For the 12 days examined, the regions where the backscatter echoes and the aurorae were produced generally coincided. In several instances identifiable backscatter echoes were observed in places where the aurora could not be detected on the all-sky-camera photographs but could be on the appreciably more sensitive meridian-scanning-photometer records. Generally, only the weaker backscatter echoes showed the poor agreement; relatively strong echoes and strong aurorae showed the best agreement. (This may be partly due to the lack of photometric coverage above 71° latitude because much of the lack of agreement occurred between aurorae and echoes produced in the 75°–80° latitudes.) This finding indicates that the backscatter method is apparently sensitive to very weak auroral disturbances.

Normal incidence of the high-frequency energy on the geomagnetic field at any distance is produced by ordinary ionospheric refraction. The high-frequency radar can, therefore, detect an auroral disturbance anywhere from overhead to far beyond the horizon, whereas very-high-frequency and higher-frequency radars can do so only in the limited region where the line-of-sight perpendicularly intersects the geomagnetic field. Thus, the high-frequency system has the considerable advantage of continuous spatial coverage along its magnetic meridian.

The results of this work show that the observed radio-wave scattering region is closely associated with the nighttime auroral belt. No appreciable diurnal difference has been found between backscatter records obtained when the ionosphere was dark or sunlit for a given level of magnetic activity. Thus, the determination of the spatial and temporal variations of the auroral belt is possible from multi-frequency high-frequency backscatter records during daylight hours, and in particular during the Arctic summer, when ground-based optical observations cannot be made.

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### The Mohorovičić Discontinuity

THIS distinctive discontinuous layer separates the crust and the mantle of the Earth and extends from 30 km to 50 km below the continents and about 10 km below sea-level in the ocean basins. It has been estimated<sup>1</sup> that this layer has a temperature of 500°–800° C at the base of the continental crust and 150°–200° C in the sub-oceanic discontinuity. This layer is also under a compressive stress of about 10 kilobars (the mean pressure at 35 km is about 10 kilobars) and it has an average density of about 4.0 g/cm<sup>3</sup> as measured by the velocities of seismic waves.

Several theories have been proposed to explain the higher density of this layer in comparison with the density of that just above it, on the basis of (a) a chemical boundary<sup>2</sup>, (b) the gabbro-eclogite phase change or Fermor's theory<sup>3</sup> and (c) the olivine-serpentine reaction as proposed by Hess<sup>4</sup>.

It has been recently reported<sup>5</sup> that a considerably higher density in clay compacts can be achieved if the pressure is present during the decomposition reaction of clays. An extension of this work was carried out to test this effect on some natural rocks and minerals usually present in the Earth's crust. The range of temperatures over which the decomposition or phase transformation occurred for each mineral was determined by the differential thermal analysis technique. In most cases, the temperature of initiation was found to lie between 500° and 600° C. Small powder compacts of these minerals were pressed in a standard hot press using induction heating and either graphite or high-temperature steel dies which functioned both as susceptor and pressing component. The specimens were pressed during the decomposition reaction (first series) and after the decomposition or phase transformation was completed (second series). The final temperatures used in all experiments were above the decomposition temperatures. This was to ensure that the decomposition reaction was completed in a short time. In the first series the pressure was applied from the start of heating, and in the second series the specimens were preheated to the temperature and soaked for about 30 min before the pressure was applied. The materials and experimental conditions used and the results obtained are shown in Table 1.

The most significant difference between this and other investigations is that, in the present case, the decomposition products (water or carbon dioxide) were allowed to escape, whereas in other experiments hydrostatic loading in a closed system was generally used, and this precluded the change of composition produced by the decomposition reaction.

It is quite significant that higher densities can be obtained if the materials are under pressure during the decomposition reaction than if pressed after the decomposition is completed. In all cases, about a 10–25 per cent increase in density was obtained in the first series. In these experiments, however, pressures of about 12,000 lb./in.<sup>2</sup> or less were used. Further experiments were carried out in order to test the effect of increasing pressure on the densification both during (first series) and after the decomposition reaction (second series) of a kaolinitic clay. Fig. 1 shows the final densities of the compacts as a function of the applied pressure for both series. The maximum pressure was limited to 25,000 lb./in.<sup>2</sup> by the experimental



Table 1

Material	Experimental conditions			Results—First series			Results—Second series		
	Applied pressure (lb./in. <sup>2</sup> )	Hot-pressing temperature (°C)	Hot-pressing time (min)	Weight loss (%)	Cold comp. strength (lb./in. <sup>2</sup> )	Bulk density (g/c.c.)	Weight loss (%)	Cold comp. strength (lb./in. <sup>2</sup> )	Bulk density (g/c.c.)
Basalt	10,500	680	10	6.8	6,900	1.96	7.20	200	1.76
Chlorite	10,500	720	10	4.6	7,000	2.24	4.90	5,000	2.01
Serpentine	12,000	800	10	12.2	12,000	2.01	12.60	2,000	1.79
Brucite	10,500	750	5	20.0	10,500	1.82	21.00	8,000	1.62
Diaspore	11,750	700	10	10.3	4,000	2.20	10.20	1,700	2.04
Bauxite	11,750	700	5	20.4	7,500	1.58	21.00	2,500	1.30
Prochlorite	10,500	850	10	12.5	22,000	2.52	12.20	9,000	2.02
Antigorite	10,500	800	10	13.5	11,500	2.35	13.50	1,050	1.90
Thuringite	10,500	650	10	9.6	14,500	2.62	9.50	2,300	2.19
Sillodite	11,750	750	10	25.8	21,000	2.22	25.60	14,000	2.20
Natural magnesite	10,500	800	10	42.6	8,400	1.66	49.00	7,000	1.59
Monmorillonite	10,500	650	10	8.5	20,500	2.22	9.00	8,000	2.04
Kaolinite	2,500	650	15	13.6	6,000	1.55	13.6	1,200	1.25

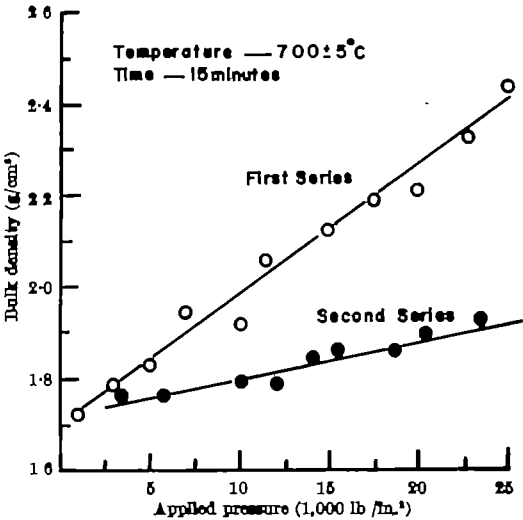


Fig. 1. Bulk density of kaolinitic clay compacts as a function of applied stress

set-up. A change in density from 1.80 to 2.50 g/cm<sup>3</sup> was obtained when the applied pressure was increased from 5,000 lb./in.<sup>2</sup> to 25,000 lb./in.<sup>2</sup> in the first series. On the other hand, in the second series the increase was only from 1.78 to 1.90 for the same increment of pressure.

On the basis of these findings, it is suggested that the higher density of the Mohorovičić layer may be due to the effect of pressure (10–100 kilobars) at the temperatures of decomposition or phase transformation where the natural minerals are unstable and are particularly susceptible to densification.

In drawing any conclusion, however, it should be noted that the density and strength reported here are those measured at room temperature and not those at high temperature and at high pressure. The nature of dense phases that can be obtained if the temperature and pressure are maintained for a very long period of time during the decomposition reaction is still not known and requires further investigation.

I thank Prof. R. M. Thompson, Department of Geology, University of British Columbia, for supplying the natural minerals.

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RADIOPHYSICS

Latitude Effect on the Variation of Spread-F Occurrence with Sunspot Number

SEVERAL investigators have studied the variation of percentage occurrence of spread-F with sunspot number. Walls<sup>1</sup> reported a decrease in the occurrence of spread-F with increasing sunspot activity at Huancayo. Considering the data for the period 1944–1951, Singleton<sup>2</sup> observed an inverse correlation between the occurrence of spread-F and sunspot number at Brisbane. Kotadia<sup>3</sup> found that at Ahmedabad spread-F occurrence decreases with increasing sunspot activity. These investigators have considered the data taken on all the days (in each month) in their studies. At Ibadan, Lyon *et al.*<sup>4</sup> have shown that spread-F on quiet days reached a maximum value for high sunspot numbers. Recently Rangaswamy and Kapasi<sup>5</sup> studied the variation of spread-F occurrence with sunspot activity in each season taking data for the five quiet days in each month for the period 1957–1962 for Trivandrum, Kodaikanal, Ahmedabad and Yamagawa. At Kodaikanal, a well-defined positive-correlation between the percentage occurrence and the sunspot number was observed by Rangaswamy and Kapasi in all the seasons, while at Ahmedabad a negative correlation was found for summer and a positive one for all other seasons. No definite correlation could be established for the data of Trivandrum and Yamagawa. All these investigators have studied the qualitative aspect of the variation of spread-F occurrence with sunspot number. The purpose of the present investigation was to study quantitatively the dependence of the spread-F occurrence on sunspot number and its variation with latitude by analysing observations from a large number of stations both in the northern and southern hemispheres.

Data from fourteen stations in the northern hemisphere and from eight stations in the southern hemisphere have been analysed. This group of stations is spread over a magnetic latitude range of 88° N. to 57° S. The percentage occurrence of the spread-F from 1800 h to 0800 h for five quiet days in each month has been taken from the published monthly *f<sub>o</sub>F<sub>2</sub>* characteristics. Data for the period 1954–63 have been used, but for certain stations only part of the period has been covered. A twelve-month running average has been taken for the monthly mean percentage occurrence for each station, following the conventional practice, in order to eliminate the seasonal variation. The values thus obtained have been plotted separately for each station against the smoothed observed Zurich sunspot number published in the Central Radio Propagation Laboratory (U.S.A.) bulletins (Part-A).

It has been noticed in all these plots that there is a clear linear variation of mean percentage occurrence of spread-F with sunspot number within a restricted range of sunspot numbers which varies from station to station. In general, the linearity is observed in the sunspot number

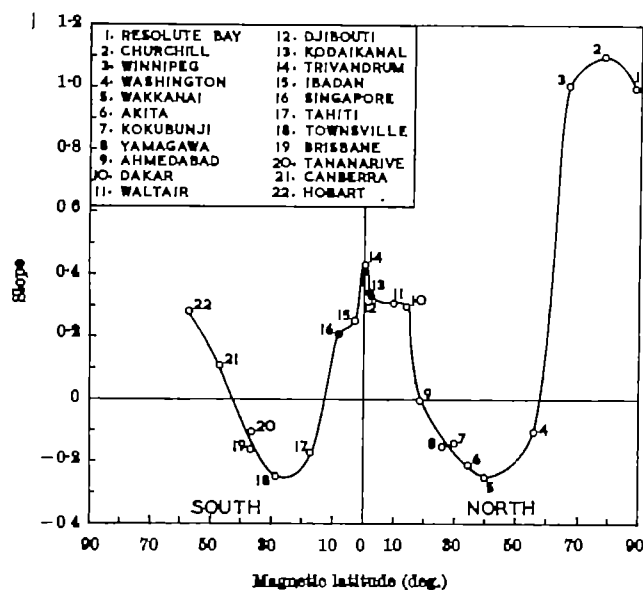


Fig. 1 Plot of spread- $F$  variation with sunspot number versus magnetic latitude

range of 40–120. It is found that the slopes of these linear portions vary widely from latitude to latitude, giving both positive and negative values. Positive slopes are obtained for the stations which show positive correlation of mean percentage occurrence of spread- $F$  with sunspot numbers, and negative slopes are obtained with those which show negative correlation. For most of the stations used in this analysis only data for either ascending or descending sunspot cycles are available and, as such, the slopes obtained from either of these cycles are used assuming that there is no difference between the slopes obtained from the ascending and descending cycles for each station. With the view of determining whether there is any systematic variation of these slopes with latitude, the slopes thus obtained from each of the plots of the individual stations have been plotted against geographic, geomagnetic and magnetic latitudes. Of the three plots, the data for the variation of slope with magnetic latitude fit best into a smooth curve (Fig. 1).

Fig. 1 clearly shows that there is a systematic latitude variation of spread- $F$  occurrence with sunspot number. For example, within the low-latitude range of 12° S. to 18° N., the slopes are positive, indicating that, for the low-latitude belt, the spread- $F$  occurrence increases with the increase of sunspot number, the effect being greater for the stations nearer the magnetic equator. The sunspot effect on spread- $F$  seems to be quite prominent for the stations close to the magnetic equator as can be seen from Fig. 1, in which the slope for Trivandrum (Mag. Lat. 0°) stands out prominently from the rest of the surrounding stations. There is a middle-latitude range of about 20°–60° in the northern hemisphere and 12°–45° in the southern hemisphere where the slopes are negative. This means that for all the middle-latitude stations in the ranges specified, the percentage occurrence of spread- $F$  decreases with increasing sunspot number. Furthermore, the slopes attain a maximum negative value for stations situated about a latitude of 40° in the northern hemisphere and about 30° in the southern hemisphere. It may also be seen from Fig. 1 that the sign of the slope changes from negative values to positive values at high latitudes both in the northern and southern hemispheres, the change being more pronounced in the northern hemisphere. It may also be noted that the curve is not symmetrical with respect to the magnetic equator. The results obtained in this investigation are in general agreement with those reported earlier.

One of us (V. R. J. K.) thanks the Indian Council of Scientific and Industrial Research for financial support of this investigation.

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## PHYSICS

### The Bath-Tub Vortex In the Southern Hemisphere

It has long been thought that water draining from a tank would rotate counter-clockwise in the northern hemisphere and clockwise in the southern hemisphere, provided other influences were kept small compared with the influence of the rotation of the Earth. This idea has only recently been tested, by Shapiro in Watertown, Massachusetts, as part of a film on vorticity<sup>1-4</sup>, and later by Binnie in Cambridge, England<sup>5</sup>. Shapiro and Binnie both acquired confidence, after surmounting difficulties in their early experiments, that the counter-clockwise rotations observed in their later experiments were due to the rotation of the Earth.

The experiment has now been performed in Sydney, Australia. For this we should like to express appreciation for assistance from the University of Sydney, Tufts University, and the U.S. National Science Foundation.

The apparatus was modelled on Shapiro's. It was a tank, 6 ft. in diameter and 9 in. high, with a central drain pipe 0.375 in. in diameter connected to a draining hose. The tank differed from Shapiro's in ways suggested by early difficulties Shapiro and Binnie experienced. It was made of ply-wood instead of metal, to reduce thermal convection. The drain pipe projected up 1 in. from the bottom, and was tapered to a sharp-edged opening; and the tank was located in a small, cement-walled basement room which had an overhead louvre but no windows, and in which both room temperature and inlet-water temperature remained within a degree of 20° C during the tests. As in Shapiro's experiments, the tank was filled by hose to a depth of about 6 in. above the orifice. The hose was directed so as to leave water swirling counter-clockwise in the tank.

Initially, the apparatus did not work as expected. In the first test, after a 60-h settling period, dust particles on the water surface showed no discernible rotation at any time during an 80-min draining period. This apparently meant that the water was moving to the centre so slowly that the viscous damping effect of the bottom was cancelling the water's angular momentum to the point where no angular momentum was perceptible in the draining fluid. To speed draining, the tank was raised to 30 in. above the floor. From then on, with the increased drop of the draining hose, drainage took about 22 min, and some form of rotation was always observed above the orifice.

Clockwise rotation was observed in all five of the later tests that had settling times of 18 h or more. During the first 10 or 12 min of drainage, no rotation was apparent. Rotation then developed as drainage progressed. In three of these tests a float 0.625 in. in diameter, a slice from a wine cork, was used to indicate rotation. It reached speeds of about one revolution in 8 sec for the runs with settling times of 18 and 20 h, and one revolution in 3 sec

in the one run with a settling time of 70 h. One revolution in 3 sec is what one would expect of a ring of particles rotating with the surface of the Earth at the latitude of Sydney, and then brought in from a diameter of 6 ft. to a diameter of 0.375 in., provided the ring conserves its angular momentum.

To provide a comparison with Shapiro's work, a floating cross made of two matchstick segments 1 in. long was alternated in one test with the cork float. Both cross and float rotated at approximately the same speed. Shapiro used a comparable cross. He reported<sup>3</sup> and filmed<sup>4</sup> rotation speeds of one revolution in about 3–4 sec. His drainage time was also comparable, that is, 20–24 min, and he also reports rotation only after about 10–12 min of drainage.

One early test did not fit the pattern of settling-time influence that emerged later from the tests. The tank, after a settling time of only 4 h 40 min, performed as if it had had 20 h of settling time. Near the end of the drainage period, a 0.5-in. patch of dust particles that had accumulated at the centre was rotating: one revolution in 8 sec, clockwise. This was the only test in which the tank had not been mostly or wholly covered during the settling period. It was an unusually windy day outside, and just before drainage dust specks on different areas of the water surface were moving in several directions at large speeds of about 1 cm/min. The test may therefore have been significantly influenced by air currents. For subsequent tests, the louvre in the ceiling, which had been blocked off, though not completely, by ply-wood sheets, was additionally covered outside with 'Pliofilm'. The door was kept closed, except briefly to allow entry, and the tank was kept mostly covered by two ply-wood sheets resting on two angle irons placed diametrically across the tank. The angle irons were usually spaced about 3 in. apart, so that surface motions could be observed between them, and the direction of these supporting beams in relation to the room was varied from test to test in an attempt to detect any remaining influence of air currents. Air currents did not appear to be a significant influence in the later tests, but it is our opinion that they are likely to have been the largest of the disturbing influences.

In one early test, after a settling period of only 13 h, the no-rotation period was followed by a period of counter-clockwise rotation, which changed to clockwise near the end of the draining period. (In this particular test it is not known how the tank had been filled.) Shapiro reports one similar test, experienced after a settling time of 4–5 h, attributed to undamped initial angular velocity residing in the upper water, while the water nearer the bottom was rotating in the direction of the Earth's rotation. In another of our tests, with a deliberately short settling time of 3 h, water drained out counter-clockwise during all but an initial 2 min of no rotation. Shapiro reports a similar result. In fact, it would seem that the results of these experiments at Sydney are quite similar to those obtained by Shapiro in the northern hemisphere, with one exception. After suitable settling periods, Shapiro observed counter-clockwise rotation. We, in Australia, observed the opposite.

These tests posed for us an unusual problem in experimental work. Normally, one does experiments in which there is some uncertainty in the expected outcome. In these experiments, however, our confidence in the idea that the Earth rotates, and in the applicability of conservation of angular momentum to masses of fluid, was probably so strong that experimental denial would have been almost inadmissible. We should have gone to unusual lengths to get the apparatus to work as expected. Realising this, we found ourselves reluctant to accept as conclusive the results we were getting, results which apparently confirmed our ideas. One can never prove, for example, that it was not some small air current which persistently maintained a circulation that gave the results

we observed, and that a quantitatively comparable, but oppositely directed, air current caused Shapiro's results. There is, in principle, an infinite number of hypotheses that can explain any set of observations. This difficulty in validation of scientific theories is not a new one and, in this instance, as in all instances, it cannot be proved that any one hypothesis is correct. Nevertheless, we have acquired confidence in the hypothesis that carefully performed experiments on liquid drainage from a tank will show clockwise rotation, if done in the southern hemisphere.

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## ENGINEERING

### Variation of Breakdown Voltage of a Positive-point/Plane Gap with Applied Voltage Waveshape

In recent years it has become a well-known experimentally observed fact<sup>1–4</sup> that the breakdown voltages of certain non-uniform field gaps are strongly dependent on the shape of the applied voltage impulses. For positive-point negative-plane gaps it has generally been found that, for standard double exponential applied voltage impulses and with gap spacings of 1–5 m, the breakdown voltage decreases with increasing rise-time, for rise-times of 100–200  $\mu$ sec, beyond which it increases again. The magnitude of the effect increases with increase of gap-length and for a 5-m gap, for example, the breakdown voltage at the minimum can be as little as 50 per cent of that for a 1- $\mu$ sec rise-time impulse.

It was suggested<sup>1</sup> that the phenomenon might be due to pre-breakdown corona mechanisms which are dependent on time and could therefore vary with rise-time of any applied impulse voltage. During the discussion on a paper<sup>5</sup> concerning the difficulties of installing a 400-kV grid-system in England and Wales it was suggested that pre-discharge processes occur for slow rise-times and cause the field to become more uniform so that the breakdown voltage increases.

During the course of an investigation of the mechanism of the long spark at CERL the pre-breakdown corona stage was investigated. It was found that the pre-breakdown coronas consisted of pulsed discharges and that the pulses carried considerable quantities of charge. Furthermore, the intervals between pulses clearly indicated that space charge fields resulting from earlier corona pulses affected the development of later pulses. This suggested the following mechanism to explain the experimental observations of the dependence of the breakdown voltage with waveshape referred to above.

For the positive-point negative-plane arrangement the corona discharge propagates across the gap by the following mechanism. An electron occurring within a certain distance of the point causes the initiation of an electron avalanche towards the point. Photons produced by the decay of molecules excited during the avalanche produce more electrons further out from the point. These latter electrons start new avalanches. Thus the corona filaments are built up until the electric field is too weak to allow further avalanche propagation.

The distance from a point electrode at which the field is  $E$ , when the applied voltage is  $V$ , is given by:

$$x = \frac{V}{E} f(D, R) \quad (1)$$

where  $D$  is the electrode separation and  $R$  is the radius of curvature of the point.

For any particular value of  $V$  the maximum distance  $x_0$  from the point at which an avalanche can be initiated, is:

$$x_0 = \frac{V}{E_0} f(D, R) \quad (2)$$

where  $E_0$  is the minimum field in which an avalanche can propagate.

Thus for a filament to propagate across the entire gap the applied voltage has a unique value:

$$V_B = \frac{D E_0}{f(D, R)} \quad (3)$$

from equation (2):

$$\frac{dx_0}{dt} = \frac{f(D, R)}{E_0} \cdot \frac{dV}{dt} \quad (4)$$

Now  $\frac{dx_0}{dt}$  is the speed with which the position of the boundary of the limiting field for avalanche propagation moves away from the point. Also  $\frac{dx_0}{dt}$  is directly proportional to the rate of rise of applied voltage which can be related to the rise-time of the applied impulse if the wave-shape parameters are known. The shape of the experimentally observed curves relating the breakdown voltage and impulse rise-time can be explained in the following manner.

(1) *Short rise-time impulses.* In this case  $\frac{dx_0}{dt}$  is large and therefore the position of  $E_0$  moves very rapidly away from the point. For breakdown to occur an applied voltage is needed of such a magnitude that a field of at least  $E_0$  is produced everywhere within the gap.

(2) *Medium rise-time impulses corresponding to the minimum breakdown voltage.* For a certain slower rise-time  $\frac{dx_0}{dt}$  will be such that in addition to the electrostatic field produced by the applied impulse there will be time for space charge fields to form due to ionization produced in the electron avalanches. Now if the positive space charge drifts across the gap at the same speed as  $\frac{dx_0}{dt}$ , and is just behind the filament tip, the electrostatic field due to the applied voltage will always be enhanced by the space charge field. Thus the field  $E_0$  can be produced by a smaller applied voltage under these conditions than in the absence of space charge. The breakdown voltage will be less than in case (1).

(3) *Slow rise-time impulses.* Beyond the value of  $\frac{dx_0}{dt}$  corresponding to the minimum breakdown voltage the space charge will drift away from the point faster than  $E_0$ . In this case the space charge field will reduce rather than increase the electrostatic field and hence quench the discharge. This inhibiting space charge then has to be removed by the applied field before a discharge can occur. As in (1) the applied field alone has to produce  $E_0$  everywhere in the gap and thus a higher

applied voltage is needed than in case (2). This propagation model is being developed further and it is intended that the complete breakdown process under all conditions will also be included.

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## METALLURGY

### Orientation-dependence of Fatigue-accelerated Creep in Aluminium Single Crystals

THE character of some of the creep-fatigue interactions in metals can be extremely complex, as reviews of the subject reveal<sup>1,2</sup>. The fact that creep rates of relatively pure metals, such as lead<sup>3</sup>, copper<sup>4</sup> and zinc<sup>5</sup>, are significantly accelerated by a superimposed fatigue stress is nevertheless well established. A sound explanation for the effect, and its associated features, is not easily advanced without more fundamental information, particularly in respect of the behaviour of single crystals. While some effects in single crystals have been examined (fatigue-activated recovery<sup>6</sup>, for example), no examination of fatigue-activated creep in single crystals appears to have been published so far. The purpose of this communication is to report some initial results derived from experiments on aluminium single crystals in which particular emphasis has been given to the orientation-dependence of fatigue-accelerated creep.

Single crystals of aluminium spark-machined to the specimen shape discussed by Andrade<sup>7</sup> were stressed in a thermostatic enclosure at 180° C by means of an hydraulic device. The experimental assembly enables the static and the alternating components of stress to be controlled and monitored separately, with digital display of the creep strain during a test. The geometry of the specimens also makes it possible to reverse the creep stress direction if required. Crystals of two orientations have been examined. The crystals of one batch were oriented for single slip, and those of the other for double slip, that is, for symmetrical <110> slip on two sets of {111} planes.

The results of the tests on both batches were remarkably similar, and there was no distinctive difference between the behaviour of crystals in the two orientations. A large fatigue-acceleration effect was observed (Fig. 1, curve A), much larger than that which arises from a static stress equal to the peak stress in the cycle (see Fig. 1, curve B), and the hardening introduced was similar to that noted previously in polycrystalline materials<sup>8</sup>; the hardening is manifest by an incubation period of zero creep following the removal of the fatigue stress, with thermal softening eventually bringing about a renewed creep (Fig. 1, curve C). As unexpectedly high values of stress were required to induce measurable creep, the onset of plastic strain in the two orientations as deduced from stress-strain measurements was particularly examined.

The results are summarized in Fig. 2; the differences between the behaviour of the two types of crystal are clearly small and, as with the creep results, no significant distinctions emerge. The possible occurrence of some double slip in the crystals oriented for single slip only

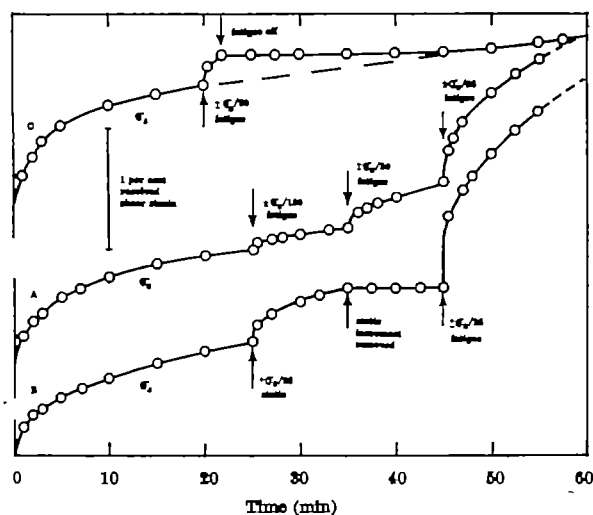


Fig. 1. The creep of aluminum crystals oriented for predominantly single slip, under a static resolved shear stress ( $\sigma_s$ ) of 55 kg cm<sup>-2</sup> and at a temperature of 180° C. The curves are displaced arbitrarily parallel to the strain axis for convenience, the scale being as indicated, and they are not significantly different from those obtained from crystals oriented for symmetrical double slip

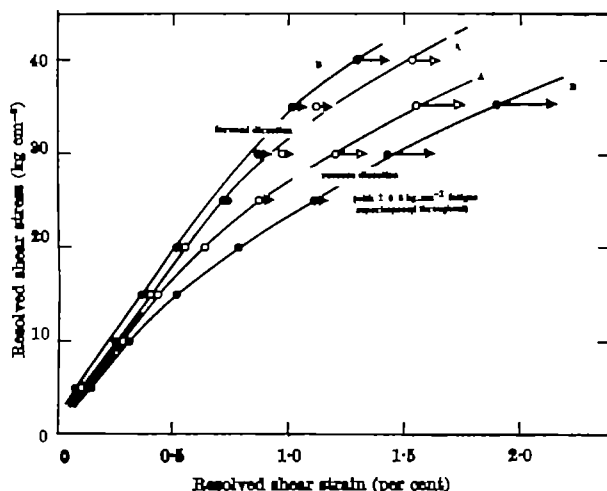


Fig. 2. The stress-strain curves of the aluminum crystals in shear, showing the behaviour of crystals undergoing double slip (A) and single slip (B) when stressed in forward and reverse directions. The arrows indicate the amount of creep occurring in 10 min under the respective stresses

cannot be ignored. This seems unlikely, but is being checked.

This particular series of experiments suggests, then, that the acceleration does not necessarily arise from the enhanced activation of cross-slip, that is, from the mechanism primarily responsible for initial fatigue damage. The point-defect generating hypothesis, which associates the acceleration with a more rapid dislocation climb, is not ruled out, but remains unsatisfactory on several counts. We are left with the observation that vibrating dislocations appear to circumvent barriers in their slip planes more easily than statically stressed dislocations, and although the feasibility of this has been commented on previously (for example, by Forsyth *et al.*<sup>9</sup>), a convincing quantitative theory is lacking. The answer may lie in the fact that slip in fatigue-activated creep may be distributed over a band of adjacent slip planes, just as it is in fatigue proper<sup>10</sup>, due to intersections between the primary dislocations and those lying in other slip systems. If the properties of these zones are the key factors, then it remains to explain why these are relatively harder in creep, when stressed statically, than statically-formed

bands are, but effectively softer if an alternating component is present.

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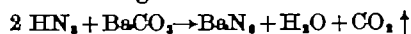
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## CRYSTALLOGRAPHY

### Preparation of Single Crystals of Barium Azide

THE need for large, optically clear crystals of barium azide of high purity for research studies has been realized for some time. It was first necessary to obtain pure barium azide powder to satisfy the requirements necessary for obtaining optically clear crystals. A method for preparing high-purity barium azide powder was developed at the Explosives Research Laboratory by Reitzner and Manno<sup>1</sup>. In this method, dry gaseous hydrogen azide was made to react with spectral grade barium carbonate in aqueous suspension. Using spectral grade acetone, barium azide was precipitated from this reaction. Table 1 gives the elemental impurities found in BaCO<sub>3</sub> and BaN<sub>3</sub>, in parts per million (atomic) and indicates that even such 'spectroscopically pure' materials contain many impurities. The results of this analysis were obtained from Battelle Memorial Institute by mass spectrographic techniques. An investigation was then undertaken to develop a technique for growing optically clear seed crystals of barium azide from which larger crystals could be obtained.

Spectral grade barium carbonate (Johnson and Matthey) was reacted with dry hydrogen azide gas from the Reitzner-Manno generator:



All reactions were carried out in polyethylene containers to eliminate impurities such as silicon and sodium from glass surfaces. BaN<sub>3</sub> was precipitated at pH 4 with an excess of spectral grade acetone. The water used in the reaction was triply distilled and de-ionized in an ion-exchange column as a purification measure. The material was filtered under nitrogen and placed in a vacuum desiccator over CaSO<sub>4</sub> to dry.

A successful method of obtaining seed crystals of barium azide was to place 50 ml. of saturated BaN<sub>3</sub> solution in water in a 250 ml. graduated cylinder. Three ml. of a 50/50 water/acetone mixture was placed on the surface of this solution by means of a long, thin capillary tube. This procedure prevented immediate precipitation of BaN<sub>3</sub> when acetone alone was placed on the surface. On top of the intermediate layer, spectral grade acetone was added to the 250-ml. mark in the graduate. The graduate was kept at room temperature and was not moved after the acetone layer had been added. Seventy-two hours later, by the diffusion of one liquid into the other, small barium azide monohydrate crystals were precipitated on the surface of the graduate in the water and the acetone levels. Crystals also appeared at the bottom of the graduate. After 10 days, when the crystals had grown to approximately 6.5 mm in length, they were

Table 1. ANALYSIS OF SPONG-FORM  $\text{BaO}_2$  AND  $\text{BaN}_2$  (Atomic p.p.m.)

Element	$\text{BaO}_2$	$\text{BaN}_2$	Element	$\text{BaO}_2$	$\text{BaN}_2$
Li	2	0.07	Ag	0.004	0.01
Ba	0.04	0.006	Od	0.004	0.03
B	0.6	0.3	In	0.02	0.006
F	0.4	0.3	Sn	0.025	0.03
Na	4	2	Sb	0.006	0.01
Mg	4	150	Te	0.004	0.02
Al	2	12	I	0.008	0.006
Si	10	2	Cs	0.004	0.006
P	1.2	2	La	0.06	0.006
S	10	70	Ce	0.02	0.008
Cl	150	0.4	Pr	0.02	0.006
K	6	40	Nd	0.12	0.02
Ca	4	6	Sm	0.6	0.02
Sc	0.6	0.1	Eu	0.04	0.12
Ti	0.15	0.04	Gd	0.12	0.2
V	0.015	0.03	Tb	0.02	0.03
Cr	0.15	0.06	Dy	0.03	0.12
Mn	0.04	0.03	Ho	0.02	0.03
Fe	0.4	3	Er	0.04	0.02
Co	0.004	0.02	Tm	0.02	0.03
Ni	0.04	0.3	Yb	0.12	0.1
Cu	0.03	1	Lu	0.02	0.03
Zn	0.03	0.25	Hf	0.1	0.02
Ga	0.003	0.06	Ta	0.03	0.3
Ge	0.2	0.02	W	0.04	0.07
As	0.06	0.12	Re	0.01	0.03
Se	0.006	0.01	Os	0.006	0.02
Br	0.003	0.06	Ir	0.002	0.01
Rb	0.003	0.01	Pt	0.02	0.02
Sr	0.03	0.02	Au	0.006	0.02
Y	0.0015	0.006	Hg	0.004	0.03
Zr	0.02	0.03	Tl	0.002	0.01
Nb	0.015	0.006	Pb	0.6	0.15
Mo	0.006	0.15	Bi	0.006	0.006
Ba	0.012	0.02	Th	0.02	0.006
Bh	0.03	0.06	U	0.0015	0.006
Pd	0.012	0.03			

taken from the solution by siphoning out the liquid. Several dozen seed crystals were obtained from each graduate by this method.

The 6.5-mm seed crystal of  $\text{BaN}_2 \cdot \text{H}_2\text{O}$  was fastened to a glass rod with an adhesive made by dissolving 'Styrofoam' in benzene. Fifty ml. of a saturated aqueous  $\text{BaN}_2$  solution was placed in a 250 ml. graduate and the seed crystal was suspended in the centre of the solution. An intermediate layer of 50/50 acetone/water solution was placed on its surface using the capillary tube. Acetone was then added to the 250-ml. mark and the graduate sealed with a polyethylene-covered rubber stopper. The graduate was kept at room temperature. After five days at room temperature (24° C), the seed could be seen to have grown. Thirty days later the crystal was taken out and found to be 12.10 mm in length.

A saturated  $\text{BaN}_2$  water solution was made and one c.c. of excess water added. The solution was placed in a graduate and the aforementioned crystal placed in its

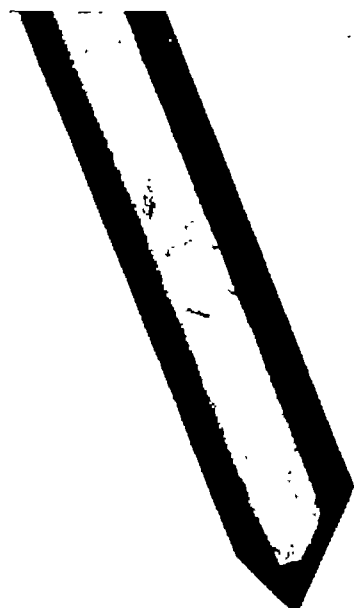


Fig. 1

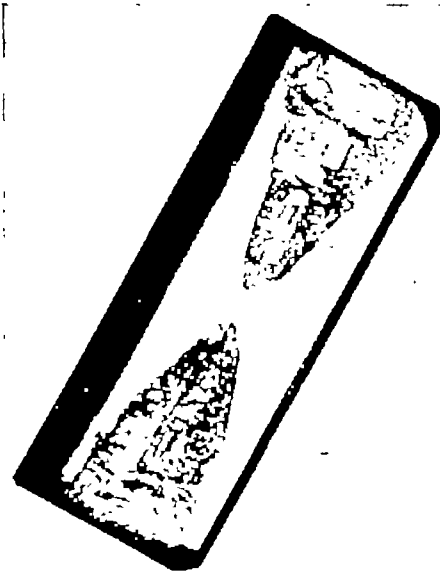


Fig. 2

centre. The intermediate layer and precipitating solvent were added as described. After 24 h, a rough surface could be observed on the crystal face as a result of its dissolving in the excess water. Several days later new growth could be observed without a line of demarcation of one growth period on another. After 30 days the crystal was extracted and measured to be 19.05 mm long by 4.76 mm in diameter.

Diffusion is the movement of molecules which in time will tend toward an equilibrium situation in which there are no concentration gradients. With the diffusion technique in the liquid phase, the most abundant crystal habit of barium azide was of the monohydrate variety observed in the water layer (Fig. 1). However, in the precipitating solvent, acetone or alcohol, small square barium azide crystals were observed (Fig. 2)\*. When an ether/alcohol mixture was used as the intermediate layer, clear flat plates of barium azide were observed in the ether/alcohol which may be a new crystal habit.

Slow cooling of saturated barium azide at 55° C in water, water/alcohol, water/methanol, water/acetone, alcohol, methanol, and acetone was found to be an unsuccessful method for the growth of good crystals. These experiments resulted in the formation of a fine powder and some decomposition of barium azide. However, further experiments utilizing these techniques should be undertaken. Slow evaporation of barium azide from water solutions produced large crystals that were in general rough surfaced and uneven. Evaporation from other solvents produced a fine powder and afterwards these techniques were dropped.

I thank Dr. T. Richter, Lieut. P. Marinkas and Dr. P. Kemney for their advice.

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### Habit of Triglyceride Crystals

We have examined the habit of purified tripalmitin crystals of the  $\beta$  form grown from the melt and a variety of solvents. The results are summarized in Table 1.

Solvent	Largest face parallel to <i>c</i> axis	Prefereed growth direction	Remarks	Fig. No.
Melt	{110} only	None	—	1
Hexane	100	<i>b</i> Axis	—	2
Benzene	100	<i>b</i> Axis	010 face on most crystals	3
Chloroform	100	<i>b</i> Axis	Large crystals	4
Acetone	{110}	—	Small 100 face visible	5

Figs. 1–5 show typical crystals from each solvent.

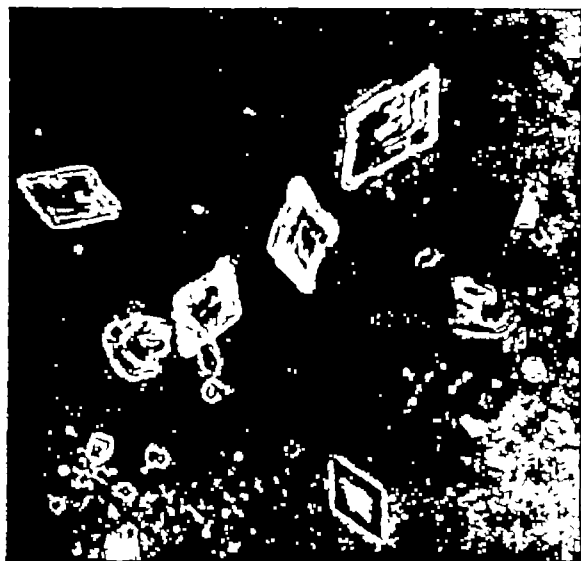


Fig. 1. Crystals of tripalmitin grown from the melt ( $\times c. 125$ )

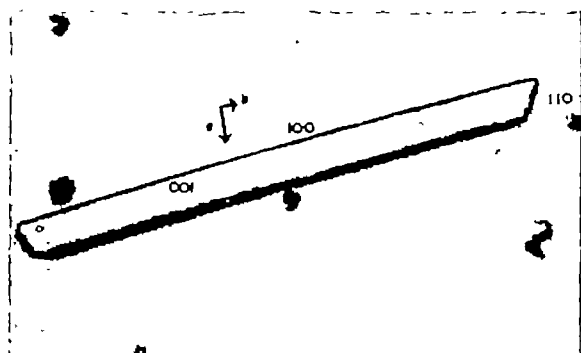


Fig. 2. Crystal of tripalmitin grown from hexane ( $\times c. 170$ )

Identification of the crystal faces is based on measurement of angles and comparison with the work of Vand<sup>1</sup> and Jensen and Mabis<sup>2</sup> for the  $\beta$  form of trilaurin and tricaprln. Tripalmitin may be expected to have a similar structure with a larger *c* axis parameter.

Thicknesses of the crystals along the *c* axis has been measured for a few crystals. This dimension is extremely small for melt grown crystals and relatively larger for solution growth. The crystals grown from acetone were about  $300\mu$  long in the *a*-*b* plane and  $15\mu$  thick along the *c* axis.

The interesting feature of these results is that crystals grown from hexane, benzene and chloroform are elongated in the *b* axis direction and bounded by large 100 faces. Crystals grown from acetone are bounded by {110} faces with small 100 faces sometimes visible and crystals grown from the melt have only {110} faces parallel to the *c* axis.

These observations are consistent with the electron microscope studies of Okada<sup>3</sup> on very small crystals, which also suggest that the crystals grown from ether are similar in habit to those grown from acetone.

Larson<sup>4</sup> has analysed the structure of trilaurin in detail and shown that in the *b* direction there is an

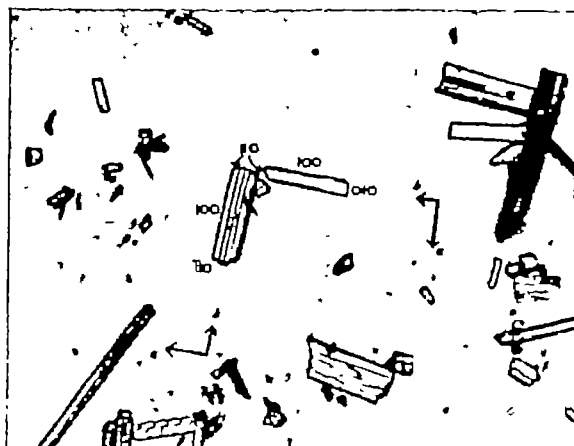


Fig. 3. Crystals of tripalmitin grown from benzene ( $\times c. 28$ )



Fig. 4. Crystals of tripalmitin grown from chloroform ( $\times c. 75$ )



Fig. 5. Crystals of tripalmitin grown from acetone ( $\times c. 150$ )

unusually short intermolecular distance and probably a considerable polar attraction. This evidently results in enhanced growth in this direction when hydrocarbon solvents are used.

Several examples of solvent effects on crystal habit have been reported by Wells<sup>5,6</sup>.

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## CHEMISTRY

### An Improved Method of calculating Particle Size Distribution from Centrifugal Sedimentation Experiments

CENTRIFUGAL sedimentation methods are widely used for determining the particle-size distribution of suspensions of fine solids in liquids. Donoghue and Boetock<sup>1</sup>



give methods for calculating the cumulative weight size distribution of a sample from the experimental figures. This method is based on filling the centrifuge with the sample under examination, as opposed to the method initially used by Marshall<sup>3</sup>, and afterwards developed by others<sup>3,4</sup>, in which the sample is introduced as a thin layer superposed on a clear medium of slightly higher density. This latter method is likely to give inaccurate results with some systems due to the phenomenon of 'streaming'. Using the former method, the particle size analysis is performed by running the centrifuge for a given time at constant speed and then measuring the amount of sample which has either sedimented to (or past) a certain radius in the centrifuge, or which remains above this radius. It is immaterial for the purpose of this note which method of measurement is used. The complete size distribution is determined by one of three methods:

(1) The inner radius of the liquid surface and the outer radius of collection are kept constant and samples are collected after varying running times of the centrifuge. (We assume the centrifuge speed is kept constant for all experiments.)

(2) The running time of the centrifuge is kept constant, as is the inner radius of the liquid, and the outer collection radius is varied.

(3) The running time of the centrifuge is kept constant as before, but the outer collection radius is kept constant for successive measurements, while the inner liquid surface radius is varied.

Donoghue and Bostock give methods for calculating the size distribution from experimental data obtained by methods 2 and 3. A simpler and more accurate calculation than that given by these authors exists for method 3 and does not appear in the literature. This can be developed on a physical basis by considering a centrifuge with a collecting plane at radius  $R$ , and filled to a radius  $S$ . We also need to define the speed of rotation of the centrifuge,  $\omega$ , the viscosity of the liquid,  $\eta$ , the density difference between solid and liquid,  $\Delta\rho$ , and the running time of the centrifuge between sample insertion and collection,  $t$ . It is assumed that all these latter conditions are constant. If the inner radius of the centrifuge suspension is decreased by a small amount  $dS$ , then the extra weight of sample introduced into the centrifuge is  $2\pi S T c dS$ , where  $T$  is the thickness of the suspension in an axial direction and  $c$  is the weight of solid per unit volume of suspension. For this extra amount of material added, all that with a particle size larger than a Stokes diameter  $D$  will reach the collecting plane at  $R$ , and all that smaller than  $D$  will be at a smaller radius than  $R$  at the end of the running time  $t$ . This diameter  $D$  is given by the equation:

$$\ln(R/S) = D^2 \Delta\rho \omega^2 t / 18\eta \quad (1)$$

The extra weight of sample deposited at a plane at radius  $R$  due to this change in radius  $dS$  of the top surface of the sample is therefore:

$$dW = -2\pi S T c dS \int_D^\infty f(D) dD \quad (2)$$

The negative sign occurs because the extra added layer causes a decrease in  $S$ . This equation applies to an apparatus where the liquid is run off and the deposited layer is retained for analysis, whereas in the type of apparatus where the over-lying liquid layer is removed for estimation of the weight of solids<sup>3</sup>, the following equation should be used:

$$dW = -2\pi S T c dS \int_0^D f(D) dD \quad (3)$$

In both these equations  $f(D) dD$  is the weight fraction of the sample lying in the size range  $D$  to  $D + dD$ , and hence  $\int f(D) dD$  is the cumulative weight percentage in the

size range stated. It is seen that the cumulative weight percentage can be obtained easily from experiments in which  $S$  is varied but all the other conditions of the experiment are kept constant. Considering equation (3), the weight of sample collected,  $W$ , is plotted against  $S$ , and the tangent to the curve found for varying values of  $S$ . This is divided by  $S(2\pi T c)$ , the bracketed term being a constant for any one sample and apparatus. The resulting figure is the cumulative weight percentage below diameter  $D$ , where  $D$  is related to the value of  $S$  at which the tangent was drawn by equation (1). A similar result can be derived for equation (2).

Equations (2) and (3) can be derived mathematically from the equation for the total weight of material collected, for example, in the case of equation (3):

$$W = \pi T c \int_0^D (R^2 e^{-2\pi D^2} - S^2) f(D) dD, \text{ where } \varphi = \frac{\Delta\rho \omega^2 t}{18\eta} \quad (4)$$

Differentiation of this with respect to  $S$  leads directly to equation (3). Ref. 1 treats the whole problem in terms of the fraction of material in the centrifuge which is collected, rather than the total weight collected, and this results in a much more complicated and less accurate method of calculation. It will be noted that the problem of calculating the size distribution when the radius of collection  $R$  is varied, that is, when equation (4) is differentiated with respect to  $R$ , is not so simple, and hence is less accurate. In this case the method of Donoghue and Bostock is as good as any other.

Donoghue and Bostock also state that no analytical method exists for the derivation of the particle size distribution of a sample when  $t$  is varied. This is correct, though with modern computers iterative methods can be developed for calculating the required results at quite low cost. In our experience this is not as accurate as the method outlined here.

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## BIOCHEMISTRY

### Fatty Acid Synthesis in Rabbit Thymus Preparations

IN connexion with our earlier studies on the lipid metabolism in human white blood cells<sup>1-3</sup>, a study on the metabolism of acetate-1-<sup>14</sup>C and malonate-2-<sup>14</sup>C by rabbit thymus preparations was initiated, in order to investigate lipid synthesis in this gland. Information from such experiments does not only apply to the physiological role of the thymus connected with lymphocyte production, but also, eventually, to other functions of this gland<sup>4</sup>.

Two- to three-month-old rabbits were killed by a blow on the head and the thymus immediately excised and chilled in ice. The glands were sliced with a razor and homogenized with 9 volumes of ice-cold 0.25 M sucrose solution in a motor-driven Potter-Elvehjem homogenizer with a 'Teflon' pestle for 1 min. The homogenate was passed through a cheese cloth and separated into two particulate fractions and the particle-free supernatant (Sb), essentially as described previously for human leucocyte preparations<sup>5</sup>. Any floating fat appearing during centrifugation was removed.

The incubation system used in these experiments is shown in Table 1. D(+) biotin was added, as it was shown that in some preparations the addition of 2  $\mu$ moles

Table 1. INCORPORATION OF ACETATE-1-<sup>14</sup>C AND MALONATE-2-<sup>14</sup>C INTO TOTAL LIPIDS OF RABBIT THYMUS PREPARATIONS

	Acetate-1- <sup>14</sup> C		Malonate-2- <sup>14</sup> C
	Incorporation mmoles per mg of protein	Ratio of radioactivity of carboxy carbon to total radioactivity	Incorporation mmoles per mg of protein
15,000g sediment	8.12	1:2.7	7.89
93,000g sediment	17.88	1:3	18.70
8b 93,000g supernatant	Exp. I 49.3	1:2	56.25
" "	Exp. II 7.98	1:2.2	35.40
" "	Exp. III 9.23		18.86

The complete system contained 60 μmoles phosphate buffer (pH 6.5), 0.04 μmoles CoA, 10 μmoles ATP, 1 μmole NADP, 1 μmole NAD, 6 μmoles cysteine, 5 μmoles Mg<sup>++</sup>, 10 μmoles KHCO<sub>3</sub>, 15 μmoles citrate, 2 μmoles biotin, 0.15 μmole acetate-1-<sup>14</sup>C, 5 μmole, or 0.15 μmole malonate-2-<sup>14</sup>C, 3:1 v/v. The volume was 1 ml. and contained 0.2 to 0.5 mg of protein. The flasks were incubated for 1 h. in air atmosphere; at the end of this period total lipids were extracted by the method of Folch *et al.*<sup>1</sup>, and hydrolyzed when needed with methanol hydrochloric acid 5:1 v/v for 7 h at 75° C under nitrogen. The fatty acids which accounted for 95 per cent of the radioactivity of the total lipids were extracted with pentane. Decarboxylation of the free acids was according to the Schmidt reaction, as described by Brady *et al.*<sup>2</sup>.

of it resulted in a maximum incorporation of acetate-1-<sup>14</sup>C. Preliminary experiments showed that CoA and ATP were the absolute requirement of the systems for the incorporation of acetate-1-<sup>14</sup>C by both particulate fractions and Sb, while Mg<sup>++</sup> stimulated the reaction and could replace Mn<sup>++</sup>. A partial requirement for NAD and NADP could be shown for the mitochondria. When Sb was treated with 'Dowex-1 Cl<sup>-</sup>' the system showed a partial requirement only for NADP.

Table 1 shows that both <sup>14</sup>C-precursors are efficiently incorporated into all tested cell fractions. The presented data for malonate-2-<sup>14</sup>C must not be taken as a measure of its rate of incorporation by the various fractions, since part of it may be decarboxylated and incorporated as acetate-2-<sup>14</sup>C, in the presence of ATP<sup>3,4</sup>.

The high percentage of radioactivity found in the carboxy carbon, after decarboxylation of the total fatty acids synthesized from acetate-1-<sup>14</sup>C by the various fractions of the cell, indicates that a large part of this substrate is incorporated through the elongation or mitochondrial system<sup>5</sup>, that is, by direct addition of one or more acetyl-CoA units to pre-existing fatty acyl-CoA units. Since this is a usual finding only for the particulate fractions<sup>6,10</sup>, the operation of the mitochondrial system in the particle-free supernatant must be due either to a peculiarity of the tissue studied, or to an extraction of the system from the particles during the separation procedure. A similar finding has been reported by Christ and Hultmann<sup>11</sup> for rabbit heart and pigeon liver mitochondria. They suggested that the major part of the lipogenic activity is actually present in mitochondria, but is extracted and appears in the centrifugal supernatant. Our data, although they may indicate a release of the mitochondrial system into the supernatant, do not show that the malonyl-CoA pathway system has a mitochondrial origin.

Table 2 shows that both particulate fractions synthesize similar fatty acids, predominantly with a chain-length of

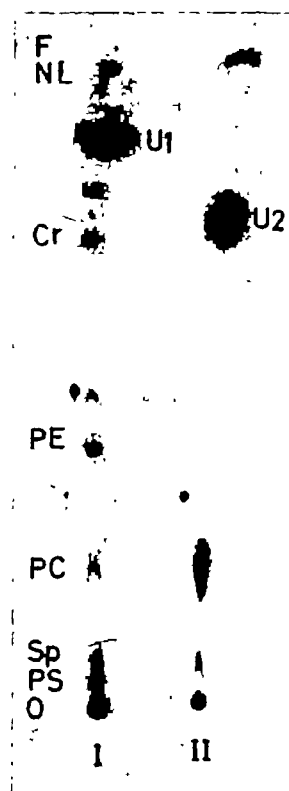


Fig. 1. Autoradiogram of thin-layer chromatogram of total lipids extracted from thymus 93,000g supernatant, incubated with acetate-1-<sup>14</sup>C (I), or malonate-2-<sup>14</sup>C (II). Solvent system: chloroform, methanol, water, 80:20:3 v/v. The abbreviations show the position where the corresponding standards, which were run simultaneously, moved. NL, neutral lipids; U, unidentified; Cr, cerebrosides; PE, PC, PS, phosphatidyl (ethanolamine, choline and serine, respectively); Sp, sphingomyelin.

18, or more, carbon atoms, which are characteristic products of the mitochondrial system<sup>8,10</sup>. The quantitative difference in the fatty acids, formed by Sb from acetate-1-<sup>14</sup>C and malonate-2-<sup>14</sup>C, is consistent with the results of decarboxylation and indicates that not all acetate-1-<sup>14</sup>C is incorporated via the malonyl-CoA intermediate. It should be mentioned here that results reported for the Sb of other tissues<sup>8-9,12-15</sup> show that malonate is mainly incorporated into palmitate. In contradiction to the foregoing, in the Sb of thymus, malonate is almost equally incorporated into myristate and palmitate. We have no explanation at the moment for this high rate of myristate synthesis.

Fig. 1 shows that when Sb from the same preparation was incubated with acetate-1-<sup>14</sup>C (U<sub>1</sub>) or malonate-2-<sup>14</sup>C (U<sub>2</sub>), the radioactivity was mainly associated with one

Table 2. FATTY ACIDS SYNTHESIZED BY RABBIT THYMUS PREPARATIONS

	Percentage of total radioactivity eluted from the column									
	< C <sub>14</sub>	C <sub>14</sub>	C <sub>14</sub> -C <sub>18</sub> †	C <sub>18</sub>	C <sub>18</sub> -C <sub>22</sub>	C <sub>22</sub>	C <sub>22</sub> :1	C <sub>22</sub> :2	C <sub>22</sub> unsaturated	> 20:4
15,000g sediment + acetate-1- <sup>14</sup> C	2.7	4.0	—	11.2	6.1	10.3	10.7	4.9	31.0	3.5
93,000g sediment + acetate-1- <sup>14</sup> C	5.3	4.7	—	3.5	5.4	11.8	8.1	4.8	33.0	3.6
93,000g supernatant + acetate-1- <sup>14</sup> C		11.4	—	25.4		21.5	15.1		12.1	14.6
93,000g supernatant + malonate-2- <sup>14</sup> C*	7.4 ± 2	24.7 ± 0.5	11.3 ± 2.1	25.5 ± 0.5	11.5 ± 3.6	7.7 ± 8.1			6.8 ± 1.4	4.8 ± 0.9

\* Average from two experiments ± deviation from the mean.

† Dashes indicate that the compounds were trapped with the C<sub>14</sub>.

Fatty acids, extracted as described in Table 1, were methylated and separated by preparative gas liquid chromatography on a Pye argon chromatograph as described previously<sup>12</sup>. Standard fatty acid esters were added routinely for identification.

fast-moving spot, which moved to different positions, depending on the precursor used. In addition, it was shown that by mild alkaline hydrolysis and silicic acid chromatography<sup>10</sup>, when Sb was incubated with acetate-1-<sup>14</sup>C, only 10–30 per cent of the labelled fatty acids were linked in ester bond to the complex lipid, whereas the opposite was true when malonate-2-<sup>14</sup>C was used as substrate. It is unlikely that the differences in fatty acids synthesized from the two precursors account for the observed difference in their utilization. Therefore, further experiments are now in progress, to elucidate the structure and significance of these lipids, which are resistant to mild alkaline hydrolysis.

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### Role of Cysteine in the Production of Fc and F'c Sub-components of $\gamma$ G Myeloma Globulins

RECENTLY, Osserman and Takatsuki<sup>1</sup> have described a group of  $\gamma$ G myeloma proteins the Fc fragment of which was completely destroyed by the action of papain. A similar protein (myeloma N) was independently examined in this laboratory. This protein was found to be susceptible to papain and trypsin<sup>2</sup>.

Immunological typing has characterized this protein as shown in Table 1. The Zu (ref. 3), V<sub>4</sub> (ref. 4) and  $\gamma$ 2c (ref. 5) groups denote different terms for a similar antigenic determinant. All enzyme-susceptible Fc fragments so far belong to this group. The results of Grey and Kunkel<sup>4</sup> indicate that monkey anti-Zu, but not rabbit anti-V<sub>4</sub>, develops an Fc component on immunoelectrophoresis. This would suggest that a more heterogeneous group of proteins is found in this H chain sub-group.

Some of the factors which might be involved in this susceptibility were now examined. Oebra *et al.*<sup>3</sup>, using a water-insoluble papain derivative, have shown that cleavage of rabbit  $\gamma$ -globulin is a two-stage process. The initial stage of the reaction involved the splitting of some peptide bonds by papain, and the subsequent reduction of the products into smaller fragments by cysteine. Hsiao and Putnam<sup>7</sup>, using mercurypapain, have shown that proteolysis of human  $\gamma$ -globulin in the absence of cysteine results in the liberation of 3.5S fragments, and a variable amount of 6.6S material. Accordingly, the role of cysteine in the proteolysis of myeloma N was examined. In our experiments, papain digestion (crystallized papain  $\times 2$ , lot No. 5575, Worthington

Biochemicals), in the absence of cysteine, was conducted in 0.1 M phosphate buffer, pH 6.0 at 37° C at a protein to enzyme concentration of 100:1 for 18 h. The immunoelectrophoretic patterns in Figs. 1a and c show the production of an Fc-like but not F'c fragment under the foregoing described conditions. The incorporation of 0.01 M cysteine into the digestion process results in the production of both the Fc and F'c precipitin bands in human  $\gamma$ G (Cohn Fr II) (Fig. 1b), but in the destruction of both these fragments in the case of myeloma N (Fig. 1d). Ultracentrifugal analysis has shown that under the former conditions, myeloma N protein digested with and without cysteine yields only 3.5 S fragments. Under similar conditions in the absence of cysteine, human  $\gamma$ -globulin and papain insensitive  $\gamma$ G myelomas yield products sedimenting at 3.6 S (80 per cent) and 6.7 S (20 per cent).

A large number of  $\gamma$ G myelomas and human  $\gamma$ G (Cohn Fr II) were studied with the aid of starch-gel electrophoresis after papain digestion. The Fc component of these proteins resolves into a series of bands which are identical as to concentration, number and migration. The sub-units of the Fab fragment are specific for each  $\gamma$ G myeloma on starch-gels.

Fig. 2 comprises human  $\gamma$ G (Cohn Fr II),  $\gamma$ G myeloma T (papain insensitive) and  $\gamma$ G myeloma N (papain sensitive) digested by papain with and without cysteine. Figs. 2A and B, respectively, show the typical distribution of Fc sub-components of human  $\gamma$ G and T myeloma proteins digested in the presence of cysteine. There are at least 9 bands of which No. 6, counting from the application slot toward the anode, stains the most intensely. C and E represent, respectively, similar proteins digested in the absence of this sulphhydryl agent. It can be seen that the number, migration and intensity of the Fc sub-components are the same, but differ from those of A and B. In contrast, D and G, respectively, show myeloma N cleaved without and with cysteine. Complete dissolution of the Fc sub-components is seen in Fig. 2G. The Fc fragment and its sub-components, which are only produced in the absence of cysteine (Fig. 2D), show no similarity to those of myeloma T or myeloma N, and human  $\gamma$ G similarly treated. In Figs. 2B and C, several bands can be seen on the anode side of the application slot. These have a similar rate of migration to native myeloma protein T (Fig. 2I) and may be identical. It is suggestive of the 6.7 S sedimenting material seen in the digests of myeloma T treated without cysteine.

The four bands in Fig. 2D, and to a lesser extent in Fig. 2G, which migrate slightly to the cathode side of the application slot, have a mobility similar to native myeloma

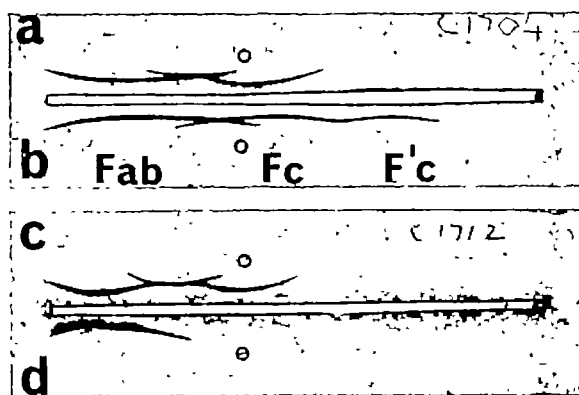


Fig. 1. Immunoelectrophoretic analysis of human  $\gamma$ G (Cohn Fr II) and myeloma N protein after digestion with papain in the presence and absence of cysteine

a, b, Human  $\gamma$ -globulin; c, d, myeloma N; a, c, digested without cysteine; b, d, digested with cysteine. Precipitin arcs were developed with a rabbit antiserum containing antibodies directed against the Fab, Fc, and F'c fragments

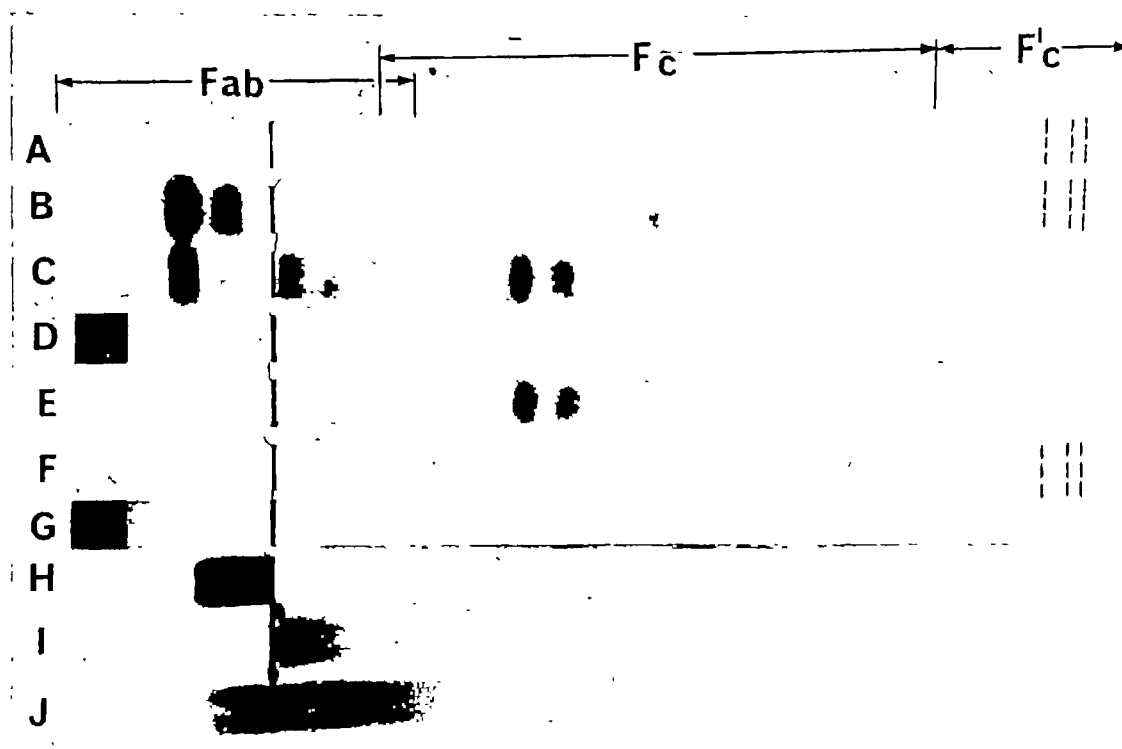


Fig. 2. Starch-gel electrophoresis of human  $\gamma$ -globulin (Cohn Fr II) and  $\gamma$ G myelomas (T and N) after digestion with papain in the presence and absence of cysteine. Human  $\gamma$ -globulin: A, F, cysteine present; B, cysteine absent; J, native protein. Myeloma T: B, cysteine present; C, cysteine absent; I, native protein. Myeloma N: G, cysteine present; D, cysteine absent; H, native protein. Fab, Fc and Fc' fragments are indicated.

protein N (Fig. 2H). Although no 6.8 S sedimenting component was seen in myeloma N treated without cysteine, this may be no more than due to concentration. In determining Svedberg constants, 0.4 per cent solutions were used as opposed to 5 per cent on the gels.

Another difference in the patterns obtained with and without cysteine in the papain digestion mixture is seen in the Fc' region. All materials digested in the absence of cysteine (Figs. 2C, D and E) are characterized by the lack of the Fc' fragment in contrast to proteolysis with cysteine (Figs. 2A, B and F) where the three typical Fc' bands appear. These observations were also confirmed by immunoelectrophoresis (Fig. 1).

Table 1. PROPERTIES OF  $\gamma$ G MYELOMA N

Physico-chemical		
$S_{20}$	6.85	
Solubility	75% soluble in saline at 1% w/v	
Sludge content	0.16% dry weight	
Mobility—agar	$\gamma$ G	
Starch (tris buffer)	Cathode	
Immunological		
	H chain	L chain
Antigenic type	Vi ( $\gamma$ 2, 2a)	K (I)
Genetic allotype	Gm (a-b-*) (a-)	Im V (a-b-)

\* The Gm(b) group was negative at 0.25 mg/ml, but positive at 5 mg/ml.

In summary, it should be noted that the number and position of the Fc' and Fc fragments of human  $\gamma$ G and papain insensitive  $\gamma$ G myelomas are characteristic for each mode of papain digestion. When proteolysis occurs in the presence of cysteine, the Fc' bands evolve and a distinct set of Fc sub-components is separated on starch-gel. However, when digestion occurs in the absence of cysteine, the Fc' sub-components do not appear and a new set of Fc sub-components is demonstrated. For each mode of digestion, the Fc' and Fc sub-components are identical in all the papain insensitive  $\gamma$ G myelomas and human  $\gamma$ G (Cohn Fr II) thus far tested.

A unique  $\gamma$ G myeloma protein has been described where the integrity of the Fc and Fc' sub-components depends

on the presence or absence of cysteine during digestion. The Fc sub-components of myeloma N can be produced by cleavage in the absence of this sulphhydryl compound, and the separated Fc sub-components show a different distribution from those described here. Short periods of digestion (less than 1 h) can also result in production of an Fc precipitin arc. The Fab component typifies each myeloma protein, and its pattern on starch-gels is independent of the action of cysteine, suggesting the primary action of this agent is on the Fc fragment. From the practical point of view one is now able to isolate an Fc fragment of papain-sensitive myelomas for further investigation.

From the aforementioned experiments, it appears that cysteine acts on some crucial disulphide bond(s) which may unfold or denature the Fc intermediates<sup>1</sup>, making the latter more susceptible to papain.

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### Specificity of Pigment Formation by *Penicillium rubrum* Stoll

THOUGH *Penicillium rubrum* Stoll can often be isolated from mould growth on organic substance and is, indeed, very widely distributed, the appearance of the red pigment at the site of the growth, which is one of its important characteristics, is not at all frequent. This fact suggests that some strict specificity exists in pigment formation by *P. rubrum*. During a preliminary study<sup>1</sup> on pigment formation by *P. rubrum* Stoll, interest was aroused by the fact that while a number of hexoses and pentoses, providing a source of carbon, gave good pigment formation, a few of them invariably failed to produce the pigment, although they served the nutritional requirements of the mould equally well. Experimental evidence indicates that the source and quantity of nitrogen, within wide limits, had no effect on pigment formation nor was it dependent on the presence of trace elements. That pigment formation was closely connected to some specificity of the saccharide molecules used as the carbon source in the culture was evident from such observations.

A close scrutiny of the conventional molecular structure of saccharides used in this study and their effect on pigment production by *P. rubrum* (Table 1) suggests that the configuration of the saccharide molecules themselves is significant and that only those hexoses and pentoses having a core of HCOH at the 4-carbon and 2-carbon respectively, taking the carbon core of D-glucose as standard, are able to produce the pigment, no matter whether they are of D- or L-variety. This obviously suggests that the formation of some specific intermediate in the biogenic pathway of the pigment synthesis is strictly dependent on the core configuration of the 'sugar' molecule.

Table 1. PIGMENT PRODUCTION BY *P. rubrum* IN MEDIUM CONTAINING 1.0 PER CENT (W/V) BACTO-PEPTONE AND 2.0 PER CENT (W/V) 'SUGARS' pH 5.3

'Sugars'	Pigment production (O.D.) 20 days	Structural characteristics
Monosaccharides		
Hexoses and hexose alcohols:		
D-Glucose	0.424	Core at 4-carbon atom*
D-Mannose	0.420	H-C-OH
D-Galactose	0.000	OH-C-H
D-Fructose	0.350	H-C-OH
L-Sorbose	0.316	H-C-OH
D-Mannitol	0.174	H-C-OH
D-Sorbitol	0.000	OH-C-H
Pentoses		
D-Xylose	0.194	Core at 2-carbon atom*
D-Arabinose	0.000	OH-C-H
L-Arabinose	0.434	H-C-OH

O.D. = Optical density (measured in 1:1 dilute culture filtrate).

\* Taking the core at 4-carbon atom in D-glucose as standard.

It is known that one of the biogenic pathways to aromatization from glucose and similar compounds is through the quinic acid-dehydroshikimic acid-shikimic acid system<sup>2</sup>. To investigate whether such a pathway also existed in aromatization to pigment formation, cultures in peptone-glucose, peptone-galactose, alanine-glucose and alanine-galactose were periodically tested for quinic acid and shikimic acid chromatographically<sup>3</sup>. A yellow spot on the paper corresponding to quinic acid in position and  $R_f$  value was obtained with glucose media but not with galactose. Any spot corresponding to shikimic and dehydroshikimic acid was, however, absent in either case. The observation of quinic acid alone and not shikimic dehydroshikimic acid in glucose media became conspicuous for the fact that quinic acid, by itself, is not considered to be in the line of direct aromatization, but it can only do so through the dehydroshikimic and shikimic acid system<sup>4</sup>. Therefore the possibility of quinic acid being formed as a casual metabolite instead of an essential intermediate could not be ruled out, and it was considered pertinent to investigate whether the addition of quinic acid to a culture

Table 2. PIGMENT PRODUCTION BY *P. rubrum* IN MEDIA CONTAINING  $\beta$ -ALANINE (0.5 PER CENT W/V) AS THE SOLE NITROGEN SOURCE AND QUINIC ACID OR GLUCOSE AS THE ONLY OTHER INGREDIENT. pH 5.3

Media	Pigment production (O.D.) in days		
	12	18	24
$\beta$ -Alanine + glucose 2.0% (w/v)	0.000	0.066	0.068
$\beta$ -Alanine + glucose 1.0% (w/v)	0.000	0.000	0.000
$\beta$ -Alanine + glucose 1.0% (w/v) + quinic acid 0.1% (w/v)	0.060	0.062	0.180
$\beta$ -Alanine + quinic acid 1.0%	0.116	0.256	0.450
$\beta$ -Alanine + quinic acid 0.5%	0.062	0.152	0.446
$\beta$ -Alanine + quinic acid 0.25%	0.022	0.062	0.270

Optical density (O.D.) was measured in 1:1 diluted culture filtrates.

could produce the pigment which was otherwise negative. This would establish the intermediate role of quinic acid as well as of conditions prevailing in the culture for its conversion to pigment. Results obtained in this direction are recorded in Table 2.

The fact that in a chemically defined medium quinic acid can supplement glucose, or can itself act as the carbon source for pigment production, sufficiently indicates the role of quinic acid as an intermediate in the biogenesis of this pigment. It was further confirmed spectrophotometrically that in all cases, either with saccharides or with quinic acid, the same pigment was produced (Fig. 1 A and B).

Identification of a compound as an intermediate in microbial metabolism, or in the biogenesis of a particular end-product, depends on the detection of the supposed intermediate in the culture and the ability of the inter-

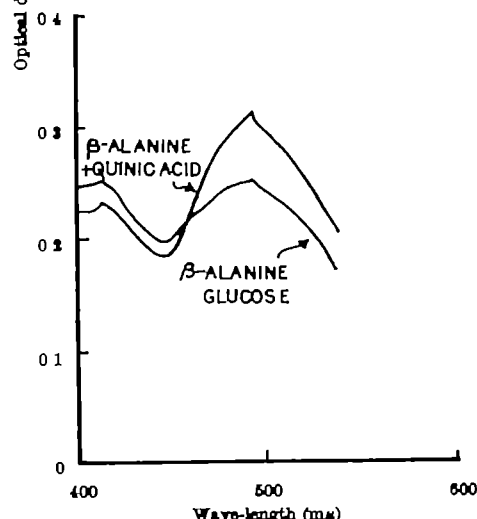
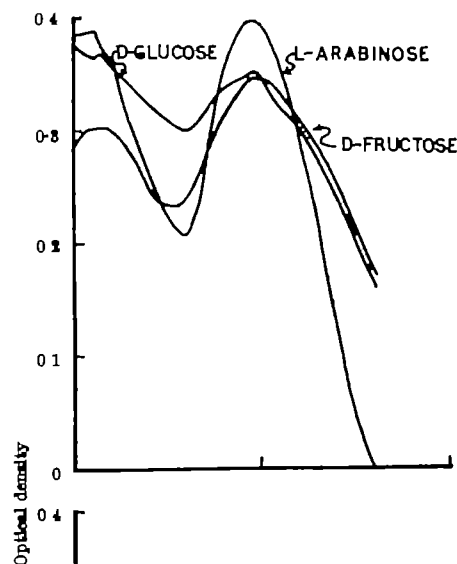


Fig. 1. Absorption curves of the pigmented culture filtrates. Media A, Bacto-peptone 1.0% (w/v), 'sugars' 2.0% (w/v). B,  $\beta$ -alanine 0.5% (w/v), quinic acid 0.5% (w/v) or glucose 2.0% (w/v). The absorption maxima of the pigmented cultures were read in a spectrophotometer at wave-lengths between 400 and 600 m $\mu$  in 10-mm cells.

mediate to support the growth of the organism to give the particular end-product<sup>1</sup>. The fact that quinic acid has been detected in the mould culture in peptone-glucose and alanine-glucose media prior to pigment formation, and that quinic acid by itself as the carbon source can form the pigment in medium containing  $\beta$ -alanine as the only other ingredient, unequivocally shows that the pathway to this pigment formation is through quinic acid.

The biogenesis of pigment in *P. rubrum* Stoll, as observed here, does not reveal the complete pathway but does indicate that quinic acid is one of the essential intermediates in the process and that, once it is available in the required concentration, pigment formation becomes a normal sequence to it. The evidence suggests that the structural specificity of the saccharides is the main determinant of quinic acid formation and consequently of production of the pigment.

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### Glucose Space of the Corn Scutellum

SLICES of the corn scutellum (*Zea mays* L., var. 'Tunka G-76') do not accumulate glucose although they take up glucose from a glucose solution at an appreciable rate<sup>1</sup>. Our results indicate that the scutellum tissue contains an intracellular glucose space the glucose content of which is in equilibrium with the bathing solution. Glucose space is defined as the volume of tissue water necessary to contain the glucose of the tissue at the concentration of the bathing solution<sup>2</sup>.

Slices (0.5 mm or less in thickness) were prepared from scutella obtained from 3-day-old, etiolated seedlings. To determine the glucose content, the slices were washed on a Buchner funnel for 2 min with cold water (1°-2°) and then extracted with boiling 80 per cent ethanol. After removal of the ethanol, the extracts were taken to volume with water, cleared with  $ZnSO_4$  and  $Ba(OH)_2$ , and analysed for glucose by the glucose oxidase method ('Glucostat', Worthington Biochemical Corp., Freehold, N.J.).

The slices took up glucose at a maximum rate of about 70  $\mu$ moles/h/g fresh weight. This rate was obtained at a glucose concentration of 0.1 M in the bathing solution. At this concentration the glucose space contained only about 8  $\mu$ moles of glucose and thus it contributed very little to the net uptake (Fig. 1). The presence of  $5 \times 10^{-4}$  M 2,4-dinitrophenol (DNP) in the bathing solution inhibited net glucose uptake by 80 per cent. However, DNP caused a doubling of the glucose space (Fig. 1). Mannose (0.1 M) also inhibited net glucose uptake<sup>3</sup> and increased glucose space (to 0.16 ml.). The mannose results (not shown in Fig. 1) were similar to but fell slightly below the results with DNP. The size of the glucose space was independent of the glucose concentration of the bathing solution in the range of 0.1-0.3 M when DNP or mannose was present. In the absence of these compounds the glucose space decreased slightly when the concentration of glucose in the bathing solution was below 0.2 M (Fig. 1). The glucose space made up 12 per cent of the tissue water when the tissue was incubated in glucose alone, 24 per cent when the slices were incubated in glucose + DNP, and 20 per cent when the slices were incubated with glucose + mannose. Finkelman and Reinhold, using segments of sunflower

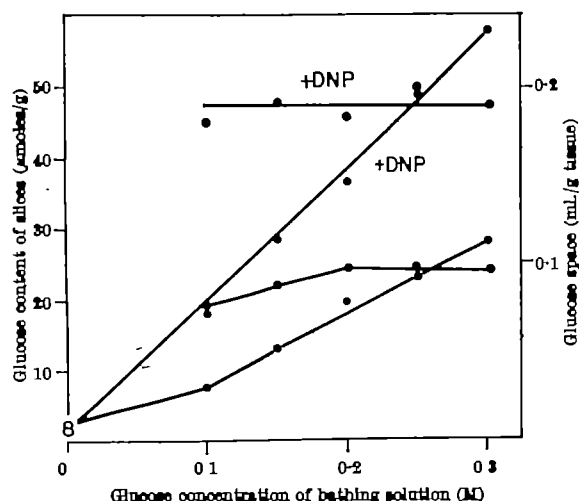


Fig. 1. Glucose content (○) and glucose space (●) of the scutellum slices as a function of the glucose concentration of the bathing solution. The slices were incubated (1 h, 30°) in glucose (bottom two lines) or glucose plus  $5 \times 10^{-4}$  M DNP (top two lines).

hypoocotyls, found that DNP brought about an increase in the apparent free space both for fructose and inulin<sup>4</sup>.

The movement of glucose into the glucose space was rapid, and equilibrium with the bathing solution was reached within 30 min (Fig. 2A). In the absence of DNP or mannose (which inhibit glucose utilization), measurement of the glucose content of the slices as a function of time indicates the rate of glucose build-up in the glucose space, not the net rate of glucose movement from the bathing solution. This is apparent from the fact that

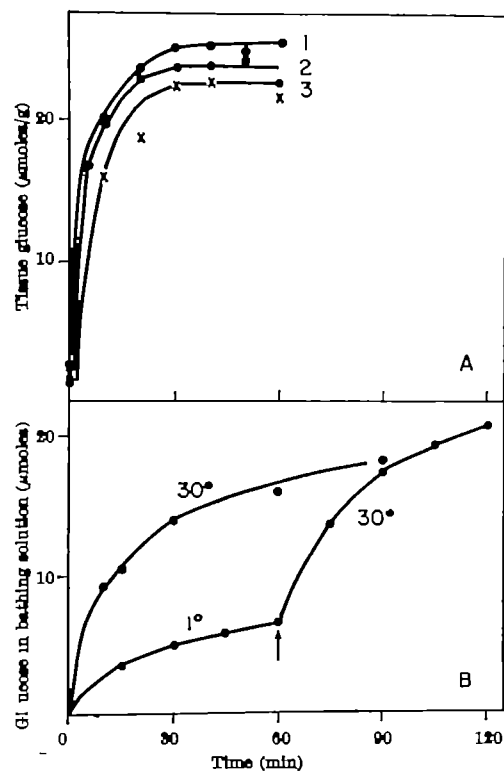


Fig. 2. A, Tissue glucose as a function of the incubation period in 0.15 M glucose +  $5 \times 10^{-4}$  M DNP (curve 1), 0.25 M glucose (curve 2) and 0.15 M glucose + 0.1 M mannose (curve 3). B, Effect of temperature on the movement of glucose from the slices into the bathing solution. Slices (1 g) were placed in 0.1 M glucose +  $5 \times 10^{-4}$  M DNP for 1 h. At the end of this period the slices were filtered out and washed on a Buchner funnel with cold water (1°-5°) for 2 min, and then placed in flasks containing a  $5 \times 10^{-4}$  M DNP solution at either 30° or 1° C. The glucose content of the bathing solution was measured at the times shown. The arrow indicates the time at which the temperature was raised from 1° to 30°.

glucose was being utilized by the slices at a rate exceeding 1  $\mu$ mole/min. The rapid rate of glucose utilization probably accounts for the break in the tissue glucose curve of Fig. 1.

Since DNP inhibited glucose utilization and increased the glucose space, it was used in the study of the movement of glucose from the slices into the bathing solution. Slices were incubated with glucose and DNP to fill the glucose space. The slices were then washed and placed in DNP and the movement of glucose into the bathing solution was measured as a function of time at two temperatures (Fig. 2B). At 30° the glucose of the slices rapidly equilibrated with the bathing solution, but at 1° there was only a slow movement of glucose into the bathing solution. The effect of temperature on the movement of glucose out of the slices indicates that there is a barrier to free diffusion. Presumably, therefore, the glucose was intracellular.

Fructose, mannose, methyl-D-glucose, glucosamine, N-acetyl glucosamine and mannitol did not inhibit the entry of glucose into the glucose space. Fructose and mannose occupied a space of similar size to that for glucose. Mannitol, which was metabolized by the slices at very low rates if at all, also entered a space similar in size to that for glucose. These results do not indicate that the movement of glucose into the glucose space is carrier-mediated.

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### Surfactant Lipids of Plant Quantasomes

THE plant sulpholipid, 6-sulpho- $\alpha$ -D-quinovopyranosyl-(1 $\rightarrow$ 1')-2',3'-di-O-acyl-D-glycerol, has been found in all photosynthetic organisms so far investigated<sup>1-3</sup> and only in such organisms. Although its biological role and its localization in photosynthetic tissues have not been experimentally elucidated, the observed relation between its occurrence and photosynthetic activity suggested a possible relation between this surfactant lipid and plant photosynthesis<sup>4</sup>.

We have now demonstrated that the sulpholipid and the lysosulpholipid<sup>5</sup> of *Lemna* are specifically concentrated in the quantasomes, the particle fraction of chloroplast first demonstrated by Park and Pon<sup>6</sup> to be responsible for the light reactions of plant photosynthesis.

*Lemna perpusilla* 6746, uniformly labelled with <sup>14</sup>C or <sup>35</sup>S, was prepared by growing it in a mineral medium<sup>7</sup> under photosynthetic conditions with <sup>14</sup>CO<sub>2</sub> or <sup>35</sup>SO<sub>4</sub><sup>2-</sup> for 3-7 days. We used *Lemna*, a small aquatic plant with leaves 1-3 mm long, as supplies of chloroplasts and various subcellular components could be easily prepared and labelled with radio-isotopes. Micro-analyses of components were made by radiochemical techniques.

Preliminary experiments with the chloroplasts isolated from *Lemna* uniformly labelled with <sup>14</sup>C revealed the virtual absence of major glycolipids, sulpholipids and galactolipids<sup>8</sup> in both the membrane (14,000g pellet) and the 145,000g supernatant fractions of sonically ruptured chloroplasts. Since most of the glycolipid content was found in its 145,000g pellet fraction, the latter could be deemed to represent whole chloroplast as far as the glycolipid content was concerned.

<sup>35</sup>S-labelled *Lemna perpusilla* was gathered by filtration and washed with 0.1 M potassium phosphate, pH 7.4. The whole plant was well ground in a chilled mortar with a small amount of the same buffer, and disintegrated by sonic oscillation at 9 kc/s for 2 min. The sonicate was centrifuged at 14,000g for 10 min, and the pellet was re-suspended in the same phosphate buffer and centrifuged at 14,000g for 10 min. This re-suspension of the pellet and centrifugation were further repeated twice. The pellet (14P) became completely colourless. The combined supernatant was centrifuged at 145,000g for 30 min. The pellet was washed once in water and collected by re-centrifugation (145P). This dark-green pellet should have contained all quantasomes of the starting plant tissues. The supernatant (145S) was completely colourless.

Each fraction was analysed by two-dimensional chromatography on Tōyō No. 51 paper, first in phenol-water (500:180 w/w), and then in *n*-butanol-propionic acid-water (142:71:100 v/v/v)<sup>9</sup>; afterwards autoradiograms were prepared. The sulpholipid content was determined by measuring the radioactivity of corresponding spots with a Type 5006 Geiger-Müller tube (Nihon Musen Co., Tokyo). Table 1 demonstrates that the sulpholipid and the lysosulpholipid are practically absent in the two colourless fractions and are specifically concentrated in the quantasome fraction which represents the major part of the lamellar structure of the chloroplast and is responsible for the light reactions of photosynthesis<sup>6,10</sup>.

Table 1. DISTRIBUTION OF THE SULPHOLIPIDS IN *Lemna perpusilla*<sup>35</sup>S

		14P	145S	145P
Sulpholipid	<sup>35</sup> S c.p.m.	570	1,980	23,800
	Per cent	2	6	92
Lysosulpholipid	<sup>35</sup> S c.p.m.	0	335	4,890
	Per cent	0	6	94

A quantasome preparation isolated from non-labelled spinach leaves by our method was analysed for its chemical composition. The results shown in Table 2 indicate the lipoprotein-like nature of the preparation. Amino-acid composition of the protein moiety was studied and was compared with those of average plant proteins: somewhat higher contents in neutral amino-acids and lower in basic acids were observed.

Table 2. CHARACTERIZATION OF SPINACH QUANTASOMES

Lipid	Weight per cent	Composition*
	45	Chlorophyll <i>a</i> : 6.4† Chlorophyll <i>b</i> : 2.4† Protochlorophyll: 5.5† C=68, H=10, N=1.3, S=0.3
Protein‡	35	
Polysaccharide§	17	C=46, H=6.9, N=0.8, S=0.0, Ash=1.2
Nucleic acid¶	3	

\* Weight per cent in each group.

† Calculated from the optical densities at 663, 644 and 624 m $\mu$  in aqueous acetone solution<sup>11</sup>.

‡ Amount recovered as amino-acids after hydrolysis with acid.

§ Hot perchloric acid-insoluble fraction minus protein.

¶ Calculated from the optical density at 260 m $\mu$  of hot perchloric acid-soluble fraction.

The plant sulpholipids are the only lipids so far described the occurrence of which is limited to tissues with one common biological activity—photosynthesis. The present findings that the sulpholipid and the lysosulpholipid are specifically located in the quantasomal or lamellar part of the plant cells further suggest the importance of the role of these surfactant compounds in the photosynthetic reactions of plants.

Preliminary experiments revealed that two galactolipids, galactosyl diglyceride and digalactosyl diglyceride<sup>8</sup>, are also concentrated in the quantasomes, whereas phospholipids and triglycerides are rather rich in other fractions, suggesting different biochemical roles for these lipids in plants.

The quantasome preparations described here may be contaminated by non-chloroplast components such as mitochondria and ribosomes. The possibility that such con-



terminating particles contain a considerable proportion of the sulpholipids is, however, very small, since our preliminary studies showed the absence of the sulpholipids in isolated mitochondrial as well as in ribosomal fractions.

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## PHYSIOLOGY

### Release of Acetylcholine from a Nerve Terminal by Electric Pulses of Variable Strength and Duration

It has recently been shown<sup>1</sup> that the release of acetylcholine from a motor nerve ending does not occur immediately when the action potential arrives at the terminal, but starts after a delay of about 0.5 msec at 20° C (and considerably later at lower temperatures<sup>2</sup>). To find out more about the relation between the electrical changes in the nerve ending and the subsequent secretion of the transmitter substance, it is desirable to apply graded electric pulses locally to the presynaptic terminal. This has been difficult to achieve because above a certain strength a locally applied depolarizing pulse initiates an all-or-none impulse in the terminal<sup>3</sup>. To overcome this difficulty we have used the substance tetrodo-toxin which is known to abolish impulses in nerve and muscle fibres

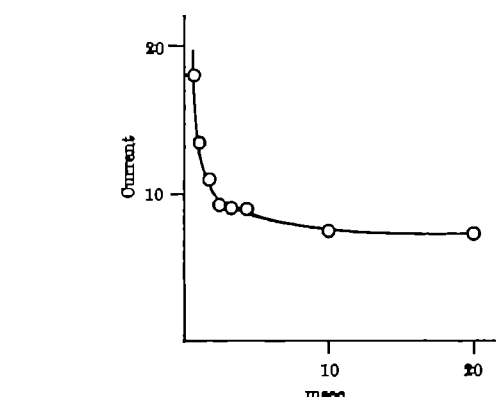


Fig. 2. Relation between duration and strength of equally effective pulses. The duration (abscissa) was varied, and the current intensity (relative units along the ordinate) was determined which caused occasional unit responses to appear at the end of the pulse. The lowest current strength (20 msec pulse) was approximately 0.15 mamp. Temperature 20° C.

without blocking the depolarizing action of acetylcholine on the end-plate<sup>4</sup>. Quite recently, Elmquist and Feldman<sup>5</sup> showed that the spontaneous miniature end-plate potentials (m.e.p.p.'s)—which are indicative of an intermittent spontaneous secretion of packets of acetylcholine from the terminals<sup>6</sup>—are not affected by tetrodo-toxin, and that their frequency can be raised, much as in a normal preparation, by increasing the external potassium concentration. Thus, tetrodo-toxin has the remarkable property, unlike other blocking agents such as curare or procaine, of paralysing the nerve and muscle fibre while leaving the special properties of the neuromuscular junction unimpaired.

The following experiments were made on frog sartorius and rat diaphragm muscles immersed in tetrodo-toxin at a concentration of  $1-4 \times 10^{-6}$  g/ml. (about a hundred times more than required to block the motor nerve impulse). Under these conditions, brief negative-going pulses applied through a micropipette (2-4  $\mu$  tip) to the surface of a nerve terminal evoked end-plate potentials which were recorded with an electrode inside the muscle fibre (Fig. 1). The response had the well-known quantal character: that is, it was composed of a fluctuating whole number of m.e.p.p.'s. With less than a certain intensity of the applied pulse, no response was observed. As the pulse strength was raised, single m.e.p.p.'s appeared in an all-or-none manner, their average number growing gradually as the intensity of the applied pulse was further increased. Similar effects were observed when the strength was kept constant and the duration increased. Finally, a 'strength-duration' curve could be obtained for a constant size of response, as in Fig. 2, showing the relation between length and intensity of equally effective depolarizing pulses. The experiments showed that depolarizations lasting no more than 100  $\mu$ sec (which is much shorter than the action potential) can be effective in releasing acetylcholine, provided they are made strong enough.

A point of particular interest was the time relation between the pulse and the commencement of the response. It has previously been shown that the start of the e.p.p. can be taken as a fairly accurate signal of the instant at which the first packet of transmitter is released<sup>1,2</sup>. There is a fluctuating delay between pulse and response, its statistical distribution in one particular case being shown in Fig. 3. With brief pulses it was consistently found that the release

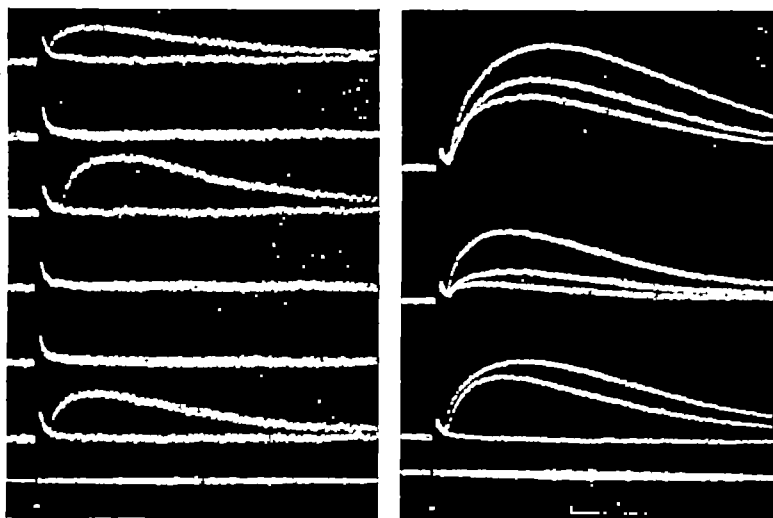


Fig. 1. End-plate potentials recorded with intracellular electrode from frog muscle paralysed with tetrodo-toxin. Temperature 7° C. Depolarizing pulses applied directly to nerve terminal. Pulse strength indicated in bottom trace of each column. Pulse duration 0.5 msec. Left column, weak current. The 3 top records consist of pairs of superimposed traces. There are 3 unit e.p.p.'s and 9 failures, in response to 12 pulses. Right column, stronger current. The 3 top records consist of 3 superimposed traces, showing one failure and 8 responses (note lower amplification).

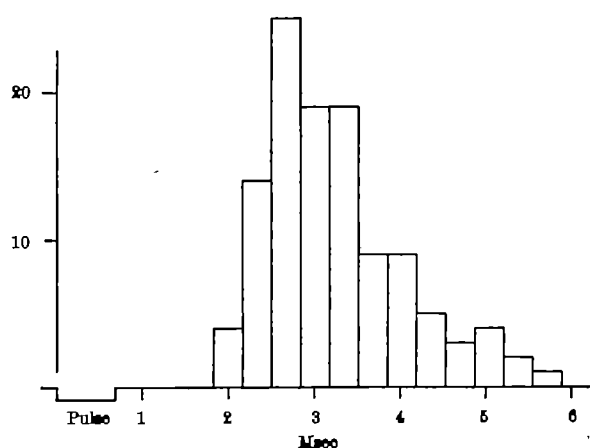


Fig. 3 Latency distribution of end-plate potentials evoked by constant current pulses of 0.68 msec duration. Temperature  $4-6^{\circ}\text{C}$ . Ordinate: number of observed responses. Abscissa: time-interval between start of depolarizing pulse and start of e.p.p.

does not begin until well after the end of the pulse; the probability of release then rises rapidly to a peak and gradually declines again within a few msec. The time-course of the probability of release after a brief pulse suggests that the secretion of acetylcholine is the end-product of a sequence of reaction steps set off by the displacement of the membrane potential. Little is known as yet about the nature of the intermediate reactions except that calcium ions must be involved in at least one of them<sup>7</sup>.

We thank Dr. F. A. Fuhrman, of Stanford University, for the gift of crystalline tetrodo-toxin.

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### Uncoupling the Sodium Pump

It is now widely accepted that the extrusion of sodium from living cells is associated with the splitting of ATP at the inside surface of the cell membrane<sup>1-7</sup>. The mechanism responsible is inhibited by cardiac glycosides and it is affected in a complicated way by potassium ions. With membrane preparations from most cells, glycoside-sensitive ATPase activity can be demonstrated only when both sodium and potassium are present<sup>8</sup>, and in red cells and nerve there is evidence that the sodium ions must be inside and the potassium ions outside<sup>6,7</sup>. This fits in with the known coupling between sodium efflux and potassium influx in these tissues, and suggests that under normal conditions the transport mechanism catalyses an exchange of sodium ions for potassium ions across the membrane using energy from the hydrolysis of ATP at the internal surface.

Under certain abnormal conditions, the transport mechanism appears to behave in a different fashion. In the absence of external potassium, red cells still show quite a large glycoside-sensitive sodium efflux, and they also show an increased sodium influx, which is partly glycoside-sensitive<sup>9</sup>. It has been suggested that this behaviour comes about when the carrier mechanism shuttles sodium ions backwards and forwards across the

membrane<sup>8,10</sup>; there is evidence for a similar 'exchange diffusion' in muscle<sup>12-14</sup> and in nerve<sup>11</sup>.

The experiments reported here were designed to elucidate the factors affecting the efflux of sodium, and its coupling to the influx of potassium and the hydrolysis of ATP, in red cells. By the process of 'reversible haemolysis'<sup>15,16</sup> cells were prepared containing ATP, magnesium, radiosodium and other constituents in pre-determined quantities, and the efflux of sodium into test solutions was measured.

Unless ATP or ADP was present, no glycoside-sensitive efflux of sodium was observed. (The ability of ADP to maintain transport is not surprising as the cells contain adenylate kinase.)

When cells were prepared containing a high concentration of sodium and a high concentration of ATP, it was found that the glycoside-sensitive sodium efflux could be abolished completely by the removal of external potassium (Fig. 1, top). Under these conditions efflux of sodium by the carrier mechanism appears to be tightly coupled to influx of potassium.

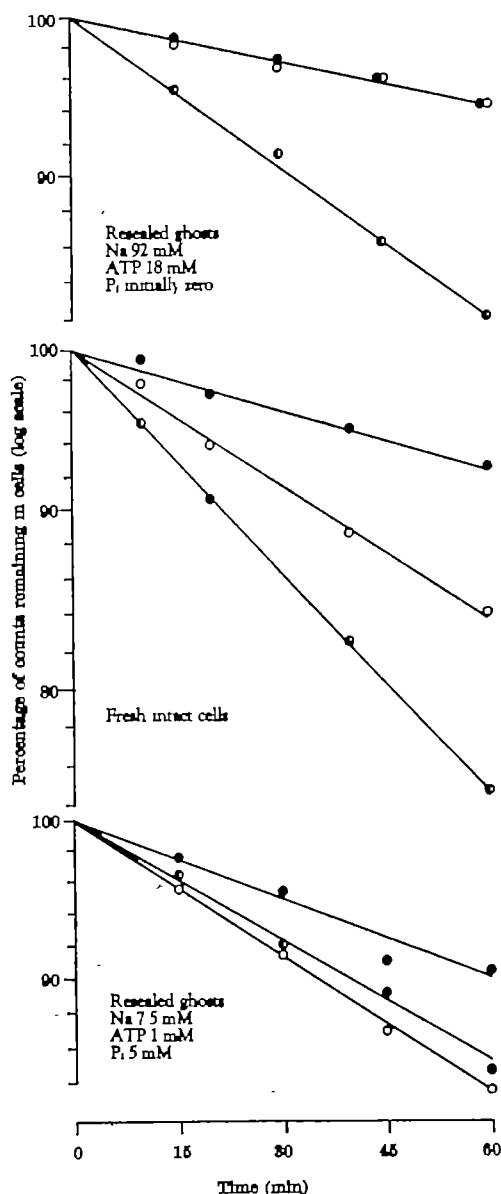
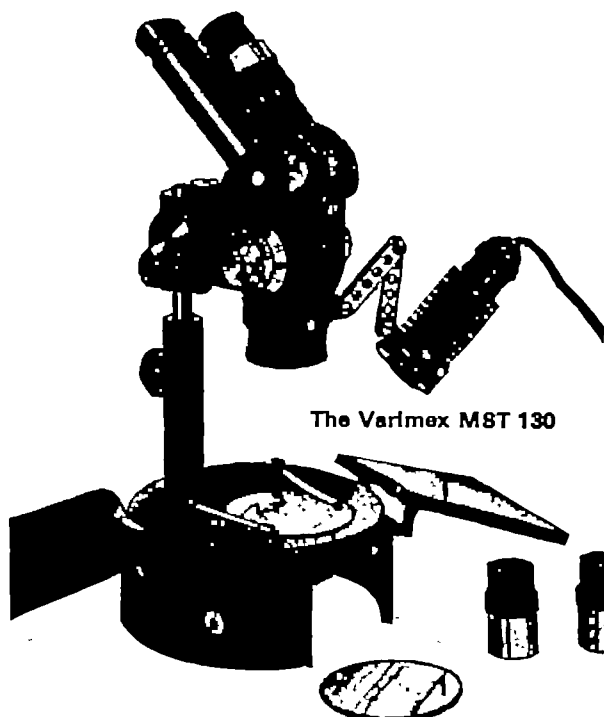


Fig. 1. Loss of  $^{22}\text{Na}$  from red cells.  $\bigcirc$ , 10 mM-potassium in incubation medium;  $\bigcirc$ , potassium absent from incubation medium;  $\bullet$ ,  $10^{-4}$  g/ml. ouabain in incubation medium.

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Partial uncoupling could be achieved either by lowering the internal sodium concentration or by incorporating enough inorganic phosphate inside the cells to give a low ATP/P<sub>i</sub> ratio (say 1/5). The 'partially uncoupled' cells showed a reduced sodium efflux in the absence of external potassium, but ouabain caused a further reduction. Fresh intact red cells loaded with sodium-24 behaved in a similar manner (Fig. 1, middle).

When cells were prepared with a low internal sodium concentration and with a low ATP/P<sub>i</sub> ratio, it was found that the efflux of sodium was slightly increased by the removal of external potassium (Fig. 1, bottom).

Whatever kind of cells were used, in a medium containing 10 mM potassium the external sodium concentration did not affect the sodium efflux except at high concentrations, when the efflux was somewhat reduced. This reduction is to be expected, because at high sodium concentrations competition by sodium ions for the potassium selective sites becomes appreciable. Quite a different effect of external sodium was observed, both in intact cells and in re-sealed cells, under conditions which allowed 'uncoupled' efflux of sodium into a potassium-free medium. Replacement of all but 5 mM of the sodium by choline reduced the glycoside-sensitive sodium efflux to zero. This effect is easily explained if the 'uncoupled' sodium efflux represents exchange diffusion of sodium across the membrane. Rather surprisingly, at external sodium concentrations near zero, a glycoside-sensitive efflux of sodium was again detectable. The significance of this is not yet understood, but in crab nerves under comparable conditions (O, K; O, Na) Baker<sup>17</sup> found that an ouabain-sensitive efflux of sodium was accompanied by a loss of amino-acids from the cell—in crab nerve dibasic amino-acids are the chief internal anions.

Although it is clear that a low internal sodium concentration and a low internal ATP/P<sub>i</sub> ratio both tend to uncouple the pump, it is not at all clear why they do. It is tempting to suggest that the low internal sodium acts by increasing the amount of energy required to expel sodium ions, and the low ATP/P<sub>i</sub> ratio acts by reducing the amount of energy available from the hydrolysis of ATP (compare the suggested role of ATP/ADP ratio in the squid axon<sup>18</sup>); but even if this is true it is difficult to think of a plausible mechanism. The resemblances between the observations reported here and recent findings in frog muscle<sup>12-14</sup> and squid nerve<sup>11</sup> suggest that, whatever the mechanism is, it is not peculiar to red cells.

When red cells are expelling sodium in exchange for potassium there is evidence that, whether the sodium movement is downhill<sup>8</sup>, on the level<sup>19</sup> or uphill<sup>20</sup>, approximately three sodium ions are expelled for each molecule of ATP split by the pump. In attempting to invent hypothetical mechanisms for the transport system, it is important to know whether the same stoichiometry holds for the 'uncoupled' flux. We have therefore compared glycoside-sensitive sodium efflux and glycoside-sensitive ATP hydrolysis in 'partially uncoupled' cells with and without external potassium. It turned out that, though ATP was required both for the 'uncoupled' efflux of sodium and for the coupled exchange of sodium for potassium, the rate of hydrolysis of ATP associated with the 'uncoupled' flux was very much less. It was, in fact, not significantly greater than zero (Table 1), but because of scatter in the results a small utilization of ATP cannot be ruled out. We have also found that both the 'uncoupled' and the 'coupled' efflux of sodium are inhibited by oligomycin. With 1 µg/ml., inhibition of both fluxes was about 48 per cent; with 10 µg/ml., the 'uncoupled' flux was reduced by 91 per cent, the 'coupled' flux by 74 per cent.

The fact that the 'uncoupled' efflux requires the presence of ATP, though it is associated with little or no hydrolysis, suggests that ATP is required for the formation

Table 1. EFFECT OF EXTERNAL POTASSIUM ON THE RELATION BETWEEN Na EFFLUX AND ATP HYDROLYSIS IN 'PARTIALLY UNCOUPLED' RED CELL GHOSTS

	External K concn. (mM)	(ATP/P <sub>i</sub> ratio = 1/5)	
		Glycoside-sensitive <sup>24</sup> Na loss (counts/min/h)	Glycoside-sensitive ATP hydrolysis (µmoles/ml sealed ghosts/h)
Exp. 1	10	474 ± 43	0.328 ± 0.004
	0	224 ± 32	0.015 ± 0.020
Exp. 2*	10	296 ± 20	0.266 ± 0.008
	0	136 ± 11	0.032 ± 0.013

\* The specific activity of the intracellular sodium was not the same in the two experiments.

of a sodium carrier which, in the absence of external potassium and under conditions that allow uncoupling, shuttles sodium ions backwards and forwards across the membrane without itself undergoing any irreversible change. If potassium is present externally, sodium is exchanged for potassium and this process presumably leads to breakdown of the carrier and utilization of ATP for its resynthesis. Two points remain difficult to explain. Exchange diffusion requires a high level of sodium externally<sup>19</sup>; even in the absence of external potassium, it occurs only when the internal sodium-level is low or the ATP/P<sub>i</sub> ratio is unfavourable energetically.

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## HAEMATOLOGY

### Relation between Gm(f) and the Structure of the γ-Globulin Molecule

THE serologically detectable genetically determined antigens Gm(a) and Gm(b) are restricted to the F<sub>0</sub> (fast) fragment of the A (Heavy) polypeptide chains of human gamma-globulin<sup>1</sup>. In contrast, Gm(b<sup>w</sup>) is present in the F<sub>ab</sub> (slow) fragment, and its serological activity seems to depend on the 'quaternary' structure of the intact gamma-globulin molecule<sup>2</sup>. Gm(f) activity also is localized to the F<sub>ab</sub> fragment of gamma-globulin<sup>3,4</sup>, and is also present in the 5S product obtained by pepsin digestion of the 7S gamma-globulin molecule<sup>4</sup> (Table 1); Gm(a) and Gm(b) activities are lost during pepsin digestion. Recent studies<sup>5</sup> indicate that Gm(f) and Gm(b<sup>w</sup>) are similar but not identical. We report here the results of experiments with anti-Gm(f) and isolated A and B chains of gamma-globulin.

Table 1. INHIBITORY ACTIVITY IN Gm(f) AND Gm(a) SYSTEMS

	Proteolytic fragments						Chains			
	7S	6S	5S	4S	3S	2S	A	B	A	B
2 normal Gm(a+f+)	+	+	0	+	0	+	+	0	0	0
1 normal Gm(a-f+)	0	+	0	+	0	+	0	0	0	0
1 normal Gm(a+f-)	+	0	0	0	0	+	+	0	0	0
2 myeloma Gm(a+f-)	+	0	0	0	0	+	+	0	0	0
3 myeloma Gm(a-f+)	0	+	0	+	0	0	0	0	0	0

A and B chains were prepared by the method of Porter<sup>4</sup> from the isolated gamma-globulins of three Caucasian normal subjects, from the gamma-paraprotein of six Caucasians with multiple myeloma, and from a pool of 20-30 African donors in a village in Gambia. (The latter preparation and the isolated A and B chains investigated in Bristol were kindly supplied by Dr. S. Cohen.) After isolation of the A and B chains, they were suspended in distilled water at concentrations of 10 and 5 mg/ml., respectively, then diluted five-fold with normal saline. To avoid precipitation the protein solution of the A chains was added to the saline and mixed rapidly. (No haemolysis due to hypotonicity or to low pH was noted in the subsequent serological tests.) The A and B chains in each instance were shown to be free of significant contamination by contralateral chain or by intact gamma-globulin as judged by agar diffusion studies with specific antisera to A and to B chains.

In additional experiments 16 gamma<sub>A</sub>, 8 gamma<sub>M</sub> and 8 gamma<sub>M</sub>-paraproteins and 16 Bence-Jones proteins, all free of significant contamination as shown by agar diffusion studies with polyvalent and monospecific antisera, were also tested for Gm(f) activity.

The serological tests utilized stock O R<sub>1</sub>R<sub>1</sub> cells and also O D - /D - - cells, stored in glycerol but reconstituted by dialysis. The cells were coated with anti-D (P.H.A.) which sensitizes for Gm(f) but not for Gm(b) (ref. 7). After washing, the sensitized cells gave strongly positive antiglobulin reactions with rabbit antisera. Anti-Gm(f) Mau, diluted 1:4 for use with the R<sub>1</sub>R<sub>1</sub> cells, and 1:20 with the D - - cells, was used to establish a Gm(f) agglutination system. For inhibition studies, suitable dilutions of A chains, B chains, or a mixture thereof, were mixed with the anti-Gm(f), left for 10 min at room temperature, and the sensitized cells then added. In the Bristol studies, the tests were performed on tiles, and the tests read macroscopically 10 min after addition of the cells. In the serological studies in San Francisco, the tests were read as outlined elsewhere<sup>9</sup> after 2 h incubation at room temperature in 10 × 75-mm test-tubes, and subsequent centrifugation for 30 sec in a serological centrifuge.

Neither A nor B chains isolated from three Caucasian Gm(a+f+) normal individuals inhibited these Gm(f) agglutination systems, whereas the A but not the B chains strongly inhibited standard Gm(a) agglutination systems. In the latter systems, inhibition by the A chains was greater than that of the intact γ-globulin, as also noted independently by Cohen<sup>8</sup> and Lawler *et al.*<sup>9</sup>. Similarly, neither A nor B chains of either Gm(a+f-) or Gm(a-f+) myeloma proteins inhibited the Gm(f) system, although the A chains of the Gm(a+) but not of the Gm(a-) paraproteins inhibited the Gm(a) agglutination systems. (Addition of B chains to the A chains did not affect the degree of inhibition of the Gm(a) test systems.)

When A and B chains derived from Caucasian Gm(f+) γ-globulin were mixed together in equimolar amounts (assuming a molecular weight of 27,000 for L chains and 54,000 for H chains), Gm(f) activity reappeared (Table 2); in contrast, an equimolar mixture of African A and B chains did not inhibit Gm(f). Further, a mixture of equimolar amounts of African Gm(f-) A and Caucasian Gm(f+) B chains did not inhibit, whereas a mixture of equimolar amounts of Caucasian A and African B chains produced partial inhibition of the Gm(f) system.

Table 2. EFFECT OF MIXTURES OF A AND B CHAINS ON Gm(f) AGGLUTINATION SYSTEM

Test system*	A chains B chains		Chain source Caucasian + African		African + Caucasian	
	Caucasian	African	Caucasian	African	Caucasian	African
(1) Man 1:4+ sensitized R <sub>1</sub> R <sub>1</sub> cells	0	+	+++	+++	+++	+++
(2) Man 1:20+ sensitized D - /D - - cells	0	+	+++	+++	+++	+++

0, Lack of agglutination = complete inhibition. +, ++, (+++), and ++++, Different degrees of agglutination.

\* Anti-Gm(f) Mau + sensitized cells + chains in saline tested for inhibition (lack of agglutination) in a solution of equimolar ratio and of total protein concentration of 8 mg/ml.

When unequal amounts of A and B chains from a Gm(f+) Caucasian donor were mixed together and assayed for Gm(f) activity, such activity decreased in direct relation to the degree of departure from equimolarity (Table 3), for example, a 1:2 mixture of A:B produced partial inhibition and a 1:3 mixture only very weak inhibition.

Table 3. EFFECT OF VARYING RATIOS OF A AND B CHAINS OF Gm(f+) GAMMA-GLOBULIN ON Gm(f) AGGLUTINATION SYSTEM

Anti-Gm(f) Mau	Molar ratio of A/B*						Saline control
	1:1	1:2	1:3	1:4	2:1	3:1	4:1
1:4 plus sensitized R <sub>1</sub> R <sub>1</sub> cells	0	+	++	+++	(+++)	+++	+++

\* Total protein of each solution is 8 mg/ml.

0, Lack of agglutination = complete inhibition.

The reported results are tentatively interpreted as supporting the hypothesis that Gm(f), like Gm(b<sup>w</sup>) (ref. 2), depends for its serological activity on the tertiary structure of the H chains; this in turn depends to a great extent on the configuration of the intact γ-globulin molecule. The connexion between Gm(f) and the A chains is further supported by recent results obtained with rabbit and primate antisera to A chain antigens<sup>10</sup> and with peptide mapping experiments of Gm(f+) and (f-) normal and myeloma proteins<sup>11</sup>. Further, Gm(f) activity was absent from 16 purified Bence-Jones proteins (at 4 mg/ml.); Bence-Jones proteins, as shown by Edelman and Gally<sup>12</sup>, are B chains devoid of A chain.

These results are of interest since the two currently defined allotypic loci in rabbits *Aa* and *Ab* define antigens present in the Heavy and Light chains respectively of rabbit gamma-globulin, and since the allotypic antigens *Aa* 1, 2 and 3 are localized to that part of the H chain which is within the F<sub>ab</sub> fragment<sup>13</sup>. Further, the six currently defined allotypic antigens *Aa* 1, 2, 3 and *Ab* 4, 5, 6 are all present in gamma<sub>M</sub> as well as gamma<sub>A</sub>-globulin<sup>14,15</sup>. Hence, it might be anticipated that Gm(f) would also be present in a portion of the A chain (presumably within the 'A piece') shared in common by the gamma<sub>A</sub>-, gamma<sub>M</sub>- and gamma<sub>M</sub>-globulins. However, in additional experiments, none of 8 gamma<sub>A</sub>- and none of 8 gamma<sub>M</sub>-paraproteins displayed Gm(f) activity at concentrations of 4 mg/ml., whereas 7/16 purified gamma<sub>A</sub>-paraproteins inhibited Gm(f) agglutination systems at concentrations as low as 0.25 mg/ml. In one Gm(f+) normal individual, the isolated gamma<sub>A</sub>-globulin was positive for Gm(f), but the isolated purified gamma<sub>A</sub>- and gamma<sub>M</sub>-globulins were not. Hence the Gm(f) antigenic determinant appears to be restricted to gamma<sub>A</sub>-globulin, and probably is localized to a portion of the gamma<sub>A</sub> A chain other than the A piece. Similar conclusions have been obtained from peptide-mapping studies<sup>16</sup>.

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## PATHOLOGY

### Sensitization to *Khaya anthotheca*

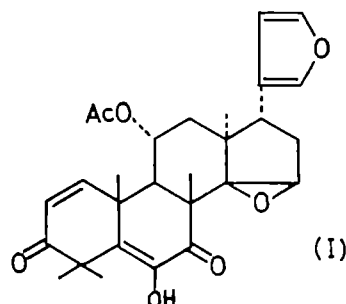
AFRICAN mahogany is a timber which has been used extensively in the furniture trade since the early part of the century. It is derived exclusively from the *Khaya* genus and commercial supplies consist principally of *K. ivorensis* and *K. anthotheca* with smaller amounts of *K. grandifoliola*<sup>1</sup>. The timber is not usually regarded as troublesome as an irritant and, indeed, only a very few cases have been reported<sup>2</sup>. It was rather surprising, therefore, to have our attention directed to an outbreak of dermatitis in a factory in which large quantities of the timber were being processed, and it was decided to investigate this more fully.

Seven or eight cases among approximately 75 employees had occurred some two years previously, and the cases recorded here, involving a similar number, occurred in the three months prior to the start of this investigation. Men engaged on finishing operations (sanding, etc.) were mainly concerned, the face, forearms and back of the hands being affected, the eyelids swelling slightly or moderately. The back and sides of the neck were not involved. This pattern suggested a direct contact sensitization to a dust or volatile agent and not a photodermatitis. The eruptions usually subsided with varying (but usually brief) periods away from work and on return the affected men were moved to other jobs and remained clear.

To determine whether one or all three *Khaya* spp. were involved in this sensitization seven employees affected were patch tested to dust prepared from authentic samples of *K. ivorensis*, *K. anthotheca* and *K. grandifoliola* and four of these to wood dust from the factory. (It is not possible to distinguish between the woods comprising the commercial *Khaya* spp. by anatomical examination of the wood alone.) The tests were carried out by the standard method<sup>3</sup> and read at 48 and 96 h. Two other men who had developed a sensitivity to African mahogany (though not employees from the same factory), who were seen as routine patients by one of us (D.S.W.), were also tested. Apart from one 'false case', negative to all woods and extracts (see following), the remaining eight reacted strongly to authentic *K. anthotheca* (three giving a strongly intensified 96-h reaction), and in four cases confirmatory positives were obtained with the actual sawdust from the factory. No reactions were obtained with *K. ivorensis* or *K. grandifoliola*.

The sawdust of *K. anthotheca* was then Soxhlet-extracted successively with light petroleum, ether, acetone and

methanol, and the crude extracts were used to patch test six men. In addition patches were also applied of the fully extracted sawdust and of anthothecol (I) (ref. 4), a pure crystalline constituent which has been isolated from *K. anthotheca* but not from the other two species<sup>5</sup>. Five out of the six men reacted to the extracts, often with delayed reactions, there being little variation in the degree of reaction obtained with each extract, though one man reacted only to the petroleum extract. In five cases the completely extracted sawdust (10 per cent in petrolatum molle) gave no reaction; in the sixth case a quickly subsiding positive reaction occurred (probably a false positive). Finally, reactions, some severe, occurred with 1 per cent and 0.1 per cent anthothecol in acetone on all six men tested.



As controls *K. anthotheca* dust was added to a routine battery of patch tests in ten patients not sensitized by the wood, and in four cases anthothecol (0.1 per cent) patches were also added. No reactions occurred in either group of tests.

From these results it is clear that *K. anthotheca* contains sensitizing substances which can be removed by solvent extraction. The tests with anthothecol, a compound found mainly in the petrol extract, show that this is one of them. However, the equally sensitizing action of the subsequent ether, acetone and methanol extracts suggests that other active constituents may be present, though the presence of traces of anthothecol in each of these extracts cannot be excluded.

The results establish without doubt that *K. anthotheca* dust can cause sensitization and that this was the cause of the trouble in the cases investigated. It should be remembered, however, that African mahogany has been used for many years, apparently without trouble. One possible reason for the present outbreak may be that the consignment contained a particularly high proportion of *K. anthotheca*. Another possibility is that logs unusually rich in the sensitizing constituent(s) may have been included in the consignment. This phenomenon is encountered in teak in which a certain variety rich in deoxylapachol has enhanced irritant properties<sup>6</sup>. The results recorded here should be considered in the light of the amount of African mahogany used annually, which must contain a considerable proportion of *K. anthotheca*. The apparent almost trouble-free use of this timber for many years may indicate that the present outbreak is non-typical, and further evidence is necessary before *K. anthotheca* can be generally regarded as a troublesome irritant timber.

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### Cathepsin A In Nutritional and Hereditary Muscular Dystrophy

DURING the past few years, several laboratories<sup>1-3</sup> have reported that the activities of many of the acid hydrolases of skeletal muscle are greatly increased in wasting diseases from different causes. Although the progressive loss of muscle mass in these conditions is generally attributed primarily to this enhanced activity of the acid hydrolases, there is little direct experimental evidence in support of this hypothesis. Furthermore, other enzymes involved in protein catabolism, such as alkaline protease<sup>4</sup> and peptidases<sup>5</sup>, have been shown to be elevated in dystrophic muscle. The normally low levels of these enzymes in skeletal muscle and the limited knowledge of their substrate specificities have hindered a better understanding of the mechanism of the wasting process.

From skeletal muscle of rabbits and chickens we have isolated a highly purified protease<sup>6</sup>, similar in its properties to cathepsin D of spleen<sup>11</sup>, which in liver has been shown to be localized in the lysosomes<sup>12</sup>. Unpublished data obtained with this purified enzyme freed of other known cathepsins show that it does hydrolyse certain muscle constituents but that, in itself, it cannot account for all the observed increase in dystrophic muscle autolysis.

This communication reports our finding that the activity of another proteolytic enzyme, cathepsin A, shown by Fruton and Bergmann to hydrolyse a number of peptides containing tyrosine or phenylalanine and glutamic acid<sup>13</sup>, is greatly increased in skeletal muscle of dystrophic animals.

Nutritional muscular dystrophy was produced in rabbits by feeding them a vitamin E-deficient diet<sup>14</sup>. Two types of control were used: animals maintained on the deficient diet but given oral supplements of DL- $\alpha$ -tocopherol, and animals maintained on stock diets. There were no demonstrable differences in enzyme-levels in either type of control. Chickens with hereditary dystrophy<sup>15</sup>, and some controls, were raised from eggs supplied by the Department of Genetics, University of Connecticut; other controls were purchased from commercial suppliers. There was likewise no difference in enzyme-levels in either type of control. All chickens were 3-4 months old when used for these experiments.

Cathepsin A levels in breast muscle of the chicken and in mixed thigh, leg and back muscles of the rabbit were measured in a 2 per cent potassium chloride homogenate (1:4 w/v) prepared in a Waring blender to which 'Triton X-100' had been added in a final concentration of 0.2 per cent. In experiments to be reported elsewhere, cathepsin A has been found to be associated with the lysosomes isolated from liver and to be fully released under these conditions. The release of tyrosine due to the enzymatic hydrolysis of carbobenzoxy- $\alpha$ -glutamyl-L-tyrosine (Mann Research Laboratories, Inc.) by cathepsin A was determined by the ninhydrin colorimetric procedure of Moore and Stein<sup>16</sup>. There was no detectable non-enzymatic release of tyrosine under the assay conditions used, nor did the substrate itself react with ninhydrin. The reaction mixture, containing 0.4 ml. homogenate, 0.2 ml. 0.2 M sodium acetate, pH 5.0, and 0.4 ml. 0.038 M substrate or H<sub>2</sub>O, was incubated at 37°-38° C for 2 h. The reaction was stopped by the addition of 1.0 ml. of 10 per cent trichloroacetic acid and the mixture was heated in a 50°-55° C bath for 10 min and centrifuged for 15 min at low speed. The supernatant was diluted 1:5 with water. Aliquots of 0.10-0.20 ml. of the diluted supernatant were used for the colorimetric ninhydrin determination, with the appropriate level of tyrosine as a standard. Protein was estimated by the procedure of Lowry *et al.*<sup>17</sup>. The results are shown in Table 1.

The data indicate that the activity of cathepsin A is significantly increased in both types of muscular dystrophy. It is noteworthy that the skeletal muscle of normal rabbits appears to be almost free of this protease,

Table 1. CATHEPSIN A ACTIVITY IN DYSTROPHIC MUSCLE  
amoles tyrosine liberated  
per mg protein

Rabbit	
Control (5)	0.012 [0-0.032]
Vitamin E-deficient (5)	0.197 [0.118-0.371]
Chicken	
Control (7)	0.125 [0.094-0.170]
Dystrophic (8)	0.292 [0.230-0.366]

The numbers in parentheses refer to the number of animals in that group and the square brackets enclose the range of values obtained.

whereas in the breast muscle of the chicken the level of this enzyme is comparatively high and approaches that seen in muscle of the vitamin E-deficient rabbit. The significance of these results in terms of the overall enzymatic hydrolysis of proteins in various species and in dystrophy cannot be evaluated until a more highly purified enzyme preparation, free of other cathepsins, has been obtained. Indeed, it is not yet known whether cathepsin A does actually have proteolytic activity or whether it is restricted in its action to the hydrolysis of small peptides. However, preliminary data obtained with a partially purified cathepsin A preparation indicate that haemoglobin, at least, is not a good substrate for this enzyme.

Our present investigations with isolated cathepsin A, as well as other proteases, are directed toward the elucidation of the role of these enzymes in protein catabolism and a better understanding of the wasting process.

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### HISTOLOGY

#### Visualization of the Nuclei of the Basal Melanocytes of the Black Guinea-pig and of Human Skin under the Bright Field Microscope

THE nuclei of the melanocytes isolated from the black guinea-pig and from human skin by the method of Shukla, Karkun and Mukerji<sup>1</sup> are always masked by the dense melanin present in the cytoplasm (Figs. 1A and 2B). A method to demelanize the cells and visualize the nuclei is reported here.

A saline suspension of melanocytes isolated by the technique of Shukla *et al.* from the skin of the dorsal surface of the black region of the ear of the spotted guinea-pig, or the peroneal surface of the leg of normally pigmented human subjects, is mounted on albuminized slides and dried at 58° C in an oven. After an hour of drying the

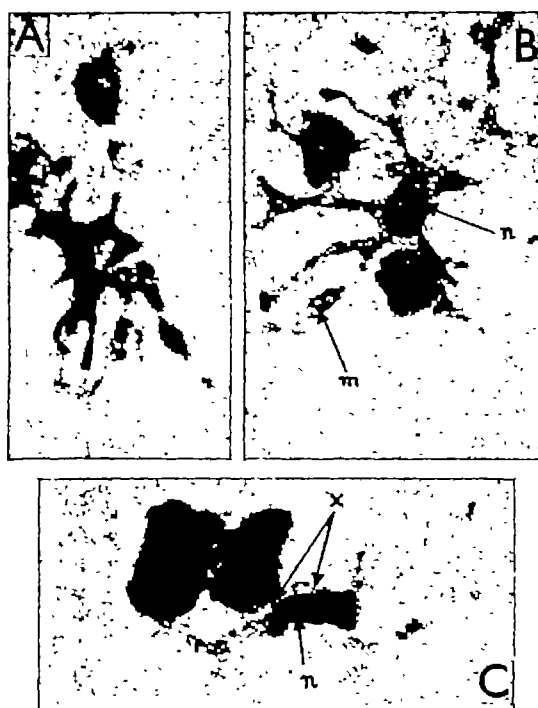


Fig. 1. The isolated basal melanotic melanocyte prepared from the dorsal surface of skin of the ear of the guinea-pig. (A) The melanocyte after DOPA reaction where the internal structures are completely masked by the densely packed melanin and premelanin. (B) The large, basophilic, egg-shaped nucleus (n) in the centre of the partially demelanized melanocyte after Mayer's haemalum stain. Melanin granules (m) are visible in the dendritic processes. (C) The antero-posterior view of the egg-shaped nucleus (n) of the completely demelanized melanocyte with two characteristic notches in its boundary wall near the anterior end, marked s in the figure. Haematoxylin-eosin stain  $\times 600$ .

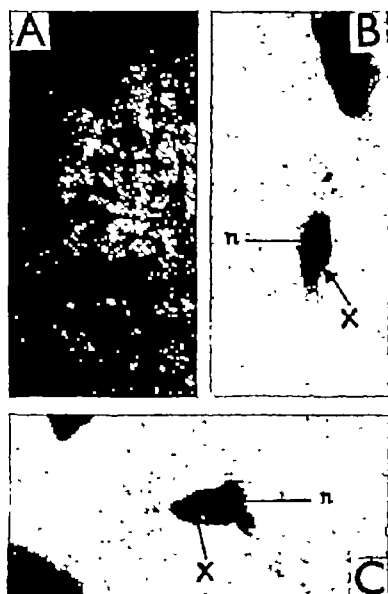


Fig. 2. Isolated basal melanotic melanocyte prepared from the peroneal surface of the leg of a normally pigmented human subject. (A) The cell after DOPA reaction where the internal structures are completely masked by dense melanin and premelanin. (B) Lateral view of the nucleus (n) in the completely demelanized cell after Mayer's haemalum stain. A notch at the anterior end is shown as s. (C) Shows the antero-posterior view of the nucleus of the melanocyte. One of the two characteristic notches at the anterior end is shown as s. Haematoxylin-eosin stain  $\times 600$ .

cells are affixed to the slides, and fixed in 5 per cent formal-saline for 30 min and washed in running water for 4 h. The slides are placed in 40 per cent peracetic acid freshly prepared by the method of Findley, Swern and Scanlan<sup>2</sup>. They are taken out after 12 h, washed in running water

for 1 h and stained by Harris's haematoxylin and eosin and Mayer's haemalum by the method of McManus and Mowry<sup>3</sup>. These are then dehydrated in ascending series of alcohol, cleared in xylol, mounted in balsam and examined under the microscope.

The nucleus of the basal melanotic melanocyte of the guinea-pig has been observed to be a constantly occurring basophilic structure in the centre of each cell (Fig. 1B). It is large in size, occupies nearly the whole of the cytoplasm of the cell and is concave in the long axis corresponding to the curvature of the parent melanocyte<sup>4</sup>. In the antero-posterior aspect, the nucleus has a smooth and regular border and is shaped like an elongated egg, the narrow end being directed towards the dendritic process which is stouter than the others (Fig. 1C). A little posterior to the narrow end there are two smooth indentations, one behind the other, on the boundary wall of the nucleus.

The nuclei of the basal melanotic melanocytes of the skin of human subjects also occur as constant basophilic structures. The concavity in the long axis of the nucleus of the melanocyte of human skin appears more pronounced and, therefore, is visualized often as a folded structure (Fig. 2B). Occasionally, the nucleus in the unfolded form is seen; it is egg-shaped, and indented like the nucleus of the guinea-pig melanocyte (Fig. 2C). It is smaller in size than the nucleus of the guinea-pig and there is a broad rim of cytoplasm around it.

Visualization of the nucleus of the melanotic melanocyte of the guinea-pig and human skin under the light microscope has led us to the study of the structure of the nucleus as related to the formation of melanin.

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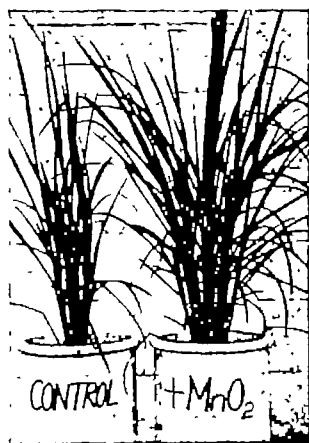
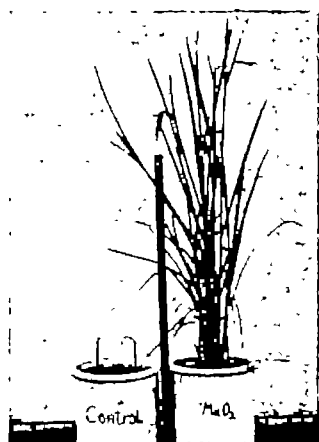
## BIOLOGY

### Manganese Dioxide as a Remedy for a Physiological Disease of Rice associated with Reduction of the Soil

A PHYSIOLOGICAL disorder of rice known as 'suffocation disease' occurs on the poorly drained soils of north-eastern Taiwan. The disease, which is characterized by a reddish-brown discoloration of the older leaves, stunted growth, root rot, and low yield, has been attributed to excessive reduction of the soil<sup>1,2</sup>. As the recommended remedy<sup>3</sup> of draining the fields to facilitate oxidation of the soil by atmospheric oxygen is not feasible during the rainy season, chemical retardation of reduction of the soil was investigated in a greenhouse experiment.

Of the five retardants of reduction examined (potassium chlorate, potassium perchlorate, sodium nitrate, manganese dioxide, and 4-chloro-3,5-xyleneol), manganese dioxide at 0.4 per cent by weight of the dry soil (the single level used) retarded soil reduction, corrected the physiological disorder in the problem soil from Taiwan (Fig. 1), and improved the growth of rice on three other problem soils (Table 1).

The benefits apparently were not a response to the micronutrient, manganese, or to impurities in the manganese dioxide because: (a) all soils had adequate concentrations of water-soluble manganese; (b) 25 p.p.m. of manganese as manganous chloride applied to the soils produced no response; (c) manganese dioxide prepared in the

Fig. 1. Tungahan silt loam with  $\text{MnO}_2$  (right), without  $\text{MnO}_2$  (left)Fig. 2. An acid sulphate soil with  $\text{MnO}_2$  (right), without  $\text{MnO}_2$  (left)

laboratory from chemically pure potassium permanganate gave a better response than commercial manganese dioxide. The higher redox potential and smaller concentrations of ferrous iron and organic reduction products in the soil solution of the manganese dioxide treatment than in the control (Table 2) suggest that the benefits were associated with retardation of soil reduction. Removal of carbon dioxide as the sparingly soluble manganese carbonate may have been an additional benefit<sup>4</sup>. The dramatic response to manganese dioxide in the acid-sulphate soil from Viet Nam (Fig. 2) may be ascribed to a slightly higher pH ( $\Delta\text{pH} = 0.2$ ), markedly lower concentrations of aluminium ( $\Delta\text{Al} = 30$  p.p.m.) and iron ( $\Delta\text{Fe} = 600$  p.p.m.) in the soil solution, and perhaps to

Table 1. INFLUENCE OF MANGANESE DIOXIDE ON THE YIELD OF RICE ON FOUR ACID SOILS IN A GREENHOUSE EXPERIMENT

Soil type	pH	O.M.† (%)	Straw (g/pot)		Grain (g/pot)	
			Control	$\text{MnO}_2$	Control	$\text{MnO}_2$
Tungahan silt loam*	5.8	3.4	26.4	55.8	18.1	43.0
Cusiguran sandy loam	4.8	4.4	74.9	85.9	69.7	74.6
Lubana clay	4.7	3.2	33.5	40.4	29.3	35.2
Acid sulphate clay	3.6	9.6	0	16.6	0	4.6

\* Soil from a site where 'suffocation disease' occurs

† Organic matter.

Table 2. INFLUENCE OF MANGANESE DIOXIDE ON EA OF THE SOIL SOLUTION AND CONCENTRATION OF WATER-SOLUBLE REDUCTION PRODUCTS IN TUNGSHAN SILT LOAM IN A POT EXPERIMENT

Weeks sub-merged	EA (mv)		Fe <sup>++</sup> (p.p.m.)		Mn <sup>++</sup> (p.p.m.)		Oxidizable matter* (m.equiv./l)	
	Control	$\text{MnO}_2$	Control	$\text{MnO}_2$	Control	$\text{MnO}_2$	Control	$\text{MnO}_2$
1	196	201	5	2	11	13	35	20
21	46	135	54	21	28	35	51	21
27	-19	106	88	7	8	22	24	24
27	-24	122	107	6	9	30	29	29

\* 0.01 N  $\text{KMnO}_4$  consumed under standard conditions.

a more favourable iron:manganese ratio than in the control.

Manganese dioxide retarded the accumulation of harmful reduction products, corrected a physiological disease of rice on a problem soil and improved the growth of rice on three acid soils. Because manganese dioxide: (a) undergoes reversible oxidation-reduction when rice soils are alternately drained and flooded; (b) is not likely to suffer appreciable losses in poorly drained soils; (c) is a relatively inexpensive commercial commodity, its use as a remedy for physiological diseases caused by soil reduction and as an amendment for acid lowland rice soils merits attention.

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### Carbon-14 Labelled Photosynthate in Wheat Root Exudates

There is abundant evidence that organic compounds are exuded from the roots of intact plants<sup>1</sup>, but the amounts are small, and to obtain sufficient material for identification even by paper chromatography it is necessary to collect exudates from a large number of plants or from one plant over a long period. To overcome these difficulties a sensitive method using radioactive labelling has been developed which enables short-term studies on the exudation by roots of individual wheat plants to be used.

Wheat seed (variety 'Gabo') was surface sterilized with calcium hypochlorite, germinated on agar plates and two seeds planted per test-tube (3.5 cm diam. × 20 cm) containing sterile Hoagland and Arnon medium<sup>2</sup> adjusted to pH 6.5 after autoclaving. The seeds were supported on glass fibre 2 mm mesh stretched over one end of a glass cylinder standing in the nutrient solution. After 5–6 days in the glasshouse, when the leaves and roots were 10–12 cm long, the solutions were checked for sterility. The plants were carefully removed from the tube together with the supporting mesh and cylinder. The mesh was cut away so that the roots were not handled, and in this way damage and contamination were avoided. The plants were then transferred to an apparatus made of 'Perspex' (Imperial Chemical Industries, Ltd.) designed to support them so that the tops could be exposed to  $^{14}\text{CO}_2$  and isolated from the roots which were immersed in sterile liquid. The apparatus was sterilized before use by soaking overnight in a solution of sodium hypochlorite. Air containing approximately 10  $\mu\text{c}$ . of  $^{14}\text{CO}_2$ , prepared from  $\text{Na}_2^{14}\text{CO}_3$  and lactic acid, was injected through a port into a tube enclosing the foliage. After 1 h in sunlight, when  $^{14}\text{CO}_2$  was almost completely assimilated, the tube was removed and the plants were allowed to photosynthesize in the normal atmosphere. The liquid around the roots (water or plant nutrient solution) was changed at intervals and aliquots were checked for sterility. Usually the solution remained sterile or showed insignificant contamination over 24-h collection periods.

In addition to exuded organic compounds the solution surrounding the roots would contain dissolved  $^{14}\text{CO}_2$ , derived from metabolism of root tissue. This was removed by adding  $\text{Na}_2\text{CO}_3$  as unlabelled carrier, acidifying the solution to pH 2–3 and bubbling air through it for 30 min. Other volatile compounds may have been removed by aeration. Approximately 80 per cent of the total counts of a solution which had not been aerated during collection of exudate were removed by this treatment. The solution was neutralized, 5 mg glucose added, then evaporated to

dryness in a silica boat in a vacuum oven at 55° C. The dry residue was combusted to carbon dioxide in a furnace and the carbon dioxide evolved collected in sodium hydroxide and precipitated as barium carbonate. The radioactivity of the barium carbonate was determined by liquid scintillation counting after suspending it in a thioxotrophic gel-toluene counting solution. The reproducibility of this method was checked using  $^{14}\text{C}$ -glucose, uniformly labelled. From the results of 5 replicates, the standard error, expressed as a percentage of the mean, was  $\pm 3.6$ .

Radioactivity first appeared in the exudate 4–5 h after the start of exposure of the tops to  $^{14}\text{CO}_2$  and continued for the final period of collection of 24–48 h. The radioactivity of the exudates collected over a 48-h period from roots in plant nutrient solution indicated that approximately 0.1 per cent of carbon-14 assimilated was exuded by the roots. The amount of exudate, as indicated by radioactivity, was increased when roots were immersed in distilled water rather than plant nutrient solution during the course of the experiment (Table 1). There are indications that exudation of  $^{14}\text{C}$ -labelled compounds from roots is affected by aeration of the roots—less radioactivity being exuded at low oxygen tensions. Several radioactive compounds were detected when paper chromatograms of the root exudates were examined by autoradiography.

Table 1. EXUDATION OF RADIOACTIVITY BY WHEAT ROOTS IN WATER AND PLANT NUTRIENT SOLUTION

Roots immersed in:	
Distilled water	Plant nutrient solution
7248* (455)†	4570* (610)†

\* Counts per 100 sec in exudate from 2 plants over 15–5 h.

† S.E.

This investigation has shown that labelled photosynthate can be used to trace the exudation of organic compounds from intact roots. Application of carbon-14 to the plant as  $^{14}\text{CO}_2$  is considered superior to the application of labelled sugars or amino-acids to leaves or cut petioles, since the normal translocated materials of the plant become labelled and there is no danger of loading the plant with unrealistic concentrations of any particular compound, a factor which might alter exudation patterns.

The use of  $^{14}\text{CO}_2$ -treated plants for root exudate studies has been reported previously<sup>3,4</sup>, but the work recorded here has shown that the technique can be used for short-term studies for which complete asepsis is unnecessary, measuring the exudation from individual plants and even from different portions of the same root. By growing plants in the presence of  $^{14}\text{CO}_2$  for longer periods it may be possible to obtain equilibration of labelling in the plant and the radioactivity exuded should then yield quantitative data of exudate components.

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### Method to study Germination of Fungus Spores in Soil

VARIOUS methods have been devised to study the germination of fungus spores in the soil<sup>1,2</sup>. These methods have the disadvantage that the spores are held under conditions different from those in natural soil. Boosalis<sup>3</sup> developed a method which eliminates the disadvantages of other methods but is rather cumbersome. The following method was found to be satisfactory for application in studies on soil-fungistasis and for determining the effect of various

soil amendments on the germination of conidia of *Helminthosporium sativum* P.K. and B. in the soil.

The soil is air-dried and sieved through a 1-mm mesh sieve to obtain a uniform maximum particle size. Both halves of a Petri dish are then filled with the soil and the surface of each levelled. To retain a smooth surface the soil is moistened by means of a spray gun operating at 5 lb./sq. in. Care must be taken not to blow holes in the soil surface by holding the spray gun too close to the soil. If an absolutely level surface is not required, the soil can be moistened by means of a pipette. The amount of water applied will be determined by the soil type and the nature of the test. The spores (in suspension) are applied by means of a pipette to the soil in the lid part of the Petri dish (Fig. 1). The number of spores must be sufficient to permit easy recovery after incubation. A disk of plastic or nylon mesh (approx. 2 mm), with a diameter similar to that of the Petri dish, is placed on the other half and pressed down firmly. The purpose of the mesh is to separate the soil surfaces and yet provide contact and air spaces. The Petri dish is then assembled as indicated in Fig. 1 and the two parts lightly pressed together to ensure contact between the soil surfaces. It is then incubated under conditions suitable for the germination of the spores.

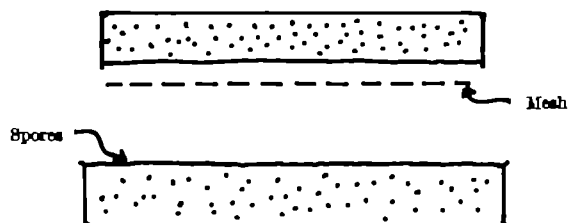


Fig. 1. Diagrammatic representation of Petri-dish assembly for tests of spore germination in the soil

After incubation, the two parts of the Petri dish are separated carefully, and a spore print is made with an agar strip<sup>4</sup> which is examined microscopically. The agar strips can also be incubated in a moist chamber to determine the viability of ungerminated spores.

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### Male and Female Influence of Adult Individuals on Undifferentiated Larvae of the Parasitic Nematode *Paramermis contorta*

PREVIOUS experimental infestations of *Chironomus tentans* larvae with the nematode *Paramermis contorta* confirmed that crowding plays a major part in determining the sex of the parasites<sup>1</sup>. Observations by Caullery and Comas<sup>2</sup> on natural populations showed that an increase of the degree of infestation results in an increase of the relative frequency of the males of *Paramermis*. It was also established that, at a given degree of infestation, the relative frequency of male *Paramermis* decreases with the increasing lengths of the *Chironomus* larvae at the moment when they are penetrated by the nematode<sup>3</sup>.

Work has now been carried out in order to establish whether, in cases of two successive infestations, the individuals which develop first in the haemocoel of the host can influence the sexual differentiation of the young individuals that penetrate successively the *Chironomus* larva.

About 4,000 larvae parasitized by a single nematode were obtained from about 10,000 larvae of *Chironomus*

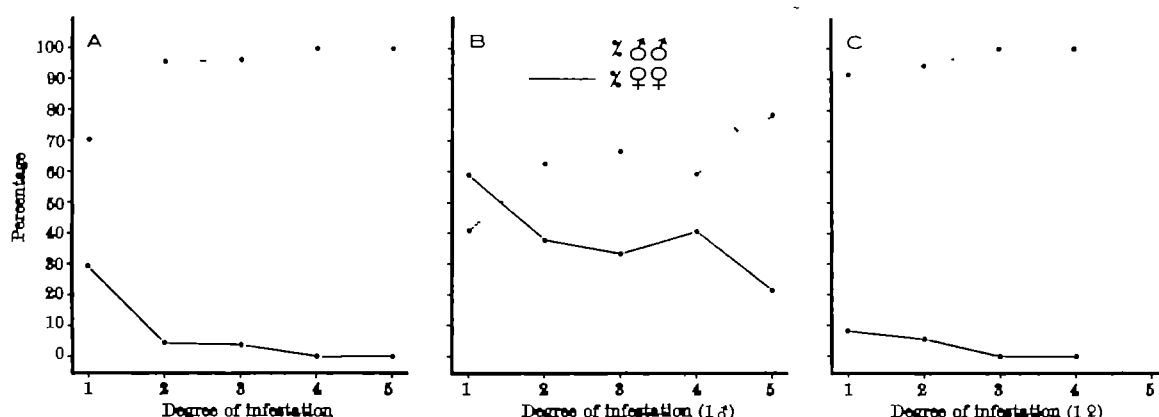


Fig. 1

*lentans* which were subjected to a first light infestation with *Paramermis contorta*. The remaining *Chironomus* larvae, which were parasitized by more than one nematode or were not parasitized at all, have not been considered in the prosecution of the experiments. Nine days after the infestation, with the parasites reaching the medium length of 13 mm, a second massive infestation has been carried out on the *Chironomus* larvae parasitized by the single nematode.

A second lot of 10,000 *Chironomus* larvae of the same age as those which were previously parasitized was subjected in the same way as the first and single massive infestation of *Paramermis*.

Repeated controls demonstrated that the death rates of both the parasitized larvae and of the nematode remained at such low levels that they could not influence in the least the results of the experiments.

All the *Chironomus* larvae were fixed fifteen days after the last infestations and were afterwards opened in order to sex the parasites.

This allowed significant differences to be established in the sex ratios of the following three groups of parasites: (1) the parasites which penetrated the *Chironomus* larvae simultaneously in the course of a single infestation; (2) the nematodes which penetrated *Chironomus* larvae which already contained a single female *Paramermis*; (3) the nematodes which penetrated *Chironomus* larvae which already contained a single male *Paramermis*. It is easy to make out at this stage the parasite which has been introduced at the first course of infestation from those which have been introduced in the second course and are much smaller.

84.53 per cent male individuals (15.47 per cent female individuals) were obtained in the first group (Fig. 1A). This group is represented by 6,595 parasites which were obtained from 4,517 *Chironomus* larvae which had been subjected to the single massive infestation eleven days after their births.

94.56 per cent male individuals (5.34 per cent female individuals) were obtained in the second group (Fig. 1C). This group is represented by 1,581 nematodes which penetrated 1,082 *Chironomus* larvae which already contained a single female *Paramermis* from the first course of infestation.

61.80 per cent male individuals only (38.20 per cent females) were obtained in the third group (Fig. 1B). This group is represented by 6,860 nematodes which penetrated 2,837 *Chironomus* larvae which already contained a single male *Paramermis* from the first course of infestation.

In the first, second and third groups (which are all perfectly comparable) the sex ratios are significantly different. A close examination shows that the percentage of females is significantly lower in the group which penetrated the *Chironomus* larvae which were already parasitized

by a single female nematode (group 3) than it is in the group of parasites which penetrated the *Chironomus* larvae in a single course of infestation (group 1). The percentage of females is, on the other hand, significantly higher in the group which penetrated *Chironomus* larvae which were already parasitized by a single male nematode (group 2) than it is in group 1. The percentage of females of group 1 is thus intermediate between the percentages of groups 2 and 3.

Such results show that a female individual of *Paramermis* which develops first influences the sexually undifferentiated larvae of *Paramermis* which penetrate, at a later time, the *Chironomus* larva by favouring their differentiation in the male direction. A male individual of *Paramermis* which develops first influences on the contrary the undifferentiated larvae in the female direction.

The following influences are thus ascertained concerning sexual differentiation of *Paramermis contorta*: (1) the degree of infestation; (2) the length of the host; (3) the sex of the parasite which first penetrated the *Chironomus* larva.

It is already known through such classic examples as those of *Bonellia*, of *Orepidula* and of *Jones* that adult females may exert a male-determining influence on younger individuals. The female-determining influence by adult male individuals has now been demonstrated for the first time.

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### A Method of Increased Sensitivity for detecting Single Antibody-forming Cells

THE plaque techniques of Jerne *et al.*<sup>1</sup> and of Ingraham and Bussard<sup>2</sup> for assaying single antibody-producing cells involve the lysis of erythrocytes suspended in three dimensions around the active cells in a supporting medium, agar or methyl cellulose. Maximum sensitivity with this system would be expected if a single layer of lymphoid cells and target erythrocytes was examined. A method has been devised which dispenses with supporting media and allows detection of cells producing antibody sufficient to lyse only 10-20 adjacent erythrocytes.

A sheet of paraffin wax 2 cm × 2 cm and 10μ thick is cut with a microtome, and a hole 1 cm diameter then punched in it with a cork borer. This sheet is dried on to a microscope slide forming a shallow 'chamber'. A suspension of the antibody-producing cells to be examined is made in Eagle's medium at 0° C, and complement and

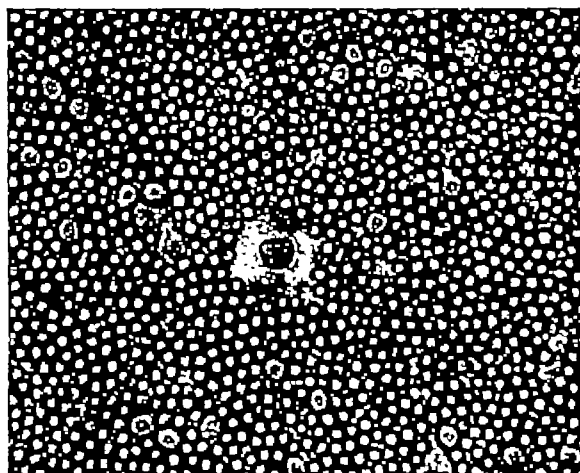


Fig. 1. Cell from the efferent popliteal lymph duct of a sheep producing a small plaque by lysis of adjacent sheep red cells coated with bacterial polysaccharide

target erythrocytes added to final concentrations of 10 per cent each. The mixture is warmed to room temperature for a few seconds, and a small drop (about 5  $\mu$ l.) is pipetted on to the glass at an inner edge of the chamber. A round coverslip, 1.3 cm in diameter, is lowered slowly on to the drop, which spreads to fill the chamber and overflows it. Excess fluid is sucked off with a filter-paper around the edges of the coverslip, which settles on to the rim of paraffin beneath, and the chamber is sealed with heated paraffin-'Vaseline'.

Plaques begin to appear in the monolayer within 1-2 min when the slide is incubated at 37° C, and a maximum number may be counted after 20 min. Most plaques do not increase in size after 1-2 h. The number of nucleated cells in a chamber of measured size is determined directly by counting several (calibrated) high-power fields under the microscope. Slides may be handled quite roughly without disturbing the monolayers.

Table 1. COMPARISON OF THE SENSITIVITY OF THE 'AGAR PLATE' AND 'FREE SUSPENSION' TECHNIQUES FOR DETECTING SINGLE PLAQUE-FORMING CELLS

Origin of cells	Hours after primary antigenic stimulus	Antigen	Calculated Plaque-formers per 10 <sup>4</sup> nucleated cells			
			'Agar plate'		'Free suspension'	
			A*	B*	A*	B*
OBA mouse spleen	96	10 <sup>6</sup> sheep rbc	1,090	1,080	2,980	2,760
OBA mouse spleen	144	10 <sup>6</sup> sheep rbc	87	40	144	134
OBA mouse lymph node	120	10 <sup>6</sup> sheep rbc	232	215	875	950
Sheep: efferent lymph duct from popliteal node	88	10 <sup>6</sup> boiled <i>Salmonella muenchen</i>	<10	<10	11,200	10,500

\* Plaques were counted by me (A) and by an independent observer (B).

The main advantages of this modified technique are:

(a) Increased sensitivity: about three times as many haemolysin producers are counted with the 'free suspension' method as are seen with the 'agar plate' technique. Using sheep red cells coated with *Salmonella* lipopolysaccharide<sup>4</sup> as the target, sheep lymph node cells secreting antibody to the bacterium were found to produce large numbers of very small (30-40 $\mu$  diameter) plaques which could not be detected by the agar plate technique<sup>4</sup> (Fig. 1). Such very small spheres of lysis in agar are probably obscured by intact red cells above or below.

(b) All cells in a chamber are unobscured, and may be examined at high magnification.

(c) The free-suspension technique detects the characteristic localized agglutination of lipopolysaccharide-coated erythrocytes around sheep lymph node cells producing antibacterial antibody in a secondary response<sup>4</sup>.

The main disadvantage of the method is that not more than about 300,000 lymphoid cells may be incubated in

one chamber. Increasing the size of a single chamber is unsatisfactory.

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## Unique Occurrence of Complemental Males in a Sessile Barnacle

ALTHOUGH most thoracic barnacles are hermaphrodite, males and females are known in some of the pedunculate species. Darwin<sup>1</sup>, who was the first to observe males, found them associated with both hermaphrodites and females in the two related genera, *Ibla* and *Scalpellum*. The females, except for lacking male organs, were similar to the hermaphrodites of related species. The males, however, were always very small and one or more were attached, either just inside the operculum or in pits on the inside of the scutum. As the association of males with hermaphrodites was unknown in the animal kingdom, Darwin<sup>1</sup> made a very careful study of the attached organisms and, by finding the characteristic scalpelliform antennae, male organs and spermatozoa, proved conclusively that they were the males of their respective species. The males attached to the hermaphrodites he called complemental males.

Darwin<sup>1</sup>, who had only six species of *Scalpellum* available for study, distinguished two types of males, both associated with either females or hermaphrodites. One type was similar in basic structure to the latter forms. This type was also found in the two species of *Ibla*. The second bore no resemblance to the female or hermaphrodite or, for that matter, to any pedunculate barnacle. These males lacked a mouth and prehensile cirri and were, when mature, mere sacs of spermatozoa. By 1908, one hundred and seventy species of *Scalpellum* had been described and intermediate types of males had been found. At this time, Pilsbry<sup>2</sup> divided the genus into four genera and, using the characters of the male as well as those of the hermaphrodite and female, he constructed a more or less natural key to the scalpelliform barnacles.

Since the monographs of Darwin<sup>1,2</sup>, many new species of both pedunculate and sessile barnacles have been described and many of the previously known species have been more fully studied, but males have never been found in any barnacles except in *Ibla* and *Scalpellum* (*sensu lato*). Consequently, we were astonished to find complemental males in a member of the sessile barnacles. In preparing a description of a new species of *Balanus*, it was noticed that frequently the rostrum, which is strongly concave internally, had a deep depression in the vicinity of the sheath. From these depressions, cyprids and males in various stages of development were recovered. Each of the barnacles to which a male was attached has an extremely long penis and, therefore, must be classed as hermaphroditic. The male is degenerate; it lacks a mouth but has vestigial cirri and typical antennules. Testes and seminal vesicles, as well as spermatozoa, are present in mature males.

This species primarily agrees with *Solidobalanus*, a sub-genus of *Balanus*, which is one of the most highly evolved of the sessile barnacles. A full description of the new species is in preparation. However, since the occurrence of complemental males in a sessile barnacle is so noteworthy, it seemed worthwhile to call this phenomenon to the attention of other cirripedologists. This work was

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## MICROBIOLOGY

### Attachment of Marine- and Fresh-water Bacteria to Solid Surfaces

DURING an investigation of the factors influencing choice of substrates by the marine amphipod *Corophium volutator* (Pallas), I observed that distilled water induced many bacteria to detach from the surfaces of particles of marine sand<sup>1</sup>. A search of the literature revealed that this phenomenon had not been reported, and that there was little information on why aquatic bacteria attach to surfaces<sup>2,3</sup>.

It was therefore decided to examine the attachment of aquatic bacteria to surfaces under experimental conditions. The experiments were conducted as follows. Marine bacteria were grown in a sea-water nutrient broth consisting of meat extract 10 g, peptone 10 g, aged sea-water 1 l.; the broth used for fresh-water bacteria was made up of beef extract 1 g, yeast extract 1 g, peptone 1 g, de-ionized water 1 l. Cultures were always used during the log phase of growth. The mixed cultures were obtained by inoculating sea-water or fresh-water into broth. Clean glass microscope slides were suspended for 3 h in a particular culture. During this time many bacteria attached. The slides were then removed one at a time and placed into different test solutions. After 1.5 h the slides were removed. When they were examined, it was found that varying numbers of bacteria had detached, depending on the nature of the test solution.

In view of my observation on the effect of distilled water on marine bacteria attached to sand grains, it was of interest to determine the influence of reduced salinity on attachment. Mixed cultures of marine and fresh-water bacteria were therefore tested as described here. Experiments were run in triplicate. The test solutions used were 100, 25, 5 and 0 per cent sea-water; these dilutions were obtained by diluting sea-water with de-ionized water.

The results of these experiments (Fig. 1) show that marine bacteria remain attached only in high salinities, whereas the peak of attachment for fresh-water bacteria occurs in 5 per cent sea-water. These results have been substantiated by others on pure strains of marine and fresh-water bacteria, including the fish pathogen *Vibrio anguillarum* NCMB 829.

Further experiments with marine bacteria have shown that sea-water can be replaced by solutions of sodium chloride of the same ionic strength as sea-water. If, however, a solution of glycerol of the same osmotic strength as sea-water is used, many marine bacteria detach. This is not true of fresh-water bacteria.

It seems, then, that marine- and fresh-water bacteria require different ionic environments before they will remain attached to surfaces.

These results have implications for a number of fields of investigation. In rivers and estuaries, marine bacteria will only remain attached to solid structures when tidal flow produces water of high salinity; this would limit them to the mouth of estuaries. Similarly, fresh-water bacteria will only remain attached up-river, where salinity is low. These are important ecological considerations; it has, for example, been suggested that organic substances adsorbed on surfaces are there in higher concentration

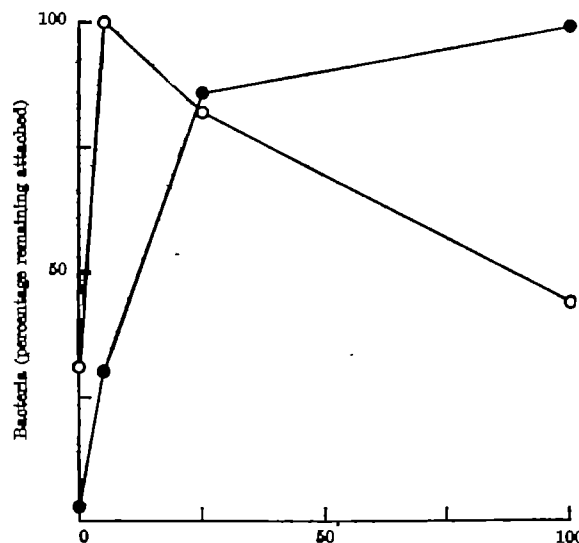


Fig. 1. Influence of salinity on the number of marine and fresh-water bacteria which remain attached to glass slides. ●, Marine bacteria; ○ fresh-water bacteria. The ordinate represents the percentage of those attached to the 5 per cent sea-water slide (fresh-water bacteria), or the percentage of those attached to the 100 per cent sea-water slide (marine bacteria). The abscissa represents the salinity of the test solution expressed as a percentage of undiluted sea-water. Each point is the mean of three values.

than in the body of the water mass, and hence are more readily available to bacteria as a food source<sup>4-6</sup>.

The results described here are also of interest in relation to the development of fouling communities. It seems probable that bacteria are among the first organisms to attach to newly immersed surfaces in the sea<sup>7,8</sup>. Fig. 1 shows that they do not remain attached in low salinities. Under these conditions, any film which developed would consist of fresh-water micro-organisms. Now it is known that the larvae of sedentary marine invertebrates, when they explore surfaces as a prelude to settlement, are sensitive to the numbers and types of attached micro-organisms<sup>9</sup>. A change in these numbers and types, caused by low salinity water, would almost certainly be detected by such larvae, and would in all probability induce them to settle elsewhere. This, and larval mortality in low salinities<sup>10</sup>, are factors which may play a part in limiting the spread of marine fouling communities into fresh-water.

It is difficult to suggest reasons for the observations reported here, for not a great deal of information is available on the way aquatic bacteria attach to surfaces, or on any differences in the physical chemistry of the cell surface between marine- and fresh-water forms; however, a report of a correlation between membrane stability and salt tolerance in Gram-negative bacteria is of interest<sup>11</sup>. Certainly mortality does not appear to be a limiting factor<sup>12</sup>. These and related problems are at present under investigation.

I thank Vera Collins and Dr. G. D. Floodgate for helpful discussion and the Royal Society for a grant for apparatus.

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# 'Lincomycin' Treatment of Staphylococcal Infection at High Altitude

EXPERIMENTAL data previously reported<sup>1,2</sup> indicate that exposure to simulated altitudes of 18,000 or 35,000 ft. reduces the resistance of mice to infection caused by respiratory challenge with airborne *Klebsiella pneumoniae*. This effect was most marked in mice kept at 35,000 ft. for 1 or 14 days.

In the present studies the resistance of mice to *Staphylococcus aureus* and the efficiency of antibiotic treatment at 35,000 ft. were determined.

The gaseous environment used consisted of 85 per cent oxygen and 10 per cent carbon dioxide. At reduced pressure, 179.3 mm Hg, this environment corresponds to sea-level-equivalent partial pressure of oxygen ( $pO_2 = 152$ ) and elevated partial pressure of carbon dioxide ( $pCO_2 = 17.3$ ). The temperature in the chamber was maintained at  $22^\circ \pm 1^\circ C$  at relative humidity 50 per cent.

Table 1. MORTALITY OF MICE INFECTED WITH *S. aureus*

Duration of exposure (days)	1 x LD <sub>50</sub>		8 x LD <sub>50</sub>		100 x LD <sub>50</sub>	
	Ambient	Altitude	Ambient	Altitude	Ambient	Altitude
3	5/10*	7/10	7/10	9/10	9/10	10/10
7	4/10	4/10	6/10	8/10	9/10	9/10
14	5/10	4/10	8/10	4/10	9/10	9/10
30	5/10	4/10	7/10	7/10	10/10	10/10
Mean %	47.5	47.5	70.0	67.5	92.5	95.0

\* Dead/total.

Swiss albino mice were kept in this environment continuously for up to 30 days. At various times they were challenged intraperitoneally with virulent *S. aureus*, strain 284 (UG-76). The dose concentrations of *S. aureus* were 1, 8 and 100 LD<sub>50</sub> per injection. The data summarized in Table 1 show that no differences were observed between the mortality of mice kept at 35,000 ft. and that of control mice kept at ambient environment.

To study the efficiency of antibiotic treatment, Swiss albino mice exposed to 35,000-ft. altitude for 3, 7, 14 and 30 days were infected intraperitoneally with 100 LD<sub>50</sub> units of *S. aureus* 284. 'Lincomycin' (the Upjohn Company) was selected as the antibiotic and was injected subcutaneously at three dose-levels: 0.5 OD<sub>50</sub>, OD<sub>50</sub> (median protective dose), and 3 OD<sub>50</sub>. The 'Lincomycin' treatment was initiated within 30 sec after inoculation with *S. aureus* and was repeated on three consecutive days for a total of four injections.

Table 2. EFFECT OF 35,000-FT. ALTITUDE ON 'LINCOMYCIN' TREATMENT OF STAPHYLOCOCCAL INFECTION

Duration of exposure (days)	<i>S. aureus</i> Control		0.5 OD <sub>50</sub>		OD <sub>50</sub>		3.0 OD <sub>50</sub>	
	Ambient	Altitude	Ambient	Altitude	Ambient	Altitude	Ambient	Altitude
3	90*	100	80	70	10	50	10	10
7	90	90	20	60	20	40	20	10
14	90	90	41	59	28	34	6	16
30	100	100	30	30	10	30	10	15
Mean %	92.5	95.0	25.2	54.8	17.0	38.5	11.5	12.6

\* Percentage mortality.

Table 2 summarizes the results. Treatment with 'Lincomycin' at 0.5 OD<sub>50</sub> or 1 OD<sub>50</sub> level was less effective in reducing the mortality of the altitude-stressed mice than of the control mice. At the 3 OD<sub>50</sub> concentration of 'Lincomycin' this difference was absent. The duration of exposure of the mice to the elevated altitude also affected the therapeutic value of the antibiotic. Increased mortality was observed in mice kept at altitude for 3 and 7 days. At 14 days the mortality was still higher than in the controls. At 30 days the mortality was almost the same in both experimental groups.

For statistical analysis ('t' test) the data from ambient and altitude experiments were pooled irrespective of the level of antibiotic or the duration of exposure. The differences in mortality between the two groups were significant at  $P < 5$  per cent.

From these exploratory experiments it appears that the efficiency of 'Lincomycin' therapy of staphylococcal infection is reduced when the host is kept at an altitude of 35,000 ft. for up to 14 days.

We thank Dr. J. Grady, the Upjohn Company, for the samples of 'Lincomycin' used in this work.

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## GENETICS

### Paucity of Albumin Variants In Man

PLASMA albumin in mammals is a protein of molecular weight about 69,000 and consists of a single kind of polypeptide chain<sup>1</sup>. Until recently, the only genetic variants of this protein recognized in any mammal have been the very rare cases of 'bis-albuminaemia' in man<sup>2,3</sup>. However, it has recently been reported<sup>4,5</sup> that some breeds of horses and zebu cattle are polymorphic (as defined by Ford<sup>6</sup>) for electrophoretically different albumins, and these findings have prompted me to enlarge and record some data indicating that certain human populations are not similarly polymorphic. This conclusion is to be anticipated from the fact that filter-paper electrophoresis of human plasma has been carried out routinely in clinical laboratories all over the world for more than a decade with only a few cases of bis-albuminaemia being reported, even though this condition gives a conspicuously abnormal electrophoretic pattern with two well-resolved components. In this context one may note that starch-gel, which has now widely replaced filter-paper as supporting medium, is not more sensitive than filter-paper for the study of albumin in undiluted plasma, because the large quantity of albumin present distorts the electric field in the gel, leading to reduced resolution. However, as noted also by Ashton<sup>7</sup>, multiple albumins can be very sharply resolved in starch gels if the plasma is diluted, and the present survey is based on this technique.

Plasma or serum specimens, diluted 10-fold with distilled water, were examined by one-dimensional starch gel electrophoresis in discontinuous buffer systems at pH 8.6 and pH 5.1<sup>8,9</sup>. Each gel contained, as a control, a sample of 10-fold diluted serum from a known case of bis-albuminaemia, in which the mobility difference between the usual (faster) and unusual (slower) components is probably due to two charge units<sup>8</sup>. The separations obtained were such that a difference as great as or greater than one-quarter the magnitude in the control would have been recognized with certainty.

Seven hundred and forty-eight specimens were examined, without a single variant being recognized. The specimens came from the following populations: Glasgow hospital patients, 548; South Indian leprosy patients, 50; East African Karamojo, 50; West African Yoruba, 50; New Guinea Highlanders, 50. Of the Glasgow sample, 202 were collected in 1961 and were analysed at pH 8.6 only; the remainder were collected and analysed in 1965. The South Indian sera were collected in 1964, and the other foreign samples were collected earlier. All the sera had been stored frozen, the oldest of them for about six years.

These results show that the observed frequency of unusual alleles determining net charge differences in human plasma albumin is less than 0.001 in a Glasgow population, and less than 0.02 in some other populations. In another random survey an allele frequency of about 0.0005 was obtained in a largely Norwegian population<sup>3</sup>. Consideration of the work of routine clinical laboratories and of other research workers suggests that these values are much higher than the real values. It thus appears that man is not polymorphic (*sensu strictu*) for alleles detectable via electrophoresis at the structural locus for albumin.

The simplest and most important of the mechanisms leading to polymorphism is heterozygous advantage and, for any locus controlling an enzyme or other protein, heterozygous advantage will usually result from increased activity or altered specificity of the protein. Such a change can be a property of only few of the possible mutations at a locus (since most will result in decrease or loss of function) and may not be possible at all loci; thus one is led to expect that many protein structural loci will not be polymorphic.

That serum albumin is one of the proteins unlikely to be polymorphic in man receives some support from the existence of an albuminaemic individuals who show no serious impairment of health due to their almost complete lack of albumin<sup>2</sup>. This suggests that albumin function(s), far from being limiting to fitness, may in certain circumstances be almost dispensable.

I thank Dr. H. E. Hutchinson for providing the Glasgow sera; Dr. A. E. Mourant, Miss S. Povey and Mr. L. J. Horton for the Indian sera; Dr. J. P. Garlick for the African and New Guinean sera, and Prof. H. Harris for the bis-albuminaemic serum.

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### Chromosomes of the Striped Indian Squirrel (*Funambulus pennanti*)

*Funambulus pennanti*, the striped Indian squirrel, is a small arboreal rodent characterized by three white stripes on its dorsal surface and two on the sides and is the predominant species of Northern India. So far as we are aware, the chromosome number of this species has not been reported. In fact, no information is available on chromosome numbers of any member of the genus *Funambulus*, family Sciuridae<sup>1</sup>. The results of an investigation into chromosome number and karyotype of this species are summarized in this report.

Bone marrow was processed for chromosome preparations by the method of Ford and Hamerton<sup>2</sup>. Each squirrel was injected with 0.5 ml. 0.025 per cent colchicine solution and killed 1 h later. Marrow from femurs was removed into hypotonic solution of 0.95 per cent sodium citrate in water warmed at 37° C. After 15 min in hypotonic solution at 37° C the cells were settled out by centrifugation at low gravity and fixed for 15 min in a freshly prepared chilled (1 : 3) mixture of glacial acetic acid and methyl alcohol. Air-dried preparations were made by the method of Rothfels and Siminovich<sup>3</sup> and stained with 2 per cent aceto-orcein. The slides were made permanent by passing them through two changes of n-butyl alcohol, and mounted in neutral canada balsam. All drawings, measurements and photographs were made from permanent slides. Only undamaged and well-spread cells with uniform contraction were selected for recording data. For chromatin length measurements, five cells were drawn with the help of camera lucida. The chromatin length figures given in the text are an average of five mitotic metaphase cells. The chromosomes were matched into pairs using relative length and arm ratio as criteria.

All but one of the 50 cells examined critically from two male squirrels available for study had 54 chromosomes in mitotic cells (Fig. 1). The only cell with a number devi-



Fig. 1. Typical metaphase plate and karyotype of *Funambulus pennanti* showing 54 somatic chromosomes.

ating from the normal had 52 chromosomes. Fig. 1 (below) shows the 54 chromosomes matched into 27 pairs and arranged in descending order of length, the pair of sex chromosomes being placed at the end. The longest chromosome in the complement was 6.67  $\mu$  in length and the smallest measurable one, chromosome 24, measured 1.46  $\mu$ . Chromosomes 25 and 26 are too small to be drawn accurately for length measurements. The 26 autosomes can be grouped into the following categories:

(A) Large chromosomes, ranging in length from 6.67  $\mu$  to 3.96  $\mu$ , include: (i) Subterminals: Chromosome numbers 1, 2, 6, 8, 11 and 13. Chromosome 11 has a satellite on the short arm. (ii) Telocentrics: Chromosome numbers 3, 4, 5, 7, 9, 10 and 12.

(B) Medium long chromosomes, ranging in length from 3.75  $\mu$  to 2.08  $\mu$ , include: (i) Subterminals: Chromosome numbers 17 and 19. (ii) Telocentrics: Chromosome numbers 14, 15, 16, 18, 20 and 21.

(C) Small chromosomes, ranging in length from 1.66  $\mu$  to 1.46  $\mu$  and less, include: (i) Metacentric: Chromosome 23. (ii) Telocentrics: Chromosome numbers 22, 24, 25 and 26.

Since the two animals examined were both males, it is difficult to say with certainty which of the sex chromosomes is the X-chromosome. It is more likely, however, that the longer one measuring 3.33  $\mu$  is the 'X' chromosome. The 'Y' then is a very short acrocentric chromosome indistinguishable from chromosomes 25 and 26.

*Note added in proof.* Since this report has been in the press, Rao and Sharda (*Cytogenetics*, 3, 342) have also reported the diploid chromosome number of *Funambulus pennanti* to be 54.

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## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Sunday, September 5—Wednesday, September 8

BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE (at Cambridge)  
—Continuation of 127th Annual Meeting.

Sunday, September 5

At 11 a.m.—Official Service in Great St. Mary's Church. Preacher, Prof. D. Nineham, Regius Professor of Divinity in the University of Cambridge.

Monday, September 6

At 10 a.m. and 2.30 p.m.—Symposium on "World Fuel and Power Resources and Needs".

At 8.30 p.m.—Prof. F. Hovie, F.R.S.: "Recent Work in the Field of Cosmology and Astronomy" (Evening Discourse).

Tuesday, September 7

At 10 a.m.—Prof. D. G. MacRae: "The Sociology of Secular Religions" (Presidential Address, Section N).

At 10 a.m.—Dr. G. F. Loodale: "Hugliens—a New Look with the Electron Microscope" (Darwin Lecture).

At 10 a.m.—Dr. M. Prutton: "Thin Films and Their Role in Computers" (Kelvin Lecture).

Wednesday, September 8

At 2.30 p.m.—Prof. H. J. Eysenck: "The Measurement of Intelligence" (Young People's Lecture).

Tuesday, September 7

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Address by Mr. John Macgregor, President of The American Society of Automotive Engineers.

Monday, September 13—Friday, September 17

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2)  
—International Conference on "The Microwave Behaviour of Ferrimagnetics and Plasmas".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

HISTORICION THEORISTIAN (graduate member of the I.E.R.M. or with equivalent qualifications, and some years experience)—The House Governor (A), Charing Cross Hospital, London, W.C.2 (September 8).

PRINCIPAL BIOCHEMIST (with a good honours degree in chemistry or biochemistry and/or Ph.D. or other higher qualification) in the DEPARTMENT OF CHEMICAL PATHOLOGY at Ashton Street, Pembroke Place, Liverpool, 3.—The Secretary, The United Liverpool Hospitals, 80 Rodney Street, Liverpool 1 (September 8).

CLINICAL PSYCHOLOGIST (with an honours degree and preferably some postgraduate experience) to give assistance to the Principal Psychologist.—The Secretary to the Board of Governors, The National Hospital for Nervous Diseases, Queen Square, London, W.C.1 (September 10).

TEMPORARY ASSISTANT LECTURER or LECTURER in ORGANIC CHEMISTRY.—The Registrar, University College of Swansea, Singleton Park, Swansea (September 11).

LECTURER (Grade II) in MATHEMATICS (Pure or Applied)—The Registrar, University Senate House, The University, Bristol, 2 (September 13).

RESEARCH ASSISTANT (with qualifications in economic geography) in the GEOGRAPHICAL RESEARCH DIVISION—Prof. R. J. Harrison-Church, London School of Economics and Political Science, Houghton Street, London, W.C.2 (September 13).

ASSISTANT LECTURER in ZOOLOGY.—The Registrar, The University, Manchester, 13, quoting Ref. 165/65 (September 15).

ASSISTANT LECTURER (preferably with special interests in the scientific aspects of geography, for example, biogeography or climatology) in GEOGRAPHY.—The Registrar, The University, Manchester, 13, quoting Ref. 157/65 (September 15).

MARINE BIOLOGIST (with a good honours degree and research experience in marine biology) in the MARINE SCIENCE LABORATORIES to conduct fundamental research on sedimentary animals or algae and to supervise the work of a technician responsible for routine tests.—The Registrar, University College of North Wales, Bangor, North Wales (September 15).

RESEARCH ASSISTANT (with a degree or equivalent qualification in physics or applied science, and preferably experience in practical electronics) in COSMIC RAY PHYSICS to join a small group working on a research project deep in the gold mines of Southern India.—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (September 15).

LECTURER (with a distinguished academic record) in PHILOSOPHY in the DEPARTMENT OF GENERAL STUDIES, University of New South Wales, Australia.—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 17).

MUSEUM ASSISTANT (with appropriate qualifications and experience) at the OXFORD AND BEXLEY MUSEUM.—The Borough Librarian and Curator, Civic Centre, Chelmsford, Essex (September 18).

CURATOR (qualified and experienced in archaeology and museum administration) of the ROYAL MUSEUM and other museums controlled by the City Council.—Town Clerk, Municipal Buildings, Canterbury, Kent (September 20).

LECTURER (with a special interest in physical chemistry) in the CHEMISTRY DEPARTMENT.—The Secretary, Royal Free Hospital School of Medicine (University of London), Hunter Street, London, W.C.1 (September 20).

LECTURER, Grade II (with special interests in electron microscopy and fine structure); and a LECTURER, Grade II (with special interests in veterinary biochemistry) in the DEPARTMENT OF BIOCHEMISTRY.—The Registrar, University Senate House, Tyndall Avenue, Bristol, 2 (September 21).

READER (with high academic qualifications, teaching experience and extensive research experience in mechanical engineering, and preferably some administrative experience) in MECHANICAL ENGINEERING (September 24); and a LECTURER (with good qualifications in mathematics and experience in teaching it in the secondary school) in EDUCATION (October 1) at the University of Queensland, Australia.—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

JUNIOR RESEARCH FELLOW (graduate, preferably with specialized training or a special qualification in sociology or anthropology) in SOCIOLOGY in the Institute of African Studies, University of Ibadan, Nigeria.—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (September 28).

CHAIR OF EXPERIMENTAL PHARMACOLOGY.—The Registrar, University of Strathclyde, Glasgow, G.1 (September 30).

CHAIR OF PHARMACOLOGICAL TECHNOLOGY.—The Registrar, University of Strathclyde, Glasgow, G.1 (September 30).

CHAIR OF PSYCHOLOGY at Queen's College, Dundee.—The Secretary, University of St. Andrews, College Gate, St. Andrews, Fife, Scotland (September 30).

COURTESY TUTOR (with several years experience as Senior or Head Technician in a University or College of Technology Department of Biology, Botany or Zoology) for SCIENCE TECHNOLOGISTS in the FACULTY of SCIENCE, The University College, Dar es Salaam, University of East Africa.—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (September 30).

LECTURER/ASSISTANT LECTURER in the DEPARTMENT of GEOGRAPHY, University of Malaya.—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Kuala Lumpur and London, September 30).

POSTGRADUATE SCHOLAR (graduate with capacity for research who wish to proceed to the degree of Ph.D. in the fields of organic chemistry or physical and theoretical chemistry) in CHEMISTRY in the RESEARCH SCHOOL of CHEMISTRY, Australian National University.—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, September 30).

RESEARCH ASSISTANT (with a degree in zoology or in forestry; previous experience in research, preferably involving field studies, would be an advantage) to work with the Lecturer in Forest Zoology on investigations into the ecology and biological control of forest insects.—The Secretary, The University, Aberdeen (September 30).

SENIOR LECTURER and LECTURER in CHEMISTRY; a SENIOR LECTURER in MATHEMATICS, and a SENIOR LECTURER and LECTURER in PHYSICS at Njala University College, Sierra Leone.—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (September 30).

ASSOCIATE PROFESSOR in the DEPARTMENT of GEOLOGY and GEOPHYSICS, University of Sydney, Australia.—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 1).

RESEARCH ASSISTANT (with a degree in a biological subject) in the DEPARTMENT of BIOMECHANICS and SURGICAL MATERIALS at the Country Section, Royal National Orthopaedic Hospital, Stanmore, Middlesex, for work concerned with a study of the biological and mechanical properties of bone and orthopaedic implants.—The Dean, Institute of Orthopaedics, Royal National Orthopaedic Hospital, 224 Great Portland Street, London, W.1 (October 10).

RESEARCH STUDENT (with an honours degree or equivalent in metallurgy or physics) in the DEPARTMENT of PHYSICAL METALLURGY for the study of the reinforcement of metals by alumina fibres.—The Secretary to the Department of Physical Metallurgy (Ref. O.J.B.), The University, Birmingham, 15 (October 11).

ASSISTANT EXPERIMENTAL OFFICER (graduate in a biological science, pure or applied, with an interest in field work) in the NEMATODE DEPARTMENT, to assist in work on nematode injurious to cereals and their control.—The Secretary, Rothamsted Experimental Station, Harpenden, Herts, quoting Ref. 1052/80 (October 15).

CHAIR of PHARMACOLOGY at the University of Singapore.—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (October 15).

CHAIR of BOTANY.—The Deputy Secretary, The University, Southampton (October 30).

LECTURER (capable of lecturing in general psychology and in psychological testing or psychological theory or vocational psychology, and with a higher degree and some teaching experience) in the SCHOOL of APPLIED PSYCHOLOGY, University of New South Wales, Australia.—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 30).

CHAIR of ELECTRONICAL ENGINEERING at the University of Tasmania, Australia.—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 31).

ASSISTANT EDITOR (with a first degree in science, preferably young university graduate wishing to make a career in scientific journalism; previous experience in editing not required) for Nature.—The Manager, Nature, Macmillan (Journals) Ltd., 4 Little Essex Street, London, W.C.2.

ASSISTANT LECTURER (interested in a research career) in the DEPARTMENT of HUMAN BIOLOGY and ANATOMY, for duties which will include teaching of gross anatomy.—Prof. R. Barrer, Department of Human Biology and Anatomy, The University, Sheffield, 10.

BIOCHEMIST, Senior or Basic Grade.—The Secretary, Althincham General Hospital, Althincham, Cheshire.

CHEMIST or BIOCHEMIST (M.Sc. in chemistry or biochemistry, or a B.Sc. honours and several years research experience), to execute research projects concerned with the metabolism of lipids in groundfish and other selected species.—The Director, Fisheries Research Board of Canada, Halifax Laboratory, P.O. Box 429, Halifax, Nova Scotia, Canada.

DISTRICT FOREST OFFICER (with a degree in forestry, and preferably experience of forest administration) to supervise a district; and a FOREST SURVEY OFFICER (with a degree in forestry and field experience in forest surveys and enumeration) in Zambia.—The Appointments Officer, Ministry of Overseas Development, Room 301, Strand House, Stag Place, London, S.W.1, quoting Ref. RO 234/132/01.

ELECTRON MICROSCOPE TECHNICIAN (with experience of electron microscopy or willing to be trained in the running of an electron microscope)—Dr. R. C. Oaking, Department of Botany, University of Nottingham, Nottingham.

FISHERIES OFFICER (with a degree in natural science and postgraduate experience) in Zambia, to assess the potential commercial production of fish by means of a statistical recording programme and to implement development projects concerned with the catching and marketing of fish.—The Appointments Officer, Ministry of Overseas Development, Room 301, Strand House, Stag Place, London, S.W.1, quoting Ref. RO 236/132/01.

**INFORMATION SCIENTIST** (graduate or equivalent) for a small team on information processing and retrieval, dealing mainly with veterinary, entomological and chemical correspondence and reports—The Personnel Secretary, The Cooper Research Station, Berkhamsted Hill, Berkhamsted, Hertfordshire.

**LECTURER (Grade IIa) IN BIOLOGY IN THE DEPARTMENT OF CHEMISTRY**—The Clerk and Treasurer, Paisley College of Technology, High Street, Paisley, Renfrewshire, Scotland.

**RESEARCH ASSISTANT** (preferably with experience in blood/gas measurements) for a new laboratory associated with the Department of Anaesthetics for work concerned with respiratory blood and gas abnormalities—The Clerk to the Governors, St. Bartholomew's Hospital, London, E.C.1, quoting Project No. 802.

**RESEARCH ASSISTANTS IN THE DEPARTMENT OF MATHEMATICS**—Prof. W. N. Eversitt, Department of Mathematics, Queen's College (University of St. Andrews), Dundee, Scotland.

**RESEARCH ASSOCIATE** (experimental physicist, preferably with some experience of scintillation counting or spark chambers) in the DEPARTMENT OF PHYSICS, for work involving developing and applying a method for investigating properties of the solid and liquid state using nuclear physics techniques—The Deputy Secretary, Westfield College (University of London), London, N.W.3.

**RESEARCH OFFICER, ENTOMOLOGIST** (male or female, with a good honours degree in entomology or zoology with specialised training in entomology, and preferably experience of insect physiology or insect control) at the Hagerman Institute for Trypanosomiasis Research for laboratory and field investigation relating to the control of tsetse flies—The Crown Agents, M. Dept., 4 Millbank, London, S.W.1, quoting Ref. M357/60362/TA.

**SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER** (graduate with a first- or second-class honours degree in biochemistry or chemistry) in the NUTRITION DEPARTMENT, to work on biochemical aspects of nutrient utilization in experimental animals—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, Berks, quoting Ref. 65/14.

**SENIOR RESEARCH ASSOCIATE IN RADIATION CHEMISTRY** to study biochemical problems of nucleic acids in collaboration with Prof. J. J. Wells—The Registrar, The University, Newcastle upon Tyne.

**SENIOR RESEARCH ASSOCIATE** (Post-doctoral) (physicist, physical chemist or chemical engineer) in the DEPARTMENT OF CHEMICAL ENGINEERING, to investigate gas phase reactions promoted by electrical discharges in continuous flow reactors—Prof. J. D. Thornton, The University, Newcastle upon Tyne.

## REPORTS and other PUBLICATIONS

### Great Britain and Ireland

Department of Education and Science and The British Council. Scientific Research in British Universities and Colleges, 1964-1965. Vol. 1: Physical Sciences. Pp. xix+368. 37s. 6d. net. Vol. 2: Life Sciences. Pp. xviii+404. 40s. net. (London: H.M. Stationery Office, 1965.) [307]

*Molecular Pharmacology*, Vol. 1, No. 1 (July 1965). Edited by Avram Goldstein. Pp. 1-112. (A publication of the American Society for Pharmacology and Experimental Therapeutics.) Subscription rates: Vol. 1, 1965, 3 issues—Institutional subscribers, 11 dollars; Private subscribers certifying that the journal is for personal use only, 5 dollars. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) [48]

Department of Scientific and Industrial Research. Memoirs of the Geological Survey of Great Britain—England and Wales. Geology of the Country around Wells and Cheddar (Explanation of One-inch Geological Sheet 580, New Series). By G. W. Green and Dr. F. B. A. Welch. Pp. x+235+5 plates. (London: H.M. Stationery Office, 1965.) 47s. 6d. [58]

The Overseas Development Institute. Aid to Education. An Anglo-American Appraisal. (Report of a Ditchley Foundation Conference held at Ditchley Park, 20-29 March 1965. Rapporteur: Peter Williams.) Pp. 52. (London: The Overseas Development Institute, in association with The Ditchley Foundation, 1965.) 2s. 6d. [58]

British Broadcasting Corporation. BBC Engineering Monograph No. 57: Drop-Out in Video Tape Recording. Part 1: The Subjective Impairment Produced by Drop-Outs. Part 2: A Simple Drop-Out Compensator for Video Tape Recorders. By W. K. B. Geddes. Pp. 15. (London: British Broadcasting Corporation, 1965.) 5s. [58]

Annals of the Library Association 1877 to 1960. Edited by W. A. Munford. Pp. 153. (London: The Library Association, 1965.) 22s.; (L.A. members 16s. 6d.) [58]

Antimalarial Prophylaxis in Primary Schools in Eastern Nigeria, February-March 1965. By J. Hay Arthur. Pp. 41. (London: Wellcome Museum of Medical Research, 1965.) [58]

Science Research Council. Radio Research 1964: The Report of the Radio Research Board and the Report of the Director of Radio Research. Pp. vi+83+4 plates. (London: H.M. Stationery Office, 1965.) 4s. 6d. net. [58]

Out of Control. By Prof. H. A. Prime. (An Inaugural Lecture delivered in the University of Birmingham on 11th May 1965.) Pp. 15. (Birmingham: The University, 1965.) 2s. 6d. [58]

The University of Leeds. Publications and Titles of Theses, 1963-65. Pp. 86. 2s. 6d. Publications and Titles of Theses, 1963-64. Pp. 97. 2s. 6d. (Leeds: The University, 1965.) [58]

The Edinburgh School of Agriculture. Calendar, 1965-1966. Pp. 67. (Edinburgh: The Edinburgh School of Agriculture, 1965.) [58]

Nuclear Studies, List No. 9. Compiled by the Science Department, The British Council. Pp. 36. (London: The British Council, 1965.) [58]

Bulletin of the British Museum (Natural History). Entomology. Supplement 2: A Reclassification of the Tribe Microgasterini (Hymenoptera: Braconidae). By G. B. J. Nixon. Pp. 254. 120s. Supplement 3: A Revision of the Ethiopian Drepanidae (Lepidoptera). By A. Watson. Pp. 177+18 plates. 84s. (London: British Museum (Natural History), 1965.) [58]

University College of Wales, Aberystwyth. Report of the Welsh Plant Breeding Station for 1964. Pp. 153+2 plates. (Aberystwyth: Welsh Plant Breeding Station, 1965.) [58]

Fluoridation. Pp. 1+14. (London: Ministry of Health, 1965.) [58]

Ministry of Housing and Local Government—Scottish Development Department. Alkali, etc. Works Regulation Act, 1906; and Alkali, etc. Works Orders, 1928-1963; Alkali, etc. Works Regulation (Scotland) Acts, 1906 and 1961; and Alkali, etc. Works (Scotland) Orders, 1963 and 1965. One-Hundred and First Annual Report on Alkali, etc. Works by the Chief Inspectors, 1964. Pp. iv+76. (London: H.M. Stationery Office, 1965.) 5s. 6d. net. [58]

### Other Countries

Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture. Comptes Rendus de Recherches, No. 33: Travaux du Comité pour l'Etude des Maladies et de l'Alimentation du Bétail (O.R.M.A.). Pp. 129. (Bruxelles: Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, 1965.) 250 francs. [167]

Australia: Commonwealth Scientific and Industrial Research Organisation. Division of Forest Products—Programme of Work 1965-66. Pp. 124. (South Melbourne: Division of Forest Products, C.S.I.R.O., 1965.) [167]

Smithsonian Miscellaneous Collections, Vol. 149, No. 4: An Endocranial Cast of the Bridger Middle Miocene Primate, *Smilodectes gracilis*. By C. Lewis Gazin. Pp. 14+2 plates. (Publication 4616.) (Washington, D.C.: Smithsonian Institution, 1965.) [167]

Institut Royal Météorologique de Belgique. Annuaire—Magnétisme Terrestre, 1964. Pp. 66. Bulletin Mensuel—Observations Ionosphériques, Mai 1965. Pp. 25. (Uccle-Bruxelles: Institut Royal Météorologique de Belgique, 1965.) [167]

University of Ibadan. Calendar 1964-65. Pp. 199. (Ibadan: The University, 1965.) [167]

Tableaux Généraux des Mémoires de l'Académie Royale des Sciences, des Lettres et des Beaux-Arts de Belgique, Supplément: 1898-1963. Par Paul Jeanlot. Pp. 159. (Bruxelles: Académie Royale des Sciences, des Lettres et des Beaux-Arts de Belgique, 1965.) [167]

Transactions of the Royal Society of New Zealand. Zoology, Vol. 5, No. 14 (February 10, 1965): Studies on the Biology of the Redfinned Bully *Gobiomorus dormitor* (Ogilby). 2: Breeding and Life History. By R. M. McDowall. Pp. 177-190. (Wellington: Royal Society of New Zealand, 1965.) [167]

United States Department of Agriculture. Agricultural Research Service. Miscellaneous Publication No. 987: A Selected Bibliography of the Oecoides—First Supplement. Pp. 44. (Washington, D.C.: Government Printing Office, 1965.) [167]

Council Permanent International pour l'Exploration de la Mer, Service Hydrographique, Charlottenlund-Slot, Denmark. 1088 Oceanographic Data List, 1958, No. 6. Pp. xiii+199. (Copenhagen: Andr. Fred. Høst & Søn, 1965.) 20 Kr. [167]

A Review of Agricultural Progress in the Nelson Land District 1950-1963. By Theodore Hagg. Pp. 89. (Nelson, N.Z.: Oarworth Institute, 1965.) [167]

Royal Ontario Museum—University of Toronto. Annual Report: 14, July 1963-June 1964. Pp. 16. (Toronto: Royal Ontario Museum—University of Toronto, 1965.) [167]

Publications of the Dominion Observatory, Ottawa. Vol. 23: Bibliography of Seismology—Author Index 1967-1963, Items 9638-15838. By R. K. Dubuc. Pp. 363-386. (Ottawa: Queen's Printer, 1965.) 1 dollar. [167]

New Zealand Forest Service: Forest Research Institute. Technical Paper No. 46: The Strength of New Zealand Radiata Pine Poles. By C. R. Hellawell. Pp. 36. (Wellington: Government Printer, 1965.) [167]

American Association for the Advancement of Science. Miscellaneous Publication 65-2: Science Books—A Quarterly Review, Vol. 1, No. 1 (April 1965). Pp. 1-56. Subscription rate: 4.50 dollars per year; Single copies 1.24 dollars. (Washington, D.C.: American Association for the Advancement of Science, 1965.) [167]

Smithsonian Miscellaneous Collections, Vol. 148, No. 3: Upper Cambrian Trilobite Faunas of Northeastern Tennessee. By Franco Rasetti. Pp. 127+21 plates. (Washington, D.C.: Smithsonian Institution, 1965.) [167]

Académie Royale des Sciences, des Lettres et des Beaux-Arts de Belgique. Index Biographique des Membres, Correspondants et Associés de l'Académie Royale de Belgique de 1789 à 1963. Pp. 390. (Bruxelles: Académie Royale des Sciences, des Lettres et des Beaux-Arts de Belgique, 1964.) [167]

Publications de l'Université Officielle du Congo à M'banza-Bénoué. Vol. 8 (Juillet 1965): Introduction à l'Etude du Développement Économique des Théories Générales. Par Akio Otschella. Pp. 156. (M'banza-Bénoué: Université Officielle du Congo à M'banza-Bénoué, 1965.) [167]

Académie Royale de Belgique. Mémoires, Tome 34: Mécanismes Physiques dans les Poudres Electroluminescentes. Par B. Goffaux. Pp. 40. (Bruxelles: Académie Royale de Belgique, 1965.) 100 francs. [167]

Transactions of the Royal Society of New Zealand. Zoology, Vol. 5, No. 17 (February 19, 1965): Studies on the Biology of the Red-finned Bully *Gobiomorus dormitor* (Ogilby). Part 3: Food Studies. By R. M. McDowall. Pp. 233-254. (Wellington: Royal Society of New Zealand, 1965.) [167]

Istituto Geodisco e Geodisco, Università di Genova. Radiogeomorfologia Investigation of Thunderstorms and Clouds of Great Electrical Activity (On Large Thunderstorms in the Mediterranean Area). (Final Technical Report.) By Mario Boscolusso et al. Pp. 16. (Genova: Università di Genova, 1965.) [167]

United States Department of the Interior. Geological Survey. Bulletin 1181-Q: Geology of the John and Williams Hill Quadrangles, Monterey County, California. By David L. Durham. Pp. iv+27+plates 1-3. Bulletin 1190-D: Barite Deposits of the Margaret District, Alabama. By H. B. Bergquist and Elizabeth F. Overstreet. Pp. iv+23+plates 1-3. Bulletin 1190-K: Barite Deposits of Virginia. By Walter C. Warren, Josiah Bridge and Elizabeth F. Overstreet. Pp. iii+17+plate 1. Professional Paper 802-A: Stratigraphy of the Pierre Shale, Valley City and Felmira Mountain Areas, North Dakota. By James R. Gill and William A. Cobban. Pp. iii+20. Professional Paper 455-G: Structural Control of Uranium-Bearing Vein Deposits and Districts in the Conterminous United States. By Frank Osterwald. Pp. iii+151+140. 30 cents. Professional Paper 493: Trilobites of the Late Cambrian Pteroccephalus Biome in the Great Basin, United States. By Allison R. Palmer. Pp. iv+106+23 plates. Professional Paper 508-B: Some Western American Cenozoic Gastropods of the Genus *Nassarius*. By W. O. Addicott. Pp. iii+24+3 plates. (Washington, D.C.: Government Printing Office, 1965.) [167]

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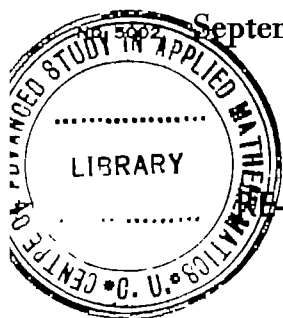
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ORGANIZATION OF SCIENCE AND TECHNOLOGY  
IN RETROSPECT

FOR all that its criticism is restrained, the new Departments and Ministries do not come too well out of the scrutiny which the Estimates Committee has given them in its third report for the Session 1964-65\*, nor particularly does the Ministry of Technology, from the evidence taken before Sub-Committee G. This is the more apparent in that the Estimates Committee is obviously unwilling to press a new Department hard. Concerning the Ministry of Overseas Development, for example, it comments on the relatively small numbers of staff who have been transferred from other Departments surrendering functions to the new Ministry and on the small size of the further staff savings anticipated in some of these Departments. Even so, the view that the savings would only be achieved after a fairly long process of internal re-organization was unacceptable to the Committee.

Here the prodding by the Estimates Committee is addressed to the Treasury, as also is a further comment on the Diplomatic Service. The present reserve of manpower is 280, representing about 4 per cent of the complement of home-based staff; however, this is expected to rise to 435 or about 6.5 per cent of complement, as compared with the 7.5 per cent fixed by the Government following the Plowden Report. The division of this reserve between home and overseas staff was admitted to be somewhat arbitrary and the Estimates Committee recommends that the total strength of the reserve should be shown separately under the heading "Common Services in the Home Organization". It also recommends closer liaison between the inspectorate responsible for reviewing overseas posts at least once every three years and the Organization and Methods Section and that interchange between the two should be encouraged.

To the scientist, as to the technologist, the interest of the Estimates report, however, lies essentially in the observations on the Departments of Education and Science and of Technology, and on the Ministry of Land and Natural Resources. However, the memorandum submitted on behalf of the Minister of Overseas Development describing the organization and staffing of that Ministry is of considerable interest and shows that the staff in some of the special units has been strengthened for 1965-66: that of the Directorate of Overseas Surveys has risen from 515 in October 1964 to 525; of Overseas Geological Surveys from 106 to 123; of the Anti-Locust Research Centre from 66 to 71. There are equally welcome memoranda outlining the functions and organization of the Ministry of Technology and of the Ministry of Land and Natural Resources, but not of the Department of Education and Science. This omission is surprising in that one of the first points of concern to the Estimates Committee was whether a clear line had been drawn between those functions appropriate to the Science side of this Department and those appropriate to the Ministry of Technology.

Staff numbers in the Science side of the Department have risen since 1964 from 107 to 124, mainly through the establishment of the Office of Scientific and Technical Information and of the new Council for Scientific Policy. The Department has now become the accounting Department for the Votes of all four Research Councils—the Medical Research Council, the Agricultural Research Council, the new Natural Environment Research Council and the new Science Research Council. It has been agreed with the Treasury, however, that the accounts of each Council, signed by its chief official, will be annexed to the Estimates of the Department of Education and Science. The Vote for the new Science Research Council, which has a staff of nearly 2,000, amounts to more than £28 million in the current financial year, and besides its responsibility for the support of university research, formerly entrusted to the Department of Scientific and Industrial Research, it is responsible for the control of the Royal Observatories and the Rutherford and Dewesbury Laboratories for Nuclear Physics. Its Headquarters staff is expected to increase from about 200 to 250 during the current financial year, but the increase is partly due to Treasury reluctance to allow recruitment of new staff pending the re-organization.

As a result, the Council faces a temporary shortage of staff which at present prevents it from carrying out more than essential day-to-day functions. The Estimates Committee's hope that this situation will be speedily rectified will be shared by scientists generally, who may also be concerned that in the meantime the present credit squeeze may have made rectification more difficult. Apart from this, however, the Committee, in this report, does not probe further into the functions of the Department of Education and Science. Behind its comments as to the vital necessity of keeping the Department and the Ministry of Technology fully informed of each other's scientific activities may be sensed a certain uneasiness as to how far a broad division of responsibility between basic research and applied research may really be practicable.

Clearly there are likely to be problems and difficulties; however, given goodwill these will be removable, but if there is any cheeseparing and misguided attempt to cut for economy it is likely that they will become accentuated. It is not, however, at this aspect of the Ministry of Technology that the Estimates Committee looked in the first instance, although it is highly critical of its organization which is regarded as top-heavy, even when allowance is made for this being a common tendency in the early stages of a new Ministry. The Ministry is at present divided into six administrative divisions, responsible for financial administration and general policy, and two departments responsible to Controllers and responsible for the scientific and technological aspects of the work of the Ministry. It is intended that there should be a partnership between the two sides of the Ministry with consultation at all levels; of the present Headquarters staff of about 500, 368 were formerly employed at the headquarters of the

\* Third Report from the Estimates Committee together with Part of the Minutes of the Evidence taken before Sub-Committee G on 14th April, 1965, and Following Dates, and Appendices, Session 1964-65—Variations in Estimates. Pp. xiv+80. (London: H.M.S.O., 1965.) 10s. 6d.

Department of Scientific and Industrial Research. A net increase of 190 in headquarters staff is expected by the end of the year and the total staff of the establishments under the Ministry is nearly 5,000.

Where the Estimates Committee is critical is in the appointment of two Controllers as well as two Deputy Secretaries, one of whom is a scientist and a former member of the scientific officer class and was assisting the Permanent Secretary in supervising the laboratories transferred from the Department of Scientific and Industrial Research. The Committee saw no reason for four such posts and shortly before the report was finally agreed learned that it had been decided that there should be only one part-time Controller responsible for all the non-secretariat functions of the Ministry. It still recommends, however, that the Treasury should further examine the need for a second Deputy Secretary.

The Estimates Committee notes that while the headquarters of the Ministry will be in London it is establishing regional offices in each of the newly formed regions as soon as the necessary staff are recruited. Three of these, in Edinburgh, Cardiff and Newcastle, were inherited from the Department of Scientific and Industrial Research, and the main function of the offices is to advise industry on the appropriate sources from which it can obtain advice and assistance. The Industrial Liaison Service, which originated in Scotland, is also being expanded and provision in the current year is expected to be £63,000 as against £35,000 in 1963-64. The Committee did not look closer either at this Service or at the functions of the regional offices; in this field of information and advice there appears to be an inherent possibility of overlap with the work of the Office for Scientific and Technical Information within the Department of Education and Science: here again close contact between the two Ministries seems essential.

More serious, however, is the Estimates Committee's criticism of the approach of the new Ministry to what Mr. Cousins has insisted on as one of its most important new functions—that of initiating technical and economic studies in depth of selected industries and processes to determine whether action is needed and if so in what direction. The four such studies so far announced involve the electronics and telecommunications industries, which were formerly sponsored by the Ministry of Aviation and the Post Office, respectively, and the computer and the machine tool industries, both formerly sponsored by the Board of Trade. The Committee was not satisfied that the teams being set up for these initial studies had the appropriate membership or that they were being set up with sufficient speed; the minutes of evidence suggest that on both points the Estimates Committee's comments are very mild. That some at least of the investigators should have had up-to-date experience of the industry in question seems a pre-requisite: that they should have been drawn mainly from within the Government service seems as indefensible as the dilatoriness with which the teams are being appointed.

Nor is this all. Apart from the uncertainty which such procedures are likely to cause in the industries concerned, the Estimates Committee has doubts as to the work of these appraisal teams overlapping or duplicating studies which are being carried out under the aegis of the Department of Economic Affairs. It would appear that the Committee was not entirely satisfied by the assurances it received as to close contact and liaison between the Ministry of Technology and the Economic Development

Committee, which is itself studying the economic performance of the machine tools industry.

The debate on the Civil Estimates for the Ministry of Technology in the House of Commons on July 14 was held immediately before the Estimates Committee's report was published, but Mr. Cousins scarcely touched on the points raised in this report dealing with the position of the Atomic Energy Authority and the National Research Development Corporation. Of the Authority's Trading Fund, he stated that sales of fuel elements, electricity and isotopes amounted to some £30 million per annum, and of hovercraft developments that some ferry services using the smaller craft were already running, while firm orders had been received for at least seventeen and possibly eight more. Generally the debate was unsatisfactory and inclined to make party points in spite of several suggestions that technology should not be a matter of party politics. However, both Sir Lionel Heald and Mr. R. Sheldon raised the question of the delay in starting the new reference library for science and invention, as well as raising the question of its location. Sir Lionel was concerned that the Patent Office should remain in London. The best part of the debate was, in fact, that in which questions of the structure of Government generally were discussed rather than the particular organization of the Ministry of Technology.

The Estimates Committee in its report also considers the Ministry of Land and Natural Resources, and while the memorandum from that Ministry, already noted, refers to the existence of a Natural Resources Planning Unit, it is curious that the Committee does not comment on the need for appropriate liaison and contact with both the Ministry of Technology and the Department of Education and Science, within the orbit of which falls the newly established Natural Environment Research Council (of which the Nature Conservancy now forms a Committee). The potentialities for overlap and confusion are as implicit as they are between the Ministry of Technology and the Department of Education and Science.

While the Estimates Committee is not entirely happy about the present arrangements, its misgivings find expression in regard to two matters only—staff and the sharing of responsibility for water between the new Ministry, the Ministry of Housing and Local Government and the Welsh Office. Welcoming, however, the statement of January 19 that the present arrangements for Ministerial responsibility are to be kept under review to see whether experience in implementing them calls for any change, the Committee makes no specific comment other than that such evidence as came before it suggests that more experience will be needed before a final decision can properly be made about ministerial responsibility for water.

On staff, it is noted that about half the present staff of 150 was seconded to the Ministry along with their former work; the total is expected to increase by 20 by the end of the financial year, partly to set up the Land Commission, partly to strengthen the section dealing with the Ministry's responsibility for water, and partly to staff the expanding work of the Natural Resources Planning Unit, which is working in close association with the new Natural Resources Advisory Committee. It is visualized that this Unit will have an administrative scientist and a professional scientist as joint heads, but it does not appear from the evidence that the structure is much more than tentative, although it is expected that the Natural Resources Advisory Committee, of which Sir Dudley



Stamp is chairman, will advise on the extent to which use could be made of the universities and other institutions. At present the established staff of the Department are on loan from their former Departments, and although the arrangement is regarded as temporary it remains to be seen whether the Department will become large enough to provide adequate opportunities for a career.

The Estimates Committee does not think that this situation should be allowed to continue for any appreciable length of time and, while the point is not pressed, the report leaves the general impression that more regard might well be paid to the point of view of staff in other new Ministries. Conditions of service can make all the difference to efficiency and enthusiasm at a time of change and experiment when a certain amount of flexibility is desirable; as a consequence some uncertainty is unavoidable. What above all emerges from this survey of the new Ministries is the undesirability of making some of the arrangements other than tentative until they have been firmly proved by experience. It is equally clear that the misgivings with which some of them are regarded are well founded and not to be dismissed as party prejudices. The Ministries would do well to heed the restrained criticism of the Estimates Committee and to respond to constructive suggestions. Especially in this matter of staff, they should spare no pains to establish full confidence and to provide the conditions of service which will encourage full and effective co-operation between departments and sections and the keenness and interest that are vital where science and technology are concerned. To persist in impeccable self-satisfaction such as Mr. Cousins presented to the House of Commons on July 14 would be fatal.

## DESALINATION OF SEA WATER

### Fresh Water from the Sea

Proceedings of the International Symposium held in Milan by Federazione Delle Associazioni Scientifiche e Tecniche and Ente Autonomo Fiera di Milano. Edited by A. Giralli. Pp. xxii + 179. (Oxford, London and New York: Pergamon Press, 1965.) 60s.

WITH the increase in world population, the spread of urbanization with its accompanying industrial development, as well as the higher standard of hygiene in the emergent nations, the demands for water both potable and industrial are ever on the increase. The supply, on the other hand, by precipitation from the sky is limited with a yearly average of some 750 mm. It is thus natural that the attention of several nations is being drawn to the problems of water conservancy and the production of potable water from the sea and other brackish or otherwise unsuitable waters. Indeed, water shortages are already manifest over many regions of the globe, and in all industrial areas water availability is fast becoming a factor of major importance in the location of factories. It has been computed that it takes 65,000 gal. of water to make a ton of steel; 5,000 gal. for every ton of sulphuric acid; while a single copy of one American Sunday newspaper consumes half a ton of water, and no less than 5 tons of water are required to make the synthetic fibres for a summer suit.

Since the production of potable water confronted shipping lines at an early date this problem has received more attention than that of water conservancy. Some seventy per cent of the annual precipitation is entirely wasted, either by evaporation or passage into drains, and eventually into rivers; thus it is to be hoped that economies in this field can be effected.

*Fresh Water from the Sea* gives an account of a symposium held in Milan in which the state of development of

desalination in a number of countries is described. These include Holland, Japan, Germany, Great Britain, Italy, Israel and the United States. Some of the conclusions result from a world-wide survey under the aegis of the United Nations Department of Economic and Social Affairs, and a very full bibliography completes the volume.

The majority of processes involve distillation, but the early form of direct heating stills has given place to flash methods as well as that using a falling film or compression with forced circulation for the vapour. Interesting formation is given by the authorities in the different countries on the chemical engineering, power sources, and distribution problems involved in the construction and operation of such plants.

While reverse osmosis is theoretically probably the most efficient process from thermal considerations, no effective plant has been constructed as yet on these lines. On the other hand, several plants utilizing electro-osmotic methods are in operation and on a relatively small scale at least are almost competitive with distillation processes. The successful development of such plants has been made possible by the fairly exhaustive enquiries which have been made into the properties of ion-exchange membranes and their technical production on a large scale. It seems doubtful whether these electrolytic plants are economic in the production of pure water, but these, combined with a subsequent treatment by ion-exchange resins, offer some hope of a successful method. The more recently developed weakly acid and weakly basic resins present the possibility of extending the ion-exchange methods from the treatment of brackish to saline waters.

Brief mention is made of some other methods by which conversion of salt to potable water might be achieved on a large scale: these include fractional crystallization of ice and its separation by centrifugal methods, the utilization of cryohydrates for a similar purpose and finally the use of solar energy for evaporation.

The various sections are written in English, German and Italian and give very readable accounts, containing but a minimum of highly technical material, of the progress which is being made in this at present important and, in the near future, very necessary field of human endeavour.

E. K. RIDGAL

## EDUCATION AND VALUES

### Education and Values

Essays in the Theory of Education. By G. H. Bantock. Pp. 182. (London: Faber and Faber, Ltd., 1965.) 25s. net.

*EDUCATION and Values* consists of a collection of essays which concern contemporary discussions and controversies in education. The author, whose outlook is predominantly literary, bases his ideas on T. S. Eliot's attempt to define a culture for the twentieth century. Prof. Bantock has an Ayer-like consideration for the nature and meaning of words, he shows a close concern for the language of education discourse and reveals some of the ambiguities and inadequacies of present-day formulations.

The subjects treated include the relation of education and society, the problem of freedom in education, the influence of educational sociologists and the role of literary intelligence, where it exists, in assisting thought in education. Of particular interest is Prof. Bantock's diagnosis of what is the trouble with so many investigations which are glorified with the title of "Research in Education". This short section could have been expanded with advantage, for his criticisms could have been extended to many investigations of a sociological nature. This is not to say that we expect the same degrees of accuracy, which are commonplace in the physical sciences, but that there must be a more intelligent assessment of subjective factors, some of a subtle nature, which are involved in researches in education and sociology. More is required than an application of suitable statistical techniques.



"The discipline of social research has not succeeded in creating a specialized language of its own; indeed, in view of the nature of its subject matter is unlike that of the physical sciences in that it deals with what already has meaning apart from that assigned to it by the scientist, it is that the social sciences will never succeed in creating a fully technical vocabulary. Since, then, the statement of research topics usually involves the use of words common in everyday usage, the prime requisite in the consideration of research problems is the conceptual clarification of the terms employed, at least to the extent that possible ambiguities are cleared up or that ostensive definitions are offered. It is disturbing to find how little modern philosophical techniques of linguistic analysis and clarification have affected our thinking about social science research; yet as a preliminary to any such research it is important at least to decide what questions involved are really conceptual and what empirical."

If Prof. Bantock's criteria are applied to much which has passed as research in sociology and education and has been published, the value of the results is seen in a much dimmed light; indeed, if it is seen at all. No doubt the large advertising firms base their schemes and expenditure on more skilful investigations. The remedy is not so much to take a leaf from the book of the more successful research scientists in the physical and so-called 'applied sciences', but to develop a more critical and subtle series of experimental techniques peculiar to the social sciences, beginning by clearing the waste-land, as Linnaeus did many decades ago in biology, by proper semantics which lead to more precise methods of description and classification.

The greater part of Prof. Bantock's book deals with the aims and philosophy of education. This is stimulating, timely and few would contradict him. His chapters constitute a warning against the technocratic state, when everybody therein ceases to be a person, as many do even now, and becomes a number of holes punched in an IBM card, stored in some state-controlled records office.

W. L. SUMNER

## IMMUNOGLOBULIN TURNOVER IN MAN

Metabolism of Human Gamma Globulin.

By Stig Bryde Andersen. Pp. x+139. (Oxford: Blackwell Scientific Publications, 1964.) 30s. net.

**A**NTIBODIES in human serum are known to be associated with at least 3 distinct but related immunoglobulins ( $I_g$ ); these are now generally referred to as  $I_gG$  (7 S  $\gamma$ -globulin),  $I_gA$  ( $\gamma_1A$ -globulin) and  $I_gM$  (19 S  $\gamma$ -globulin). These proteins may carry identical combining specificities but their biological effects are modified by differences in their distribution in the body. For example, the three classes of antibody occur in widely different relative amounts in various tissue fluids and secretions; they also vary in their transplacental passage from maternal to foetal circulations and in their capacity to attach to various tissues, including the skin. In addition, the immunoglobulins differ in their rates of overall synthesis and catabolism, and in their sequence of synthesis during the course of development and after antigenic stimulation. In pathological states the different immunoglobulins may react independently and this is seen most strikingly in proliferative disorders of the lymphoid system such as myelomatosis and macroglobulinaemia.

A comprehensive account of what is known of the distribution and metabolism of immunoglobulins in health and disease would form a welcome contribution to the immunological literature. Dr. Andersen's book is far more limited in scope. He describes results obtained with one class of immunoglobulin, namely  $I_gG$ , using a single experimental technique to measure overall distribution and turnover in a large series of patients. The labelling of  $I_gG$  with

<sup>125</sup>I was carried out by techniques which have been developed and meticulously tested by Dr. A. S. McFarlane, and the quantitative interpretation of data is based on analytical methods elaborated by Dr. Christine Matthews. An appendix contains an outline of several alternative methods of analysis and includes a discussion of the significance of the iodide pool in calculations of iodine labelled protein turnover. Results are presented for hospitalized patients considered normal and for two other groups of subjects with increased and reduced levels of  $\gamma$ -globulin respectively. The findings re-emphasize the fact that hypergammaglobulinaemia is always associated with an increased rate of  $\gamma$ -globulin synthesis and seems never to result from reduced catabolism. Similarly, hypogammaglobulinaemia is usually the result of underproduction, although it may be due to abnormal loss of  $\gamma$ -globulin through kidney or gut sometimes aggravated by an increased rate of  $\gamma$ -globulin catabolism.

This book does not constitute a down-to-earth practical guide for anyone wishing to set up the techniques employed in labelled protein turnover experiments. However, there is a detailed bibliography and the data illustrate the clinical applications of a method which has elucidated some aspects of the exceedingly complex problem of  $\gamma$ -globulin metabolism.

S. COHEN

## THE SMOKER'S DILEMMA

Smoking, Health and Personality

By Prof. H. J. Eysenck. Pp. 166. (London: Weidenfeld and Nicolson, 1965.) 25s. net.

**T**HE hypothesis that lung cancer and the habit of smoking cigarettes may both be due to a common genotype was first suggested by R. A. Fisher. Eysenck has now pressed into the service of this 'constitutional hypothesis', as it is known, the assumption that the common genotype is an extraverted personality. He defines an extravert as a person who gives appropriate answers to such questions as: "Do you usually stay in the background at parties and get-togethers?" "Do you find it hard to really enjoy yourself at a lively party?" "Can you usually let yourself go and enjoy yourself a lot at a gay party?" "Do you hate being with a crowd who play jokes on one another?" and he claims that strain differences in rats similar to those in man can be established with respect to extraversion and introversion. (One wonders how many housewives and boilermakers have sufficient time to go to enough parties to enable us to decide whether they are extravert. It is also rather hard to imagine how a rat is expected to conduct himself at a gay and lively get-together or what a rat's joke would consist of.)

By his dogma that the lung cancer linked with cigarette smoking is due to innate extraversion, Eysenck would have us believe that the genetic composition of many countries, especially in urban areas, has changed uniformly towards extraversion during the past half-century. Furthermore, he assures us that "men are very slightly more extraverted [than women] although the difference is too slight to mention". If so, and if lung cancer is a disease of extraverts, why should the lung cancer sex ratio have changed from 1.5 to 1 in 1930 to more than 6 to 1 in 1961?

He begins with a discussion of methods of study in microbiology and bacteriology, topics in which it is unusual for a psychologist to display special competence. This is followed by an evaluation of epidemiological investigations which, he asserts, "can give wrong conclusions as readily and as easily as correct ones"—a dictum which is demonstrably either trivial or untrue. He goes on to consider alleged criticisms of the smoking-lung cancer hypothesis. Here, guidance from a medical statistician would have helped him to avoid an error in stating the difference between expected and observed

mortality from lung cancer. On p. 40, when it is a question of a correlation between smoking and lung cancer, Eysenck (quoting Thurstone) describes a correlation coefficient as a confession of ignorance. On p. 80, when referring to his questionnaires, he declares approvingly that the similarity between two traits is assessed by "a mathematical technique known as correlation". Moreover, while he insists that correlation is no evidence of causality when dealing with 'smoking' he has no hesitation in interpreting correlation as causality when incriminating 'smoke'. For in Chapter 7 he seems to abandon extra-version and brings forward a "logical indictment of smoke". But he soon leaves smoke and comes down in favour of insufficient enzymatic fermentation in the processing of cigarettes as the cause of lung cancer.

As for breaking the habit of smoking, the method to use is "quite obviously to have punishing consequences follow the smoking of a cigarette *before* the rewarding consequences have any chance to establish themselves". This can be achieved by the aid of an apparatus called a ventilator heater which blows hot air at the smoker "while he pronounces the auto-suggestive phrase 'I want to give up smoking'". Eysenck concludes: "There is no reason why society should be allowed to poison our lungs, but every reason why we should be allowed to poison our own if we want to".

JOHN COHEN

## STIELTJES'S MOMENT PROBLEM

### The Classical Moment Problem

And some Related Questions in Analysis. By N. I. Akhiezer. Translated by N. Kemmer. (University Mathematical Monographs.) Pp. x+253. (Edinburgh and London: Oliver and Boyd, 1965.) 70s.

EQUIMOMENTAL systems show that different line-density distributions may have the same mass, centroid (first moment) and moment of inertia (second moment). Stieltjes (1894) asked if a knowledge of all the higher-order moments would allow and imply a unique density; more precisely, given a sequence  $\mu_k$  of numbers, can a non-decreasing function  $\sigma(u)$  be found such that:

$$\int_0^\infty u^k d\sigma(u) = \mu_k. \quad (k=0,1,2, \dots)$$

The problem established the concept of the Stieltjes integral, and gave scope for Stieltjes's mastery of continued fractions. A little earlier, Tehebychev (1873) had discussed a similar problem: given the length, mass, centroid and second moment of a line segment, to find bounds for the mass of any sub-segment; more precisely and more generally, given:

$$\int_a^b u^k f(u) du, \quad (k=0,1, \dots, n-1)$$

to determine bounds for:

$$\int_a^b f(u) du$$

for variable  $v$  in  $(a, b)$ . Tehebychev's interest lay chiefly in the probability application.

Various extensions can be made: the interval may be replaced by a set of points, or extended to  $(-\infty, \infty)$ . Essentially, the main questions are: Does a solution exist; if so, how many significantly different solutions are there; and how can they be constructed?

The field remained relatively unexplored until just after the First World War, when in a burst of activity the first full discussion and solution were given by Hamburger, quickly followed by Nevanlinna, M. Riesz, Hellinger, and Carleman. The problem was seen to have connexions with many branches of analysis; thus Hausdorff, in two masterly papers, showed the relation with summation methods. Later, the development of functional analysis and linear

operators provided new ways of looking at the problems, and new fields for applications and extensions. While many questions remain, so much has been done to codify the theory that the time is ripe for a broad survey. Prof. Akhiezer, who has himself contributed largely to recent progress in this domain, provided such a survey in his 1961 book, of which we now have a welcome translation in the new Oliver and Boyd "University Mathematical Monographs". After a necessary preliminary chapter on sequences and the related orthogonal polynomials and Jacobi matrices, he gives the main results of Hamburger, M. Riesz, and Nevanlinna, often using the present-day language of abstract spaces and functionals, and then deals with the moment problem as an instance of the spectral theory of operators; here the reader must know at least the elements of modern operator theory. The final chapter discusses a trigonometric moment problem, related to Carathéodory's problem about the conformal mapping of the unit circle on a half-plane when a correspondence between two point-sequences is prescribed; this leads on to certain continuous analogues of the moment problem.

The reader who is content to study the main text will obtain a sound and precise knowledge of the principal themes; but to master the subject he will be well advised to spend time and effort on the "addenda and problems" which complete each chapter, for these are not drill examples, but serious (and sometimes difficult) developments and extensions of the main text, relegated to a secondary position simply to reduce bulk. The young research student who appreciates abstract analysis while not despising manipulative skill should find here matter and questioning to his taste.

T. A. A. BROADBENT

## KANTIAN STUDIES

### Experience and Its Systematization

Studies in Kant. By Nathan Rotenstreich. Pp. vii+178. (The Hague: Martinus Nijhoff, 1965.) 21 guilders.

IN this work, the traditionally high standard of the house of Martinus Nijhoff is well maintained, and the author need have no reservations about the desirability of offering a selection of 'pivotal issues' which have become definitive for Kantian scholars. The master spent practically his whole life in his native city of Königsberg analysing empirical knowledge, and erecting on this ratiocination a systematic structure, which, at least in principle, involves ethics and aesthetics. It is a measure of the stature of this intellectual adventure that only comparatively recently has the suggestion been made that these axiological studies might eventually become subject to the power of symbolic logic. If ever they do, then Prof. Rotenstreich will have provided a suitable springboard for the transition, since the leap could scarcely be made from the *Critiques* in their original form, including, as they do, a number of inconsistencies and ambiguities.

For Kant, formal or general logic is almost a calculus for thought without regard to the differences which its objects present, whereas transcendental logic deals with origins—actually of our *a priori* knowledge of objects. This is the point of contact with the Husserl school, and the development of phenomenology, including the realization of empathy. If there is one word which expresses this connexion it is *Anschauung*, or intuition. Kant epistemologically allows time preference over space, although in terms of "true no matter what" they are supposedly equal. If, on the other hand, the criterion is ontological (following Parmenides and Spinoza), then the order is reversed. Twentieth-century physics was in fact to eliminate this distinction in the well-known relativistic prophecy of Minkowski. All of which goes to show the value of a re-assessment of Kant's technique.

In a discussion of the primacy of practical reason, it appears that immortality and God are not implied by

action, whereas duty must contain certain empirical concepts. Strangely parallel with this, it may be mentioned that there is a recorded conversation between F. W. H. Myers and George Eliot (when her *Middlemarch* had just appeared in book form), which took place in the Fellows' Garden at Trinity College, Cambridge. She pronounced God to be inconceivable, immortality to be unbelievable, and "yet how peremptory and absolute" was duty. Whatever may be thought of the first two assertions, it is manifest that Kant's practical sense was subconsciously underlying the Victorian emphasis on 'must'.

The appendix may be styled, in brief, 'from Schopenhauer to Heidegger', and contains an appraisal of the position of the Marburg school, and a reference to Vaihinger's philosophy of *Als ob* ('As if'). Off the mainland of Europe, the latter discipline has been sadly neglected, to the detriment of our grasp of certain fundamental aspects of natural science. Kant's purpose is moral, whereas Vaihinger's aim is practical. His fictions are conscious fictions, forged to secure orientation.

Such questions as these, and many others, are well displayed. There are a few failures to achieve idiomatic English, but considering how closely the author's thesis is argued, the reader gets a clear picture of the Kantian mode.

F. I. G. RAWLINS

## FOUNDATION OF MODERN NETWORK THEORIES

### Tensor Analysis of Networks

By Gabriel Kron. Pp. xxix+635. (London: Macdonald and Co. (Publishers), Ltd., 1965.) 70s. net.

DR. KRON has been, and to a certain extent still is, a centre of controversy, but his genius is now acknowledged and his reputation firmly established. The experience of many years has proved the immense value of his pioneer work to engineering practitioners and teachers as well as theorists. His recent researches are too advanced for most engineers to understand, and some years must elapse before an assured and durable assessment can be made of his total contribution to knowledge.

His first imaginative proposal, made about 30 years ago, was to apply the tensor calculus to electrical engineering. It was brought to the attention of his fellow engineers by a series of articles in the *G.E. Review* entitled "The Application of Tensors to the Analysis of Rotating Electrical Machinery", later revised and republished in book form. Meanwhile he had written *Tensor Analysis of Networks*, a book published shortly after the series ended. Kron refers to these two books as *A.T.E.M.* and *T.A.N.*; they form the basis of all his subsequent work. Their publication immediately evoked vehement and extensive criticism, a little of which was perhaps justifiable, for it could scarcely be expected that so unusual a work would command universal assent; we cannot all accept Kron's approach in its entirety. But much of the criticism was destructive, ill-informed and grossly unfair. Even among those who approved and admired the work there were some who regarded it as an academic exercise of no practical value. If this had been so, the two books would have been isolated publications, whereas they proved to be forerunners of many books by various authors on this topic. For what eventually vindicated Kron and established his high reputation was the belated realization that his methods had enormous practical and pedagogic usefulness. They worked. Many experienced engineers now believe that the application of tensors to electrical engineering was a major advance that ranks Kron with Steinmetz and Heaviside.

Clearly both *A.T.E.M.* and *T.A.N.* are important books and should be available to all who propose to study this subject. They were published in the United States and unfortunately have been out of print for some years. It is therefore gratifying to see *T.A.N.* reproduced by British

publishers. As it is a reproduction without revision it is probably sufficient to say that its title effectively indicates its scope. The author includes an astonishing amount of material, so much that the beginner may well be bewildered by the wealth of information offered. Kron says that this volume scarcely scratches the surface of the vast possibilities in the subject, but the scratch is deep enough for most of us to be going on with.

The only new part in the book is the 16-page introduction included at the expense of the original introduction. I should have preferred to see the retention of the original with the new introduction added at the end as an epilogue, for two reasons: one is that for new readers the old introduction is understandable without knowledge of what is in the rest of the book, whereas the new one is not; the other is that, for all of us, both are of great interest, and comparing them brings out the progress of thought over a quarter-century. The new introduction looks to the future, and Kron has some scathing things to say about those who confuse linear graphs with electric networks, which is something like confusing diagrams with vector space configurations, or matrices with tensors.

Kron's achievements assure us of his genius, but the new introduction reveals parts of his personality. Among other traits we see a man gifted with unusual imagination, great industry and intellectual courage of a high order. Both opponents and admirers should note that he expressly disclaims mathematical rigour in his proofs. Like Heaviside, he is guided by an amazing intuition. His aim, he says, is to get definite answers to definite engineering problems; he rightly claims that his methods do just that.

The publishers are to be commended for their enterprise in republishing this notable book; I hope that its reception will encourage them to do the same with *A.T.E.M.*

W. J. GIBBS

## PSYCHOSOMATIC RESEARCH

### Psychosomatic Research

A Collection of Papers. By J. J. Groen *et al.* Pp. x+318. (London and New York: Pergamon Press, 1964.) 80s. net.

THE psychosomatic hypothesis takes two forms. According to one, certain types of people, showing measurable personality traits, are more likely to suffer from specified diseases than others. According to the other, certain life experiences of an emotional kind are liable to predispose people to develop certain specified diseases. It is widely believed that these psychosomatic theories are of recent origin, and spring from the work of Freud and the early psychoanalysts; this is not true. Hippocrates put forward explicitly views of a psychosomatic kind and these were widely held in the Middle Ages; indeed psychosomatic theories were flourishing during the first half of the nineteenth century and were applied even to such disorders as cancer and tuberculosis. Pasteur and the success of scientific medicine temporarily eliminated these hypotheses, but more recently they have been re-introduced by psychiatrists, although even now their reputation among critical experts is not high. This low standing of psychosomatic research is due in part to the great difficulties involved in measuring personality or quantifying the life circumstances which are supposed to contribute to the development of disease; in part it is due to the low level of rigour and experimental expertise shown in many published investigations.

*Psychosomatic Research*, edited by an eminent Dutch physician who has done much work in this field, is a set of reprinted articles from his laboratory at the Wilhelmina Gasthuis in Amsterdam, originally published in a variety of journals by him and his collaborators. Groen explains at the beginning of the book that "progress in modern medicine was largely due to the introduction of exact methods of thinking, the application of physics and

chemistry to the study of disease, and the introduction of an experimental and quantitative approach to clinical problems. The human mind, however, . . . had not been similarly explored and it was chiefly through the lack of suitable methods of research that psychiatry had lagged behind". This is an interesting statement because it reveals an unawareness on the part of the author that many experimental and quantitative methods for the study of the mind have in fact been developed by psychology, and that the failure of psychiatry to become scientific has been largely due to the fact that these methods have not been applied.

Groen and his colleagues have tried hard to overcome the difficulties of their task, and to apply experimental and quantitative methods, but they have done so only in a half-hearted manner, and the level of the work is not always as high as it should be. Sometimes, as for example in the study by Valk and Groen on the electric resistance of the skin during induced emotional stress, the statistical treatment of the data is too tenuous to be acceptable, the experimental arrangement is rather primitive, and much of the argument is rather anecdotal. It is true that the research was done more than fifteen years ago, and that it would have been nearer the average standard of experimentation then than it would now, but in a book published in 1964 one must judge the contents in terms of the present level of sophistication. In another paper, Barendregt has studied the effect of group psychotherapy by using the Rorschach test, which in spite of the ingenious way in which he has managed to overcome some of the inherent difficulties and subjective weaknesses of this projective device can scarcely claim to be an appropriate tool for experimental analysis. In addition the statistical argument presented is faulty; three hypotheses are tested of which two are clearly insignificant even on a one-tailed test. One is barely significant at the 1 per cent level on the one-tailed test, but being one out of three must be regarded as not supported by the evidence. Barendregt quite incorrectly combines the *P* values for all three hypotheses and arrives at the conclusion that his data reject the null hypothesis.

It would be wrong to conclude that there is nothing of interest in this book. Groen and his collaborators have tried hard to lift the subject of psychosomatic research from the quagmire in which psychoanalytic speculation has landed it, and many of their investigations are intriguing and suggestive. It is questionable whether they are important enough to merit resurrection and publication in book form at the rather high price of 80s.; it seems likely that only specialists will feel that whatever the price this book is essential to them.

H. J. EYENROCK

## UNCONTROLLED INSECT PHYSIOLOGY

### The Physiology of Insecta

Vol. 2. Edited by M. Rockstein. Pp. xvi+905. List Price 235s.; subscription price 211s. Vol. 3. Pp. xiv+692. 178s. 6d. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.)

THESE two weighty volumes complete Rockstein's book on *The Physiology of Insecta*, the first volume of which was published in 1964 (*Nature*, 205, 116; 1965). I cannot claim to have read the 1,600 pages in the critical manner which would have been a pleasure with a shorter book; but the same difficulty has beset the editor, who has failed adequately to collate the various contributions. This shows itself most obviously in the index, which not only mis-spells the names of many insects, but often lists the same insect under two generic names. There is no indication that *Anagasta* is the same insect as *Ephestia*, *Lucilia* the same as *Phaenicia* and so on. In the text identical data for a species are quoted twice, under differ-

ent names, and the larva and adult of the same species are under different genera in the same table (Volume 3, p. 116, Table 1). One author, whose name is unaccountably printed in italics in the text, has also been elevated to insect rank and appears with them in the index to Insecta. Indexing deserves more serious consideration than this if a work is to be used for handy reference. Separate indexes to authors, insects and subjects are a good feature; a combined subject index in the last volume would have partially overcome the disadvantages of the awkward subdivision of the work. It is a pity that the bibliographies do not include titles: the time saved the reader justifies the increased cost.

The editor has grouped his subjects into three sections, which unfortunately do not match the volumes. Biology, development and ageing (Section A) was finished in Volume 1, which also had seven chapters from Section B on the insect and the external environment. Volume 2 completes this part with six further chapters. Markl and Lindauer consider insect behaviour in 130 pages, of which as many as 50 are devoted to the mechanism of orientation. Lindauer then follows this with 65 pages on social communication. The chapters on migration by Johnson, terrestrial locomotion by G. M. Hughes, swimming by Nachtigall and flight by Pringle are all much shorter.

Section C, "The Insect and the Internal Environment—Homeostasis", has eight chapters in Volume 2 and eleven in Volume 3. Huber writes on neural integration and Graham Hoyle discusses the neural control of skeletal muscle. Hoyle's Fig. 1a on p. 410 is quite unnecessarily repeated by Maruyama on p. 453 in an article on the biochemistry of insect muscle. Maruyama apologizes for not discussing the controversial topic of the mechanism of muscular contraction.

Sacktor's 100-page chapter on the energetics and metabolism of muscle uses, without any explanation, all the usual biochemist's abbreviations for the many substances which come into his story. This seems unwise if the book is to be useful to the general reader. Chetfurka follows in a similar vein with two long chapters on the metabolism of carbohydrates, nitrogenous and lipid compounds. House completes a useful account of nutrition and digestion in less than a hundred pages.

Volume 3 deals with various aspects of blood in three chapters by Jones, Florkin and Jeuniaux, and Grégoire. There is an informative chapter on salt and water balance and excretion by Stobbs and Shaw. Briggs writes on immunology and Perry on insecticide resistance. Locke, Hackman and Ebeling have three chapters on the cuticle. All use data from Baker's study of the waxes of the Mormon cricket. Ebeling quotes this in detail in his text and the other two each have tables summarizing it to different degrees. The work ends with two chapters on respiration by Miller and by Keister and Buck.

Most of the illustrations are good and some useful new diagrams are included. Some have been reduplicated and some (for example, Volume 3, p. 30) show nothing and should have been replaced by drawings.

The book is extremely patchy. Contributors cannot have seen the chapters by their co-authors on closely related topics. The duplications and omissions show that the editor has exercised quite insufficient control. The work as a whole has the character of *Recent Advances in Insect Physiology*. It cannot stand as a definitive text of reference. Even though many of the articles are individually excellent they do not between them cover the field adequately.

A book of this length and character cannot be recommended for student reading. Research workers whose interests are covered by one or more chapters will find it helpful. The articles would, however, have been more useful separately than bound up in these three volumes, which together weigh 9.5 lb.

G. C. VARLEY

### Nuclei and Radioactivity

By G. R. Choppin. Pp. ix + 150. (New York and Amsterdam: W. A. Benjamin, Inc., 1964.) Cloth back, 5.50 dollars; paper back, 2.15 dollars.

*NUCLEI and Radioactivity* is one of a series of short monographs primarily intended for inclusion in a first-year university chemistry course. It covers the field commonly described in the United States as nuclear chemistry—nuclear forces, radioactivity, the radiations and their detection, accelerators and the production of radioactive species, and the study of nuclear species and reactions, including fission. The final chapter, however, is devoted to an outline of the applications of radioactivity in chemical, biochemical and technical studies. Brief appendices on the biological effects of radiation and a table of isotopes are included, as well as a glossary.

All this is a great deal to attempt within such a brief monograph. Inevitably much must be discarded and opinions will differ on what can be regarded as expendable. A chapter is devoted to fission, culminating in an outline of the principles of a reactor; yet the delayed neutrons are not mentioned and the impression is given that an uncontrolled reactor will explode like a bomb.

An outline of nuclear spectroscopy, the shell model and of the different modes of nuclear reaction is included; but the procedures by which decay schemes are unravelled are not described and one has an uncomfortable feeling that some students will find that nuclear chemistry is too completely disconnected from the rest of their subject.

Nevertheless, it is certainly desirable that the chemistry graduate should be familiar with the whole of the contents of this book and I am by no means sure that this is yet true in Britain. A. G. MADDOCK

### Teilhard de Chardin

*The Man and his Theories.* By Abbé Paul Grenet. Translated by R. A. Rudorff. (Profiles in Science.) Pp. 176 + 15 illustrations. (London: Souvenir Press, Ltd., 1965.) 21s.

THE Abbé Grenet has written a concise and well-balanced book and has set out the many strands of Teilhard de Chardin's energy and intellect. The author deals with his scientific work, his philosophy and his theology. He shows how the writer of *The Phenomenon of Man* and *The Future of Man* exercised such a great influence in the field of prehistoric archaeology. No doubt Teilhard has been extravagantly praised in recent years: "He has emerged as one of the century's most remarkable prophetic thinkers, an Aquinas of the atomic era" and "some people rank him as the greatest thinker-prophet of the twentieth century".

Although Teilhard himself seems to have arrived at a satisfactory synthesis of his science, philosophy and theology, his superiors in his Catholic order seem to have been less happy about some facets of his thought. He was ordained a priest in 1911. He taught physics and chemistry, but his true interest was in geology and palaeontology. In 1923, he was sent to help with excavations in Central China, where he found traces of Palaeolithic man, a prelude to the discovery of Peking Man six years later. From that time much of his life was spent in China, and all his scientific work and his prehistoric studies were related, through his deep religious feeling, to the origin and future of mankind. The theological implications of his reflections on evolution led his superiors to forbid the publication of much of his non-technical work. As a Jesuit priest he remained obedient to his order but nevertheless he freed and inspired the imaginations of others working on palaeontology and prehistoric archaeology. He was able to build up the strongest personal relations with all the major workers in his field. The citation of his promo-

tion as an officer of the d'Honneur read: "in the realms of palaeontology and geology he may now be considered one of the glories of France". Teilhard de Chardin concludes with some revealing extracts from his own writings, commencing with letters from the War front in 1917. It is well illustrated and has been ably translated by R. A. Rudorff. W. L. SUMNER

### Stability of Nonlinear Control Systems

By Solomon Lefschetz. (Mathematics in Science and Engineering: a Series of Monographs and Textbooks, Vol. 13.) Pp. xi + 150. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 60s.

STUDY of the stability of a linear dynamical system with linear control is a fairly well-organized domain, since the full linearity permits use of standard algebraic techniques. But with non-linear control, even of a linear system, these techniques lose their efficacy. Here, thanks to Soviet mathematicians in the first place, the direct method of Liapounov has been exploited; some five years ago, the Rumanian mathematician Popov produced two remarkable theorems on sufficient conditions for stability, using convolution and Fourier transforms. This has prompted further work in the United States, with Lefschetz himself, Kalman and LaSalle making important contributions.

Lefschetz gives a terse description of this work, the high points being Popov's theorems and some complements; the book has the master's customary vitality, illuminating phraseology, and unerring selection and emphasis of critical points in the argument. Of course, the reader is expected to be sophisticated, and reasonably industrious in supplying details; but knowledge assumed is not more than the amount of matrix theory and transform methods normally contained in a good honours degree course. Thus the young mathematician, contemplating research in a field which is likely to yield large dividends, should find the present monograph an efficient survey and guide, bringing him right up to the front line of advance. T. A. A. BROADBENT

### The Structure of Atoms

By J. J. Lagowski. (Classic Researches in General Chemistry.) Pp. vii + 120. (Boston and New York: Houghton Mifflin Company, 1964.) 1.95 dollars.

THE purpose of the series of paperbacks of which *The Structure of Atoms* is a member is to give first-year students of science a glimpse of the crucial discoveries through the eyes of the discoverers. In this book passages from original papers are set in a narrative which describes the development of the essential ideas in our modern picture of the atom, presenting each discovery against the background of contemporary ideas and knowledge.

The present work achieves its purpose very satisfactorily. It takes the reader through the evidence for the existence of atoms from chemistry and from the physics of gaseous discharges (quoting from Dalton, Avogadro and J. J. Thomson). A reasonably full account is given of the discovery of radioactivity and isotopes (quotations from, *inter alia*, Becquerel, the Curies, A. S. Russel, F. Soddy and H. G. J. Moseley) and of the nuclear atom (Rutherford). The final chapter deals with the extra-nuclear structure (Bohr). For a short book the coverage is full, though one wished that Marie Curie's recognition that radioactivity is an atomic phenomenon had been discussed explicitly and not merely mentioned incidentally.

This book provides an opportunity to glance at some of the most important publications of the modern age for students who might not otherwise be able to read them. It should also interest those whose primary studies are in fields other than science. [The late] K. E. B. JAY

## INTERACTION OF TECHNOLOGIES

AWARDS BY THE SHELL CHEMICAL CO., LTD., AND THE BRITISH ASSOCIATION FOR THE  
ADVANCEMENT OF SCIENCE

THE results have recently been announced of the world-wide award being sponsored jointly by Shell Chemical Co., Ltd., and the British Association for the Advancement of Science on the theme of "The Interaction of Technologies". The judges have decided that the main prizes should be shared between the authors of two papers, of equal merit—Mr. J. W. S. Hearle and Mr. L. W. Boxer—and they have both received a prize of 150 guineas.

Among the members of the panel of judges were Sir Robert Robinson, F.R.S., Mr. G. B. R. Feilden, F.R.S., Dr. D. O. Martin, O.B.E., and Mr. L. J. F. Brimble (editor of *Nature*).

The prizewinning papers were read at the recent British Association meeting at Cambridge.

The Award is being offered annually for seven years and the terms of reference for the 1965 Award were as follows: "It is acknowledged that there are divisions and barriers between different technologies at the moment and it is agreed that these impede scientific and industrial progress. We believe that there is more common ground between these existing technologies than is normally conceded".

The first of the prize-winning papers follows. The second will be published in the next issue of *Nature*.

## INTERACTION OF TECHNOLOGIES

By L. W. BOXER

Counsellor and Head of the Technical and Economics Division, European Nuclear Energy Agency, Organization for Economic Co-operation and Development, Paris

THE conjunction of a rapidly increasing interest in the role of science and technology in modern society, and the relative multiplicity of practitioners in the field to-day compared with only 40–50 years ago, means that it is becoming increasingly difficult to say anything new when attempting to explore a topic as complex as the interaction of technologies. Notwithstanding this dearth of originality, I feel compelled at the outset to restate the basic tenet—however trite—that the eternal riddle of the relationship between the forces within Nature and the forces within man has never been more pertinent in the history of human civilization than to-day, when we find ourselves precariously balanced at the fulcrum between man-manipulated natural forces of immensely constructive and destructive potential.

It is also general knowledge that, until recent times, discoveries of the nature of our environment and the evolution of scientific thinking had had relatively little impact on the organization of society, and that changes which resulted in human attitudes were confined to minority groups consisting chiefly of natural philosophers and theologians. With the advent of the industrial revolution, the expansion of manufacture and trade—stimulated by frequent wars—the situation changed rapidly in those countries whose political institutions and whose resources in manpower and materials were in a balanced relationship for such development.

These facts have long been recognized and need only to be repeated to emphasize the fact that we are already beyond the turning point from when man's growing knowledge of the eternal forces of Nature were still in step with his capacity to relate the implications of such knowledge to his contemporary needs. To-day it is generally conceded that the two factors are very much out of step and that the individual—whether scientist or layman—is beginning to experience a growing sense of personal impotence in adapting to the avalanche of scientific and technical knowledge, and a growing concern over the great tracts of isolation which appear to exist between areas of intellectual activity.

Unfortunately the fragmentation of scientific thought and activity cannot be conceived in such a way that the situation presents itself as a rough jig-saw puzzle, where it is only necessary to relate the boundaries of the fragments to each other in order to perceive the correspondence

between their profiles. Obviously, discoveries are yet to be made, and these may alter the intellectual shape of many areas of scientific investigation, and reveal fundamental links which were previously unsuspected, as was the case in the relationship between physics and chemistry in the nineteenth century, and as is now the case with chemistry and biology. The lack of such links, however, constitutes a series of gaps in knowledge rather than barriers or divisions between branches of knowledge and practice, and there is evidence that the barriers which have become insinuated between various branches of scientific activity have their origin in human weakness rather than in the incompleteness of human knowledge of the natural order.

Even a cursory examination of the history of science and natural philosophy will immediately reveal the falsity of denying the principle of common ground between all branches of science and their application in the technologies. It becomes immediately evident that only in recent times has the growing body of scientific discovery and application made it increasingly difficult and finally impossible for any single individual to practise science to the full extent of contemporary knowledge, as was feasible for his historical predecessors. Compartmentalism then appears in its true perspective as a purely arbitrary expedient to enable groups of individuals to concentrate in depth on a programme of education and application in a particular branch of science. However necessary it may be in the interests of progress to pursue a particular investigation in depth, it is tediously necessary to bear in mind the old maxims that a horizon is invisible from the bottom of a mine shaft, and that scientific knowledge and truth are the eternal parents of all technological offspring. Just as it may seem incongruous to project barriers between the technologies, it would be equally incongruous to examine a family illness without examining not only the interrelationships of the children, but also their joint relationship with the parents. This leads me inevitably to the conclusion that in any examination of the interaction of technologies, a good deal of attention should be given to the interaction of technologists, both within the scientific community and with society in general. It might, therefore, be more profitable to place particular emphasis on the complex humanistic elements of the contemporary situation, rather than to



attempt to repeat the common scientific principles which underlie all technological activity, and to arrive at purely abstract conclusions.

But before doing so it would be relevant to direct attention to a particularly prominent example of failure of one technology to interact with others. It is astonishing to observe the comparative isolation of the knowledge gained in the workings of the conscious mind and of the revelations of man's role as a rational being, in other words, of his capacity for objective evaluation of the relative significance of applying scientific thought and discovery in a developing society. A second example is the lack of cohesion between the current situation in science and technology, and knowledge and experience gained in recent years of the forces which control and influence the development of society. So to begin with, there are two technologies which have so far been largely missed out of the interaction altogether, namely psychology and sociology, and which could otherwise provide us with some insight to self-knowledge and the behaviour of human beings in their social environment. This is a significant oversight in a period in which there are frequent professions of awareness of the narrowness of mind and prejudice engendered in us by our educational and social background. Is it not likely, then, that man is frequently attempting to exercise highly skilled judgment of mind over matter without a fuller understanding of the mechanism of the mastery of mind over mind? If, then, it may be postulated that the present impression of discontinuity within the scientific and technical community is a purely behavioural phenomenon, then it may be relevant to attempt an appraisal of the current thinking habits acquired by scientists and technologists as a fundamental factor in this enquiry.

It is facile to repeat that the instinctive human regard for the preservation of the intellectual and material interests of the individual is the root cause of the formation of autonomous clans within the scientific and technical community, which are uniquely absorbed in the importance of their own affairs, and interested in their relationship with other clans primarily with the view of maintaining the preservation of relative status. This platitude misses the point, first, that for practical reasons arising from human limitations recent advances have made it essential to provide autonomy in different branches of science in order to achieve a desirable degree of organizational efficiency, and secondly, that practically all professional groups to-day are faced with overwhelming difficulties in communication not only between other specialized groups but also within their own speciality. There is the perpetual problem of determining the optimum proportion of total working time which can and should be devoted to keeping up to date, even within a limited field, and the way in which such time can be spent to the best advantage. A great deal of discrimination is now needed to discern the personal usefulness and relevance of the enormous amount of material which is now available through the media of publications and organized meetings. Some progress is being made on the physical problems of information retrieval and information transfer; but is factual knowledge in itself enough without an individual readiness to see oneself as an integrated, rather than an isolated, entity within the scope of human learning? If it were possible to sum up the inestimable number of gaps in knowledge and understanding between individual people with a similar tradition of scientific training and discipline, the total might even surpass in magnitude the much-publicized single gap between science and the humanities—which have recently been presented as the 'two cultures'—and where again the recognition of the inherent problems does not seem to have progressed beyond general expressions of awareness, to the accompaniment of a good deal of unconstructive argument.

The very terms 'science' and 'the humanities' in themselves are a typical demonstration of human wilfulness

in creating distinctions in fields of learning. 'Science' has been adopted as a convenient term for grouping the manifold studies of animate and inert matter, and the forces which influence its behaviour; and 'the humanities' has become an equally convenient term for grouping the branches of study of human activities. But is it not beyond the bounds of human capability to assert that these two arbitrary concepts are entirely unrelated and can never become integrated within a continuous intellectual perception of reality? It is inevitable, therefore, that the barriers created by our present basic thinking habits should be reflected in a certain lack of communication between many of our institutions. Indeed, contemporary psychological attitudes among many practitioners of science and technology probably present the most formidable problem in the effort towards achieving greater unity in these fields. We have to reaffirm to ourselves that science and technology must be maintained as branches of learning, and must not deteriorate into becoming attitudes of mind.

Perhaps the most obvious topical issue is the rigid distinction between scientists and engineers or technologists, which is aggravated to some extent by the unfortunate connotation of scientific activity into the categories of 'pure' and 'applied', from which the inference is often drawn that exercise in the abstract field is an intellectually clean occupation as opposed to a supposed vulgarity associated with activity in the material field, particularly where commercial considerations are important. The root of this distinction can probably be traced back to more than 150 years ago when craft technique first began to be used on an industrial scale in advance of the direct application of science, so that technology originally became established in industry as practical craftsmanship with very little tradition for intellectual training. The scientific approach was most likely regarded with some suspicion, and was only adopted to the extent that it was able to produce a practical and profitable result.

It is understandable that, in such circumstances, scientists would feel that the aims of industry would have little real connexion with the aims of science, and that industry would in fact tend only to impose restrictions on scientific intellectual liberty. Times have changed, however, and with the enormous growth of competitive industry, the evolution of skilled industrial workmen from artisans and craftsmen to engineering scientists and technologists has been every bit as rapid as the evolution in scientific knowledge. Furthermore, the training of engineers in the technological applications of science has become just as rigorous as that of scientists, who can now no longer claim a monopoly over objective and creative thinking, nor justify an attitude of condescension towards the more pragmatic aspects of their art.

Nevertheless, as we all know, the academic isolation of the scientist from the engineer, however unrealistic and harmful to human development, still persists in 1965 and is actually fostered by a cult of snobbery which is as equally indefensible as the isolationism itself, and which it would be hypocritical to ignore. The outdated images of white coat and greasy overalls, intellectual and non-intellectual, are still unconsciously or even consciously preserved, prompted perhaps by a pseudo-aristocratic and anachronistic attitude towards the 'anathema' of the industrial revolution. Social distinctions between scientists and technologists in 1965 are just as artificial, as any such distinctions have always been between composers and musicians, playwrights and actors, sculptors, painters and architects or in any field which requires a duality of origination and interpretation for its expression. The one is the essential complement of the other and there is no foundation whatsoever for any kind of distinction which attempts to compare two different kinds of mentality of equal quality, but trained in different orientations. In spite of the diffidence which still surrounds the mention of the whole delicate question of class relations in Britain,



the social image of applied science could well be a significant factor in explaining why so many engineering places are now going begging in such British universities as provide them.

In effect, then, the professional engineer in modern society is still classified by many as being associated with craft industry with a non-scientific tradition, and indeed he still shares the generic title 'engineer' with to-day's skilled or semi-skilled workman who has not sought the rigorous educational training and intellectual discipline necessary to achieve professional competence. The situation is now further complicated by the introduction of a new category of specialists, the 'technicians', whose function and formation lie somewhere between that of the two arbitrarily defined 'engineers', although there is no reason for supposing that their relations with professional engineers cannot be modelled on those developed between fully qualified specialists and their technical assistants in other professions, as, for example, in medicine. In Britain, recognition in terms of a more prestigious title such as 'chartered engineer', and more enlightened teaching to place the functions of the professional engineer in perspective with those of people of lesser skill and training but who are nevertheless free to borrow the same generic title, may help to raise the status of the engineering scientist in the public mind. The recent decision to grant fellowships of the Royal Society to more engineers and applied scientists generally is a welcome recognition of overall contributions of technology to the advancement of science. Nevertheless, the social nuance still continues to be an insidious element in the pseudo-intellectual flux of forces which tend to isolate scientists and engineers from each other.

The seriousness of all the psychological obstacles which block incentive towards free communication should not be underestimated in any attempt to reconcile the two camps of abstract and practical activities. How then will it be possible for both sides to arrive at a mutual understanding, at the individual level, of the problems encountered in these two basic forms of scientific activity, and to gain an appreciation of the nature of the creative thinking which has to be applied in order to make their particular contributions to progress? There is, of course, excellent scope for improvement in interdisciplinary relations in many large research establishments where closely co-ordinated teamwork between scientists and engineers is essential for the development of the sophisticated experimental techniques which are vital to success in certain fields of advanced research, notably in the structure of matter and in radio astronomy. Furthermore, the rapidly increasing use of computers is likely to make an important contribution to fundamental investigations in cybernetics, as well as liberating the whole scientific and technical community from a multitude of time-consuming mental chores, and providing more scope for initiative and for inventive thinking.

It is perhaps through such media that the best conditions are created for a balanced interaction between science and various branches of technology, always provided that organizational structure is also geared to this objective, and that management is sufficiently enlightened to appreciate the importance of encouraging a balanced relationship between science and engineering in achieving a high quality of contribution to further knowledge. However, situations in which people of different specialist training are able to work together in a healthy jumble of practices are still rare, and the vast majority of scientists and engineers are destined to spend most of their working lives in the relative isolation of laboratories, universities, design offices, production sites, etc.

Here the problem is again basically one of the practical means of communication, but like most men dedicated to a profession, the first concern of scientists and engineers will be to do a specific job to the best of their ability. It would be unfair and unrealistic to expect people with

primarily sectarian interests to take the initiative in establishing the necessarily complex means of communication and in formulating the broad lines of policy designed for a general rapprochement in the different sectors of the scientific and technical community. Surely the responsibility here lies with management, which is uniquely placed to take the horizontal view, and which is becoming of increasing importance with the present high degree of organization in science and in science-based industries, particularly with the general tendency away from individual research and development effort to co-ordinated teamwork.

Nevertheless, managers themselves are not always exempt from the sin of narrowness of interest, although awareness is half the battle—the most comfortable half. The already growing interest in the relatively new science of management is an encouraging sign, as supplementary training of this kind is bound to enlarge the field of interest and sympathy between individuals charged with different fields of responsibility, and may help also to achieve a more rational distribution of skills and aptitudes.

In spite of the financial and practical difficulties, the principle of 'sabbatical' leave might be applied more generously and more generally to encourage individual incentive and capacity to go on learning, and to promote a general realization of the need for education to be a continuing process throughout the life of the individual. The inconvenience and temporary loss of manpower would surely be more than compensated by the general stimulus which would result from the successful coaxing of management into a professional approach to its responsibilities, and the possibility for specialists at all executive levels to bring themselves up to date in their own fields, and to realize where current advancements in knowledge have produced scientific and technological 'fall-out' in allied fields of activity. The value of this kind of movement of personnel between industry, universities and research establishments has already been demonstrated on a modest scale, and there is now not only a justification but an urgent need to enlarge the practice both within and between national boundaries.

Furthermore, the successes of CERN in the field of fundamental research in the structure of matter, of ENEA, Euratom and the IAEA in nuclear science and technology, of ESRO and ELDO in space research and engineering, and of the FAO and WHO in the support and conservation of human life have all demonstrated the scientific and technical as well as the political value of international co-operation, and such organizations deserve every future encouragement as a means towards the objective of greater unity in science.

Nevertheless, both national survival and the stimulus for technical advancement depend on competitive trading, and it is therefore inevitable that commercial secrecy should present certain obstacles to a free flow of information in industrial circles. It would be quite impossible to quantify the resultant losses represented by patents, secret design information and closely guarded manufacturing techniques in terms of impediments to scientific and industrial progress, or of the inefficiency engendered under the cloak of such commercial secrecy. This will only become apparent in retrospect when each sector of industry has been able to determine what is to be gained by less restrictive policies on the flow of knowledge and information, and whereas industrial federations have done much to promote co-operative progress, the further encouragement to share industrial skills and resources must rely on Government initiative.

In this respect, and in view of the significance of expenditure on a national scale in the overall pattern of the alliance between scientific and technological development, Government can play an important part in sponsoring the closest contact between national scientific research programmes and industrial development work. However, the achievement of good relations between the public and

private sectors is often adversely influenced as a result of a Government's own commercial activities in certain scientific and technical fields.

It is an unfortunate fact that in the public mind, and particularly since the advent of nuclear energy, the men and women in the ranks of scientific and technical community still tend to be regarded as something apart from the run of ordinary mortals, even though as individuals they share the disadvantages of being at the mercy of their personal prejudices and quirks of temperament as all human beings. Nevertheless, the dispassionate objectivity of the scientific outlook, and the readiness—in the face of undeniable evidence—to admit the possibility of being wrong, rouses strong (even if inarticulate) emotions of hostility among those who are devoted to the perpetuation of fantasy, and who respond by equating science with inhumanity. There is ample evidence that the scientific community is becoming increasingly conscious of its responsibilities to society and is willing to meet those responsibilities in terms of twentieth-century reality. Many scientists and engineers have also come to realize that the source of ideas has become of secondary importance to the influence behind the expression of ideas, and they are now more prepared to compete for positions in which their professional activities are subordinated to their political functions.

A great deal still remains to be done to improve the public image of science and to remove public suspicion of the morality of scientific pursuit, and there is still scope for further exploitation of all the powerful media now at our disposal to stimulate frequent and persistent public argument and informed discussion of the issues affecting the relationship of scientists and engineers with each other, and with society at large, including dispassionate analysis of the human motivations which impede improvements in relations.

So much for short-term solutions in counteracting the consequences of our self-created problems. There is still one fundamentally important factor in the evolution of thinking habits which, in the longer term, presents a much greater problem in the development of science and technology, namely, education. There is no doubt that nowadays many young people in the later stages of their basic education, when faced with the seemingly irrevocable choice of a life vocation, are still puzzling over the fundamental problem as to whether education is the key to a way of life or the means of earning a livelihood. Students and the public in general are continually being reminded of an apparently insatiable demand for more and more scientists and technologists, to continue the economic growth rates which have become the principal objective of all Governments.

On the other hand, relatively little is known either about the degree of efficiency with which the specialized training of scientific and technical personnel is already employed in a great diversity of organizational structures, or to what extent such specialized training is essential prior to a specific professional activity. But when, in spite of possibly unfavourable social prospects, and faced with the exigencies of getting a job, the would-be scientist or engineer poses his candidature without having been equipped to view the whole scope of science in broad perspective, he is so overwhelmed by the potential breadth of his studies, and by the vast accumulation of knowledge to be acquired in depth, that not unnaturally he seeks refuge in the specialization for which he has shown the greatest aptitude under the restricted conditions of his earlier formation, to the everlasting detriment of his future capacity to appreciate the problems in other fields of learning. We have arrived, then, at the paradoxical situation where collectively man knows more now about himself and his environment than ever before in history, but is incapable of providing himself individually with more than a fragmented appraisal of this accumulated knowledge, before plunging into the relative mental

security of a particular pattern of specialist thinking (always assuming that he still chooses to go on thinking at all).

Recent history shows us that educational reform has been a continuous process throughout more than a century and at a rate which has been more or less in correspondence with social and economic evolution. However, to-day our experience of the rapid changes in civilized society in the past few decades, and our consequent ability to forecast the trends of the future, should provide us with an unprecedented opportunity to anticipate the kind of educational pattern which will be needed in the future, and the nature of the reforms which must therefore be put in hand to-day. Once again, fundamental rethinking is required, beginning with a thoroughly objective reappraisal of the relevance of the present content of education and teaching methods to the needs of modern society, and a critical analysis of the basic criteria in education which should be afforded to everyone, even though this might involve the relegation of some sacred pillars of learning which have masqueraded for years under the guise of logic and memory training media.

The disruption caused by radical changes need not be enormous and could be introduced in such a way as to take due account of the inertia of the present educational machinery. A golden opportunity now exists in Britain, where the ground has already been broken by the establishment of the comprehensive schools. Governmental initiative would, of course, be vital and could take the sponsorship of experiments in a number of provincial centres in which a specially designed course of secondary education would be dovetailed into a special degree course at selected institutes of further education. The aim should be to direct secondary and tertiary studies away from a strict subdivision into relatively isolated subjects, and to place particular emphasis on the evolution of ideas over the history of civilization, and an understanding of the factors which controlled their success in their contemporary settings. Detailed and lively instruction in the history of natural philosophy and religion, and their relation to the evolution of scientific thought would place the concept of entirely separate cultures in a perspective of total irrelevance to a unified concept of cultural activity. The development of mathematical philosophy, technique and application (including ultimately the elements of logic as embodied in computer theory) would then fit into these studies as part of a natural sequence. The special secondary course should also include English and at least one other modern language (taught by the most up-to-date language laboratory techniques) with particular emphasis on those aspects of language which limit the communication of ideas between people of different nationalities. Furthermore, the importance of self-knowledge and the awareness of human physical and intellectual limitations could be revealed to some extent by a study of the fundamentals of physiology and psychology. An equally essential tributary to this main stream of thought would be a study of historical influences on art, music and literature, and in order to gain some insight into the problems of modern Western society, the elements of economics should complete the list.

Such a special secondary course should perhaps give first priority to the philosophical treatment of the whole range of the syllabus, technological aspects being introduced as consequential and sequential, and left for further development in the tertiary course which would make provision for the study of one or two subjects in depth in addition to the continuation of the broad basic programme previously outlined. Each student would thus have an opportunity of creating a personal concept of the full scope of the intelligence of civilized man over the centuries, which is essential for a balanced view of the contemporary scene and for the difficult task of choosing the subsequent emphases in one's personal further education. Such a programme will seem over-ambitious and hopelessly

impractical if judged by present concepts of what is vital and what is useful for an educated person to know. Even if an optimum solution could be found, it is fairly inevitable that the completion of some kind of specialized study course and the resultant feed-back of the individual's educational background into society would add one or two years to the present time-scale of formal education. But given the resources, is this such a great sacrifice in the long term in order to achieve a society which has a greater consciousness of its scope, together with a balanced perspective of specialist activity and its contribution to human progress?

The idea is far from being new. Plato is quoted as saying the following in his famous *Republic* about the

students of his equally famous Academy: "They will be offered all at one time the sciences which they have studied at random in their adolescence, so that they can get a broader view of the relationships between these sciences and by this means become acquainted with the true nature of reality".

In the fourth century B.C. it was, of course, possible to read the output of practically all the contemporary thinkers—a task which is beyond human capability to-day. Nevertheless, it would be ironic if, more than twenty-three centuries later, man has become so overwhelmed by the revelations of his own intellectual industry that he finally becomes the slave, rather than the master, of knowledge.

## THE NATIONAL PHYSICAL LABORATORY, TEDDINGTON, MIDDLESEX

ON April 1, 1965, the National Physical Laboratory, together with most of the other stations of the former Department of Scientific and Industrial Research, became part of the new Ministry of Technology, and on the same day the National Chemical and National Physical Laboratories were amalgamated under one director. This year, therefore, for the first time, it was the combined Laboratories that were open for inspection during open days which were held on May 19–21, when 330 items were on display. Some 3,570 visitors attended during the first two days, while on the final day visits were made by more than 1,200 sixth-form pupils from 62 schools. No lectures were arranged, but two films were shown: one, *Ship Shape*, dealt with the work of Ship Division, and the other, *Precise Measurement in Engineering*, with the metrological work of Standards Division.

It is convenient to consider the various Divisions of the Laboratory as constituting a number of groups, each group having common interests. Thus there are two Divisions—Aerodynamics and Ship—concerned essentially with the movement of a solid body through gaseous or liquid media. Four others—Applied Physics, Chemical Standards, Light, and Standards—are interested in accurate measurement and standardization. Autonomics and Mathematics devote much of their effort to automation and computers, while Basic Physics and Metallurgy are concerned with the investigation of materials. The common interests between groups of Divisions were apparent from the items on display during the open days.

In Aerodynamics Division, research has been in progress for several years on base flows. This type of flow occurs behind wings or bodies, moving through the air, which are not shaped so as to converge to a sharp edge at the rear but are truncated earlier, leaving a bluff rearward-facing area or 'base'. Similar flows exist behind downstream-facing steps in a wall (the outer skin of an aircraft, for example) past which air is flowing. The problem is of considerable practical importance because the air pressure which acts on the rearward-facing area is usually low, making a contribution to the total drag of the aircraft or vehicle.

One of the Division's exhibits illustrated various methods which have been explored for reducing the drag arising in this way. One method at present being investigated consists of hollowing out the base, so that at the rear of the wing or body there is a thin-walled cavity with its opening facing downstream. The walls of the cavity are ventilated so as to allow air to bleed through into the cavity from the outer surface. This has the effect of alleviating the low pressures acting on the base. The preliminary results are encouraging, and further research on the method is in progress.

Two separate lines of research into methods of calculating the development of turbulent boundary layers are being pursued, with the ultimate objectives of improving estimates of the surface-friction drag of aircraft and ships, and predicting the undesirable phenomenon of separation of the flow from the surface. One method relies on an empirical correlation of nearly all existing data and should be more reliable than the more narrowly based existing methods; the second method uses the equation for the flow of turbulent kinetic energy to obtain a relation between the velocity and turbulent shear stress and their spatial derivatives, which is then solved simultaneously with the boundary-layer equation and the continuity equation on the KDF9 computer. This method requires a fairly small quantity of empirical information about the turbulence structure, which is being obtained as part of a wider experimental programme using a special wind tunnel. Experimental work is also being done on surface pressure fluctuations under turbulent boundary layers, which cause structural vibration, fatigue and noise.

The effort devoted to non-aeronautical aerodynamics is steadily increasing. One interesting investigation in this field concerns the collapse of cooling towers under wind loading conditions. This has been investigated using scale-models in the compressed air tunnel in which the increased air density raises the impact pressure of the airstream sufficiently to buckle specially prepared thin metal models (wall thickness 0.005–0.012 in.). Associated mathematical analyses have been made in the Mathematics Division. Work is also in progress on the aerodynamic stability of suspension bridges, with special reference to the Severn Bridge. Tall buildings, chimney-stacks and other tall, slender structures represent other examples where the natural wind can introduce loadings, particularly those of an oscillatory nature, for which no adequate provision is made in current *Standard Codes of Practice*. Wind-tunnel investigations have been undertaken to ensure that no unacceptable oscillations will occur on the General Post Office towers for London and Birmingham. Another interesting investigation, at present in progress, is that for the World Trade Center, a 1,350-ft. twin sky-scraper development for Manhattan, New York, which when built will be the world's tallest pair of buildings. On show also was a landscape model of a section of the Lancashire–Yorkshire motorway, on which wind-tunnel tests had been made in order to investigate the airflow with special reference to snow drifting in a deep cutting.

Another aspect of the problem of drag was illustrated by a hydroelastic model of a fully cavitating propeller in Ship Division.

The blade sections of fully cavitating propellers must have thin leading edges to avoid high cavitation drag with consequent loss of power and low efficiency. Despite the use of high-strength materials such as stainless steel, fully cavitating propellers with thin leading edges have failed structurally in service, and experiments are being made to determine the steady and unsteady stress levels in such propellers. Unsteady stresses occur mainly because a propeller has to operate in a non-uniform wake.

In order to predict full-scale steady-state stresses from a model propeller, the deformation under load of the model must be similar to that of the full-size screw. This condition may be closely simulated by making the model from a more flexible material; here this is an epoxy resin with a suitable filler added to adjust the elastic modulus to the required value.

The strains at selected positions on the blades are measured by means of very thin electrical strain-gauge rosettes attached to the blade surfaces. The energization of the strain-gauge bridges and the resulting output signals are controlled and measured from outside the tunnel by means of slip-rings and leads passing along the shaft into the propeller. Stress-levels in the blades are then calculated from these strain measurements.

The Division has recently also been investigating the potentialities of passive tank-type roll stabilizers to supplement the activated types at present commonly fitted to ships. Most encouraging results have been obtained. The passive ship roll stabilizer has certain advantages over the activated type. It requires no instrumentation, moving parts, or power supply, its action depending on the free motion of water from side to side in a rectangular tank.

The tank is incorporated in the ship structure as high as possible and usually extends from side to side using the maximum beam of the ship. The depth of water in the tank is such that at the resonant response frequency of the vessel the water moves from side to side with the same period of oscillation as the ship but lagging the ship motion by a 90° phase angle. The quantity of water transferred is such as to create large damping of the motion.

The design and development of the system are both simple and economic, and a scale model of the vessel to which it is to be fitted is used to prove the design. A demonstration of a typical installation in a ship model showed the principle of the stabilizer and the reduction in roll angles possible with such a system.

The Radiology Section of the Applied Physics Division which is concerned with the establishment of all types of standards in the field of radiation physics was able to exhibit this year the new 3-MV Van de Graaff positive ion accelerator. The acquisition of this machine considerably widens the range of the Section's activities. It will permit the establishment of standards of neutron flux density which are required for the calibration of neutron detectors and for the measurement of neutron reaction cross-sections. The machine accelerates beams of protons, deuterons or  $\alpha$ -particles which may be directed along one of three drift tubes to bombard targets of suitable material. One such drift tube leads to a target situated in a low scatter environment in which the source of neutrons is at least 20 ft. away from all walls, ceiling and floor, the essential equipment being supported on a light open-mesh grating (Fig. 1). By suitable choice of particle, bombarding energy, target material and angle of detection, monoenergetic neutrons can be obtained at many energies from a few keV up to 20 MeV. A second drift tube, leading to another area of the accelerator hall, will enable a beam of deuterons to be rapidly oscillated between two targets situated in a large graphite moderator. At a point midway between these targets a well-thermalized neutron flux of about  $10^7$  n/cm<sup>2</sup>/sec and having a very small gradient over a volume of about 100 cm<sup>3</sup> should be obtained. By this means it is hoped to estab-

lish a standard thermal neutron flux density standard which will be used to calibrate flux measuring foils; alternatively, a small beam of neutrons can be extracted from the moderator to calibrate neutron detectors for protection measurements.

Work in the Acoustics Section of the same Division lies largely in the field of psychoacoustics, and much interest was attracted by an exhibit showing the preliminary results from the latest in a series of subjective experiments designed to gain information on the relation between noise-levels and environmental factors on people's reactions to specific classes of noise, in this case aircraft noise. The tests were carried out at Farnborough during the SBAC Show in 1964. One object was to examine the validity of rating scale procedures for tests of this sort and to refine the techniques for subsequent use. One of the principal findings is that noisiness judgments depend not only on the noise-level received, but also on the listeners' inference about the noisiness at source. No significant difference was observed, however, between judgments of landing and take-off noise at corresponding sound-levels. The tests, for which 150 subjects took part, were spread over five afternoons. On the first afternoon the subjects were divided into four psychologically matched groups by means of their responses to 'annoyance' and 'semantic differential' questionnaires administered by the Applied Psychology Research Unit of the Medical Research Council. The aircraft noise tests occupied the remaining four afternoons during which the four groups were situated at indoor and outdoor locations variously sited with respect to the flight paths. The results accord closely with those of a smaller-scale experiment carried out at the time of the 1961 SBAC Show for the Wilson Committee on Noise, and their validity is now believed to be high as a result of the balanced-design cross-checks in the present series.

The new Chemical Standards Division, formerly the Division of Chemical Physics of the National Chemical Laboratory, illustrated work in chemical thermodynamics

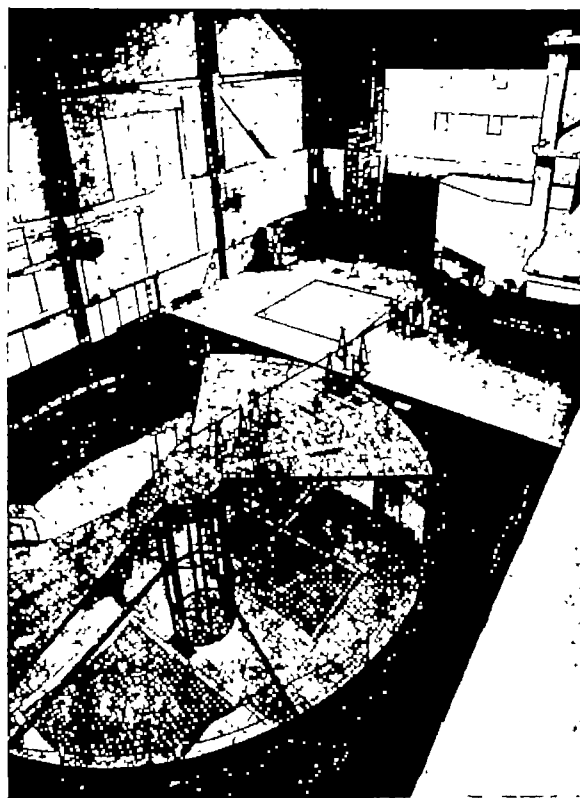


Fig. 1. General view of the Radiology Laboratory, showing a drift tube leading to the open-mesh grating forming the low scatter environment

and molecular spectroscopy, the two main items on its programme.

Thermal properties (heat capacities, heats of transition, heats of fusion, triple points) of substances are required in the chemical industry for the development of the best production methods. Heat capacities of 12°–473° K are being measured to a high degree of accuracy by adiabatic calorimetry. The apparatus exhibited has been largely automated. In each of the four sets of equipment, the temperature of a shield isolating the calorimeter is automatically maintained within a few thousandths of a degree of that of the calorimeter. Heat inputs to the calorimeter are calculated from voltage observations recorded automatically on paper tape. Temperature increases are measured to a thousandth of a degree by means of platinum resistance thermometers and an automatic a.c. resistance bridge, readings of which are also recorded on tape. All the observations are processed by digital computer.

Values of Gibbs energies and heat contents of substances are needed by chemical engineers engaged in plant design; values can sometimes be obtained by measurement of equilibrium constants over a temperature-range. Apparatus used for the determination of equilibrium constants for the gas-phase hydrogenation of three ketones and an aldehyde was shown; the vaporized compounds, mixed with hydrogen, were passed over a catalyst held at a known constant temperature and the products analysed by gas chromatography. The results confirmed the values of certain thermally derived thermodynamic quantities and data inaccessible by other methods were secured, for example, a value for the entropy at 298° K of *s*-butyl alcohol, which could not be derived from low-temperature calorimetric studies because the alcohol did not crystallize.

Important information concerning the vibrations of some molecules can be obtained from the study of their absorption spectra in the infra-red and far-infra-red regions. Examples of the use of infra-red, far-infra-red and Raman spectra to obtain fundamental frequencies were shown. The results are used to calculate thermodynamic values, for example, the heat capacity of industrially important compounds. These calculations are complementary to measurements of the same quantities and permit extension of the results to a wider temperature range.

Two of the most interesting items on display in Light Division were a colour temperature meter and thin film components for the visible and ultra-violet.

A very simple and convenient colour temperature meter has been developed which can detect differences of 1° K between incandescent tungsten filament lamps. This instrument can be used in the National Physical Laboratory and similar laboratories to inter-compare standard lamps.

The colour difference detector is a sector disk with yellow and blue filters revolving in front of a photocell. With incident light at a certain colour temperature, the a.c. output from the cell will be zero; but any slight change of colour temperature will produce an a.c. signal which can easily be greatly amplified for detection on a meter. If, as is probable, the light is not at the 'matching' colour temperature, it may be made so by sliding across a coloured wedge. The displacement of the wedge required for a null signal is then a measure of the colour temperature of the light, provided that the spectral distribution is similar to that of a full radiator.

Extensions of this principle could produce an extremely simple and sensitive colorimeter which could be used in industry to detect deviations from colour specifications in goods such as textiles, and hence, possibly, to control the process automatically.

Work is proceeding on the development of a very efficient polarizing beam-splitter which consists of a glass cube split on a diagonal, the interface bearing an interference filter comprising many alternate thin layers of

magnesium fluoride and ceric oxide. This reflects only light of one direction of polarization, light of orthogonal polarization being transmitted. Samples made so far give an efficiency of separation of the two beams of more than 90 per cent.

Such beam-splitters find application in interferometry, where they can give efficiencies of four times those given by conventional beam splitters, and where the two beams being 'labelled' by their polarization can be manipulated separately without interference.

Thin film components for the vacuum ultra-violet are needed in space research, plasma physics and other spectroscopic applications. Most materials used in such components absorb strongly below 2000 Å, and work is in progress to determine the conditions necessary for efficient components.

Aluminium mirrors protected by thin layers of magnesium fluoride have given reflectivities of more than 80 per cent at 1216 Å, but deteriorate with time. It is hoped that further work will disclose the reason for this.

The application of lasers in the field of accurate measurement was illustrated by an exhibit in Standards Division.

For many years engineers' reference end bars have been measured accurately by means of wave-lengths of light, but such measurements have been limited to short bars by lack of complete purity in the colour of the light from the usual sources. The advent of separated isotopes, notably mercury-198 and krypton-86, permitted the production of purer radiations; but even then measurements could only be extended to 1 m with difficulty. The helium-neon laser, on the other hand, emits almost perfectly monochromatic light the wave-length of which has been measured at the National Physical Laboratory in terms of the krypton-86 radiation defining the metre. This practically removes the length limitation, and, in conjunction with other properties of laser radiation, extends the possibilities of measurement by light waves far beyond the usual measurement of end bars. A precise length-measuring machine using a laser has been designed and constructed using standard engineering techniques which will automatically measure the position errors of the lines on a scale with an accuracy in the region of 0.2 µm. Although there may be up to a thousand lines, their measurement takes only a few minutes; whereas by the classical methods a corresponding measurement used to occupy many hours. Moreover, the results are automatically recorded on paper tape and the subsequent analysis carried out by digital computer. Further developments will include application of the machine to metrological gratings and other types of scale, and refinement of its accuracy to better than 0.1 µm by closer control of ambient conditions.

Another exhibit which attracted attention in the same Division was an instrument designed for the easy and accurate measurement of distances up to one mile. This instrument known as *Mekometer II*, and believed to be the first of its kind in the world, can be regarded as a truly electronic tape-measure.

The device utilizes a light beam which is polarization-modulated in the ultra-high-frequency region. This measures the distance from the instrument to a reflector placed at the far end of the line. The modulation wave-length is conditioned by specially designed and constructed cavity resonators filled with dry air but allowed to acquire atmospheric temperature and pressure. By these means the velocity of light is excluded from the basic measuring equations and the results indicated by the device are scarcely affected by the state of the atmosphere. The accuracy is better than one part in one hundred thousand. Distance is indicated on dials directly in feet and the whole process takes but a few minutes whereas existing methods take hours. The first tests have been made using the National Physical Laboratory 800-m (984-ft.) base and the results obtained from *Mekometer II* differ from the true length by less than 0.005 ft. It is hoped to

improve the difference to  $\pm 0.002$  ft. later in the year. A commercial licence to manufacture has been granted.

Considerable attention is being given in Autonomics Division to the possibilities of the automation of industrial processes, and visitors to the Division were able to see an automated small-scale distillation column in operation. The column, designed to separate a mixture of ethanol and water, is now coupled to a Ferranti *Hermes* digital computer, and direct digital control of top product composition has been achieved. The equipment is being used to investigate techniques for estimating the dynamic characteristics of the process, and for achieving adaptive and optimal control. The computer accepts signals from temperature, flow-rate and product composition transducers. The last of these is a newly developed instrument based on the use of very-high-frequency resonators immersed in the liquid. Control is exercised by the computer over input heater power and reflux rate. Alterations can be made in the feed composition.

A steady-state model, based on the materials balance equations for the column, has been programmed in the computer to provide predictive control of the process to an optimum steady state. A dynamic model is derived in the computer by applying to the control variables small, specially designed binary perturbations. By cross-correlation of the resulting signals from the column transducers with respect to the applied perturbations the computer derives estimates of parameters describing the dynamic behaviour of the column. These estimates are continually updated to keep pace with temporal changes in the dynamic behaviour and they therefore define an adaptive model for use in more sophisticated control techniques. Programming for full adaptive control is well advanced and will include investigations of hill-climbing methods and of techniques based on dynamic programming.

Another problem being investigated in the Division is that of information retrieval. The retrieval of scientific information is now a formidable, but important, task. It presents many challenging problems which, with the possibility of mechanising many of the routine operations, are receiving much attention.

A mechanical system is being developed in which documents are indexed and enquiries are expressed in terms of descriptors. Each descriptor is a ground of associated key-words, the grouping being based on statistics of word co-occurrence in documents. During the retrieval process a comparison is made between the set of descriptors assigned to each document and the set used in formulating the request. Documents for which a perfect correspondence is found are retrieved, but it is also worth retrieving documents that have been assigned most, but not all, of the required descriptors. The retrieved items are therefore rank-ordered according to their degree of correspondence with the request.

The standard of retrieval which has already been achieved entirely by computer is illustrated by the following example. An enquiry for information on 'magnetostrictive band-pass filters' was made with reference to a collection of 12,000 abstracts covering electronics, computers and aspects of physics and geophysics pertinent to radio communication. The five abstracts which follow were at the top of the rank-ordered output:

(1) Electromechanical filters for use in telecommunication equipment. Includes descriptions of construction and performance of reed type magnetostrictive and piezoelectric filters.

(2) A practical electromechanical filter. Details of materials and fabrication techniques are given for narrow-band torsional filters.

(3) A theoretical analysis of the torsional electromechanical filters. The mechanical properties of a torsional system are expressed in terms of electrical equivalents. By introducing a transducer transfer ratio with the dimensions of charge a method is developed for the design of electromechanical filters based on equivalent electrical

networks. Pass band ripple spurious modes and transducer matching are discussed.

(4) Surface magnetostatic modes and surface spin waves. The general existence of surface wave modes in the frequency region above the spin wave band is shown.

(5) Image parameter theory for mechanical quadrupoles in compressional or torsional oscillation. The equivalence of the parameters of a mechanical system transmitting compressional or torsional oscillations and the parameters of an electrical transmission line is established. The design of mechanical filters is described and design formulae with response characteristics are given for the basic filter sections.

Much of the pioneer work in the design and application of computers was carried out in the Mathematics Division, which now has a *KDF9* in addition to its older machines *Ace* and *Deuce*. For many years the Division has concentrated on developing techniques and programmes for facilitating the scientific applications of computers. These have been used not only on the Division's own research in numerical analysis, applied mathematics, theoretical physics and engineering problems but also on work for the Laboratory as a whole and for outside organizations. The attention of visitors was directed to a variety of such studies. These included the calculation of stresses in concrete cooling towers, which was associated with experimental work on show in the Aerodynamics Division, and work on the mathematical design and analysis of ship hulls.

For any shape and size of cooling tower, the stresses induced by the wind and by the tower's own weight can be computed by solving a set of rather complicated differential equations. For routine office work, however, it is more convenient to refer to data sheets or graphs which summarize the results relating to suitable skeleton sets of shape parameters. The design of cooling tower shells for the Central Electricity Generating Board is now based entirely on data sheets derived from the method developed at the National Physical Laboratory.

The problem of mathematical ship fairing has been successfully treated; mathematical surfaces are fitted to the detailed measurements of a ship's hull taken from the naval architect's design drawings, and these can be used to simplify the construction of the ship. In addition, improved hull forms have been estimated following a statistical analysis of model tests which has produced an empirical relationship between resistance and suitably chosen geometrical parameters of the hull shape.

The work in Metallurgy Division on high-field superconductors, besides constituting a field of research, is an illustration of the part the National Physical Laboratory can and does play in undertaking from time to time the technical direction of national projects financed by Government.

The technological importance of superconductors has been recognized by the formation of a national Superconductivity Advisory Committee of which Dr. N. P. Allen, superintendent of the Metallurgy Division, is the chairman. This Committee is responsible for co-ordinating the research and development effort throughout the country formerly on behalf of the Department of Scientific and Industrial Research, and now of the Ministry of Technology.

The area in this field in which the Metallurgy Division is itself engaged is concerned with endeavouring to elucidate the relation between the metallurgical properties of superconductors and the ability of certain of these materials to sustain large current-densities in high magnetic fields. To this end, niobium has been selected for investigation since it exhibits the required behaviour in relatively low magnetic fields, thus making the necessary experiments somewhat simpler to perform.

The relation between the deformation of single crystals of niobium and their superconducting properties has



previously been investigated in the Division, and this work has now been extended to heavily deformed polycrystalline niobium. Cold-swaged niobium wire has been progressively annealed for fixed periods of time at temperatures of 200° C, 500° C, 700° C, 900° C and 1,100° C, and measurements of magnetization, critical current, and restoration of resistance taken from 4.2° K to 1.5° K after each heat treatment. A peak in the mechanical hardness for the 500° C treatment is matched by peaks in the various critical fields as defined by magnetic and resistance measurements, and it is believed that strain-ageing provides pinning centres which prevent the motion of magnetic flux through the material.

An additional piece of work involving the examination of the superconducting properties of sintered mixtures of copper and niobium was motivated by the present interest in adding large quantities of copper to superconducting wire to mitigate the effects of 'degradation' and 'flux jumping' when the wire is wound into coils. The properties of the resulting compacts are found to be strongly dependent on the distribution of the niobium particles in the sintered mixture, in addition to its density and composition.

Another interesting item on display in the Division was a goniometer stage adjustable over a wide range of angles, designed and built at the Laboratory for use in the *B.M.6* electron microscope, together with examples of the much increased information it has been possible to obtain as a result.

When a metal deforms at normal temperature the stress increases continuously with the amount of deformation. This is the phenomenon of work hardening. The enforced change of shape is accommodated by blocks of metal sliding over each other on certain preferred atomic planes (slip planes), a process facilitated by movement along these planes of a particular type of atomic defect known as a dislocation. During deformation the number of dislocations may increase from  $5 \times 10^7/\text{cm}^2$  to more than  $1 \times 10^{10}/\text{cm}^2$ , and as they move they interact with each other, tangling together to form an irregular cellular structure (Fig. 2). Moreover, the degree of strengthening

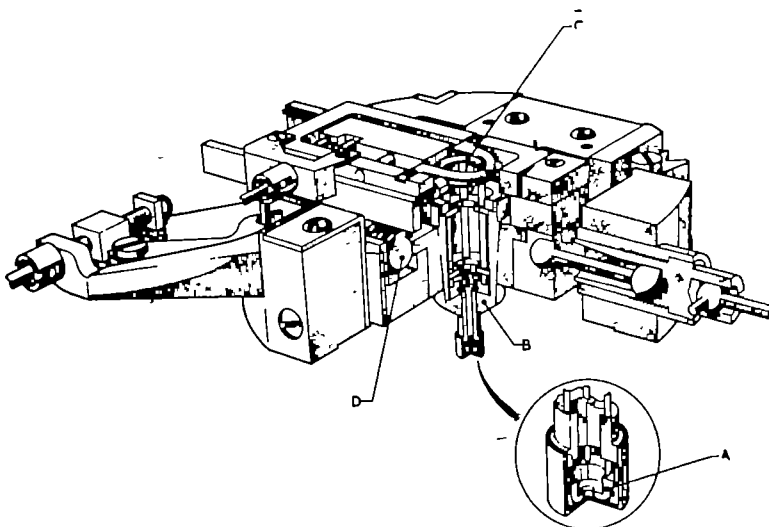


Fig. 2. The goniometer stage, adjustable over a wide range of angles, designed and built in the Laboratory

produced during deformation is known to be proportional to the square root of the number of dislocations present. It is therefore necessary to obtain as much detail as possible about the dislocation tangles so as to find out which types of dislocation are interacting with each other and why strength should be dependent on their number.

The technique for distinguishing between the dislocations makes use of a result obtained from electron diffraction theory, that a dislocation produces no contrast, that is, its image can be made to disappear, when electron diffraction conditions are such that the diffracted beam lies normal to the displacement field of the dislocation. This displacement field characterizes the type of dislocation; it is called its Burgers vector, and hence by knowing the diffraction conditions when a dislocation disappears its type can be determined.

Until now a bright field technique has been used in which the specimen is viewed in the usual manner using the directly transmitted beam. Under this condition, however, in order that the disappearance condition shall be unambiguous the diffraction conditions have to be arranged so that apart from the transmitted beam only one other diffracted beam operates. This is normally difficult to achieve.

A considerable improvement has now been made by the use of a high-resolution dark-field technique in which the specimen is viewed using a chosen diffracted beam instead of the transmitted beam. Two beam conditions are no longer essential though it is necessary to be able to tilt the electron beam so that the desired diffraction spot may be brought to the centre of the microscope. This has been achieved by the use of a rotatable beam tilt device. To vary the diffraction conditions it is also necessary to tilt the specimen inside the electron microscope so that it can be made to lie at a variety of angles with respect to the electron beam. Since the commercial specimen tilt device only permitted this within an angular range of  $\pm 5^\circ$ , the new goniometer was designed (Fig. 3). The specimen which is mounted between special grids, 2.3 mm in diameter, is placed in the hemispherical ball A which is tilted by the action of the three small push-rods housed in the cartridge B. The movement of the push rods is controlled by the swash plate C which is motor driven from outside the microscope. The specimen can also be rotated through  $360^\circ$  in its own plane by action of the worm and wheel D, so that, by a combination of rotation and tilt, the specimen can be accurately manipulated to lie in any plane within the angular range of  $\pm 30^\circ$  with respect to the electron beam. Some success has already been obtained with this goniometer in determining the

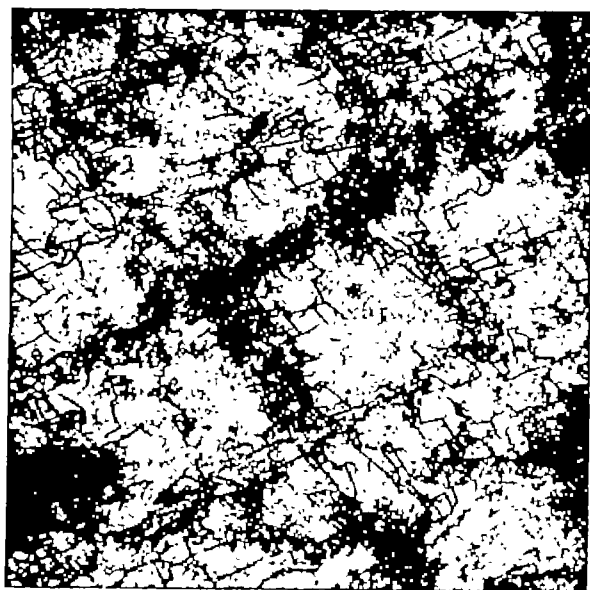


Fig. 2. A tangle of dislocations in iron ( $\times c. 10,000$ )



Burgers vectors of dislocations in iron and an iron alloy deformed over a range of temperatures.

At the open days last year, the Basic Physics Division released news of the discovery of a powerfully stimulated emission source in the sub-millimetre region at 0.34 mm wave-length. This year visitors were able to see the progress made in the development of this discovery into a practical laboratory source. The device is a gas discharge maser utilizing a rotational transition in the ON molecular radical, and has been given the name *Terairon* derived from the frequency of the radiation emitted. Discharge tubes only 3 ft. in length give useful amounts of power, and a commercial model with a portable power-pack is now being developed in co-operation with an industrial organization. Although the device is still at an early stage of development it is believed that great value is to be derived from making a powerful sub-millimetre source commercially available as soon as possible. At the Laboratory, the *Terairon* has already been used to measure errors in the lead screw of a lathe, and for dielectric measurements. The source promises also to be of value in teaching institutions because the wave-length is particularly suitable for demonstrating interference and diffraction phenomena.

A new field of research—Non-linear Optics—has recently been opened up in Basic Physics Division, and one of the items on display showed the results so far. The basic objective is to devise a modulator which will mix together optical and microwave signals in such a way that the output is a monochromatic signal the frequency of which equals the sum of the frequencies of the two incident waves. This follows work at low frequencies by Bührer, Baird and Conwell<sup>1</sup>. Present experiments are aimed at producing an efficient mixer in which the microwave energy is used to the best advantage. The system uses a tunable resonant cavity in which a circularly polarized 10 Gc/s microwave field is set up, and it is expected to

obtain complete frequency conversion with a microwave input of 10 W or less.

The High Temperature Group in Basic Physics Division is concerned with the behaviour of elements and compounds at elevated temperatures. Species, different in composition from those which exist at normal temperatures, often exist under these conditions and may be condensed to form new materials at normal temperatures. Gaseous reactants may be heated to 5,000°–20,000° K in the familiar d.c. discharge and radio frequency induction plasma torches which were exhibited. The RF plasma torch was used to demonstrate the oxidation of a mixture of metal halide vapours in oxygen plasma to form mixed oxides of small particle size and controlled stoichiometry. These devices are unsuitable, however, for reactions in which solid feed materials must be used since heat transfer from plasma generated in these ways is generally only sufficient to melt the particles.

A rotating plasma furnace was also exhibited in which the plasma of a d.c. arc column is expanded by tangential bombardment from cool gas molecules to occupy a volume of approximately 2.5 l. Solid feed materials may be vaporized in this device. Although arc expansion by this principle has been demonstrated before with low-current (~10 amp) discharges, the device exhibited is the first to include an electrode configuration by which high-current (~400 amp) expanded discharges may be stabilized at atmospheric pressure for long periods of time. The power (~20 kW) dissipated in this device and the residence time of particles fed into the plasma are great enough to enable solid feed materials to be vaporized completely. It is intended to use this furnace in fundamental studies of vaporization in plasmas and for a variety of chemical syntheses which involve high-temperature species.

H. A. SLOMAN

<sup>1</sup> Bührer, O. F., Baird, D., and Conwell, R. M., *App. Phys. Letters*, 1, 46 (1962).

## OBITUARIES

### Prof. Wilson Smith, F.R.S.

PROF. WILSON SMITH, emeritus professor of bacteriology in the University of London, died on July 10 at the age of sixty-eight. Wilson Smith's life-work in bacteriology may be considered as in three succeeding phases. His reputation was built on his early research, when he was a full-time worker under the Medical Research Council. Later, though by no means giving up his own investigations, he became an excellent teacher; and he finally devoted more and more time, as an elder statesman, to help in guiding scientific policy.

He was born on June 21, 1897, in Great Harwood, Lancashire, and always remained something of a Lancashire man. He went to school there at Accrington Grammar School and served with the 107th Field Ambulance during the First World War from 1915 until 1919. He then entered as a medical student at the University of Manchester, qualifying in 1923.

After a short while in general practice, he felt the call of bacteriology and took the course for the diploma in bacteriology at Manchester under Prof. Topley. He not only obtained the diploma but also married one of Topley's young demonstrators. He then came to London, joining the staff at the National Institute for Medical Research, then at Hampstead.

Those were days when filter-passing viruses, as we then called them, were first becoming a subject of serious study. Wilson Smith took part in the exciting developments in this field, working at first under S. R. Douglas on vaccinia virus. Then, in 1933, came his most important contribution to knowledge. He and I were making an all-out endeavour to find a virus in influenza: even as we planned,

I fell sick with the disease and Smith carried on, inoculating my filtered throat washings into various laboratory animals. Guinea-pigs, rabbits and mice were injected, mostly intramuscularly or subcutaneously; and nothing happened. Then came the turn of ferrets, available because of their use by Laidlaw and Dunkin in current work on distemper. Somehow inspired, he this time put some of the washings up the ferrets' noses and two days later one developed nasal discharge and sneezing. This first step led on to an immense amount of work on the 'flu virus and subsequent developments kept him interested for the rest of his life. Discovery of the susceptibility of ferrets to 'flu entailed the building of a 'ferret hospital' with 32 isolation units at Mill Hill. Laidlaw, Smith and I made daily clinical rounds of our ferret 'patients' every morning for some years, working in the laboratory at Mill Hill in the morning and returning to the Institute at Hampstead for other work in the afternoons. It was duly revealed that mice as well as ferrets could be infected, that neutralizing and complement-fixing antibodies developed in the sera of both man and ferret, that our virus was related to that of Shope's swine influenza virus and that a formalin-inactivated vaccine could be used to immunize. All this came out of the team-work: particular contributions made by Wilson Smith himself were to show that the virus would grow in fertile hens' eggs and in tissue culture.

In 1939 Wilson Smith left Mill Hill to fill the chair of bacteriology in the University of Sheffield. With the outbreak of the Second World War it was a difficult time to begin a new kind of life. Besides his teaching duties, he was put in charge of local public-health bacteriology

and of the penicillin treatment of local Service casualties. He had the unpleasant experience of having his house collapse on top of him as a result of an air-raid; fortunately he was in a cellar and was unharmed.

Soon after the War, in 1946, Wilson Smith returned to London to the chair of bacteriology at University College Hospital Medical School, where he remained until his retirement in 1960. At University College Hospital he stimulated much further work, particularly on influenza and poliomyelitis. He showed that some host components became integrated into the structure of influenza virus particles, but his conclusion that this incorporation is of basic importance in virus evolution is somewhat doubtful. Wilson Smith's reputation as a teacher and investigator rests mainly on his clarity of thought and utterance, his critical faculty and his very high standards of accuracy.

These qualities proved invaluable in his later years when he came to be much in demand as a member or chairman of numerous committees of the Medical Research Council and other bodies. He was a member of, the Medical Research Council from 1961 until his death. Several of these committees were concerned with virus vaccines—for influenza, poliomyelitis and measles. Sir Harold Himsworth has written of Smith: "The development of the safe and effective vaccines now available in this country is in no small measure due to his own efforts".

On his retirement he became consultant adviser to the Microbiological Research Establishment at Porton. While here he edited a book on *Mechanisms of Virus Infection*, of which he wrote the introductory chapter himself.

Wilson Smith was elected a Fellow of the Royal Society in 1949 and delivered its Leeuwenhoek Lecture in 1957. He became a Fellow of the Royal College of Physicians in 1959. The University of London gave him a Graham Gold Medal in 1960. His very high standards contributed greatly, through his own work and that of those whom he taught, to the advance of virology in its rapidly developing years. At the end, when he developed a progressive and debilitating disease, he showed great courage in extracting the last ounce of energy from himself in the service of medicine. Though his enthusiasms had their ups and downs, he had a keen sense of humour. He was an enthusiastic violin player and derived much enjoyment, and some profit, from buying and selling old violins.

He leaves a wife and two married daughters.

C. H. ANDREWS

Prof. B. Lindblad

BERTIL LINDBLAD was born on November 26, 1895, at Örebro, Sweden. After studying at the University of Uppsala, and later becoming an assistant at the Observa-

tory there, he joined the Stockholm Observatory. He became director of the Observatory in 1927 and held this office at the time of his death.

Lindblad was an important pioneer in the determination of absolute magnitude from studies of stellar spectra and also in the development of the kinematics and dynamics of our own Galaxy. Following on the work of Adams and Kohlschutter, who demonstrated that the difference between giants and dwarfs could be seen in stellar spectra, Lindblad showed that the cyanogen bands were an important criterion of absolute magnitude. He derived absolute magnitudes from his own scheme of spectral classification, and used these absolute magnitudes, combined with the available apparent magnitudes and proper motions, to give distances and transverse velocities. He was able to show by means of these data that the motions were consistent with his concept of the Galaxy as an assemblage of rotating sub-systems, the rotation and dispersion of the internal velocities varying with the degree of flattening of the sub-systems. He considered the high-velocity stars as forming one of these sub-systems, and explained the tendency of the directions and motions of these stars to avoid the hemisphere, the centre of which is in the galactic plane at longitude 60°—in modern terms, to avoid the direction in which the Galaxy rotates. Lindblad demonstrated that the phenomenon of star streaming was connected with galactic rotation, and that the direction of the preferential stream was approximately the centre of the galactic centre. Lindblad is therefore an important figure in the development of stellar dynamics from the early work of Schwarzschild and Eddington to the later work of Oort, in which the notion of galactic rotation is fully recognised in galactic kinematics. He later extended his interests to the dynamics of galaxies in general, and wrote many papers about the development of spiral galaxies.

While Lindblad's work on stellar luminosities was very practical in character, and consisted of determining luminosities from the cyanogen band and from an extended scheme of spectral classification which he himself developed, his work on galactic rotation and his researches on spiral structure were extremely mathematical, and in many topics which are of interest to-day in connexion with theories of the Galaxy it will be found that the leading ideas are contained in Lindblad's work.

His influence over Swedish astronomy and Swedish astronomers was very great and he was extremely well known in international circles—he was president of the International Astronomical Union from 1948 until 1952. He was also president of the International Council of Scientific Unions from 1952 until 1955. He received the Gold Medal of the Royal Astronomical Society in 1948.

RICHARD WOOLLEY

## NEWS and VIEWS

Commonwealth Advisory Committee on Advanced Education (Australia): Dr. I. W. Wark, C.B.E.

DR. I. W. WARK has been appointed chairman of the Commonwealth Advisory Committee on Advanced Education (Australia). Dr. Wark is at present a member of the Executive of the Commonwealth Scientific and Industrial Research Organisation. The Committee will make recommendations to the Commonwealth Minister in Charge of Commonwealth Activities in Education and Research as to the distribution of funds available for support of both capital and recurrent expenditure for projects recommended by institutes or colleges or State Governments and accepted by the Commonwealth as suitable subjects for support in colleges of advanced

education. The Commonwealth's decision to assist the States in developing advanced education facilities arose from its consideration of the report of the Committee on the Future of Tertiary Education. 'College of advanced education' is the title chosen for new tertiary colleges which will provide courses requiring an entry standard equivalent to university entrance and providing a diploma qualification on completion of such courses. These colleges will in many cases be developed from and around the tertiary sections of existing technical colleges in the States, and one of the Committee's principal tasks will be to encourage their development, not only in technology but also in the liberal arts. The Commonwealth's assistance to these colleges will be to strengthen and expand their diploma courses, but it does not intend to support finan-

cially their conversion into universities under another name.

Dr. Wark's appointment is a full-time one, and he will be assisted by a small number of part-time members. Dr. Wark has been a member of the Executive of the C.S.I.R.O. since 1961 and was formerly director of that Organization's Chemical Research Laboratories and the chief of its Division of Industrial Chemistry.

#### Principal of Chelsea College of Science and Technology: Prof. M. R. Gavin

PROF. MALCOLM ROSS GAVIN, professor of electronic engineering and head of the School of Engineering Science in the University College of North Wales, Bangor, since 1955, has been appointed principal of Chelsea College of Science and Technology and the vice-chancellor-elect of the new University. Prof. Gavin was educated at the Hamilton Academy, Glasgow, and the University of Glasgow, from which he graduated in mathematics and natural philosophy in 1929. He was awarded a D.Sc. degree by the University of Glasgow in 1949. After an early career in school teaching, he joined the Research Laboratories of the General Electric Co. (now the Hirst Research Centre) at Wembley. In 1947, he entered the Inspectorate of the Scottish Education Department. During 1950-55 he was head of the Department of Physics and Mathematics in the Birmingham College of Technology, where he was also vice-principal during 1953-55. Prof. Gavin was then appointed to the University College of North Wales, Bangor, where he has since built up a School of Engineering Science noted for its progressive approach to the education of applied scientists. He is a member of the Science Research Council and vice-president of the Institute of Physics and the Physical Society. He was a member of the Electronic Research Council of the Ministry of Aviation during 1960-64. Prof. Gavin has been closely associated with Chelsea College since 1964 as a member of the Academic Advisory Committee set up by the Governing Body in consultation with the University Grants Committee. Prof. Gavin succeeds Mr. C. C. Hentschel, who retires in September 1965 after thirty-four years, full-time service at the College. Mr. Hentschel became principal in 1962 on the death of Mr. N. M. H. Lightfoot, having previously been vice-principal since 1961 and head of the Department of Botany and Zoology since 1952.

#### Pathology in the University of Liverpool:

Prof. H. L. Sheehan

At the end of September, Prof. H. L. Sheehan will retire from the George Holt chair of pathology in the University of Liverpool to which he was appointed in 1946. At the time of his appointment he was already an authority on the pathology of pregnancy and the puerperium and had shown that necrosis of the anterior lobe of the pituitary gland, which commonly occurs during obstetrical shock, is a cause of chronic ill health in women that have survived an episode of obstetrical shock. During his tenure of the chair in Liverpool he clarified the clinical picture of post-partum hypopituitarism, now known as Sheehan's syndrome, and contributed monographs and many papers on the morbid histology and experimental pathology of renal cortical necrosis and hydronephrosis. His enthusiasm for clinical endocrinology and the importance of endocrine factors in cancerous conditions led to the establishment in Liverpool of an Endocrine Unit with Prof. Sheehan as director, and gradually the problems of neoplasia became one of his main interests; he plans to devote his official retirement to cancer research. Prof. Sheehan's effectiveness as a lecturer and his willingness and ability to lecture in foreign languages led to many invitations to lecture abroad; he has given many lectures in Europe on both sides of the Iron Curtain and in Australia, New Zealand and the United States. He is to

begin his 'retirement' with a series of lectures in South America.

#### Prof. J. R. Anderson

DR. J. R. ANDERSON has been appointed to succeed Prof. H. L. Sheehan in the George Holt chair of pathology in the University of Liverpool. Dr. Anderson graduated B.Sc. (1939) and M.D. at the University of St. Andrews (1955), gaining a University Gold Medal for his thesis on "Immune Antibodies in Haemolytic Anaemia". He was the first to produce in the rabbit haemolytic disease of the new-born that closely patterned human disease, and these studies led to extensive investigations on auto-immune disease in man, a field in which he is a recognized authority, with more than 30 publications mainly on the so-called collagen diseases. His work on non-tuberculous Addison's disease has helped to identify the site of formation of various adreno-corticosteroids. After initial experience in pathology with Prof. D. F. Cappell in Dundee, Dr. Anderson served for three years as a graded pathologist in the Royal Army Medical Corps, chiefly in west and north Africa. In 1947 he returned to rejoin Prof. Cappell, now in Glasgow, as lecturer and became senior lecturer in 1954 after a Rockefeller fellowship with G. H. Whipple and L. E. Young in Rochester, N.Y. Dr. Anderson is a lucid and interesting lecturer who has had a wide experience of undergraduate and postgraduate teaching and examining; his publications (with Dr. Lennox) on the use of multiple choice papers (1957) and on selection procedures in the light of students' examination performance (1964) have attracted much attention. His extensive experience of hospital diagnostic pathology has given him an enviable reputation as a sound opinion in such matters, which is much sought by his clinical colleagues.

#### Director of Research, Marconi Company:

Mr. G. D. Speake

MR. G. D. SPEAKE has been appointed director of research in the Marconi Company. Mr. Speake was formerly chief of research at the Great Baddow Research Laboratories, and development manager for the Radar Division. This new appointment will enable Mr. Speake to concentrate on the task of reorganizing and strengthening the research activities of the Company. George David Speake was born in 1919 in Newport, Shropshire. He was educated at Adams Grammar School, Newport, and took his degree in physics at St. Catharine's College, Cambridge. He joined the Marconi Company in 1950 and was engaged in Radar Systems Research until 1954, when he was appointed chief of the Vacuum Physics Section of the Research Division. Two years later he became chief of the Microwave Physics Section, and in 1960 he was appointed deputy chief of research under Dr. E. Eastwood. In 1962, when Dr. Eastwood was appointed director of engineering and research for the Company, Mr. Speake became chief of research, and he has remained in this position until this new appointment. Before joining the Marconi Company, Mr. Speake was a flight-lieutenant in the Technical Branch of the Royal Air Force, and, later, instrument manager in the Plastic Division of Imperial Chemical Industries, Ltd.

#### Government Advisory Committees

IN a written answer in the House of Commons on February 28, the Prime Minister gave Mr. P. Dean a list of 241 advisory committees, most of which had been set up during this century, and more than half of which had been established since the Second World War. Since that question was raised, the Advisory Council on Scientific Policy has become an executive body, a Social Research Council has been established, and so also an Advisory Committee for Scientific and Technical Information. Accordingly, Mr. Dean raised the question of Standing Advisory Committees in an adjournment debate on

July 28. He suggested that while these committees had an important part to play in Britain's system of government, particularly in regard to scientific and technical advice, it was desirable to take a closer look at the part they play, the way in which they were developing and their constitutional significance. Mr. Dean said that besides increasing in number, the scope of the Committees was tending to widen so that they dealt increasingly with major aspects of policy and advised on a much broader front than many of the early committees, which were largely technical or specialist. He challenged the necessity for the reports of many of these committees to be confidential, citing some obvious examples of discrepancy.

Mr. N. MacDermot, the financial secretary to the Treasury, who replied for the Government, agreed that advisory committees were a valuable institution of government, but did not think that at present there was any burning issue calling for official examination of the scope and activities of these committees. If a review of their activities was desirable, he thought that the Estimates Committee might be a suitable body to undertake such an investigation, unless it was desirable to appoint an outside body. On the cost of these committees, Mr. MacDermot said that the total cost in remuneration and expenses was about £150,000 a year, in addition to the secretarial services provided for them, which cost a further £280,000. There had been no change in the policy regarding publication of reports, but he pointed out that care should be taken to avoid confusion between the role of Ministers, on one hand, and that of the advisory committees, on the other. Whether the advice was published or not was the responsibility of Ministers on whom responsibility for policy also rested: publication of the advice tended might sometimes exert pressure on Ministers. He thought it was undesirable to make it an invariable rule that the advice of committees must be published, as we might thereby find ourselves deprived of some of the freedom we now had in obtaining expert opinion. Neither Mr. Dean nor Mr. MacDermot referred to the study of advisory committees published in 1960 by Political and Economic Planning or to the earlier study of the use of "Committees in Government", published in 1955 by Prof. K. C. Wheare.

### The Nature Conservancy

In a written answer in the House of Commons on August 2, Mr. A. Croxall, the Secretary of State for Education and Science, stated that he had approved proposals submitted by the Natural Environment Council for senior headquarters staff serving the Nature Conservancy. The next director of the Conservancy would be a scientist appointed at the upper level of the chief scientific officer grade (£4,700) and would be supported by a deputy director for the organization and planning of research, and a deputy director for conservation and management. The former would be a scientist at deputy chief scientific officer level and the latter either a scientist at the same level or an administrator at assistant secretary level. These proposals were prepared by a working party, headed by the chairman of the Council, Sir Graham Smith, and were endorsed by the Council and carried the full support of the chairman of the Conservancy.

### Towards a Manpower Grid

There is a growing realization that, in raising productivity from the recent levels of 1.5-2 per cent to the 3.5 per cent national target, efficient utilization of industrial manpower plays a critical part. International comparison with other leading industrial countries implies that British management is wasteful in its use of manpower. Viewed from the other angle, restrictive working practices make worse a regressive situation. The apparent full employment of the British economy is, in fact, a

spurious interpretation of the real employment situation. Present high employment is falsely bolstered by the over-manning of the profitable sectors of the industry, and retention of labour in industries that have ceased to be fully economic. The degree of real under-employment that this involves is variously estimated; however, qualified observers consider that the elimination of it would release between 10 and 15 per cent of the industrial work force for re-deployment into other work. If Britain is to remain internationally competitive during the next decade, her industry will have to make real inroads into this manpower problem. An article by Michael Hall, manager (recruitment and manpower), Esso Petroleum Co., Ltd., directs attention to the need for more efficient instruments and methods of manpower planning at all levels in industry and at the national level (*Personnel Management*, 47, No. 372; June 1965). Without improved data sources or manpower forecast techniques, it is difficult to see how the massive labour movements, between skills, localities of employment or between employers, that the present economic analyses imply to be necessary can be achieved.

### Education of Engineers in Western Germany

In a lecture delivered at the summer meeting of the Institution of Mechanical Engineers at Wiesbaden, Western Germany, in June, Dr. G. Branken described the education of professional engineers in Western Germany. He began with the school system which feeds the technical schools, whether Technische Mittelschule, Technikerschule or Technische Hochschule. One of the two qualifications of a professional engineer is the Diplom-Ingenieur, obtained after third-level engineering education in Technische Hochschule, normally by a four-year course. Practical training in industry is a prerequisite condition of entry. In 1964, there were 55,000 students in the nine Technische Hochschulen and 4,500 graduates, compared with 13,500 graduates from the 122 Ingenieurschulen with their 52,472 students in three-year courses, and 8,000 graduates from the 220 Technikerschulen with their 30,000 students, which provide day courses of a year and a half and 3-4 years in evening classes for technicians. Most of the professional engineers are later placed in design and planning; only 10 per cent are placed in research and development. The academic education of engineers at Technische Hochschulen has been extended by one to two years over the past fifteen years, although at most of the Hochschulen the courses are still four years (*Proc. Inst. Mech. Eng.*, 179, Part 4; 1964-65).

### Institution of Production Engineers

The *International Journal of Production Research*, published by the Institution of Production Engineers, is a quarterly periodical now beginning its fourth year, and in the latest issue an editorial note deservedly chronicles achievements since its inception (4, No. 1; 1965). The University, Birmingham 15, and 10 Chesterfield Street, London, W.1). First, this *Journal* is truly international in that its advisory board consists of distinguished members from many parts of the world, and it now circulates in forty countries; secondly, it is a useful link between research workers through which progress may be reported and new ideas generated; thirdly, it covers fields of study to-day recognized as constituent parts of production engineering. Among the papers in the present issue are: "The Ductile-Brittle Transition when Machining Perspex", by R. F. Scrutton, which identifies the conditions under which 'Perspex' can be machined in a ductile manner avoiding troubles often incurred with this and other brittle plastic materials; "A Case Study of Programmed Instruction in a Self-Correcting Teaching System in an Apprentice Training School", by B. T. Dodd, concerning accelerated training methods designed to reduce the period of craft apprenticeship from five to four

years; "Work Sampling using Extended Observations", by E. N. Corlett and R. H. Hollier, describing 'snap sampling' as applied to estimations of proportions of time spent in activities by operators and machines, but with an extended technique to provide idle time distributions, and the method involved; "Mechanics of Chip-breakers", by T. L. Subramanian and A. Bhattacharyya, deals with the problem of chip disposal now more acute with the introduction of high-speed carbide and ceramic tools, and presents a physical explanation of behaviours of the cutting variables encountered; W. A. Morcos contributes an article on the complex problem of "Interchangeability of Screw Threads: a Study of the Effective Diameter Equivalent of Flank Angle Errors for a Basic Taper Thread", with appropriate mathematical treatment and new methods of determination; a paper on "Classification of Pacing", by K. F. H. Murrell, explains the part played by tolerance in the classification and the relationship between tolerance and machine rate. With each contribution an appropriate synopsis in both French and German is included.

#### A. P. Sloan Foundation

THE report of the A. P. Sloan Foundation for the two years 1963-64 records commitments authorized by the Trustees of 30.4 million dollars. In support of basic scientific research, 5 million dollars went to the Massachusetts Institute of Technology to establish a special institutional research fund, and 1 million dollars to four mid-continent universities to strengthen their science facilities and teaching. The level of expenditure in the Foundation's fellowship programme for basic research in the physical sciences was increased from 1.2 million dollars to 1.4 million dollars, beginning in 1965. For the academic year 1963-64 there were 144 Sloan Research Fellows in 49 institutions in the United States and in Canada, compared with 155 in 48 institutions in September 1964. A grant of 1 million dollars was authorized for the new Mathematics Centre at Stanford University and one of 5 million dollars for Massachusetts Institute of Technology to establish a pioneering programme of continuing education for experienced engineers. Virtually all the Foundation's grants for cancer research (totalling 1.4 million dollars) went to the Sloane-Kettering Institute for Cancer Research in New York and to affiliated programmes; however, grants were also made for research in glaucoma and urethritis and an initial grant for a programme of research in otology. The Sloane National Scholarship Program now embraces 45 universities and colleges and totals 1.28 million dollars: a special grant of 500,000 dollars was made to the United Negro College Fund in 1963, as well as 200,000 dollars to other projects for strengthening educational opportunities for Negro students. A grant of 1 million dollars was made to Stanford University to complete a new Centre for its Graduate School of Business, and other grants in the same field were to the Columbia University Graduate School of Business, the Tuck School of Business Administration of Dartmouth College, and the Sloan School of Management of the Massachusetts Institute of Technology. Grants for economic research and education (mainly to the National Bureau of Economic Research) exceeded 1 million dollars and commitments for improving the public understanding of science totalled 275,000 dollars. In June 1963 a grant of 100,000 dollars was made to the United States Churchill Foundation for scholarships and fellowships.

#### Education In Forestry

UNDER the title *Education in British Forestry* a report of the fifth discussion meeting organized by the Society of Foresters of Great Britain has been issued as a supplement of the Society's journal, *Forestry* (Pp. 74. London: Oxford University Press, 1965. 7s. 6d.). The discussion

was held at Cirencester during January 8-10, 1965, and the three sessions dealt with the education of foresters; the education of forest officers; and with forestry education for the wider field. Besides Prof. M. V. Laurie's opening address and the text of the ten papers contributed, the report includes summaries of the discussion in each session and Mr. J. J. Lawrie's closing remarks in which he referred to responsibilities towards Voluntary Service Overseas and to the opportunity afforded by the conference, "The Countryside in 1970". There was some frank criticism of British forestry education below university level and a resolution was passed recommending the Council of the Association, in co-operation with the Forestry Commission and other interested bodies, to investigate the establishment of institutions suited to both State and other forestry interests. At the university-level the meeting was impressed by advances in recent years, by the scope of present curricula, and by the lively outlook.

#### Scientific Societies In India

*A Survey of Scientific Societies in India*, by A. Rahman, N. Sen and N. R. Rajagopal of the Survey and Planning of Scientific Research Unit, has been published by the Council of Scientific and Industrial Research, New Delhi (Pp. 40. New Delhi: Council of Scientific and Industrial Research, 1965). Data were collected on the organization and role of such societies with the view of correlating them with the scientific and technological development of the country. Prior to 1930 there were few such societies and of the 27 listed 22 were established for specialized fields, 10 being concerned with engineering and technology. During 1931-50 the number of societies increased three-fold in the physical sciences and in the medical and biological sciences, and doubled in engineering and technology; this is attributed largely to the development of a base for research in the country. From 1951 onwards the trend to establish more scientific societies continued to increase, and is marked by the establishment of such highly specialized societies as the Indian Cancer Society, the Indian Society of Haematology, the Indian Society for Helminthology, the Institute of Telecommunication Engineers, the Indian Society for Rayon Technologists, the Indian Leather Technologists Association and the Institute of Information Scientists. The societies are heavily concentrated in cities like Calcutta, New Delhi, Bombay, Bangalore and Madras, where 118 out of 150 are located and only 54 per cent have a membership of 100 to 500. Only the Institution of Engineers, India, with 42,887, has more than 5,000 members. Almost 90 per cent issue periodicals and more than 31 per cent receive financial support from the Central Government, about 80 per cent from State Governments and about 18 per cent from industry. Of the 103 societies giving figures for income and expenditure, about 36 per cent had annual incomes by 1962 of 10,000-50,000 Rs., and some 7 per cent an income of less than 1,000 Rs. The total membership of 115 societies out of the 150 is about 95,000 compared with a total of postgraduate scientists, medical graduates and engineering graduates of about 188,000.

#### Journal of Labelled Compounds

THE first number of the new quarterly *Journal of Labelled Compounds* contains eight original contributions, one German, three French and four English, with English summaries, together with a section containing abstracts of 67 papers dealing with the synthesis of labelled compounds and related compounds (1, No. 1, January-March, 1965. Pp. 1-92. Brussels: Presses Académiques Européennes, 1965. Annual subscription: B.F.I.250; 25 dollars). The editor of the *Journal* is J. Sirchia of Euratom (Brussels) and there is an editorial board consisting of twenty members. The abstractor is H. Dworschak of the Information and Documentation Centre, Euratom. Two of the

articles, written by members of the Division of Pure Chemistry, National Research Council, Canada, deal with deuterated organic compound synthesis, and two, by members of the University of Rome, with self-radiolysis of tritium-labelled compounds. L. Pichat, M. Herbert and F. Aubert of Saclay describe a new method of preparation of glycerol  $1-^{14}\text{C}$  and glyceric acid  $1-^{14}\text{C}$ . W. G. Verley and C. Gerday of the University of Liège discuss a method for the evaluation of the radiochemical purity of the labelled isomer of an amino-acid which they have applied to tritiated L-phenylalanine. Only a small sample is required but very minute contaminants can be detected.

#### Publications of the International Atomic Energy Agency

THE International Atomic Energy Agency, in addition to its many scientific activities, is now one of the largest publishing enterprises in Vienna. It has recently completed its sixth year of publication of literature dealing with the peaceful uses of atomic energy. In 1959 its output was two volumes in the *Proceedings* series, one in the *Safety* series and four technical directories, in addition to those publications prepared for free distribution, but to-day its average annual production consists of about twenty volumes of the proceedings of scientific meetings, six technical directories, several bibliographies and numerous technical reports. Two scientific journals, *Atomic Energy Review*, a four-language periodical published at irregular intervals, and *Nuclear Fusion*, a quarterly devoted to plasma physics and power from the fusion of light nuclei—together with the monthly *IAEA Bulletin* which describes the activities of the Agency in more general terms—are also published. Some publications are issued in multilingual form, others in each of the four working languages—English, French, Russian and Spanish—of the Agency. Since 1963, when printing and binding equipment was installed, the Agency has printed and bound all its own publications except the journal *Nuclear Fusion* which is separately managed in Vienna by a scientific editor and supervised by an editorial board. An up-to-date catalogue, *Publications in the Nuclear Sciences 1965*, listing all the priced publications of the Agency, either currently available or in the press, is available free of charge on request (Pp. 99. Vienna: International Atomic Energy Agency, 1965). It supersedes all previous catalogues and supplements. The titles are grouped under general subject headings: biology, medicine and agriculture; health, safety and waste disposal; physics, plasma physics and electronics; chemistry, geology and raw materials; reactor physics and reactors; industrial applications; economics; and law; corresponding to the separate activities of the Agency. They are listed in inverse order of publication, with the most recent first. Each item, in addition to the title and author, gives a summary of the contents, the language or languages, and the retail price in both dollars and sterling. Those publications which are also part of a series publication of the Agency are listed again in the "Series Index" which forms the second half of the catalogue. A list of sales agents and a title index complete the catalogue.

#### Bibliography on the Commonwealth and Its Constituent Countries

No. 45 of the *Special Subject Lists* issued by the Library Association, which is entitled *The Commonwealth To-day*, is a select bibliography on the Commonwealth and its constituent countries (By A. J. Horne. Pp. 107. London: The Library Association, 1965. 24s.; L.A. Members 18s.). There is a foreword by Sir Kenneth Bradley and the list is admirably printed and arranged. It includes no periodicals and only a few government publications, being limited mainly to books published in recent years and still in print. That qualification is doubtless the reason for some surprising omissions, and it is the shortcomings

of the book trade to-day that are displayed rather than weaknesses in selection. Titles are arranged on a geographical basis, by continent and country, and Mr. Horne has supplied an excellent and useful bibliography. While there are undoubted advantages in limiting a bibliography to books in print, the disadvantages of this practice have probably never been so marked as at the present time.

#### Bibliography from the Institute of Nutrition, Budapest

INSTEAD of the year-book, the Institute of Nutrition Budapest, has published a bibliography listing the titles of the various publications, etc., produced by its scientific members during 1964 (*Bibliography of Publications 1964*, Institute of Nutrition, Budapest, Hungary. Pp. v+21. 1965). The sources from which the titles are drawn include papers published in Hungarian and other journals, books, dissertations for degrees, contributions at congresses and lecture notes. The 81 headings cover a wide range of subjects such as the fatty acid composition of milk powder, radioactivity in milk, the application of polarography to amino nitrogen and oxytetracyclines, vitamin status and protein requirements in humans, food colour, food poisoning and hygiene. Each heading in the bibliography is given in three languages including English.

#### Synthetic Emerald

ATTEMPTS at the synthesis of many gemstones have a long history in appropriate technical literature, including emerald; latterly, beryllium and its compounds have assumed considerable economic importance in fields unsuspected only a few years ago. Scientists in the Hughes Aircraft Co., Culver City, California, have been working on synthetic emerald production since 1961 with an encouraging measure of success; the spur for this work stemmed from the need for large high-quality single crystals of emerald that could be grown rapidly; in the field of solid-state physics, for example, they find use in such devices as millimetre-wave masers. An interesting account of this work, brief but informative, is presented in the house journal *Vectors*, a quarterly publication by Hughes Aircraft Company (7, No. 1, 1965). Hitherto what is known as the flux-fusion process for synthetic emeralds has been used, but the Hughes scientists have developed a flame fusion technique which "... can produce an emerald 'boule' (pear-shaped mass of a substance formed synthetically in a special furnace) in a matter of several hours, as compared with the many months required by the flux fusion process". The flame fusion process involves beryllium oxide, a highly toxic substance, and special isolated laboratory facilities are requisite because of the poisonous vapours evolved; the other oxides concerned are those of aluminium and silicon, the proportions (by weight) being given as: beryllium, 16 per cent; aluminium, 18 per cent; silicon, 67 per cent. A minute quantity of chromium oxide is added to the mix to provide the emerald colour. The mixture is placed in one-gallon jars, rolled on a ball mill rack for 8 h, then sintered at  $1,050^{\circ}\text{C}$  for five days. The resulting powder is fed to the flame fusion unit comprising an oxy-hydrogen burner capable of a temperature of  $2,800^{\circ}\text{C}$  and appropriate cooling facilities. "The powder mixture, fed in from a hopper at the top, flows through the centre tube of the burner accompanied by a stream of oxygen to a ceramic pedestal where the boule grows". These Hughes emeralds are about 0.5 in. long and 0.25 in. diameter, blue-green in colour. X-ray photos show them to be indistinguishable from those of natural emeralds. These man-made gems are, incidentally, produced at a cost of not more than one-fourth to one-third that of the natural stones.

#### Sugar Research In Mauritius

THE annual report of the Mauritius Sugar Industry Research Institute for 1964 is the record of a very credit-



able effort (Pp. 117+xxxii+xxii. Reduit: Mauritius Sugar Industry Research Institute, 1965). However, 1963 was an unfortunate year for Mauritius and the report shows up clearly the great risks involved in tropical agriculture. The area of land under sugar cane was much the same in 1964 as in the previous year, but "A combination of negative factors, dominated once more by the occurrence of two cyclones, were responsible for an overall sugar production of only 520,000 tons, which was below that of the previous year by 24.2 per cent". Among the negative factors stands out the dangerous fact that "Two major diseases, gummosis and leaf scald, which had been totally eradicated from commercial fields, reappeared in epidemic form and are believed to be caused by new and more virulent strains of the pathogens". Only to the continuous work of the Research Institute and its constant search for disease-resistant new cane varieties is it due that "A varietal replacement programme can be designed which should not adversely affect the present sugar potential of the island". The great majority of the report is devoted to the cultivation of the sugar cane and to its diseases and pests; only 15 pages deal with problems of sugar manufacture and a single article (3 pages) mentions an unpromising by-product.

#### The Linnean Society of London :

##### Foreign Members

DR. E. I. WHITE, president of the Linnean Society of London, announced at the general meeting on April 22 that the following foreign members had been elected: Dr. S. M. Bukasov (U.S.S.R.); Prof. A. Frey-Wyssling (Switzerland); Prof. C. L. Hubbs (United States); and Prof. J. Leonard (Belgium).

##### Awards and Medals

THE following medals were awarded: The *H. H. Bloomer Award and Medal*, to E. C. Wallace; *Linnean Gold Medals* to Dr. J. Hutchinson and Dr. J. Ramsbottom.

##### Officers

THE following officers were elected: *President*: Dr. E. I. White; *Treasurer*: The Earl of Cranbrook; *Botanical Secretary*: Mr. J. P. M. Brenan; *Zoological Secretary*: Dr. H. G. Ververs; *Editorial Secretary*: Dr. J. Smart.

##### Vice-Presidents

THE following were elected vice-presidents for the session 1965-66: The Earl of Cranbrook, Prof. C. T. Ingold, Dr. R. W. J. Keay and Dr. Doris M. Kermack.

#### Society for Applied Bacteriology : Officers

At the annual meeting of the Society for Applied Bacteriology the following officers were elected: *Honorary President*, Dr. Joan Taylor; *Honorary General Secretary*, Dr. J. R. Norris; *Honorary Meetings Secretary*, Dr. Ella M. Barnes; *Honorary Treasurer*, Mr. G. E. Jones; *Honorary Editors*, Dr. F. A. Skinner and Mr. G. Sykes; *Honorary Publications Manager*, Mr. A. H. Walters; *Members of the Committee*, Dr. F. W. Beech, Miss Vera G. Collins, Dr. Ann W. Cooper, Dr. Christina M. Cousins, Mr. B. M. Gibbs, Dr. Elizabeth M. Harper, Dr. R. M. D. Keddie, Mr. D. A. Shapton and Dr. P. D. Walker.

#### Perkin Centenary Awards, 1966-1967

APPLICATIONS for the award of Perkin Centenary scholarships are invited by the Perkin Centenary Trust. Awards, tenable for one or two years, and renewable for a further year at the discretion of the Trustees, are offered to candidates employed in an industrial firm or other institution concerned with the manufacture or the application of colouring matter, for study at a university or technical college. Two types of award are available, some to the value of £100, which are to be used in conjunction with a local education authority grant, and

others to a value of £400, which are intended for students whose normal grant, inclusive of fees, would be less than about £300. Perkin travel grants are also available to teachers concerned with the study of any aspect of the manufacture or the application of colouring matter. Applications for the scholarships must be made by May 1, 1966, and for the travel grants by December 31, 1965. Further information can be obtained from the Secretary, the Perkin Centenary Trust, c/o the Chemical Society, Burlington House, London, W.1.

#### The Journal of the Franklin Institute Premium

*The Journal of the Franklin Institute* has established a special Journal Premium of 1,000 dollars. It is to be awarded annually to the author of the outstanding paper published in the *Journal* during the preceding year. The award will be presented, in general, to the recipient of the Louis E. Levy Medal. Established in 1923, the Levy Gold Medal is awarded by the Franklin Institute "to the author of a paper of special merit published in the *Journal*, preference being given to one describing the author's experimental and theoretical researches in a subject of fundamental importance". *The Journal of the Franklin Institute* was established in 1826 and is the oldest continuing scientific publication in the United States. It accepts contributions in all traditional branches of mathematics and the physical sciences, pure and applied, as well as in interdisciplinary fields or composite sciences that combine the philosophies of two or more disciplines. Consideration for the Journal Premium will be given for the first time to papers published during the calendar year 1965.

#### Announcements

PROF. G. A. OLAH, research scientist with the Eastern Research Laboratory of the Dow Chemical Company, has been appointed professor of chemistry and chairman of the department at Western Reserve University.

AN international conference on "Elementary Particles" will be held in Oxford during September 19-25. Further information can be obtained from the Rutherford High Energy Laboratory, Chilton, Didcot, Berkshire.

THE autumn meeting of the Institute of Metals will be held in Grenoble during September 19-25. Further information can be obtained from the Secretary, Institute of Metals, 17 Belgrave Square, London, S.W.1.

A SYMPOSIUM on "Electromagnetic Flow Measurement" will be held in the University of Warwick on September 24. Further information can be obtained from the Administrative Officer, School of Engineering Science, University of Warwick, Coventry.

A SYMPOSIUM on "Chemistry of Plant Pigments", organized by the Phytochemical Group, will be held at the University of Aberdeen during September 23-24. Further information can be obtained from the A. H. Williams Research Station, Long Ashton, Bristol.

AN exhibition of modern packaging methods and machines for the textile industry will be held at the Northern Ireland Linen Research Institute, Lisburn, during September 14-16. Further information can be obtained from the Linen Research Institute, Lambeg, Lisburn, Co. Antrim.

**CORRIGENDUM.** In the article entitled "Fundamental Equations governing the Hit Probabilities associated with the Release of a Pattern or Group of Weapons using a Single Aimpoint" by Dr. E. R. Terry, which appeared on p. 1135 of the June 12, 1965, issue of *Nature*, equation (10), which reads " $(\xi, \eta) = \dots$ ", should read " $f(\xi, \eta) = \dots$ "; moreover, equation (21) should read:

$$S_j^{(n)} = \sum_{k=j}^n \binom{k-1}{j-1} Q_{n,k} = \sum_{k=j}^n \binom{k}{j} Q_{[n,k]} \dots$$



## GAS CHROMATOGRAPHY

AN informal symposium organized by the Gas Chromatography Discussion Group of the Institute of Petroleum was held on March 12, 1965, in the Edward Lumley Hall of the Royal College of Surgeons, Lincoln's Inn Fields, London, with Mr. D. H. Desty in the chair. The symposium, which was preceded by the annual general meeting of the group, opened with Dr. D. W. Hill formally welcoming the visitors on behalf of the Royal College and describing some of the applications of gas chromatography in his own field.

In the first paper, entitled "Temperature Control in Gas Chromatography", presented by Dr. H. V. Carter (Perkin Elmer, Ltd.), the desirability of good temperature control was stressed particularly in the case of thermal conductivity detectors where a change in temperature as small as  $0.001^\circ\text{C}$  produces visible base-line shifts. In his discussion on types of heating systems, Dr. Carter stated that when the main consideration is negligible temperature gradient at low cost, a vapour jacket is most suitable although it has the disadvantages that the temperature is affected by pressure ( $0.4^\circ\text{C mm}^{-1}$ ) and a change in level is not conveniently made. When very tight temperature control is essential direct electric heating using the column itself as the element is suitable particularly for temperature programming because there is no time lag. Although this system is probably the best available, the user is restricted in his choice of column since its resistance must lie between defined limits. Moreover, temperature gradients are difficult to control if the column has variable thickness, although this can be overcome by removing metal from the column or varying the insulation where necessary. Proportional, on-off and programmed systems were discussed and illustrated by reference to commercial instruments and this was followed by detailed evaluation of the air-bath. While temperature control is difficult and acceptably low-temperature gradients are only obtainable with very high air velocities there is very little restriction to the dimensions of the oven and access to the column is generally very good. A method of calculating temperature gradients was described and shown to give values slightly lower than those obtained in practice. Dr. Carter concluded his paper with a brief review of the methods used for sensing changes, and the merits of thermocouples, bimetal strips, thermistors and platinum resistance thermometers were compared. Discussion of this paper evolved mainly around the geometry of systems and insulation problems, during which Dr. Carter discussed in some detail the properties of the platinum resistance thermometer and mention was made of the good oven control obtained by using a bimetallic strip as a transducer.

The second paper, on "Errors in Integration in Gas Chromatography", by Mr. W. A. Wiseman of Gas Chromatography, Ltd., was given in his absence by Dr. A. B. Littlewood. Integration errors were divided into two classes, fundamental and instrumental. It was pointed out that the area ( $A$ ) of a chromatographic peak can be measured by integrating the signal ( $E$ ) due to the detector with respect to time:

$$E = k_0 + (k - k_1)y + k_2y^2 \quad (1)$$

$$A = k_0x + (k - k_1)z + k_2\int(dx/dt)dt \quad (2)$$

where  $x$  is a measure of the detector signal (for example, charge) and  $y = dx/dt$  (for example, current).

The first and third terms of equation (2) are responsible for the sources of error in the method, the constant  $k_0$  being responsible for those incurred due to incorrect setting of the base-line, and  $k_2$  for those due to non-linearity of the detector control. The constant  $k_0$  is a function of  $a/h$ , where  $a$  is the decrease in peak height  $h$  due to incorrect

setting of the base-line: in order to obtain accurate results  $a/h$  must be only a few parts per thousand. Large errors, which increase with the width of the peak, are caused by a drifting baseline and together with the error caused by incorrect setting of the base-line represent the largest error found in integration. Errors due to non-linearity of the detector increase with increasing peak height as does the ratio of the height to the area. If accurately reproducible samples are injected into the system at differing output sensitivities using a mixture giving a number of different peak heights, the ratio of dissimilar peaks should be constant. Peaks showing up early in the analysis occur at maximum rate at which impulses appear leading to an inherent, but small, error in the analysis.

Dr. Littlewood then considered errors in instrumentation. Integration is initiated by the appearance of a peak and this may be registered either when (1) the base-line exceeds a certain minimum, or (2) the slope exceeds a certain minimum; in both cases, however, a slight error is introduced at the beginning and end of a peak. In general, slope initiation is better than base-line initiation but difficulty is encountered when studying wide shallow peaks and a mechanism combining both modes of initiation was suggested as worthy of consideration. When integration is applied to partially resolved peaks meaningful results can only be obtained when there is a deep valley between them. In the ensuing discussion references were made to the importance of the right rate of counting, that results were calculated and not measured directly for non-linear integrators, that normalizing techniques should be used for accurate evaluation of results and that electronic integrators eliminate many of the errors mentioned.

Mr. R. Gittins (W. G. Pye and Co., Ltd.) introduced his paper on "The Evaluation of Performance of Flame-Ionization Detectors" by commenting on the value of the development work carried out by instrument manufacturers. Because of the difficulties involved in sampling very small masses the performance of high-sensitivity detectors had been studied using a vapour dilution vessel containing the sample under investigation dissolved in a non-volatile liquid (compare Fowles, I. A., and Scott, R. P. W., *J. Chromatog.*, 11, 1; 1963). Detector response was found to be independent of air flow above 400 ml.  $\text{min}^{-1}$  (below this value linearity occurs over a narrow range of concentration due to incomplete combustion) and independent of applied voltage above 20 vol. (45 vol. used in practice). The hydrogen to carrier gas ratio was found to exhibit optimum values.

"Dual Channel Gas Chromatography" was the title of a paper presented by Mr. P. Jenkins (Wilkins Instrument and Research A.G.) in which, after a résumé of the useful combinations of gas chromatography and other techniques was given, the complementary nature of the electron capture and flame-ionization detectors was described and illustrated by examples obtained with an instrument incorporating both. By splitting a column effluent into two streams, one to feed each detector, simultaneous though different chromatograms could be obtained: thus a sample of butyl phenol gave one peak (butyl phenol) on the flame ionization trace and five other peaks (impurities) on the electron capture trace: a refinery stream gave propane and propene on the former and oxygen, carbonyl sulphide, carbon disulphide and carbon dioxide peaks on the latter. The electron capture detector is unpredictable in its response to chemical compounds and is probably not linear although under standard conditions the ratio of peak heights flame ionization to electron capture may be used as an aid to identification. Problems of instrumentation and design were discussed with particular reference to the choice of voltage for the electron capture detector and considerable

interest was shown in the ability of the detector to respond to oxides of nitrogen.

The final paper, "Abnormal Characteristics of the Ionization Cross-section Detector", was presented by Mr. P. F. Washbrooke (The M.E.L. Equipment Co., Ltd.). After discussing briefly the main characteristics and modes of operation of ionization detectors, Mr. Washbrooke reported fully on cross-section types and the behaviour with reference to (1) the selection of carrier gas, (2) flow rate of carrier gas, (3) temperature, (4) linearity of response, and (5) predictability of sensitivity. A micro cell had been constructed in which the electrode arrangement was a cascade system in which the tritium electrodes were off-set with respect to each other in such a manner that the effective radiation surface area was increased with very little increase in cell volume. Hydrogen and nitrogen had been found satisfactory as carrier gases: helium and argon, although they could be used, responded more readily to other ionization effects (for example, drift effects). Response variations due to voltage changes had been considered and in particular it was found that unless oxygen in nitrogen carrier gas was subjected to suitably high voltages it responded to an electron capture effect. Detector response was found to be dependent on the rate of mass input, independent of carrier gas flow rates between 10 and 100 ml. min<sup>-1</sup> and independent of temperature up to the safe working limit of tritium (200° C). The linearity of response, which depends on energy losses in the primary radiation stages,

is linear over many orders of magnitude for tritium as well as strontium-90 if the interelectrode path is small compared with that of a  $\beta$ -particle, and it is important to note that the detector can handle sample sizes considerably larger than any other type. Predictable relations have been obtained between peak area responses per millimole and values calculated from molecular cross-sections for *n*-paraffins, alcohols and monocarboxylic esters, but large deviations occur for aromatic hydrocarbons, aliphatic ketones and halogenated hydrocarbons. When quoting responses the radiation source should be given, as differences of up to 16 per cent are obtainable for cross-sections examined by different radiations. Changes in temperature may also affect the response characteristics. Mr. Washbrooke concluded his presentation by stating that the cross-section ionization detector is ideal for on-stream monitoring in preparative-scale gas chromatography when used with hydrogen or nitrogen carrier gas in order to minimize electron drift effects. The discussion was opened by Dr. H. Boer, who spoke of the anomalous behaviour of helium as carrier gas and mentioned his own version of a micro cross-section detector which had given excellent results for the detection of low boiling compounds. The remainder of the discussion was concerned with the use of the detector in the analysis of town's gas, the advantage of using argon as carrier when permanent gases were to be detected and the saturation of helium carrier gas with water to remove drift effects.

D. R. BROWNING

## INTERNATIONAL ATOMIC ENERGY AGENCY

AT its meeting in February, the Board of Governors of the International Atomic Energy Agency gave approval for the nearly one hundred projects of assistance by Agency experts and equipment in thirty-eight countries to be financed from Agency funds under the 1965 Programme. This is in addition to the work financed under the United Nations Expanded Programme of Technical Assistance. The number of requests for experts and equipment continues to increase, largely because several member states have now set up research reactors and established laboratories under bilateral arrangements. For 1965 the estimated cost of the provision of experts and equipment from Agency resources is 874,000 dollars, of which about one-third is for equipment and supplies. The work covers a wide range; about thirty projects deal with the production of radioisotopes and their application in medicine, agriculture, hydrology, food preservation and industry, and the remainder are concerned with reactors, health and safety, instrumentation, special branches of chemistry and physics, and the prospecting and processing of raw materials.

In Africa, the Congo is enlarging its research reactor and laboratories to form a regional centre and the Agency is providing an expert on nuclear electronics and a radiobiologist. Ghana is completing the construction of an experimental reactor at its nuclear research institute near Accra and will be assisted by a reactor programme

specialist and a nuclear physicist. Morocco's geological service is to be helped by the introduction of geochronological methods, and Tunisia is to receive advice on aerial prospecting for nuclear raw materials. Senegal, Rhodesia and the United Arab Republic are to be given help on the use of radioisotopes.

Equipment consisting of a pulsed neutron source and a pulsed neutron logic unit, together with assistance in reactor physics, are to be given to Argentina, and in Chile a biophysicist will advise on the development of electron spin resonance and its applications. Bolivia, Brazil, Mexico, Peru and Uruguay are also to receive expert assistance and advice. In Europe and the Near East, Afghanistan, Greece, Iran, Israel, Lebanon, Portugal, Turkey and Yugoslavia will all be visited by various nuclear scientists. In South-east Asia and the Far East, Burma will be sent standard works on nuclear physics and engineering; Cambodia and Ceylon advice and equipment relating to the use of radioisotopes; the National University Hospital in Taiwan, China, advice on the use of its cobalt teletherapy unit; Thailand an entomologist and equipment to undertake the study of the use of radiation for the control of insect pests; and India, Pakistan, Hong Kong and Viet-Nam experts for agricultural and food preservation research and assistance in teaching programmes.

S. WHINTROUB

## THE AMERICAN OYSTER

FOR some forty years Dr. Paul Galtsoff has served as shellfish biologist with what is now the U.S. Fish and Wildlife Service. Of recent years, while holding the post of senior scientist in that Service, he has been engaged on the production of a comprehensive account of the American oyster, *Crassostrea virginica*. He has had the initial benefit

of the widest experience of both laboratory and field investigations, his work on reproduction in *C. virginica* is classical while, as he tells us, he has studied oysters and related bivalves off every coastal State in the United States, in the Hawaiian Islands, in the Gulf of Panama and on Margarita Island, Venezuela.

The final result of his long experience and more recent intensive study of the literature—one hopes to his own great satisfaction but most certainly to that of all who are interested in oysters in particular and in bivalves and indeed marine biology in general—has now been published\*. Occupying an entire volume of the *Fishery Bulletin*, it is almost 500 pages long with 400 text figures, a wealth of references and an excellent index.

The species of *Crassostrea*, which include the Portuguese and Japanese oysters, *C. angulata* and *C. gigas*, are the most important of all edible bivalves. *O. virginica* in particular has been the subject of extensive studies covering every aspect of anatomy, physiology and ecology. All this, with everything relevant concerning *Ostrea edulis* and other species of 'flat' oysters, is here recounted. As Dr. Galtsoff states in his preface, it is written for the benefit of "biologists, administrators of oyster resources of various States, public health officers, students of marine biology and oyster growers", and thus he has been at great pains to "present the facts and discuss various theories in the simplest language". In this aim he has

\* United States Department of the Interior. *Fishery Bulletin of the Fish and Wildlife Service*, Vol. 64: *The American Oyster Crassostrea virginica Gmelin*. By Paul S. Galtsoff. Pp. 41 + 480. (Washington, D.C.: Government Printing Office, 1964.) 2.75 dollars.

been highly successful. This is an eminently readable as well as an exceptionally well-illustrated work.

The only criticism that strikes me in a book that has given me so much pleasure is some personal regret that Dr. Galtsoff has not apparently taken much note of recent work on the formation of the ligament—the inner layer being derived from the mantle isthmus (referred to as the ligamental ridge), and the outer layer from the outer fold of the mantle margin at either end. Surprisingly, he is also unable to find any clear reason for the presence of the promyal chamber which is one of the generic differences between *Crassostrea* and *Ostrea*. But surely this is a necessary consequence of the elongation characteristic of the former which involves a ventral-ward movement of the adductor and so restricts the exhalant region. This is certainly the case in the even more elongated *Mallois*, possibly the only other bivalve with a promyal chamber (personal, unpublished observation).

This account of the American oyster represents a major addition to the literature on the marine Bivalvia and so to marine biology. It is hard to see how it could have been done better and it only remains to congratulate and thank Dr. Galtsoff and all concerned with its production, not least the U.S. Department of the Interior which has given us so much for a mere 2.75 dollars. C. M. YONGE

## ANALYSIS OF QUEUEING AND RENEWAL WITHIN HUMAN SYSTEMS

By PROF. JOHN H. FRENSTER  
Rockefeller Institute, New York

THE analysis and integration of the diverse quantitative data of human physiology have been greatly facilitated by the recent development of such mathematical techniques as systems analysis<sup>1,2</sup>, control theory<sup>3,4</sup>, queueing theory<sup>5</sup>, and renewal theory<sup>6</sup>. Each of these techniques focuses on the abstract mathematical forms rather than the concrete physical features of the system under examination<sup>7</sup>. The aim of such analysis is the identification of the dependent and independent variables within a particular system, the determination of the quantitative relations between these variables, and the discovery of how such relations themselves change and are controlled during health and disease, rest and activity, hypertrophy and atrophy within individual patients.

For the purpose of analysis, a system may be defined as an integrated assembly of components which, by their interaction, effect some characteristic change on a substrate presented to them<sup>8</sup>. Implicit in this definition is the concept of a substrate flow of matter, energy or information first being presented to the system as an input load, then undergoing some characteristic transformation within the system, and finally emerging from the system as an output. Because of the flow or traffic characteristics of such substrates, they are recognized as throughputs, and the systems effecting their transformation as throughput systems<sup>9</sup>. Most, if not all, of the human systems of medical interest have been shown to be examples of throughput systems<sup>9</sup>, each with its own specific type of throughput units and with its own characteristic transformation of such units<sup>9</sup>.

In the identification of the variables within such human throughput systems, three general categories of independent variables have been recognized<sup>9</sup>. These are the input load of throughput units presented to the system for transformation, the available capacity of the system to effect transformation of these units, and the resistance opposing the exit of the transformed units from the system. By contrast, the output rate of transformed units from the system is a dependent variable, being determined by the interaction of the input load, the

system capacity, and the resistance to output from the system<sup>9</sup>.

Under pathological circumstances the system capacity may decrease or the input load or the resistance to output may increase<sup>10</sup>, so that the rate of arrival of throughput units into the system will exceed the rate of departure of transformed units from the system. In such a situation, the capacity of the system may become saturated and limiting, and waiting lines or pools of arriving throughput units may accumulate before the active service channels of the system (Fig. 1). This congested state of the system can be conveniently analysed by a deterministic form of queueing theory<sup>4,7</sup>.

The length in units ( $L$ ) of the waiting line formed in the buffer storage area of such a congested system is the sum of the initial waiting line-length ( $L_0$ ) and the integral of the difference in the rates of arrival ( $A$ ) and departure ( $D$ ) of throughput units from the system during the time-interval being examined.

$$L = L_0 + \int (A - D) dt$$

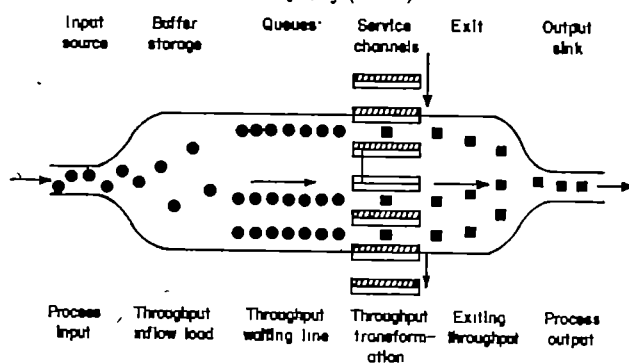


Fig. 1. Analytic model of queueing and renewal within human throughput systems. Throughput units awaiting transformation form waiting lines or pools before the service channels. An orderly and continuous turnover of service channels is mediated by a renewal mechanism which permits adaptive changes in the number of existing channels. Not all the existing channels need be in an active state nor be operating at a maximal rate of transformation per channel.

Within most human systems, a waiting line of some finite length exists in the normal state<sup>6</sup>. The presence of such a normal waiting line permits the smooth continuous flow of throughput units that is characteristic of these systems. In pathological circumstances, in which either system capacity is reduced or system input load or resistance to output is increased<sup>9,10</sup>, the length of the waiting line increases to exceed some value ( $L_{path}$ ) that is characteristic for each system, and which has been recognized statistically as a boundary value<sup>7</sup> correlating clinically with the transition from latent disease to overt disease<sup>8</sup>. Individual systems in which the length of the waiting line exceeds the value of  $L_{path}$  under basal conditions are recognized as being in a state of overt failure<sup>8</sup>. Conversely, systems in which the length of the waiting line under basal conditions is less than the value of  $L_{path}$  are recognized as being either in a state of health or in a state of latent failure<sup>8</sup>. The clinical use of load-tolerance tests<sup>11</sup> has been useful in distinguishing and quantitating these states in the health-disease continuum<sup>8</sup>.

If under basal conditions a test input load is administered to a particular system at a rate ( $A_{test}$ ) which is just sufficient to lengthen the existing waiting line length ( $L$ ) to the known boundary value ( $L_{path}$ ), and if  $D_{test}$  be the additional system output resulting from such a test load beyond that of the existing system output  $D$ , then:

$$\Delta L = L_{path} - L = \int (A_{test} - D_{test}) dt$$

where the magnitude of the test administration rate ( $A_{test}$ ) is a quantitative measure of the load-tolerance of the system at the existing levels of system capacity, input load, and resistance to output<sup>7</sup>. Such load-tolerance of a human throughput system is a dependent variable determined by the degree to which the available system capacity is in excess of that needed at the existing basal levels of input load and resistance to output<sup>8</sup>. In latent disease, the load-tolerance of the affected system is less than in the normal state, while in overt disease load-tolerance is reduced to zero and usually assumes a negative value<sup>7</sup>. A state of health within a particular system is thus characterized by a high load-tolerance within that system, and by measuring its load-tolerance, the magnitude of health or disease of a throughput system may be quantitatively determined<sup>8</sup>.

The capacity of a human throughput system is usually not constant, but rather is variable and responsive to the demands placed on the system<sup>9,12</sup>. Thus, pathological increases in the length of the system waiting line or decreases in the rate of system output often result in adaptive increases in the capacity of the system<sup>13</sup>. These throughput system changes are mediated by super-imposed control systems which continuously sample the throughput system waiting lines and output rates<sup>7</sup>, as well as communicate changes in other distant systems. Adaptive increases in system capacity are achieved by either an acceleration, an activation, or a hypertrophy of existing service capacity<sup>7</sup>. In the context of queuing

theory<sup>4,7</sup>, acceleration involves a decreased service time needed to effect a unit transformation within a service channel; activation involves a conversion of existing idle service channels to the active state; and hypertrophy involves a net increase in the total number of existing service channels (Fig. 1).

The service channels of nearly all human throughput systems are being constantly formed and broken down in an orderly turnover that can be conveniently analysed by renewal theory<sup>4</sup>. Hypertrophy of system capacity could be achieved during such channel turnover by either an increased rate of new channel formation or by a decreased rate of old channel breakdown. It is now evident that either or both of these renewal mechanisms are used by diverse human throughput systems in the adaptive hypertrophy of their system capacity<sup>13</sup>. The net increase ( $\Delta N$ ) in the number of service channels in such a hypertrophy response is a dependant variable determined by the rate of new channel formation ( $F$ ) and the rate of old channel breakdown ( $B$ ) within the system during the time-interval being examined:

$$\Delta N = \int (F - B) dt$$

Conversely, when system waiting lines decrease or when system output rates increase for a sustained period, these renewal mechanisms effect a corresponding atrophy of the system capacity, with  $\Delta N$  assuming negative values, the effect of such adaptive atrophy being to restore the balance between system capacity and the reduced demand placed on the system<sup>8</sup>.

Such adaptive changes within human throughput systems play a decisive part during the onset, course and therapy of many disease states<sup>8,14</sup>. A quantitative analysis of the queuing and renewal mechanisms which underlie these adaptive changes permits both greater insight<sup>13</sup> and more effective control<sup>11</sup> of these pathological states.

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## GAS CLOUDS IN DENSE STAR CLUSTERS: A POSSIBLE QUASAR MODEL

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IN order to account for the large energy input required by intense extragalactic radio sources, I suggested that the collective formation of a cluster of more massive stars would result in numerous Type II supernova explosions in a limited period of time<sup>1</sup>. Following the discovery

of light flashes<sup>2</sup> in the light curve of the quasar 3C 273, S. A. Colgate and I adapted this picture also for quasars<sup>3</sup>, and proposed that the flashes resulted from the enhancement of the light output of the Type II supernovae when their ejected envelopes interacted with circumstellar gas

of density about  $10^{-18}$  g/cm<sup>3</sup>. Greenstein and Schmidt<sup>4</sup> then published their analysis of the emission lines from 3C 48 and 3C 273, from which they concluded that 3C 273 was a gas cloud of density  $\sim 10^{-17}$  g/cm<sup>3</sup> and dimensions  $\sim 1$  parsec which was held in place by a large unseen mass. It is the purpose of the present article to discuss more extensively the properties of such gas clouds in dense clusters of stars, and to see if the observed properties of quasars are compatible with them. The convention will be adopted that all the visible quasi-stellar objects<sup>5</sup> will be called quasars, and the intense radio-emitting objects will be called radio quasars.

Greenstein and Schmidt<sup>4</sup> have presented arguments based on line strengths to show that the electron temperature in 3C 48 and 3C 273 cannot be too large; their calculations were carried out with the assumption  $T_e = 1.68 \times 10^4$  °K. Oke<sup>6</sup> has given reasons for preferring a temperature about ten times as high as this; but he states that the lower temperature is not ruled out if much of the optical continuum emission is due to synchrotron radiation. In this discussion I shall follow the analysis of Greenstein and Schmidt with their lower temperature assumption.

The emission lines in 3C 48 and 3C 273 are very wide. If this is interpreted as resulting from a highly turbulent state of the gas, then the typical turbulent velocities in the two sources are 2,000 and 3,000 km/sec respectively. Consequently the turbulent motions are highly supersonic. Therefore shock phenomena must be widespread throughout the gas clouds and one would expect large fluctuations in the temperature. The single temperature assumption of Greenstein and Schmidt should therefore be regarded as giving results that can only be qualitatively correct.

It will be a crude but useful approximation to calculate the structure of a gas cloud with the assumption that it is in hydrostatic equilibrium in the gravitational potential well of a star cluster, but with the pressure being the turbulent pressure. I shall go further and assume that the turbulent motions scramble the lines of force of any initial magnetic field and build up the field strengths until there is approximate equipartition of energy between gas turbulence and turbulent magnetic fields. Then:

$$\frac{1}{2} \rho v_t^2 \sim \frac{B^2}{8\pi}$$

where  $\rho$  is the gas density and  $v_t$  is a typical turbulent velocity. The effective pressure is then taken to be:

$$P \sim \frac{1}{3} \rho v_t^2 + \frac{B^2}{8\pi} = \frac{5}{6} \rho v_t^2$$

The equations of hydrostatic equilibrium are:

$$\frac{dP}{dr} = - \frac{GM(r)\rho}{r^2}$$

$$\frac{dM(r)}{dr} = 4\pi r^2 \rho_s$$

Here  $\rho$  is the gas density and  $\rho_s$  is the matter density in stars, taken to be much greater than the gas density. I shall assume that the matter density in stars can be approximated near the centre of the cluster as uniform, independent of  $r$ . Therefore:

$$M(r) = \frac{4}{3} \pi \rho_s r^3$$

$$\rho = \rho_c \exp(-\alpha r^2)$$

$$\alpha = \frac{4\pi G \rho_s}{6 v_t^2}$$

Here  $\rho_c$  is the gas density at the centre of the cloud. The total mass of the gas cloud is:

$$M_g = (\pi/\alpha)^{3/2} \rho_c$$

The densities and dimensions of the gas cloud models for 3C 48 and 3C 273 can now be determined from the line emission strengths discussed by Greenstein and Schmidt<sup>4</sup>. In their approximations the volume emissivity of the H $\beta$  line is:

$$E(\text{H}\beta) = 3.0 \times 10^{-23} N_e^2 \text{ ergs/cm}^3 \text{ sec}$$

Here  $N_e$  is the electron number density which I may write:

$$N_e = N_0 \rho / \mu$$

where  $N_0$  is Avogadro's number and  $\mu$  is the mean molecular weight per electron (which I take to be 4/3). The total luminosity of H $\beta$  is then:

$$L(\text{H}\beta) = 1.09 \times 10^{43} \frac{\rho_c^2}{\mu^2} \left( \frac{\pi}{2\alpha} \right)^{3/2}$$

Similarly, for  $N_e \gg 10^4$  cm<sup>-3</sup>, the emissivity of the forbidden lines of O II is:

$$E(\text{O II}) = 3.42 \times 10^{-31} N_e$$

and the total luminosity is:

$$L(\text{O II}) = 2.06 \times 10^{43} \frac{\rho_c}{\mu} \left( \frac{\pi}{2\alpha} \right)^{3/2}$$

The forbidden lines of O III yield similar relations:

$$E(\text{O III}) = 2.16 \times 10^{-18} N_e$$

$$L(\text{O III}) = 1.30 \times 10^{43} \frac{\rho_c}{\mu} \left( \frac{\pi}{2\alpha} \right)^{3/2}$$

Greenstein and Schmidt modified these expressions to match relative line strengths in planetary nebulae. However, it is evident that the excitation conditions are quite unlike those encountered in planetary nebulae, so the modifications of Greenstein and Schmidt have not been used here.

For 3C 273,  $L(\text{H}\beta) = 8.8 \times 10^{43}$  ergs/sec and  $L(\text{O III}) = 3.1 \times 10^{43}$  ergs/sec. From these one obtains the formal solution:

$$\rho_c = 1.28 \times 10^{-18} \text{ g/cm}^3$$

$$\alpha = 3.69 \times 10^{-17} \text{ cm}^{-2}$$

Thus in this solution the gas densities come out significantly higher than in the consideration of Greenstein and Schmidt, and the range of densities is quite consistent with that required for the enhancement of light flashes<sup>8</sup>. The characteristic radial distance in the gas cloud is:

$$\alpha^{-1/2} = 1.65 \times 10^{17} \text{ cm}$$

This distance is about 0.05 parsecs.

The turbulent magnetic field in 3C 273 is given by:

$$B \leq (4\pi \rho_c)^{1/2} v_t = 38 \text{ gauss}$$

This magnetic field will stiffen the gas very much against supersonic compression. Consequently the possibility exists that the gas cloud can undergo a radial vibration through the medium of this magnetic field. The characteristic period  $\tau$  of this vibration would be roughly the characteristic radial distance divided by the Alfvén velocity. The Alfvén velocity is:

$$v_A = B/(4\pi \rho)^{1/2} \sim v_t$$

Hence  $\tau \sim \alpha^{-1/2}/v_t = 17$  years. This value of the period is remarkably close to the observed period<sup>9</sup> of 13 years for the general variability of the light curve.

However, it is not sufficient that there should be radial vibration in order to produce a variation in optical emission. The cloud is transparent at optical wave-lengths and the recombination times are orders of magnitude less than the vibration period. Consequently the heating mechanism should also depend on the density of the gas.

The total mass  $M_g$  of the gas cloud is  $1.6 \times 10^4 M_\odot$ . The stellar density is:

$$\rho_s = 5\alpha v^2 / 4\pi G = 2 \times 10^{-11} \text{ g/cm}^3 \\ = 2.9 \times 10^{11} M_\odot / \text{pc}^3$$

This is an exceptionally high density of stars. We do not know the total mass of stars, but if the foregoing density extends out to 0.1 parsec, twice the characteristic radial distance, then a star cluster of  $10^4$ – $10^5 M_\odot$  would be involved. Many people would prefer to call an isolated star cluster of this mass a small galaxy.

For 3C 48,  $L(\text{H}\beta) \approx 6.4 \times 10^{43}$  ergs/sec and  $L(\text{O II}) = 3.3 \times 10^{43}$  ergs/sec. The formal solution of the equations then gives:

$$\rho_s = 1.38 \times 10^{-18} \text{ g/cm}^3 \\ \alpha = 2.36 \times 10^{-3} \text{ cm}^{-1} \\ \alpha^{-1/2} = 6.5 \times 10^{18} \text{ cm} \approx 2 \text{ parsecs} \\ B < 0.83 \text{ gauss} \\ M_g = 1.06 \times 10^4 M_\odot \\ \rho_s = 5.6 \times 10^{-18} \text{ g/cm}^3 = 8.3 \times 10^7 M_\odot / \text{pc}^3$$

In this case the star density is more normal, but there are already  $3 \times 10^8 M_\odot$  within the characteristic radial distance and  $2 \times 10^{10} M_\odot$  within twice that distance. Consequently there is definitely a mass of galactic order associated with the gas cloud in 3C 48.

We now consider sources of energy for heating these gas clouds. Such energy must be available in a form which continually imparts supersonic velocities to the quasar gas. Three possible energy sources may be considered: (1) supernova explosions; (2) stellar collisions; (3) interaction between stars and gas.

Supernova explosions of Type II eject envelopes containing several solar masses and  $10^{44}$ – $10^{45}$  ergs of kinetic energy<sup>1,2</sup>. These envelopes will impart supersonic motions to the gas with which they interact. The characteristic interaction time will be  $\sim 10^3$  years for 3C 48. The rate of occurrence of supernovas depends on the mass distribution of the stars and whether or not they were formed concurrently. With a collective stellar formation process there will be a period of  $10^4$  or  $10^5$  years in which there may be several supernova explosions per year in a cluster with  $10^8 M_\odot$  and a reasonable mass distribution. This would give an energy input of  $10^{44}$ – $10^{45}$  ergs/sec, which is in the quasar range of energy emission.

An energy source from stellar collisions has been suggested by Gold, Axford and Ray<sup>3</sup>. For simplicity consider that all stars in the cluster of radius  $R$  have the same mass  $M_s$  and radius  $R_s$ . Then the number of collisions per sec is:

$$N = \frac{1}{2} \frac{\rho_s^2}{M_s^2} (\pi R_s^2) v_s \left( \frac{4}{3} \pi R^3 \right)$$

Here  $v_s$  is the typical stellar velocity, which I take also to be a typical relative collision velocity. From the virial theorem I find:

$$v_s = \left[ \frac{9G\rho_s}{20\pi} \right]^{1/2} R \\ N = 6.4 \times 10^{-4} \frac{\rho_s^{3/2} R_s^2 R^4}{M_s^2}$$

For purposes of illustration let us take  $M_s = 2 \times 10^{34}$  g and  $R_s = 10^{12}$  cm, corresponding to  $10 M_\odot$  stars. For 3C 273 we also take  $R = 3 \times 10^{18}$  cm. Hence  $N = 2.3 \times 10^{-1} \text{ sec}^{-1}$ . However, only about  $10^{-3}$  of these collisions take place in the quasar gas. The stellar velocity is  $1.3 \times 10^8$  cm/sec ( $R$  was chosen sufficiently large that  $v_s$  would be significantly greater than the turbulent velocities of  $3 \times 10^8$  cm/sec). This velocity is supersonic with respect to the interior stellar thermal velocities, but most collisions will be non-central. Thus a few per

cent of stellar kinetic energies may be ejected as gas in each collision. Hence, within the quasar gas there is likely to be an energy input of  $10^{44}$ – $10^{45}$  ergs/sec. However, if  $R = 3 \times 10^{17}$  cm, the collision rate and stellar velocities each drop a factor 10 in the quasar gas, so that the energy input would drop by a factor  $10^3$ . Collisions would then supply only a small part of the energy output of 3C 273.

It is evident that collisions will not be important in 3C 48.

From the foregoing illustration it is evident that the stars will pass with supersonic motion through the gas. Hence, as in the case of the Earth in the solar wind, bow shocks will stand off in front of the stars. For simplicity we may make the rough estimate that the gas will be accelerated to the stellar velocity out to about two stellar radii, and the gas within one stellar radius will be accumulated by the star. The energy given to the gas per second per star is then:

$$\frac{3}{2} \pi \rho_s R_s^2 v_s^3$$

In 3C 273, with  $R = 3 \times 10^{18}$  cm, this imparts to the gas about  $10^{37}$  ergs/sec per star, or about  $3 \times 10^{44}$  ergs/sec in the quasar gas. The energy input to 3C 48 would be considerably less. While this mechanism seems unlikely to be providing the energy input for these radio quasars, it might readily suffice for ordinary quasars which probably have somewhat smaller total luminosities.

While the two quasar models discussed here are transparent in the optical region, they are opaque to free-free absorption in the radio region. Hence, the flat radio emission spectrum must be emitted from outside the quasar gas region. However, Dent<sup>10</sup> has found that the 8,000 Mc/s flux from 3C 273B is variable and must be emitted from a region of only a few parsecs dimension. Hoyle and Burbidge<sup>11</sup> have discussed a synchrotron emission model in which this is possible; their magnetic fields and particle energy distributions are consistent with the model of the 3C 273 gas discussed here.

Sandage<sup>8</sup> has noted that ordinary quasars are much more abundant than radio quasars and may be somewhat less luminous. If we assume that the radio quasars are powered by supernova explosions, then this situation can be understood in terms of the preceding energy discussion. In this picture energetic particles are created in the supernova explosions and provide radio emission in the magnetic field system surrounding the quasar. But after supernova activity dies out, star-gas interactions will continue to power the denser quasars for optical emission. The less-dense quasars would become very dim but would brighten when a supernova explodes. This may have happened in 3C 2 (ref. 12). However, on this picture the total optical emission lifetime of denser quasars may be considerably longer than has been suspected.

As Oke<sup>9</sup> has noted, much of the optical continuum radiation from 3C 273 may be synchrotron emission. This would lend support to the idea that it is mainly powered by supernova explosions. At the same time, variations in the light output would accompany radial oscillations owing to the variation in magnetic field strengths.

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# GEOLOGICAL HISTORY OF THE SLIEVE GAMPH MOUNTAINS, WESTERN IRELAND

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WORK now in progress into the structural and metamorphic history of the southern or Slieve Gamph part of the Ox Mountains in Counties Sligo and Mayo has yielded important information bearing on the Caledonides of Ireland. This article contains a preliminary account of these results which help to present a more complete picture of the structural and metamorphic phases operative during the Caledonian orogeny.

The area under discussion lies between Lough Eskey to the north-east and Pontoon at the southern end of Lough Conn to the south-west. The early work of R. G. Symes<sup>1,2</sup> showed the presence of a central body of granitic gneiss extending along the length of Slieve Gamph as far as Lough Talt and bounded on the north-west and south-east sides by metamorphosed sediments. In the Pontoon area Symes recognized that while the normal granite was greyish there was a distinct pink to red coloration which could be correlated with faulting. M'Henry<sup>3</sup> recognized a succession of quartzite, conglomerate, limestone, shales or schists and pebbly grits. The pebbly grits he correlated with beds of similar lithology 10 miles to the west at Westport. He further recognized that the granitic core of Slieve Gamph had invaded an earlier basic intrusion and had itself been affected by "intense Earth stresses which resulted in a milling out, shearing and banding of all three varieties of rock". The age of injection of the granitic material was regarded as early Devonian on the basis that the metamorphosed sediments were Ordovician or early Silurian in age. M'Henry's contribution to the knowledge of Caledonide geology is important in that he was one of the first workers to recognize that its metamorphic history was polyphase.

**Regional setting.** A typical Upper Dalradian sequence has been thrust over both the Slieve Gamph pluton and its meta-sedimentary envelope from the north-west, in a series of thrust slices (Conloon and Sheeans Thrusts) separated by a marked imbricate zone. Between the Conloon Thrust and the Sole Thrust a further imbricate zone involves the Slieve Gamph pluton and its envelope. The dating of these thrust movements as post- $F_1$  and pre- $F_2$  is based on the divergence and lack of correlation of  $F_1$  and  $F_2$  structures between the thrust slices and the fact that  $F_1$  structures may be traced directly across the whole area without any breaks at the thrust planes.

Coarse current bedded sandstones, conglomerates and green silts which in part can be shown to be thrust over the Dalradian at the south-west end may on the basis of somewhat poorly preserved spore material be in part Middle Old Red Sandstone<sup>4</sup>. Visean Carboniferous sediments overstep on to all these older rocks and at some stage have probably covered the whole of the Ox Mountains<sup>4</sup>.

**Sequence of events in the Slieve Gamph igneous complex and its envelope.** Four

distinct lithological types are found within the complex (Fig. 1):

- Trondjemite ( $D$ ).
- Porphyritic granodiorite ( $G_1$ ).
- Biotite adamellite ( $G_2$ ).
- Micrographic granite veins ( $G_3$ ).

Sharp contacts have been observed between  $D$  and  $G_1$ ,  $G_1$  and  $G_2$ , and also between veins of fine-grained  $G_2$  and  $G_1$ . A contact between  $G_2$  and  $G_3$  has not been found due to poor exposures in the critical localities. A small boss of  $G_3$  is exposed some 5 miles to the north-east of Lough Talt at Lough Eskey and it is considered probable that the exposed areas of  $G_2$  represent part of a larger igneous body at depth.

The following structural and metamorphic sequence of events has been deduced.

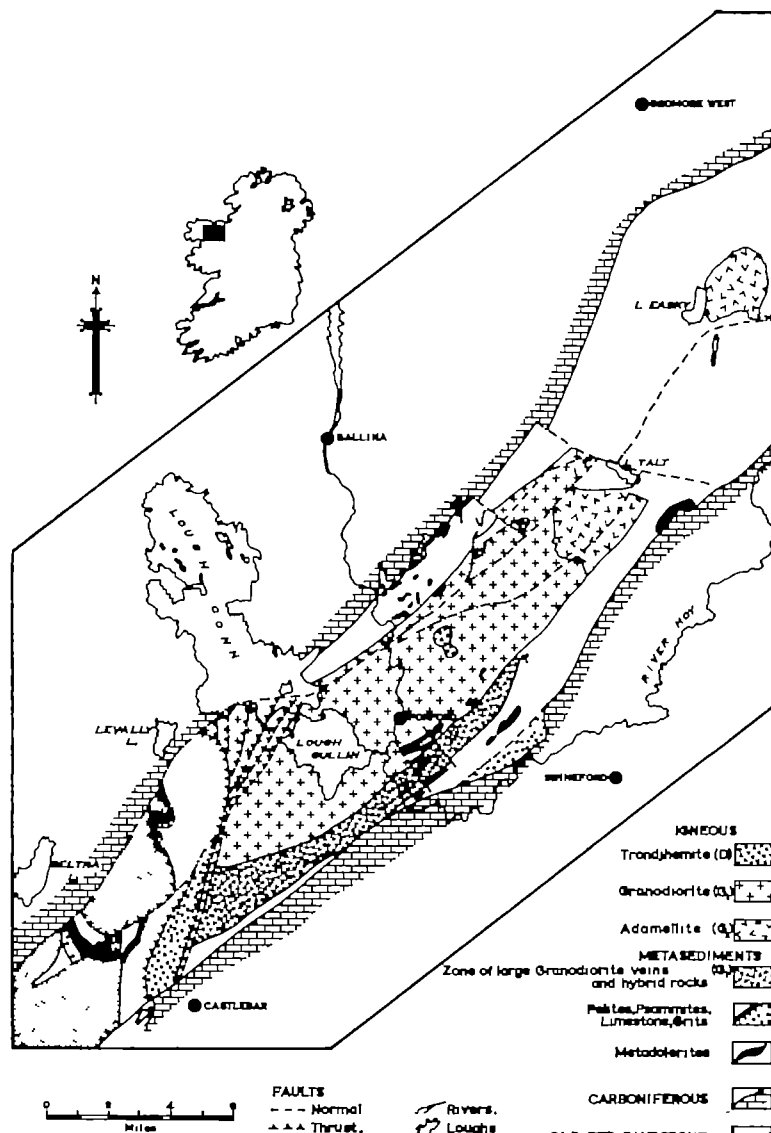


Fig. 1. Geological map of the Central and South-west parts of the Ox Mountains, Co. Mayo, Eire



(1) Deposition of the sediments of the envelope, probably Dalradian.

(2) Intrusion of a suite of basic sills and dykes.

(3)  $F_1$  movements. Folding occurred on a large recumbent scale and was associated with sliding which is well exposed to the south-west of Lough Talt. In this area part of the overturned limb of a large recumbent fold trending east-west and probably closing towards the north is present. Biotite appears to be the only new mineral developed during this phase.

(4) There then followed a period of static metamorphism in which garnet was developed which often shows trails of the  $F_1$  fabric. Felspathization occurred with the development of oligoclase and local migmatization.

(5)  $F_2$  movements. The  $F_2$  folds are tight with a steep axial plane trending between  $040^\circ$  and  $070^\circ$ . A strong penetrative axial planar foliation due to the growth of muscovite and a prominent lineation is developed. The  $F_2$  tectonic slides of the Lough Talt area were folded. The post- $F_1$  static growth of garnet was continued during this phase with the development of synkinematic overgrowths on the earlier crystals. At some stage of the deformation splite and pegmatite veins were intruded. It has not been possible to ascertain their exact time position.

(6) The  $F_2$  folding was followed by a second period of static metamorphism during which the most prominent minerals formed were staurolite, garnet and tourmaline.

(7) *Intrusive phase.* This phase in the history of this area started with emplacement of a trondjemite (D) and the main parts of which now exposed lie north-east of Foxford. The evidence leading to this position is twofold: firstly the trondjemite may be seen to cross-cut the  $F_1$  folds, and secondly both the trondjemite and the granodiorite ( $G_1$ ) are in places affected by all subsequent movement phases. The emplacement of (D) was followed fairly closely by that of the main mass of granodiorite ( $G_1$ ).

(8) Subsequent plastic deformation of D and  $G_1$  produced a shear foliation (trending  $050^\circ$ ) and a lineation. It is considered that these structures are associated with the intrusion of the Adamellite ( $G_2$ ) and that the whole intrusive complex and the envelope moved upwards as one plastic unit, but that the igneous rocks were in the more plastic condition. Evidence of this movement is to be found particularly on the southern side where  $F_2$  fold limbs are often partially sheared. There is also an extensive development of 'pull-apart' structures and pseudo boudins (Fig. 2).

*Contact metamorphism of the envelope by  $G_1$  and  $G_2$ .* It is difficult to separate the contact effects of either the  $G_1$  or  $G_2$  igneous phases from the slightly earlier static regional metamorphism. The extent of the aureole is generally about a quarter of a mile from the contact but widens towards the south-west where a series of large  $G_1$  veins invade the country rock of the envelope. To the north the contact zone is cut out because of later ( $F_2$ ) faulting. Within the zone biotite is replaced by fibrolite, and staurolite is altered to a micaceous mass which in places has gone to fibrolite and iron ore. The biotite of the country rocks also becomes increasingly foxy-brown in colour towards the contact, and flakes of muscovite have grown at random across the early schistosity. Near Foxford and around Pontoon a series of pink microcline pods are found in the metasedimentary enclaves and the nearby  $G_1$ —this is thought to be due to a late stage potash metasomatism connected with the igneous intrusion and predating the late plastic movements.

(9)  $F_3$  movements. The  $F_3$  folds occur as a series of kink bands and close, often tight, folds trending between  $000^\circ$  and  $050^\circ$ , with a strain slip cleavage. There was little new mineral development at this stage apart from a phase of retrogressive metamorphism affecting garnet and tourmaline, both of which have been altered to chlorite. Mesocrenulations corresponding to  $F_3$  can be traced right across the igneous complex.

The probable culmination of the  $F_3$  movements was large-scale major thrusting from a north-westerly direc-

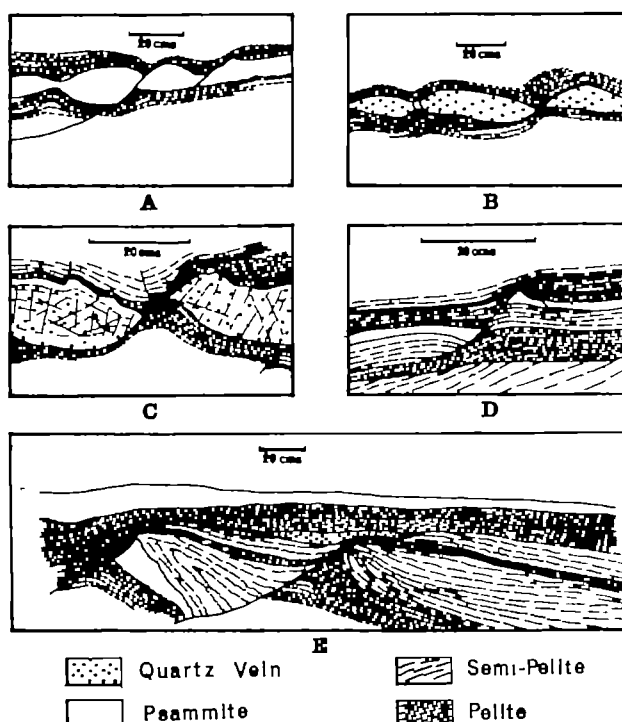


Fig. 2. 'Pull-apart' structures in metasediments. Diagrams A-E show the variations of lithology associated with these structures. Note the constancy of the angle of the surface of disruption.

tion, which brought the typical upper Dalradian sequences of the Croaghmoyle Hills (south-west Ox Mountains) into their present position\*. It was a deep-seated movement bringing up serpentine along parts of the thrust planes, particularly just north of Castlebar.

(10)  $F_4$  movements. The  $F_4$  folds are represented as a series of small kink bands and crenulations which trend between  $140^\circ$  and  $180^\circ$ . The style of folding varies from open to close and in places the intersection of  $S_3$  and  $S_4$  (that is, the axial surfaces of the  $F_2$  and  $F_3$  folds) have produced meso-scale conjugate folds with a gentle plunge towards the north-east. On the larger scale the strike of the  $F_2$  folds are rotated through approximately  $20^\circ$  (that is,  $050^\circ$ – $070^\circ$ ) in the Lough Talt area, and very open folds are found.

(11) A minor phase consisting of an emplacement of a series of pegmatites and aplites.

(12)  $F_5$  movements. The  $F_5$  folds are a poorly developed series of kink folds trending roughly north-south with a vertical plunge.

(13)  $F_6$  movements. These late movements consist of an earlier thrust fault followed by north-east to south-west trending faults (? normal) which have displaced the outcrop of the igneous complex. Small drag folds are associated with both sets of faults. It is probable that the movement history of these faults was extremely long and complex, particularly in the case of the Lough Conn Fault which separates Old Red Sandstone and metamorphic rocks on the south side from Carboniferous on the north side. They were probably initiated in Devonian times and extended well into Carboniferous times—when they had a pronounced effect on sedimentation. For example, in Glen Hest and Glen Nephin immediately to the north-west of the Lough Conn Fault at least 1,500–2,000 ft. of Carboniferous conglomerates and sandstones occur against the fault. Four miles to the north-west on the other side of the glens the maximum thickness resting unconformably on the Dalradian of Nephin is 100–150 ft., while on the southern margin of the metamorphic area less than 50 ft. are to be found near Castlebar.

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# LOWER CRETACEOUS FLUVIATITES IN THE LEVANT

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THE occurrence of a clastic series, varying in thickness, at the base of the Lower Cretaceous succession in the Levant has been known for a long time. These predominantly sandy strata, unconformably overlying the Jurassic and occasionally even older formations, extend from Sir in northern Lebanon<sup>1-3</sup> southwards, through Israel<sup>7-10</sup> and Jordan<sup>11-14</sup>, to Sinai and Egypt.

In Israel the Lower Cretaceous formations crop out in many localities, nearly always along the cores of folds and along tilted-block structures. In the south the entire section is exposed and consists mainly of variegated sandstones of the so-called 'Nubian facies'<sup>15</sup>. In central and northern Israel, it is mainly the upper part of the Lower Cretaceous, usually calcareous and shaly, which is exposed. The lower, Nubian facies sandy series appear here at two localities only, Rami and Qiryat Shemona (Galilee).

The distribution of the Lower Cretaceous strata reported from Jordan (*op. cit.*) follows a very similar pattern. In the Lebanon and Anti-Lebanon mountains of Lebanon and Syria, where the intensity of folding is greater than in Israel and Jordan, the Lower Cretaceous is fully exposed in many areas. The stratigraphical section is very similar to that exposed in north of Israel and Jordan and consists of an upper, calcareous and marly part (Albian-Aptian) and a lower sandy group, the so-called 'Grés de base' (*op. cit.*) (Neocomian?). Some of the previous investigators assumed that the Grés de base formed in an estuarine environment, while many others believed them to be of an aeolian origin.

The examination of the sedimentary structures exposed within the sandstone quarries of Qiryat Shemona indicated that the Lower Cretaceous clastics were deposited in shallow water, probably less than 10 m deep, stirred by currents flowing to the north-north-west.

The strata are heavily cross-bedded (60-80 per cent). The sets are nearly always plane parallel or sub-parallel and are grouped in thick co-sets (Fig. 1). The basal surfaces show slight erosional irregularities. The sets vary in thickness from 8 to 50 cm. The inclination of the layers is

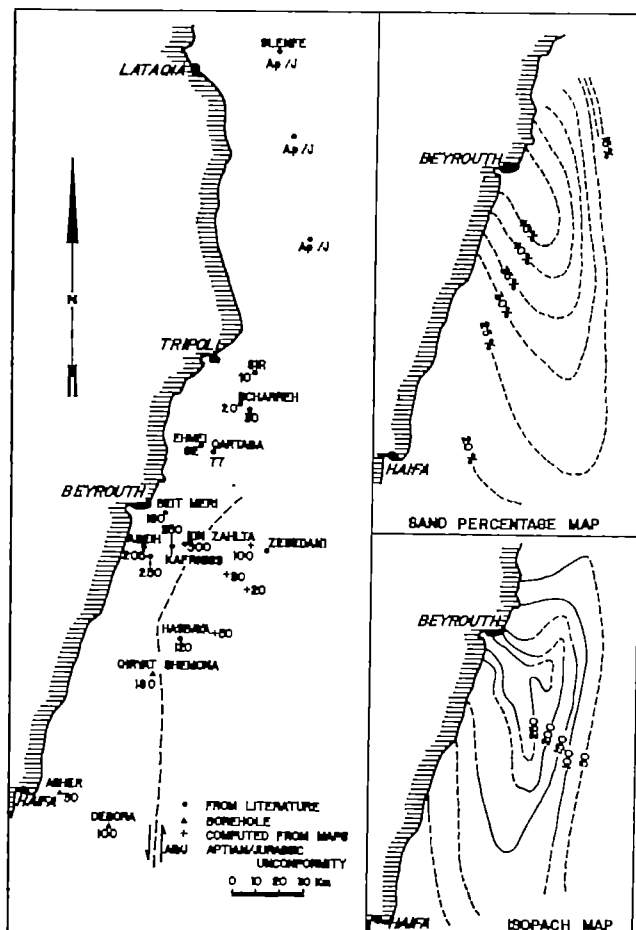


Fig. 2. Distribution of the Lower Cretaceous sandstones in the Levant (Karcz, Golan, 1964)

moderate to strong. After the removal of tectonic tilt (usually between 10° and 20°), about 50 per cent of the cross-sets show dips exceeding 20° and approximately 10 per cent show dips exceeding 30°.

Though in most cases the cross-bedding is tabular, some trough-sets are occasionally encountered. These sets are inclined to each other (15°-20°); the layers are strongly concave and approach the spoon shape. The contacts are strongly erosive.

The cross-bedding is accompanied by numerous occurrences of ripple-drift and graded bedding. The ripples are 1-2 cm high, with ripple index of 0.5-0.5. They are notably asymmetric to the north (index 1.6-2.1). In places a gradual transition from the ripple-drift régime to that of cross-bedding may be observed. The occasional rather irregular lenses and bands of shale with plant remains result either from fluctuations in flow velocity or result from sediment-transport lag.

The current directions as indicated by the re-tilted cross-bedding dips and by the ripple-drift point north-north-westwards (Fig. 1). Twenty per cent of the current-azimuths point between 320° and 340°, 20 per cent between 340° and 000°, and a further 30 per cent point between 000° and 020°.

The Qiryat Shemona and Rami exposures represent but a very small fraction of the Lower Cretaceous outcrops in the northern Levant, but, since no other detailed litho-

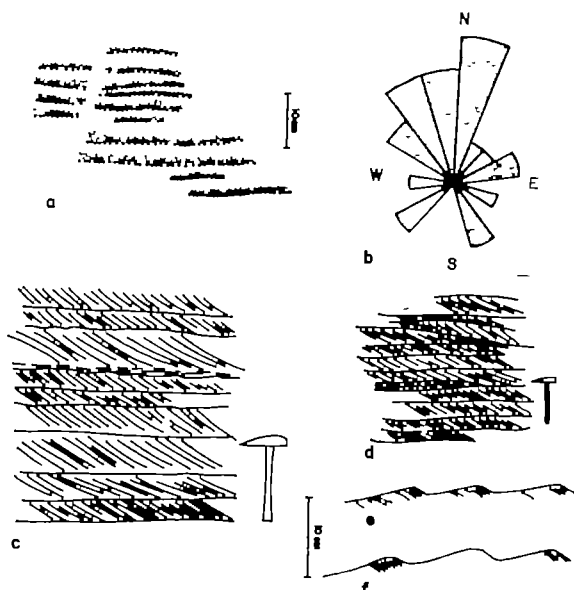


Fig. 1. Sedimentary structures in the Grés de base. a, Repeated graded bedding; b, orientation of the palaeocurrents (equal area rose diagram); c and d, cross-stratification; e and f, ripple-profiles

structural information from the adjoining countries has ever been published, I feel that a brief discussion of the possible palaeogeographical significance of the results is justified.

Fig. 2 shows the thicknesses of the Grés de base compiled from various sources. Though the information is incomplete and the stratigraphic correlations postulated by the various investigators are often tentative and somewhat ambiguous, yet the data are sufficient to present a general picture of the distribution of sands. Accordingly with the strike-slip theory of the Jordan Rift origin<sup>14-19</sup> all the points on the map, located east of the rift, were shifted 70-75 km southwards and then isopachs were drawn.

Fig. 2 suggests that we are dealing with a channel or river-like elongated area of sand deposition with a north-north-west direction of transport. The Beyrouth area, where the amount of sand is the highest, may represent an area closer to the river-mouth. It may also, however, represent an accumulation of sand close to a river-bend or close to a junction of two or more streams.

It is highly probable that, in the Beyrouth area, the north-north-west-oriented palaeocurrent pattern gives place to a more westwardly oriented one. North of Beyrouth the currents probably flowed to the south and south-west.

The Lower Cretaceous sandstones of central and southern Israel and Jordan are believed to belong to a different area of sand deposition.

It should be stressed that the picture presented in the maps of Fig. 2 may be misleading:

(a) The Lower Cretaceous clastics overlie unconformably the older strata.

(b) The absence of sand north of Tripoli, where the Aptian marls and limestones overlie the Jurassic, may be due to truncation or sedimentation-break, as opposed to the thinning out of the sands on the south and west, where they are replaced by shale, volcanics and limestones.

(c) A faulting phase is known to have occurred in the late Jurassic times. It is possible, therefore, that the sandstones were laid on a pre-existing block relief.

However, it should be obvious that the palaeogeographical picture suggested here is intended only as a temporary working hypothesis.

It is hoped that this hypothesis will be checked and re-examined by geologists working in Lebanon and Syria, whose results, when published, will make further correlations possible.

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## THE OMEGATRON: A USEFUL TOOL FOR ARGON ISOTOPE INVESTIGATION

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POTASSIUM-ARGON age measurements on young rock samples depend on the accurate determination of the extremely small volumes of argon-40 which are trapped in the rock. This radiogenic argon-40, evolved from the decay of radioactive potassium-40, is usually measured using the conventional 90° or 120° direct focusing type of mass spectrometer. We have recently constructed and used successfully an omegatron, a mass spectrometer of high sensitivity and low-background noise which works on the cyclotron principle and is ideally suited for this particular work.

The omegatron device was first described by Sommer *et al.*<sup>1</sup> and later modified by Alpert and Buritz<sup>2</sup> to give a simpler design. The omegatron head being used in the work recorded here is one designed by Philips, Ltd., Aachen, for use in measuring partial pressures (Fig. 1). Ions formed in a magnetic field by electron bombardment will describe circles the natural frequency of revolution

of which is given by the cyclotron frequency. A radio-frequency field applied perpendicularly to the magnetic field will accelerate ions with this same frequency of oscillation and their path will become an Archimedes spiral. These resonant ions can be trapped by a suitably placed collector and detected by a sensitive amplifier. Non-resonant ions describe spiral paths with pulsing radii and do not reach the collector.

The theory of an idealized omegatron (neglecting electrostatic fields and space charge effects) was formulated by Sommer *et al.*<sup>1</sup>, who gave the expressions for resolving power, time of flight, path length and final energy of the ions. In an idealized omegatron no electric fields exist and the collection of the resonant ions is complete, but in a real omegatron of the type described by Alpert and Buritz there are always fields present which can cause some of the resonant ions to be lost. These fields are caused by: (a) space charge effects due to the electron beam and the ions present; (b) the electrodes of the omegatron which can become charged; (c) leakage fields from the accelerating plates; (d) the small positive field which must be applied to prevent drift of the ions in the direction of the magnetic field.

These fields can be of the same order of magnitude as the applied electric field and hence large variations in the ion collection can result. The Philips head is identical to that designed by Sommer *et al.* when viewed in a direction perpendicular to the magnetic field (Fig. 1), but in addition it has two side plates CC' to which a large negative voltage can be applied. This negative voltage extracts non-resonant ions, reducing the space charge effects, and together with the RF voltage can be adjusted to obtain a complete collection of resonant ions<sup>3</sup>, and give the tube a reproducible sensitivity. The value of this voltage

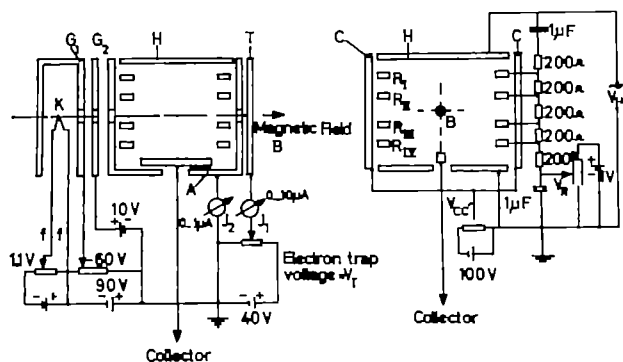


Fig. 1. Schematic diagram of the omegatron.

depends on the number of guard rings used to keep the RF field uniform and the positive voltage,  $V_R$ , which prevents the drift of ions in the direction of the magnetic field. The electrode assembly forms a cube of side about 2.5 cm and is arranged with the electrodes as in Fig. 1.  $J_s$  is a sensitive meter used in the setting up procedure and  $H$  the plate to which the RF voltage is applied. An account of the operating characteristics have been described by Krummenacher<sup>4</sup>. The whole assembly can be degassed to 400° C and the metal electrodes to 900° C by induction heating if need be. Using Balzer's ultra high vacuum pumping equipment an overnight bake at a temperature of 200° C is found quite sufficient to obtain a pressure reading in the range of  $1-2 \times 10^{-10}$  mm of mercury. At this pressure the only significant peaks observed are those at masses 28 and 44.

The voltages to the electrodes are provided from a stabilized supply and the emission regulated in the normal way using the feedback from the trap as a control.

An all-metal argon purification line similar in construction to that described by Miller<sup>5</sup> (Fig. 2), but with several modifications, is connected via a metal tap directly to the omegatron. An automatic loader was devised whereby several samples can be loaded at once. These samples rest on stainless steel rods, which can be withdrawn with a magnet into short side-arms, allowing the samples to drop in the metal fusion crucible. With this technique the atmospheric contamination due to air adsorbed on to the crucible and quartz support can be largely removed, and the contamination due to that on the sample greatly reduced. This automatic loading device is particularly useful for whole-rock measurements, but can also be adapted for separated minerals by making small glass phials to contain the samples. It also has the advantage that the system need not be opened between measurements, thus reducing the atmospheric argon content of the final gas sample and making determinations easier and quicker.

The gas sample obtained from the rock mixed with a sample of enriched argon-38 (the 'spike') of known volume is prepared for admission to the head as described by Miller and Brown<sup>6</sup>. As an extremely low emission

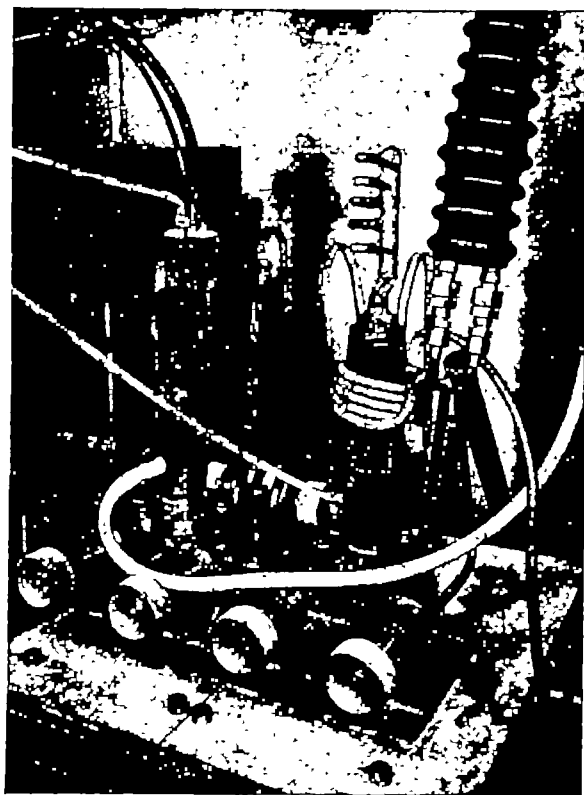


Fig. 2

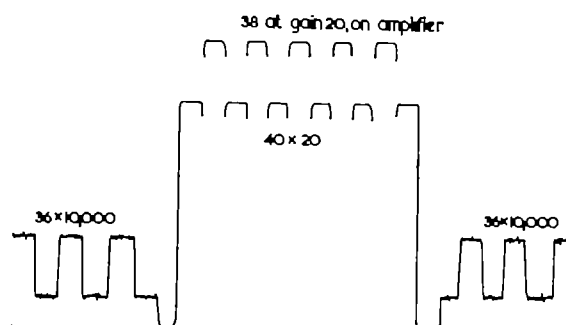


Fig. 3. A typical scan obtained by manual adjustment of the controls

current is used (1-3  $\mu$ amp) and the gas evolution of the apparatus can be made small, no significant change in pressure occurs over a period of several hours, after the gas sample has been admitted and the tap to the pumps closed. Hence the static method of analysis can be used in which the gas sample is isolated from the pumps and the appropriate masses scanned with their peak heights remaining constant. This produces a great increase in sensitivity over the more usual dynamical method where the gas sample is drawn into the mass spectrometer through a tap with a small leak, isotopic fractionation occurring, and the original composition of the sample determined by extrapolating back to the time the tap was opened.

The peaks can be scanned either automatically or manually. Using the static method, manual scanning was found to be preferable to automatic scanning as the peaks were quite stable in the resonance condition and could be measured for a considerable time, reducing any errors caused by fluctuations in the emission, due to mains transients. The resonant peaks can be located by adjustment of the oscillator frequency or the magnetic field. After some experiments it was found that the numbered scale of the dial on the electro-magnetic control could be used as a setting for the different masses and provided a more sensitive adjustment. Fig. 3 shows a typical scan using a field of approximately 9 kilogauss and a fixed frequency of 350 kc/s. The peaks remain constant in height even after a period of several hours. We have also found that the response of the machine is linear with the size of the argon sample provided the pressure does not exceed  $2 \times 10^{-8}$  mm of mercury.

It has frequently been reported that with a Reynolds type of machine the sample being analysed can become contaminated with the previous sample. Reynolds<sup>7</sup> suggested that ions could be driven into the walls of the tube and cause this memory effect. We have not experienced this difficulty, presumably because complete collection of resonant ions is achieved and hence none of the ions can reach the walls. In fact, the sample can be completely pumped away and the background restored to its original state in a few minutes.

As previously described, the voltages can be adjusted to give a complete collection of resonant ions. This resonant ion current is not critically dependent on these voltages provided the RF voltage is high enough for all resonant ions to reach the collector, and hence the isotope ratios obtained remain constant unless a large variation in  $V_{CC'}$  or  $V_R$  occurs. This is a great advantage over the 180° type of machine in which the ratios are normally critically dependent on the applied voltages<sup>8</sup>, and the 90° or 120° type where fringing magnetic fields cause non-ideal conditions in the source. Figs. 4 and 5 show the working ranges of  $V_{CC'}$  and  $V_R$  for resonant ions of argon-38. It is interesting to note that for values of  $V_R$  between 0.2 and 0.5 V non-resonant and resonant ions can be collected, for it is a property of an omegatron that total ion current can be measured in the absence of an RF field. This occurs when the potential of the ion source becomes equal to the potential of the ion collector due to space charge effects.

The omegatron has no mass discrimination effects when adjusted correctly because complete collection of resonant ions is achieved. We have carried out many determinations of the atmospheric argon isotope ratios and have obtained values of:

$$^{40}\text{Ar}/^{36}\text{Ar} = 295.6 \pm 1.5$$

$$^{36}\text{Ar}/^{38}\text{Ar} = 5.26 \pm 0.06$$

This agrees very favourably with the results obtained by Nier<sup>1</sup>, whose values were  $296.0 \pm 0.53$  and  $5.32 \pm 0.01$  respectively.

For argon isotope work it is essential that the 40, 38 and 36 peaks are completely resolved. Sommer *et al* have shown that for an idealized omegatron of similar size to ours, complete separation of one mass unit at mass 40 should be achieved. In the instance of the Cambridge

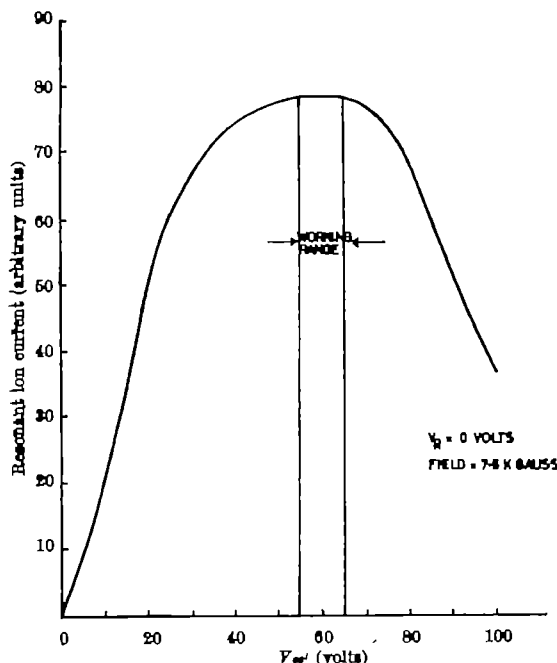


Fig. 4. Resonant ions of argon-38

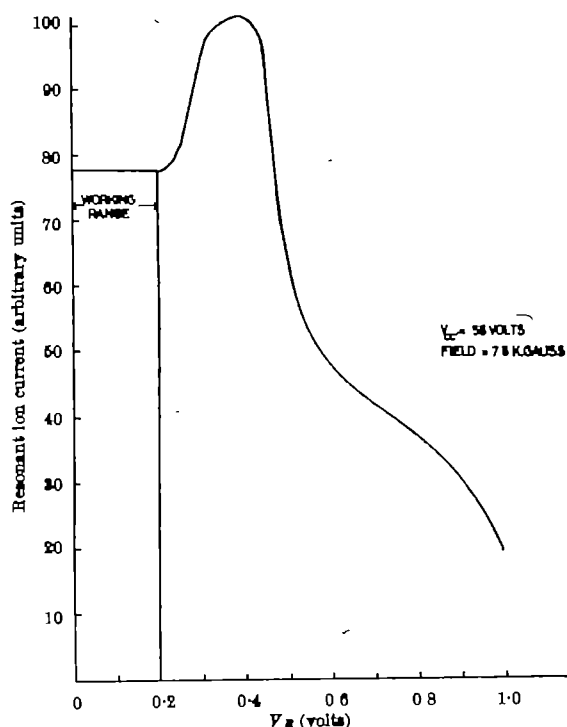


Fig. 5. Resonant ions of argon-38

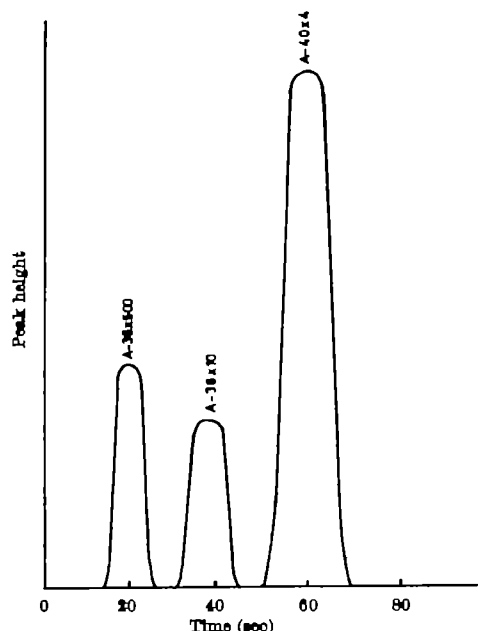


Fig. 6. Resolution scan of atmospheric argon mixed with 'spike' argon

omegatron, complete separation of one mass unit would appear only to be possible up to mass 30; however, the tailing of the 38 on the 36 peak is less than 1 part in 50,000 and completely undetectable. A typical scan of atmospheric argon mixed with 'spike' 38 clearly shows the complete separation of the three isotopes (Fig. 6).

The sensitivity clearly depends on the volume of apparatus and was not adjusted to a maximum as excessive sensitivity would be a disadvantage in dealing with the rocks at present under investigation. In our present system a gas sample of  $10^{-10}$  c.c. N.T.P. when let into the head from the last section of the argon-line will produce an ion current of  $1 \times 10^{-10}$  amperes. Using a  $10^{10}$  ohm input resistor in the vibron head of the amplifier this represents a voltage drop of 1 V at unit gain of the amplifier. At maximum gain 0.1 mV is easily detectable against the random background noise level and consequently it is possible to detect a volume of  $10^{-10}$  c.c. N.T.P. with the omegatron adjusted in its present condition. This is comparable, if not better, than the sensitivity achieved by other machines using an emission current that is at least 10 times as large.

The omegatron is not only an elegant and efficient mass spectrometer but has also been shown to be ideally suited for argon isotope work. It has several important advantages over the conventional machine, having no mass discrimination, a linear response, and being highly sensitive. With the low-emission currents used and the small volume of the detector head, pumping down time is rapid and the low ultimate pressures obtained can be maintained for long periods when the system is isolated from the pumps. It is also cheap and straightforward to operate and could prove invaluable to a research department interested in potassium-argon work at low ages.

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# ANAESTHESIA AND TEMPERATURE EFFECT MECHANISM IN NEUROMUSCULAR BLOCKING BY N<sup>+</sup> QUATERNARY DEPOLARIZING DRUGS

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It has been shown that the lowering of the muscle temperature in animals increases the magnitude and duration of the action of depolarizing neuromuscular drugs<sup>1</sup> such as decamethonium (DM) and succinylcholine (SM). This is related during anaesthesia to the interactions between acetylcholine (ACh) and the aforementioned drugs. A similar variation occurs in human beings, where a fourfold increase of the blocking activity of DM is observed at the moment of maximum activity, when a temperature lowering of about 4° C is used<sup>2</sup>.

The time necessary for the action of tetramethylammonium (TMA) on nerve-sartorius preparations<sup>3</sup> was found to increase by a factor of 1.5 for 10° C of cooling. Ing and Wright<sup>4</sup> have pointed out that "if the Arrhenius formula:

$$v_2 = v_1 \exp(\mu(T_2 - T_1)/2T_2T_1)$$

is assumed, where  $v_1$ ,  $v_2$  are velocities at temperatures  $T_1$ ,  $T_2$  degrees absolute respectively,  $\mu$  will have the values of 6426 and 7336 which are too high for a purely physical process and suggest that a chemical process is involved in the action of quaternary ammonium salts".

The work of Bigland *et al.*, however, would seem to eliminate from these chemical processes the hydrolytic phase.

On the other hand, Lonsdale and Milledge<sup>5</sup>, from consideration of the decamethonium temperature effect of ref. 2, and the X-ray crystal structure determination of hexa- (HM) and deca-methonium dibromide dihydrates, put forward the two following hypotheses:

(i) That the capacity of the N<sup>+</sup> quaternary group for electrostatic binding with the receptors can be dismissed, because in the crystal structure, "the distances from N to other atoms not in the same chain are all greater than 4.26 Å. The N atoms are so well shielded that it is difficult to see how they could take part in any specific attachment respect of the curare-like 'pharmacological' action of the methonium salts."

(ii) "That increased efficiency of action on cooling is easily understood in terms of numerous van der Waals attachments, since these will be sensitive to thermal vibration, especially of the more mobile methyl group."

In agreement with published views<sup>6</sup> that "if the same rules apply to the forces acting in the solid state as those in the adsorption state, these rules would be particularly applicable between the blocking agents and the receptor sites . . .", an intensive examination of the foregoing facts and hypotheses was carried out by me with evidence from the overlapping fields of physical chemistry, crystallography and pharmacology, as a result of which different conclusions are reached. Of these, the first three directly concern the mechanism of the temperature effect in neuromuscular blocking activity by DM and TMA, whereas all five conclusions are related to the specific equilibrium of forces acting in the adsorption and solid state of several drugs.

(1) The solubility data of methonium derivatives in polar and non-polar solvents indicate that in these compounds the coulombic forces, even if reduced, are more significant than the van der Waals forces; in the tetra-alkyl salts the influence of the van der Waals forces grows with the length of the alkyl radicals.

(2) Electrolytic, thermochemical and radiodecomposition data show that the N<sup>+</sup> quaternary group behaves as if the positive charge were at its centre.

(3) An important factor in explaining the increased blocking activity of DM for small decreases in the tem-

perature of the receptor membrane is its very steep solubility gradient with temperature in water. A similar factor should act in the regulation of the temperature effect of the blocking activity of TMA.

(4) Although there is sometimes a tendency, in the crystallographic field, to neglect distances of more than 4 Å between differently charged ions, it is shown that these coulombic forces acting over long distances play an important part in both solid and adsorption states.

(5) The positions in which pentamethonium and hexamethonium in relation to a flat receptor surface possess maximum coulombic and van der Waals binding energies are shown in Fig. 1. The number of van der Waals contacts are different from those suggested in Table 1 of ref. 4. The mechanism of the blocking activity of the bis-N-methylpyrrolidinium series is discussed later in this communication. Evidence in support of the above conclusions is as follows:

**Physico-chemical examination: solubilities.** There is evidence that, in aqueous solution, the tetraalkylammonium ions appear to be unhydrated with a low surface charge<sup>7</sup>, and that the interactions of the alkyltrimethylammonium<sup>8</sup> and alkylammonium<sup>11</sup> with enzymes have a hydrophobic character.

The presence of a low surface charge, unexplained in ref. 9, and circumvented in ref. 27, can be accounted for by the dipoles induced by the N<sup>+</sup> charge on the surrounding four alkyl groups.

This is equivalent to a dielectric constant that weakens the attachment by coulombic forces, and, therefore, the amount of crystal lattice energy of the N<sup>+</sup> quaternary salts will approach that of the hydrogen-bonded type of crystal lattices. Thus, this new hydrophobic type of hydrogen bond is highly relevant to the adsorption by the biological membranes of N<sup>+</sup> quaternary groups, and is in agreement with Pauling's views<sup>12</sup> that, in nervous transmission and muscle contraction, "... the hydrogen bond is the only strong and directed intermolecular interaction which can come into operation quickly".

The presence of a positive charge on the methyl of the N<sup>+</sup> quaternary group has been suggested by Thomas and Marlow<sup>13</sup>, but only on speculative grounds from the properties of NH<sub>4</sub><sup>+</sup>. Their views have been opposed by Belleau and Lacasse<sup>14</sup>, who argue that the presence of residual positive charges would confer a degree of hydration to the N<sup>+</sup> quaternary group. However, their statement is not borne out by the experimental electrolytic evidence<sup>9</sup> or that of nuclear magnetic resonance (Canepe, F. G., and Mooney, E. F., unpublished results), from which it appears that, in aqueous solution, the three methyls of the N<sup>+</sup> groups of acetylcholine, hexamethonium and decamethonium rotate about the chain N-C axis. Thus, this physical evidence of the absolute hydrophobic character of the N<sup>+</sup> quaternary group in dilute aqueous solution represents it as a dynamic equilibrium between the CH<sub>3</sub> and the water dipoles, and confirms the earlier observations reviewed by Ing and Wright<sup>6</sup>. From the solubility data (Table 1), and the N<sup>+</sup> group radii, it can be assumed that the reduced coulombic forces are predominant in both the TMA ion and trimethylmethonium<sup>9</sup> series.

The ionic limiting conductances  $\lambda_0$  for tetra- and penta-<sup>15</sup>, hexa- and decamethonium<sup>16</sup>, and tetraalkylammonium ions<sup>17</sup>, show that the methonium compounds are highly ionic in solution. Halliwell and Nyburg<sup>18</sup> have reviewed the thermochemical evidence, from several

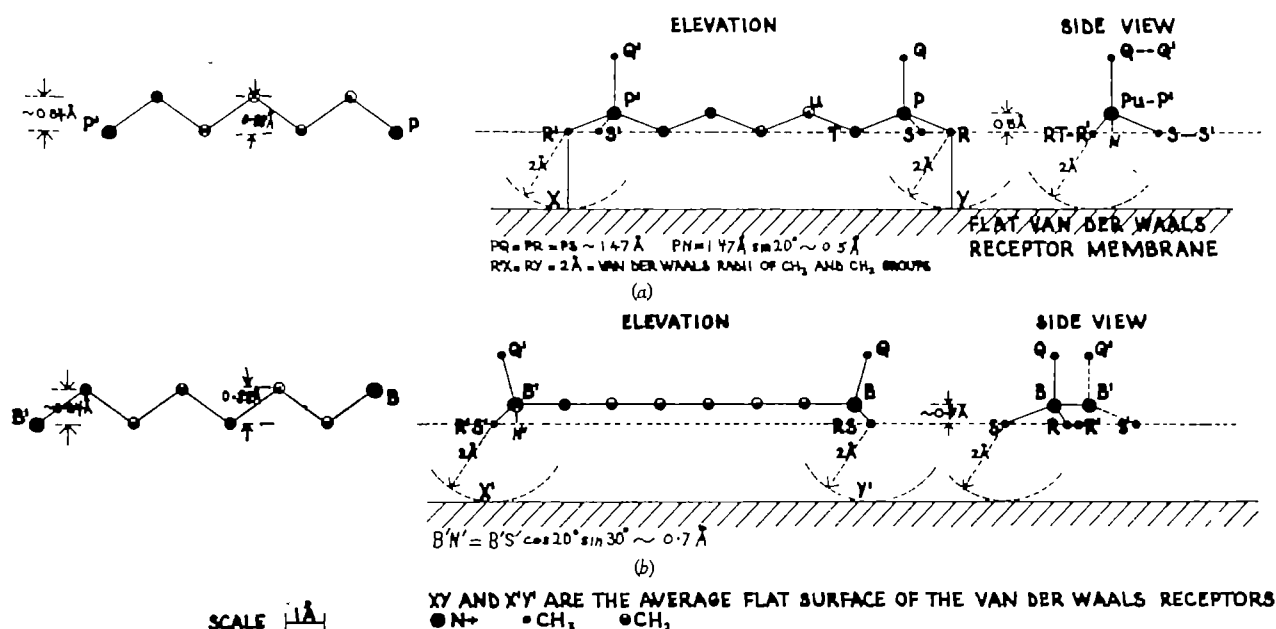


Fig. 1. Maximum binding energy in the adsorption state. (a) For pentamethonium chain, (b) for hexamethonium chain

authors, on the enthalpies of hydration of  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Rb}^+$ ,  $\text{Cs}^+$ ,  $\text{Ph.N}_4^+$  and  $\text{NMe}_4^+$ .

Plotting the absolute enthalpies of hydration against the effective ionic radii of respective ions gives a simple curve. Hence we observe that evidence given in refs. 9 and 18 indicates that the  $\text{NMe}_4^+$  behaves as if the positive charge were at its centre. Moreover, the close relationship that appears to exist between the betaine and choline chains in the mechanism of their radiation decomposition<sup>18</sup> brings further evidence for the state of the  $\text{N}^+$  quaternary group.

**Blocking activity of decamethonium and tetramethylammonium temperature effect.** The increase of the solubility of decamethonium iodide in water from 22° to 100° C is fiftyfold, an increase for each °C equivalent to 64 per cent of the initial solubility of 22° C. This extraordinary behaviour in a polar medium such as water, the nearest to the physiological medium, is a more relevant factor to the diminished neuromuscular blocking activity of DM, when the temperature increases by a few degrees, than the hypothesis of van der Waals attachment suggested by Lonsdale and Milledge<sup>4</sup>.

Although the kinetic equilibrium of the adsorption state phase between the receptor membrane charges and those of the  $\text{N}^+$  groups of decamethonium takes place in a hydrophobic layer-medium, it experiences through its boundary equilibrium with the surrounding aqueous or near-aqueous physiological phase the temperature solubility's variation of decamethonium.

Paton and Zaimis<sup>19</sup> have shown that some aspects of decamethonium adsorption state behaviour at the receptor membrane are acetylcholine-like. Wilson and Cabib<sup>21</sup>

have found that acetylcholine adsorbability (the reciprocal of the Michaelis-Menten constant) on the receptor membrane does not vary with temperature in the interval 15°–30° C, which can be extrapolated to 5°–35° C. Now the attachment of the  $\text{N}^+$  groups of Ach and decamethonium to the membrane is mainly electrostatic. Therefore, we are justified in using the electrostatic criteria rather than the kinetic considerations. Then, the foregoing findings of Wilson and Cabib<sup>21</sup> are in accordance with the experimental fact that in the solid state the variations of the long-range coulombic forces with temperature are much smaller than those of the short-range van der Waals forces, that is, the latter very high distance specificity is very sensitive to thermal displacements produced by variations of temperature. The larger Michaelis-Menten constants observed for increased substitution of hydrogen for the  $\text{OH}_2$  of the  $\text{N}^+$  group of Ach are an indication of increased hydration, that is, repulsion from the hydrophobic receptor surface.

In conclusion, it is reasonable to assume that, for decamethonium, the variations of the Michaelis-Menten constants with small variations of temperature are much smaller than the correspondingly high variations of its solubility, the latter factor being therefore responsible for the temperature effect of its neuromuscular blocking activity, despite the solution of the drug being diluted.

The solubility gradient (between 20° and 100° C), and the increase of blocking activity for 4° C of cooling, of TMA and DM should stand in similar ratios, 6.2/50 and 0.6/4 respectively.

If Ing and Wright<sup>3</sup> have firmly rejected the importance of solubility in the temperature effect, and on curariform

Table 1 IONIC CONDUCTANCE AND AQUEOUS SOLUBILITIES OF SOME  $\text{N}^+$  IODIDES IN g/ml.

Solvent	Methonium <sup>a,7</sup>			$\text{N}^+$ tetra-alkyl <sup>8</sup>			
	( $\text{CH}_3$ ) <sub>5</sub> Pentameth.	<sup>+</sup> ( $\text{CH}_3$ ) <sub>6</sub> Hexameth.	<sup>+</sup> ( $\text{CH}_3$ ) <sub>7</sub> Decameth.	<sup>+</sup> ( $\text{CH}_3$ ) <sub>4</sub>	<sup>+</sup> ( $\text{C}_2\text{H}_5$ ) <sub>4</sub>	<sup>+</sup> ( $\text{C}_3\text{H}_7$ ) <sub>4</sub>	<sup>+</sup> ( $\text{C}_4\text{H}_9$ ) <sub>4</sub>
25	54.3	49.4	42.1	44.92	22.66	23.4	17.0
H <sub>2</sub> O at 22° C		All solubilities in g/ml.			All solubilities in g/ml.		
H <sub>2</sub> O at 100° C	2	1/1.8	1/10	0.02	0.45	0.186	0.007
C <sub>2</sub> H <sub>5</sub> OH at 20° C	5	8.3	5	0.059			
At 78.5° C	1/900	0.3/1,000	1/100				
Boiling chloroform, ether, acetone	1/40	1/890	1/8				
	< 0.002	< 0.002	< 0.002	Chloroform 20° C	0.015*	0.546*	2.11*

\* Weight per g of solvent.



activity<sup>22</sup>, this was probably because the available data on the curariform activity of the trimethyl alkylammonium cations were incorrect, as can be seen by comparing their evidence with that reviewed by Bovet and Bovet-Nitti<sup>23</sup>.

It is well known that Ach activity is enhanced by cooling. Because Ach has a solubility gradient (data yet to be published), the postulated mechanism for the temperature effect of DM and TMA can be applied to Ach, which is also in agreement with the additivity of their depolarizing cholinergic effects.

Much lower concentrations of Ach are required at neuromuscular junctions for producing similar effects to those of the foregoing drugs<sup>24</sup>. As the formation process of Ach is not altered by them, the accumulation of Ach at the junction will compensate partially for the speed of elimination of DM or TMA, enhancing the duration of their blocking activity.

An increase in drug concentration in the membrane is produced by cooling; the magnitude of the block will be increased if a greater number of available receptors are occupied; the excess of the drug will affect the duration.

Bigland, Goetzee, MacLagan and Zaimis<sup>1</sup> suggest that "cooling influences the process by which a long-lasting depolarization of the motor end-plate interrupts neuromuscular transmission", but no mechanism is given. The above statement becomes clearer if the solubility gradient mechanism is introduced as a process. These authors also suggest that the temperature effect mechanism of the N<sup>+</sup> quaternary depolarizing drugs can be compared with that acting during the depolarizing action of an excess of K<sup>+</sup> ions at the receptor membrane. This view must be considered with great caution because:

(i) The aqueous solubility gradient of KCl is less than a quarter of that of TMA iodide, that is, temperature effect variations are within experimental errors if cooling is 3°–4° C only.

(ii) The K<sup>+</sup> ions are not hydrophobic, unlike the N<sup>+</sup> group.

(iii) There is an enzyme mechanism that regulates the concentration of K<sup>+</sup> ions on both sides of the receptor membrane.

Finally, from the solubility data of Table 1 it follows that a possible temperature effect operates on the ganglion-blocking activity of non-depolarizing drugs which should be less marked for the hexamethonium than for the pentamethonium substrate—their action being competitive.

**Forces in the adsorption state.** It is known that the blocking activity of the (CH<sub>3</sub>)<sub>n</sub>N<sup>+</sup>—(CH<sub>2</sub>)<sub>n</sub>—N<sup>+</sup>(CH<sub>3</sub>)<sub>n</sub> series is a function of *n* and of the receptor membrane properties<sup>25</sup>.

Since 1962 I have accumulated evidence showing that this blocking activity is also a function of the distribution of the coulombic forces surrounding the N<sup>+</sup> quaternary groups at the receptor membrane surface. For example, in Fig. 1 it can be observed that the extended methonium chains with an uneven number of CH<sub>2</sub> have their two positive charges along one side (PP') of the chain's plane, whereas the even chains have their positive charges along the diagonal (BB') of the chain's plane; PP' and BB' are axes of electrostatic attachment. Thus, when extended, the adsorbed methonium even chain will have either of its sides BB' parallel to the plane of the receptor, with only four CH<sub>2</sub> van der Waals contacts.

If, and only if, the bulky N<sup>+</sup> terminals of the extended hexamethonium chain could rest inside cavities 0.7 Å deep, nine to ten van der Waals contacts would be possible and not 3 + 3 as calculated in Table 1 of ref. 4. These cavities would not fit other chains of different lengths, such as pentamethonium, which has seven contacts in Fig. 1, that is, the sum of the van der Waals and the coulombic binding energies is maximum when the electrostatic axis PP' is about 0.5 Å from the flat receptor's surface. There are only four contacts if PP' is 0.84 Å distant from the receptor's surface, that is, the

sum of the electrostatic and van der Waals binding energies is minimum if during the adsorption state the chain's plane is perpendicular to the receptor. These contacts differ from those given in ref. 4, Table 1 (2 + 3 and 3 + 3), which are valid only for the crystalline state, and not for the adsorption state where the influence of the electrostatic axes of types PP' and BB' must be considered relative to the receptor plane. By the latter method it is possible to explain the differences in ganglion-blocking activity of the trimethylmethonium series and the polymethylene bis-N-methylpyrrolidinium series.

In ref. 4 the probable chain length of the methonium bis-cations in the physiological medium<sup>24</sup>, and the summation of the non-indifferent energy barriers of 800 cal/mole for each of the bond rotations, from a skew to a *trans* position, necessary in order to stretch fully the odd or even methonium chains at the receptor site as required in their hypotheses, are ignored, as also are the forces necessary to carry out this work at the receptor membrane.

**Crystallographic examination.** In the pentamethonium iodide crystal structure<sup>26,27</sup>, the expected N<sup>+</sup>—...I<sup>−</sup> maximum distance is the sum of the N—C bond = 1.47 Å plus the van der Waals radii of CH<sub>3</sub> = 2 Å and I<sup>−</sup> = 2.15 Å, then N<sup>+</sup>—...I<sup>−</sup> max = 5.62 Å. The maximum N<sup>+</sup>—...I<sup>−</sup> distance should diminish about 0.5 Å when a hard anion is attached between two methyls and about 0.9 Å when attached inside the cavity at the intersection of the van der Waals spheres of the three-methyl group.

Therefore, if the recorded N<sup>+</sup>—...I<sup>−</sup> distances, for one N<sup>+</sup> group only, are 4.43, 5.14, 5.19, 5.28, 4.66, 4.51 ± 0.14 Å, they are all well within expectations; they indicate the presence of twelve coulombic forces per chain, which significantly increase, for example, the angles C<sub>1</sub>N<sub>1</sub>C<sub>2</sub> and C<sub>2</sub>N<sub>1</sub>C<sub>3</sub>, and diminish the angles C<sub>1</sub>N<sub>1</sub>C<sub>4</sub> and C<sub>1</sub>N<sub>1</sub>C<sub>5</sub> (see ref. 25, page 106, Fig. 7a1).

Pressman, Grossberg, Penoe and Pauling<sup>28</sup> have evaluated coulomb interaction energy between the charged haptene group and the antibody's N<sup>+</sup> quaternary group for *r* = 7 Å, which is well above the value of 4.26 Å of ref. 4.

I have not considered the binding energy due to the increased van der Waals distances between C—O of different chains because of their weakness in relation to the long-range coulombic forces, the energy of the former being a function of 1/*r*<sup>6</sup> as compared with 1/*r* for coulombic binding energy (see Fig. 1, page 149, ref. 26).

In ref. 26 the standard deviations of the inter-atomic distances are given as *r*<sub>1</sub>—...I<sup>−</sup> ± 0.02 Å, *r*<sub>1</sub>—...N<sup>+</sup> ± 0.14 Å, *r*<sub>1</sub>—...O ± 0.17 Å and *r*<sub>0</sub>—...O ± 0.23 Å. Neglecting the Madelung constant, it is simple to obtain an approximate assessment of the reduced coulombic binding energy *E*(1/*r*<sup>2</sup>), in terms of the twelve observed N<sup>+</sup>—...I<sup>−</sup> distances per pentamethonium chain.

Alternatively, as is implicit in the hypotheses of Lonsdale and Milledge<sup>4</sup>, a rough estimate of the binding energy (excluding the van der Waals energy) could be ascertained by means of the more numerous but less-accurate distances *r* of the type I<sup>−</sup>—...CH<sub>2</sub>, CH<sub>2</sub>—...CH<sub>2</sub>, CH<sub>2</sub>—...CH<sub>2</sub> as a function of 1/*r*<sup>2</sup>, where 1 < *n* < 6 and the forces involved change for a given type of *r*. A list of the I<sup>−</sup>—...O ten distances ≤ 4.15 ± 0.17 Å for a whole pentamethonium chain is given in ref. 25, page 109, the smallest being *r* = 3.75 ± 0.17 Å.

**Pharmacological examination.** In ref. 4, in accordance with Table 1, it is stated, from consideration of pharmacological tests carried out on one type of tissue only, that the mechanism of the blocking activity for the *n*-methonium chains "is rather dependent on the extreme CH<sub>2</sub>—...CH<sub>2</sub> distances on each side of the chain and on the number of possible van der Waals contacts within those extremes". Also the critical distances of optimum ganglion and neuromuscular blocking activity are given as 10 Å and 15 Å and the conclusion drawn that the blocking activity of molecules with CH<sub>2</sub>—...CH<sub>2</sub> distances different from the above would be small because 'easily detachable'.

It should be noted, however, that pempidine, which is considerably shorter than 10 Å, is a highly active ganglion-blocking agent of protracted action<sup>22</sup>. Although changes on the extreme OH<sub>1</sub>...OH<sub>2</sub> distances are produced when two C<sub>2</sub>H<sub>5</sub> groups are substituted for 2OH<sub>1</sub> in each of the N<sup>+</sup> terminals of tetra, penta and hexamethonium<sup>23</sup>, the activities of the di-substituted tetra-, penta- and hexa-derivatives in the cat superior cervical were 100, 125 and 75 units instead of 1, 65 and 100 units of activity before the substitution. In the guinea-pig ileum<sup>24</sup>, the new activities were 25, 100 and 100, instead of 5, 75 and 100.

The critical distance of 15 Å for the optimum blocking activity at the neuromuscular junction<sup>4</sup> is no less arbitrary than that of 10 Å for ganglion-blocking activity. Paton and Zaimis<sup>25</sup> have shown that for different types of membrane the maximum blocking activity ranges between DM to dodecamethonium and that DM's activity varies greatly according to the species of animals tested:

DM activity in cat > rabbit > monkey > mouse > rat

$$\frac{\text{DM activity in cat}}{\text{DM activity in rat}} \approx 200$$

A similar di-substitution produces very different effects in the ganglionic and neuromuscular blocking activities of hexamethonium<sup>26</sup> and decamethonium<sup>27</sup>. This difference of adsorption behaviour, and the fact that only the latter depolarizes the receptor membrane, cannot be explained by weak van der Waals forces alone, but could be if to them were added the predominant action of the reduced coulombic forces.

The combined effects of these forces provide a rational explanation of the very different blocking mechanisms of, for example, pempidine, trimethylalkylammonium series, trimethylmethonium series, polymethylene bis-N-methyl pyrrolidinium series and the substituted derivatives

mentioned above, which will be dealt with in a separate paper under preparation.

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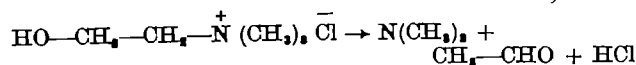
## RELATIONS BETWEEN ACETYLCHOLINE, CHOLINE AND BETAINE CHAINS IN AQUEOUS SOLUTION

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### Radiation Decomposition Mechanism of Betaine and Choline Chains

CRYSTALLINE orthorhombic choline chloride (Ch.Cl) at room temperature is very sensitive to ionizing radiation<sup>1</sup> and the choline ion breaks down mainly into trimethylamine and acetaldehyde<sup>2</sup>:



Lemmon *et al.*<sup>3</sup>, after investigating the effects of ionizing radiation on Ch.Cl and its analogues, concluded that: (i) choline bromide is about one-third as sensitive as the chloride. None of many other analysed analogues, including choline iodide (Ch.I), shows abnormal radiation instability; (ii) Ch.Cl susceptibility to radiation damage is a function of its crystal structure, as shown by its contrasting stability in solution; (iii) the high values of molecular decomposition per 100 eV indicate a chain mechanism for the solid-state reaction.

The crystal structure of the orthorhombic phase of Ch.Cl was published by Senko and Templeton<sup>4</sup>. Data on the cubic disordered reversible phase, stable over 73° C, were given by Collin<sup>5</sup>, who suggested that the transition to the disordered cubic phase and the subsequent increased stability to radiation furnished further evidence that the

decomposition was highly stereospecific. From their crystallographic evidence the authors were not able to find a mechanism which accounted for the foregoing facts.

Serlin<sup>6</sup> reported that for γ-radiation the decomposition of crystalline Ch.Cl per 100 eV absorbed was higher at 50° C than at 20° C, but at 160° C the solid Ch.Cl was much more stable than at room temperature.

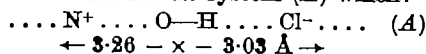
Finally, Lemmon *et al.*<sup>7,8</sup>, although still unable to find an explanatory mechanism, produced the following relevant evidence from their isotopic investigations of the radiation decomposition of crystalline Ch.Cl: "(i) the carbinol group of the ethanol moiety becomes the aldehyde group of the resultant aldehyde; (ii) no hydrogens are transferred to or from the trimethylamino-group; (iii) the hydrogens of the ethanol moiety are highly mobile; (iv) intermolecular hydrogen transfers take place".

Because X-ray diffraction data obtained under irradiation conditions provide a reliable quantitative picture of the behaviour of the Ch. and Cl<sup>-</sup> ions during the collection of the diffraction measurements, I have made an intensive examination of the combined crystallographic evidence from the structures of tetramethylammonium (TMA)<sup>9,10</sup> halides, muscarine iodide<sup>11</sup>, pentamethonium iodide<sup>12</sup>, choline chloride<sup>4</sup>, hydroxylammonium chloride<sup>13</sup>, hydrogen chloride monohydrate<sup>14</sup> and acetylcholine bromide (Canepa, Pauling and Sorum, work in preparation). As a

result of this examination, a mechanism was found for the radiation decomposition of Ch.Cl, the attenuated decomposition of Ch.Br, the insensitivity of Ch.I to radiation, the temperature effect on the rate of their decomposition and a similar mechanism for the radiation decomposition of betaine hydrochloride, based on the following evidence:

Ch.Cl orthorhombic structure can be defined as an infinite molecule with cations and anions bound by a mixture of weak electrostatic forces and even weaker hydrogen bonds. The dipole moment of the  $\text{CH}_3$  groups<sup>15,16</sup> acts as a dielectric constant reducing the coulombic forces between the  $\text{N}^+$  quaternary group and the  $\text{Cl}^-$  anions and will be dealt with in a later communication.

In the published Ch.Cl structure<sup>4</sup>, because the  $\text{Cl}^- \dots \text{O}$  distance is 3.03 Å, instead of 2.95 Å as in hydrogen chloride, 2.91 and 2.99 Å in tropolone hydrochloride, and 2.99 Å in hydroxylammonium chloride, the conclusion was reached by Senko and Templeton that the hydrogen bonds, if present, were very weak. Nevertheless, a calculation with their published co-ordinates (see Figs. 1 and 2) shows the angle  $\text{C}_4\text{C}_3\text{O} \approx 102.5^\circ$ , instead of the expected  $108^\circ$ – $110^\circ$ , and the  $\text{N}^+ \dots \text{O}$  distance 3.26 Å only, as compared with the shortest of the six  $\text{N}^+ \dots \text{Cl}^-$  electrostatic co-ordination distances: 4.08, 4.22 and 4.28 Å; moreover the non-bonded  $\text{C}_1 \dots \text{C}_2$  carbons, 3.3 Å apart instead of a minimum of 4 Å, are the seat of repulsive forces. The foregoing three factors indicate a very significant electrostatic binding between the  $\text{N}^+$  group and the alkyl oxygen. A comparative calculation with the public co-ordinates of muscarine iodide gives an even shorter  $\text{N}^+ \dots \text{O}$  distance of 3.07 Å, and the shortest of its six  $\text{N}^+ \dots \text{I}^-$  distances as 4.49 Å, suggesting a stronger electrostatic binding in the  $\text{N}^+ \dots \text{O}$  system of muscarine. Thus, there is in Ch.Cl structure a balance of forces between the pulls exerted by  $\text{N}^+$  and by  $\text{Cl}^-$  on the oxygen in the electrostatic system (A) which:



accounts for the longer  $\text{N}^+ \dots \text{O}$  distance (3.26 Å), that is, weaker electrostatic binding, and for the longer  $\text{O}-\text{H} \dots \text{Cl}^-$  (3.03 Å).

A comparison of the  $d-r$  values of Ch.Cl and TMA.Cl in Table 1 shows that repulsive forces between anions increase with the ionic co-ordination (see Ch.Cl and TMA.Cl) but not with anionic radii (see TMA halides). Therefore, it is likely that the 11 per cent increase of unit cell volume during the Ch.Cl transition from orthorhombic to the cubic phase is due to the disorder of the new phase and to a change from the distorted six-fold to the distorted eight-fold electrostatic co-ordination, the latter increasing the value of  $\text{N}^+ \dots \text{Cl}^-$  distance from 4.08 to, say, 4.38 Å as in TMA.Cl (see Table 1).

This decreased binding energy in the expanded  $\text{N}^+ \dots \text{Cl}^-$  distances of Ch.Cl cubic form allows a stronger  $\text{N}^+ \dots \text{O}$  electrostatic interaction which should cause the  $\text{N}^+ \dots \text{O}$  distance to decrease from 3.26 Å to at least 3.07 Å. This is the  $\text{N}^+ \dots \text{O}$  distance in the six-fold distorted muscarine iodide and possibly in the four-fold co-ordinated acetylcholine chloride. Because of the increased  $\text{N}^+ \dots \text{Cl}^-$  and decreased  $\text{N}^+ \dots \text{O}$  dis-

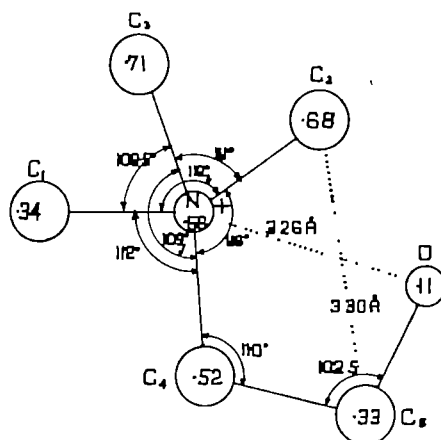


Fig. 1.  $\text{Ch}^+$  in Ch.Cl orthorhombic phase.  $\delta$  atomic co-ordinates given.  $\text{C}_1 \dots \text{C}_2$ , 2.65 Å;  $\text{N}^+ \dots \text{C}_1$ , 2.68 Å;  $\text{O} \dots \text{C}_1$ , 2.45 Å;  $\text{C}_3-\text{O}$ , 1.99 Å

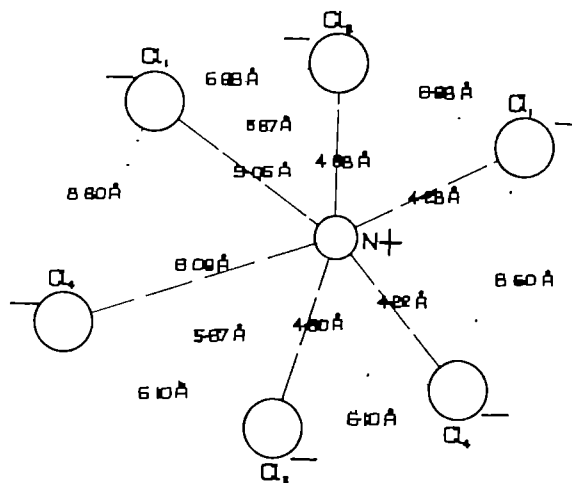


Fig. 2.  $\text{N}^+ \dots \text{Cl}^-$  and  $\text{Cl}^- \dots \text{Cl}^-$  distances in Ch.Cl orthorhombic phase

tances a weakening of the hydrogen  $\text{O}-\text{H} \dots \text{Cl}^-$  is implied in the expanded unit cell of cubic Ch.Cl.

The consequence of the foregoing is the greater stability of the Ch.Cl cubic phase to radiation decomposition. This will be better understood after a close examination of the atomic thermal displacements under X-ray irradiation in the compounds listed in Table 2.

The temperature factor is  $B_i = 8\pi^2 \langle u_i^2 \rangle$ , where  $\langle u_i^2 \rangle$  represents the isotropic mean square displacement of the atoms in the X-irradiated crystal structure and  $i = \text{O}, \text{N}, \text{Cl}$ , etc., depending on the chemical composition of the structure. Relevant also to the radiation decomposition mechanism is the greater mobility of the hydrogen atoms (higher values of  $B$ ) relative to X-irradiated heavier atoms which occur in most X-ray crystal structures.

If the thermal displacements  $\langle u_i^2 \rangle$  of the orthorhombic Ch.Cl (see Table 2 following) were really isotropic, then the maximum additive displacements of  $\text{N}^+ \dots \text{Cl}^-$ ,

Table 1

	Ch.I	Ch.Br. orthorhombic	Ch.Cl	Ch.Cl cubic	TMA.Cl tetragonal	TMA.Br tetragonal	TMA.I [tetragonal]
First part							
No. molecules per cell	4	4	4	4	2	2	2
Unit cell volume = $V$	877.4	817.7	703.0	857.0	334.8	333.1	362.5
Shortest distance ( $\text{N} \dots \text{halide}$ ) = $d$	—	—	4.08	4.38 ?	4.38	4.37	4.56
Halide radius	2.15	1.96	1.81	1.81	1.81	1.96	2.16
$d-r$	—	—	2.27	2.57 ?	2.57	2.42	2.40
Electrostatic co-ordination	—	—	6	8	8	8	8
Second part							
Cationic volume + interstices = $v$	—	—	—	—	—	—	—
( $V/4$ - anion volume)	177.12	173.88	165.91	180.41	58.86	52.23	48.4
$v_0 = \text{halide} - \text{molecule}$	0	-8.74	-11.21	+12.29	+10.466	+8.83	0
Degree of $\text{N}^+ \dots \text{halide}$ compression	—	Partial	Marked	—	—	—	—

Table 2

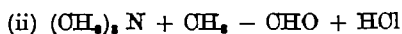
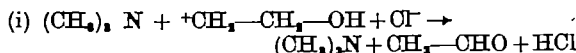
Ocholine chloride orthorhombic at room temperature	Atom	Cl	O	N	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>5</sub>
	B in Å <sup>2</sup>	5	6	4	5	7	5	7	7
	w in Å	0.252	0.276	0.225	0.207				
Hydrogen chloride monohydrate at -25° C	Atom	Cl <sub>1</sub>	Cl <sub>2</sub>	O <sub>1</sub>	O <sub>2</sub>	H			
	B	0.035	-0.007	0.041	-0.016	5.0			
	w	0.0214				0.195			
Hydroxylammonium chloride NH <sub>2</sub> OHCl	Atom	Cl	O	N					
	B	1.5	1.5	1.5					
	w	0.138	0.138	0.138					

N+....O and O....Cl- would be  $u_{Cl} + u_N \approx \pm 0.48 \text{ Å}$ ,  $u_N + u_O \approx \pm 0.5 \text{ Å}$ ,  $u_{Cl} + u_O \approx \pm 0.53 \text{ Å}$ .

The evidence as to the relevance of thermal displacements in the oscillatory behaviour of the loosely bound tetrahedral system (A) under irradiation is provided by the resultant products of the radiation decomposition.

The already strained bond N—C<sub>4</sub> of 1.6 Å length, joining the two branches of the gauche OH<sup>+</sup> chain, is stretched further if the atoms C<sub>3</sub> and O of one branch close up towards N<sup>+</sup> and C<sub>5</sub> on the other branch due to thermal displacements assisted by the high energy of the γ-radiation (see Fig. 1).

This compresses the N+....O and O<sub>3</sub>....C<sub>5</sub> distances so that the N—C<sub>4</sub> bond breaks. The increased N<sup>+</sup> induction on the O—H group repels the highly mobile hydrogen atom towards either the hydrocarbon chain (i) or the Cl- (ii).



In either, a pressure wave is set up and transmitted through the highly compressed orthorhombic structure of Ch.Cl (volume 763 Å<sup>3</sup>) due to the van der Waals forces originating from the molecules produced in the radiation decomposition because these require a van der Waals space greater even than that available in the expanded cubic phase of Ch.Cl (volume 857 Å<sup>3</sup>).

The already existing thermal displacements, the γ-radiation and the new pressure wave further compress all the N+....O and C<sub>3</sub>....C<sub>5</sub> systems in the contiguous Ch<sup>+</sup> chains, resulting in additional decomposition of the chain reaction type.

### Acetylcholine Conformation in Aqueous Solution

The great stability to irradiation of the Ch<sup>+</sup> cations in the aqueous solutions of Ch.Cl is accounted for by:

(i) The coulombic forces between the hydrophobic N<sup>+</sup> group and the hydrated Cl- anions are reduced as compared with that in the crystalline state, since besides the OH<sub>2</sub> dipoles surrounding the N<sup>+</sup> there are the water dipoles around the Cl-. Thereby the N+....Cl- distances in solution are greater than in the crystalline phases.

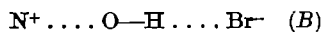
(ii) This strengthens the binding energy of N+....O, shortening its distance to at least 3.07 Å as in muscarine iodide crystal structure, leaving the O—H system too weak to compete with the water molecules for the Cl- anions. Because in aqueous solution the chain Ch<sup>+</sup> does not contain the irradiation sensitive oscillatory system (A) it is far more stable to the γ-rays. Moreover, in the aqueous medium the high-energy γ-radiation impact on the Ch<sup>+</sup> chains can be kinetically absorbed by recoil movements of these cations.

(iii) This Ch<sup>+</sup> chain stability towards irradiation in solution is the first strong indirect evidence relating the forces acting on Ch<sup>+</sup> in the solid state to those acting on Ch<sup>+</sup> in solution. Thus the N+....O distance of Ch<sup>+</sup> in solution is similar to that of Ach. in Ach.Br crystal structure and gives to both cations because of their hydrophobic N<sup>+</sup> group a quasi-zwitter ion or quasi betaine character only in aqueous solution. This completes the

analysis of the infra-red evidence of Ach. in aqueous solution of Canepa and Mooney<sup>17</sup>, where a ring conformation due to the N<sup>+</sup> group and the carbonyl oxygen of Ach. was ruled out.

The N+....O conformation in the Ach. solid state and in aqueous solution is relevant to its adsorption at the cholinergic receptor and its hydrolysis. This provides another striking example of the relations between the forces acting in the solid state and those acting in the adsorption state<sup>18</sup>: I shall deal with these in a subsequent communication.

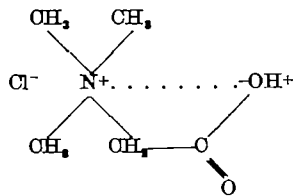
**Decomposition of Ch.Br compared with the stability to irradiation of Ch.I.** It can be seen from the first part of Table 1 that the repulsive forces between the anions co-ordinating the N<sup>+</sup> group in the TMA halides decrease when the radii of the anions increase; as a consequence, the volume of the rigid cationic TMA<sup>+</sup> group, plus inter-ionic interstices, is minimum for TMAI. Conversely, the second part of Table 1 shows that in the crystalline state the available amount of cationic volume in the Ch. halide is maximum for Ch.I and minimum for Ch.Cl. It follows from the negative values of  $\delta v$  that the N+.... halide compression is greater for Ch.Cl than for Ch.Br; therefore, the corresponding N+....O distance in the oscillating system (B):



is intermediate between 3.26 and 3.07 Å, that is, the N+....O bridge joins the branches of the gauche chain of Ch<sup>+</sup> in the Ch.Br crystal structure with a rigidity intermediate between that of Ch.Cl and that of Ch.I, which accordingly reduces the Ch<sup>+</sup> thermal displacements and with it the rate of radiation decomposition of Ch.Br.

The value of the temperature factor  $B = 8\pi^2 u^2$  is minimum when the Ch.Cl molecule is in the ground state (zero point energy), and increases steadily with the temperature for a given crystalline phase. The almost zero thermal displacement at liquid air temperature explains the stability of Ch.Cl in such conditions, whereas the growth of the rate of radiation decomposition must coincide with rising temperature.

It is possible to suggest the decomposition mechanism of betaine hydrochloride on similar lines to (B); the oxygen charge, being located between the CH<sub>3</sub> groups, where their dielectric constant is a minimum, is strongly attracted towards the centre of the N<sup>+</sup> group.



I thank Dr. C. G. Smith for his help and advice.

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# CHLOROGENIC ACID: FURTHER EVIDENCE FOR ITS ANTIGENIC AND ALLERGENIC ACTIVITY

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PREVIOUS investigations<sup>1-3</sup> in these laboratories demonstrated that chlorogenic acid was an important allergenic constituent of green coffee bean. This simple phenolic compound, which is the depside of caffeic and quinic acids (Fig. 1), accounts for 6-8 per cent of the dry weight of the green coffee bean. Coffee workers who developed asthma or rhinitis on exposure to finely dispersed coffee dust showed wheal and erythema responses when tested intradermally with chlorogenic acid, and positive Prausnitz-Kustner passive transfer reactions were obtained when chlorogenic acid was injected into human skin sites sensitized with the sera of the allergic workers. Furthermore, it was demonstrated that free chlorogenic acid was capable of inducing the formation of circulating antibodies in animal species<sup>4</sup>.

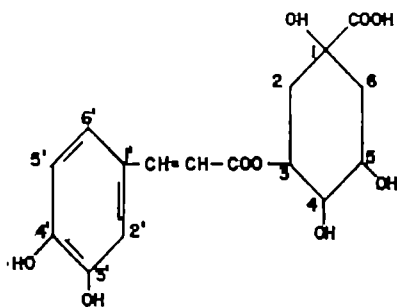


Fig. 1. Chlorogenic acid

Recently all these findings were challenged by Layton *et al.*<sup>5,6</sup>, who reported that a 'pure' chlorogenic acid prepared in their laboratories was inactive when tested by passive cutaneous anaphylaxis in monkeys sensitized with the sera of individuals allergic to green coffee bean. Moreover, it was suggested that the immunological activity of chlorogenic acid described by us was attributable to trace amounts of a protein impurity which had not been removed during its isolation from plant sources. In their criticism, however, Layton *et al.*<sup>6</sup> omitted reference to the fact that in an earlier publication<sup>4</sup> from these laboratories it was stated that synthetic chlorogenic acid (prepared by one of us, L. M. S.<sup>7</sup>) was found to be highly active on both direct and passive transfer skin testing in man. The purpose of the series of experiments recorded here was to prepare chlorogenic acid-protein conjugates, to investigate their antigenic and allergenic properties, and to provide additional evidence for the immunological capacity of chlorogenic acid.

Two types of hapten-protein conjugates were synthesized. In the first procedure, chlorogenic acid was cross-linked by formaldehyde to the amino-groups of human serum albumin (HSA) by the Mannich reaction (Fig. 2) using the conditions recommended by Fraenkel-Conrat<sup>8</sup>. About 15-18 molecules of chlorogenic acid were coupled per molecule of HSA in this reaction. The conjugates prepared by the Mannich reaction will be referred to as conjugates I for the remainder of this article.

In the second procedure an attempt was made to reproduce the biological conditions for the conjugation, as they might occur in living tissues. Although no precise information regarding the metabolic fate of chlorogenic acid is yet available, a hypothetical mechanism for the

*in vivo* conjugation of an *o*-diphenol such as chlorogenic acid can be postulated. Phenol oxidases which are widely distributed throughout mammalian tissues catalyze the dehydrogenation of *o*-diphenols to *o*-quinones, which in turn have been shown to combine readily with the free amino-groups of proteins<sup>9</sup> according to the scheme outlined in Fig. 3. Therefore, the second type of chlorogenic acid-protein conjugates was prepared by the reaction of chlorogenic acid with HSA in the presence of phenol oxidase for 2-5 h at pH 6-2; these conjugates will be referred to as conjugates II. It was deduced, by measuring the decrease of free amino-groups of HSA, that as many as 40 hapten molecules were coupled per molecule of HSA.

The chlorogenic acid used to prepare these conjugates was either a synthetic sample<sup>7</sup> or a commercial sample of plant origin supplied by the Aldrich Chemical Co., Milwaukee, Wisconsin. The latter sample of natural chlorogenic acid was recrystallized three times from water in our laboratories, and was found to be free of detectable nitrogen on microanalysis performed by the Schwartzkopf Laboratory, Woodside, New York. Both types of conjugates were freed of any unreacted chlorogenic acid by extensive dialysis and column chromatography on 'Sephadex G-25'. Phenol oxidase (mushroom tyrosinase) was purchased from the Nutritional Biochemical Corporation, Cleveland, Ohio.

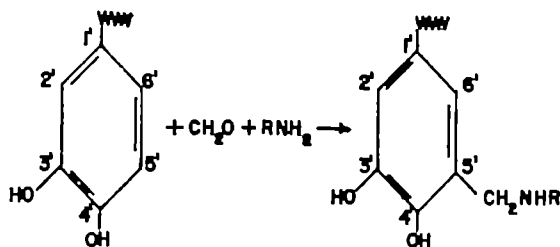


Fig. 2. Coupling of chlorogenic acid with formaldehyde

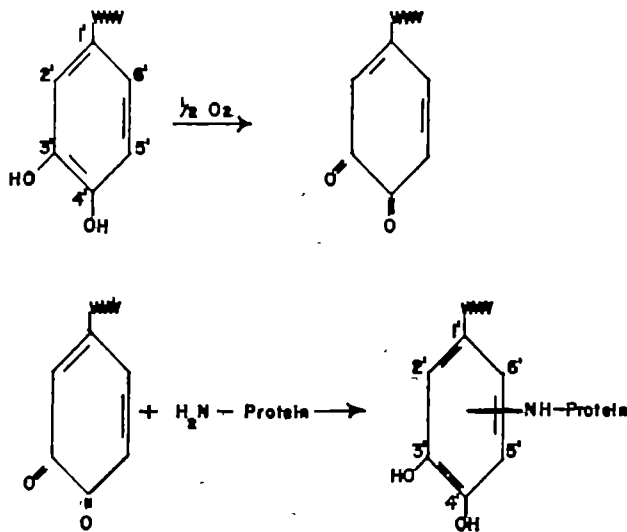


Fig. 3. Coupling of chlorogenic acid to proteins in presence of phenol oxidase

Rabbit antisera were prepared against both types of conjugates, as well as against a solution of free chlorogenic acid and an aqueous extract of green coffee bean. Human sera were obtained from coffee workers with respiratory allergy to green coffee dust. The antisera were produced against the two types of chlorogenic acid-HSA conjugates were absorbed with HSA prior to their use in immunochemical experiments.

The results of immunodiffusion experiments in agar gel are illustrated in Figs. 4-6. It will be noted that conjugate I gave a single precipitin band with the rabbit antiserum to conjugate I, with the antiserum to free chlorogenic acid or with the antiserum to green coffee (Fig. 4). Furthermore, the three precipitin bands coalesced, indicating a reaction of identity for the three antigen-antibody systems. It would thus appear that chlorogenic acid is an important antigenic constituent of the green coffee bean. The immunodiffusion patterns illustrated in Fig. 5 demonstrate complete cross-reactivity between conjugates I and II when tested against their corresponding antisera; this finding provides further evidence that the antigenic determinant groups are identical for both types of conjugate. From the patterns illustrated in Fig. 6, it is evident that multiple precipitin bands were formed when green coffee extract was tested against antiserum to green coffee. It is also apparent that one of the constituents of green coffee has an antigenic determinant group in common with conjugates I and II, as well as with free chlorogenic acid. Moreover, all the precipitin bands shown in Figs. 4-6 were specifically inhibited with both synthetic and nitrogen-free natural chlorogenic acid, except for some

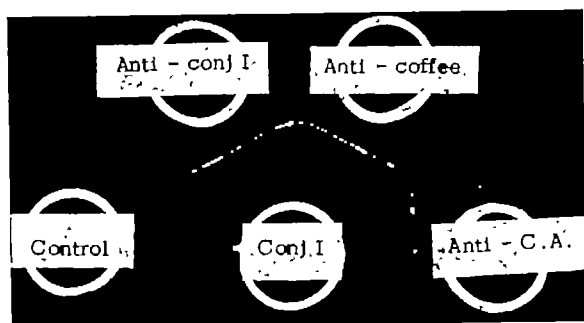


Fig. 4. The reaction of identity among rabbit antibodies to chlorogenic acid (Anti-C.A.), to the extract of green coffee bean (Anti-coffee) and to conjugate I (Anti-conj I), when tested with conjugate I (Conj I). Normal rabbit serum was placed in the well marked as 'control'.

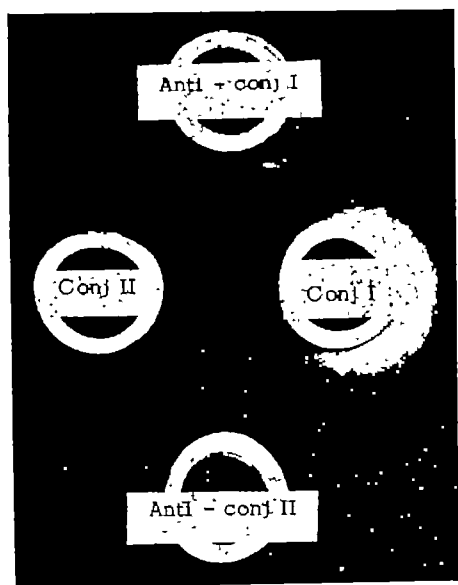


Fig. 5. Antigenic cross-reaction between conjugates I and II when tested with the corresponding rabbit antisera.

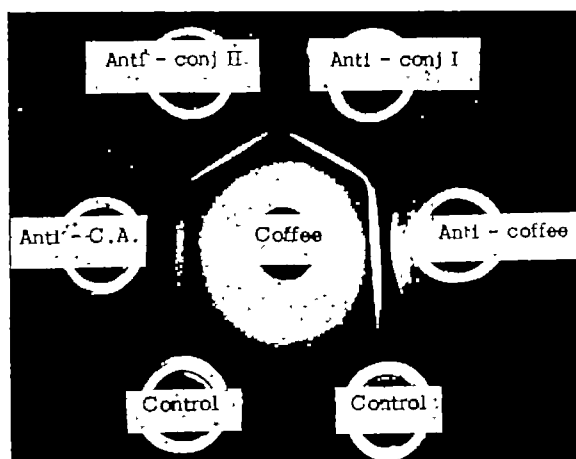


Fig. 6. Precipitin patterns obtained in agar gel between extract of green coffee bean and antisera to chlorogenic acid (Anti-C.A.), to conjugates I and II (Anti-conj. I and Anti-conj. II) and to the extract of green coffee bean (Anti-coffee). Normal rabbit serum was used as 'control'.

extra bands representing the multiple green coffee extract-antibody system (Fig. 6). The extra bands observed in this system may well be due to the protein antigens which elicited the PCA reactions in monkeys in the experiments of Layton *et al.*<sup>11</sup>.

Both types of conjugates were antigenically active when tested against rabbit and human antisera by the BDB haemagglutination techniques. Titres ranging from 8,000 to 1,000,000 were obtained with rabbit antisera to both types of conjugates, or to free chlorogenic acid, or to green coffee; titres ranging from 80 to 640 were obtained with human sera from individuals allergic to green coffee. All the haemagglutination reactions could be specifically inhibited with either of the two conjugates.

Conjugates I and II, as well as two newly prepared samples of synthetic chlorogenic acid, produced wheal and erythema reactions on direct intradermal testing in patients allergic to green coffee bean and on passive transfer testing in normal volunteers sensitized with the sera of these patients.

It has been expected in the present series of experiments that *in vitro* (Fig. 2) and *in vivo* (Fig. 3) conjugation of chlorogenic acid with HSA occurs by coupling at the C'5 position on the chlorogenic acid molecule, as demonstrated for similar catechols<sup>12</sup>. To substantiate this assumption and in an attempt to block the reactive position in chlorogenic acid, involved in the coupling with proteins, chlorogenic acid was reacted with diethylamine by the Mannich reaction (Fig. 7). Indeed, the resulting mono-substituted chlorogenic acid derivative, the composition of which was confirmed by elemental microanalysis, failed to elicit wheal and erythema reactions when tested by direct or passive transfer skin testing in humans at concentrations of 10-500 µg/ml. On the other hand, synthetic chlorogenic acid was highly skin active at these concentrations. It may thus be inferred that diethylamine reacted with chlorogenic acid at the same site at which protein conjugation occurs, and that blocking of this site with a monomeric group results in the loss of the ability of

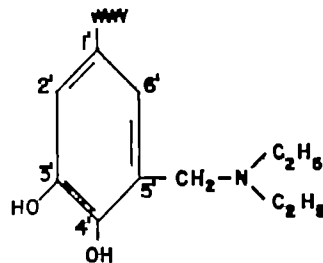


Fig. 7. The product of the coupling reaction of chlorogenic acid with diethylamine by formaldehyde.

chlorogenic acid to become incorporated into a polyhaptenic skin-active protein conjugate.

It might be argued that the purified commercial samples of chlorogenic acid used for the preparation of conjugates or for the immunization of rabbits with free chlorogenic acid might have still contained some 'active' impurities. However, it does not seem likely that this contaminant would have been selectively linked to HSA in the Mannic reaction, or in the reaction catalysed by phenol oxidase, and that antibodies could have been formed exclusively against it. Furthermore, it is inconceivable that antibodies to the hypothetical contaminant would have combined with the synthetic chlorogenic acid leading to the inhibition of the precipitin bands in agar gel.

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Health and Welfare, Ottawa, the National Institute of Allergy and Infectious Diseases, U.S. National Institutes of Health, and the Life Insurance Medical Research Fund.

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## EVOLUTIONARY SIMILARITIES BETWEEN PANCREATIC PROTEOLYTIC ENZYMES

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IT is now generally agreed that the amino-acid sequence of a protein is determined by the nucleotide sequence of its parent gene, so that comparison of the sequences of related proteins can provide clues about their genetic origins. If we compare similar proteins from different species we may hope to trace the evolutionary relationship of the species, since viable mutations which become established in a population will have evolutionary stability, and can act as markers to identify familial resemblances. Comparison of species differences in insulin, haemoglobin and cytochrome *C* have laid the foundation for such studies. However, an alternative approach is to compare different proteins from the same species or individual. Considerable homologies between the amino-acid sequences of these different proteins might indicate a common evolutionary ancestor. For example, certain mammalian peptidases and esterases are distinguished by their capacity to react with diisopropyl phosphorofluoridate (DFP) at a unique serine residue in the active centre. The amino-acid sequence immediately surrounding this active serine residue is remarkably similar in all these enzymes. One hypothesis is that these proteins may have arisen by multiplication of some common ancestral gene, followed by independent mutations to allow divergence of sequence and ultimately of enzymatic specificity. Homologies in their amino-acid sequences would therefore reflect the vestiges of their common ancestor. Alternatively, the similarities in active centre sequence of these serine enzymes might merely represent structures essential for the catalytic activity. In this case they could arise by convergent evolution from genes of different origin. Only if the homologies of sequence were found to be very extensive could this hypothesis be eliminated.

**Sequence homologies in trypsin and chymotrypsin A.** Recent determinations of the complete amino-acid sequence of bovine chymotrypsinogen A (ref. 2) and almost all the sequence of trypsinogen<sup>3</sup> have lent support to the hypothesis of a common evolutionary ancestor. It is instructive to observe that comparison of a few peptides from partial acid hydrolysates of these proteins had originally indicated considerable homology<sup>4</sup>, but further investigation tended to minimize the significance of this<sup>5,6</sup>.

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Only when the complete sequences are compared can the true homology be seen<sup>7</sup>, as shown in Table 1, where the structures are so written as to maximize similarities. (A correction (Hartley, B. S., unpublished results) to the sequence of chymotrypsinogen A (ref. 2) is included in Table 1. Positions 212–216 in this sequence<sup>8</sup> should read Ile.Val.Ser.Try and not Ile.Val.Ser.Ser.Try, so that in effect Ser-215 is deleted and the protein contains only 245 residues.) One hundred residues are identical in trypsinogen and chymotrypsinogen A, equivalent to 40 per cent of the sequence, and the homologies occur in blocks of sequence throughout the peptide chains.

One can extend the comparison to include amino-acid side-chains which are chemically similar and may therefore reflect common features in the tertiary structure: for example, lysine and arginine; aspartic and glutamic acid; asparagine and glutamine; serine and threonine; phenylalanine, tyrosine and tryptophan; leucine, isoleucine and valine. On this basis 127 residues are similar or identical, which is 51 per cent of the sequence. This degree of homology is very close to what we find between the  $\alpha$ - and  $\beta$ -chains of human haemoglobin, where 44 per cent of the residues are identical or 53 per cent similar when compared on the foregoing criteria. Yet the haemoglobin chains are remarkably similar both in activity and in tertiary structure. We must realize that trypsin and chymotrypsin have widely different specificities, and one would expect that the evolution of these different activities would accelerate the rate at which mutations could be established in the proteins. The homology between trypsin and chymotrypsin is therefore the more impressive, and might imply that these proteolytic enzymes lie relatively closer in the evolutionary scale to their common ancestor than do the haemoglobin chains, and that some of the homologies reflect elements in the primary structure which contribute to the configuration of the catalytic site but not the specificity site.

**Disulphide bridges of trypsin and chymotrypsin A.** This hypothesis is supported by the evidence of the five disulphide bridges of chymotrypsinogen A (refs. 8 and 9) and the six disulphide bridges of trypsinogen<sup>10</sup>. Radical differences in the order in which the half-cystine residues were linked would imply differences in the configuration of these proteins, whereas similarities might help to define common elements of tertiary structure. A 'diagonal'



paper electrophoretic technique proved very useful for identifying and isolating cystine-bridged peptides from peptic digests of chymotrypsinogen *A* and trypsinogen, and allowed us to determine the bridges shown in Fig. 1.

Four of the five bridges of chymotrypsinogen (Fig. 1, *A*, *B*, *C* and *D*) are exactly homologous in position with four bridges of trypsinogen, and the amino-acid sequence around these four bridges is very similar in both proteins. One bridge of chymotrypsinogen (Fig. 1, *E*) must obviously differ, since the amino terminal half-cystine (Cys-1) of chymotrypsinogen is deleted in trypsinogen and Cys-123 is replaced by serine. Instead, trypsinogen has a neighbouring half-cystine (Cys-129) which forms a different bridge (Fig. 1, *F*). The sixth bridge of trypsinogen (Fig. 1, *G*) is homologous neither in position nor in sequence. We see that the substantial similarities in sequence of trypsinogen and chymotrypsinogen are accompanied by substantial similarities in the disulphide bridges.

**Bovine chymotrypsinogen *B* and porcine elastase.** Are the homologues of sequence and of disulphide bridges found in other pancreatic proteinases? The 'diagonal' paper electrophoretic technique of Brown and Hartley<sup>8</sup> makes the purification of related sets of cysteine acid peptides relatively easy, and by this means we have been able to determine the amino-acid sequence around the five disulphide bridges of bovine chymotrypsinogen *B* (ref. 11) and the four disulphide bridges of porcine elastase<sup>12</sup>.

Chymotrypsinogen *A* and chymotrypsinogen *B* are found together in approximately the same amount in the pancreatic juice of a single cow<sup>13</sup>, and the two zymogens are similarly activated by trypsin to yield chymotrypsins of very similar activity and specificity<sup>14</sup>. Yet the two zymogens differ appreciably in amino-acid composition<sup>15</sup>, so that one is led to suspect gene-doubling, as with the  $\beta$ - and  $\gamma$ -chains of haemoglobin, for example. The amino-acid sequence around the five cystine bridges of chymotrypsinogen *B* is shown in Table 1. We see that, as expected, the homologous sequences are bridged identically to those of chymotrypsinogen *A* (Fig. 1). In this case, the occasional differences in amino-acid sequence are more illuminating. Since the proteins are so similar in activity, it is unlikely that amino-acid side-chains which differ appreciably in chemical character between *A* and *B* play an important part either in the activity or configuration of the catalytic site. In a sense, chymotrypsinogen *B* carries 'built-in chemical modifications' of chymotrypsinogen *A*, so that certain parts of the chain can be excluded from 'the active site'. This applies to residues 128, 136, 138, 168 and 205. Thus, although the Cys-137 to Cys-204 bridge (Table 1, *D*) lies very close to the active centre serine (residue 198), the amino-acid residues adjacent to it can be altered without affecting the activity of the enzyme. The sequence shown in Table 1 accounts for 71 of the 246 residues of bovine chymotrypsinogen *B*. Eight differences in sequence are observed, so that by extrapolation we may expect about 10-15 per cent of the total residues to differ from chymotrypsinogen *A*. Analogy with the evolution of haemoglobins<sup>16</sup> would lead us to conclude that the two bovine chymotrypsinogens lie closer to their common evolutionary ancestor than the  $\beta$ - and  $\alpha$ -chains of human haemoglobin (27 per cent differences) but further than the  $\beta$ - and  $\delta$ -chains (6 per cent).

Porcine elastase is another pancreatic proteolytic enzyme with a different substrate specificity from either trypsin or chymotrypsin<sup>17</sup>. However, it too reacts at a unique serine residue with diisopropyl phosphorofluoridate, and the amino-acid sequence immediately adjacent to this reactive serine is the same as that in trypsin and chymotrypsin<sup>18</sup>. The 'diagonal' disulphide bridge technique has already allowed us to conclude that it has a cystine-bridged histidine sequence homologous to that of trypsin and chymotrypsin, which appears to be a second component of the active centre<sup>18</sup>. We have purified this enzyme, and find that it has four disulphide bridges,

Table 1. AMINO-ACID SEQUENCES OF PORCINE ELASTASE (*E*) AND BOVINE TRYPSINOGEN (*T*), CHYMOTRYPSINOGEN *A* (*CA*) AND CHYMOTRYPSINOGEN *B* (*CB*)<sup>\*</sup>

\* Residues which are identical in any two of elastase, trypsinogen or chymotrypsinogen are underlined. Chemically similar residues are in normal type. Differences are shown in italics. In chymotrypsinogen *B*, residues which differ from chymotrypsinogen *A* are shown in bold type. Disulphide bridges are lettered *A-G* as in Fig. 1. Asx indicates aspartic acid or asparagine and Glx stands for glutamic acid or glutamine. The 'overlap' between residues 188 and 189 of elastase is uncertain.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
<i>E</i> :															
<i>T</i> :															
<i>CA</i> :	CYS	Gly	Val	Pro	Ala	Ile	Gln	Pro	Val	Leu	Ser	Gly	Leu	Ser	Arg
<i>CB</i> :	CYS	Gly	Val	Pro	Ala	Ile	Gln								
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
<i>E</i> :															
<i>T</i> :	Leu	Val	Gly	Gly	Tyr	Thr	CYS	Gly	Ala	Asn	Thr	Val	Pro	Tyr	Gln
<i>CA</i> :	Leu	Val	Gly	Asp	Gln	Gln	Ala	Val	Pro	Gly	Ser	Trp	Pro	Trp	Gln
<i>CB</i> :	Leu	Val	Gly	(Asx, Asx, Gln)											
	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
<i>E</i> :															
<i>T</i> :	Ser	Leu	Asn	...	...	...	Ser	Gly	Tyr	His	Pro	CYS	Gly	Gly	Ser
<i>CA</i> :	Ser	Leu	Gln	Asp	Leu	Thr	Gly	Pro	His	Pro	CYS	Gly	Gly	Ser	Leu
<i>CB</i> :	Ser	Leu	Gln	Asp	Leu	Thr	Gly	Pro	His	Pro	CYS	Gly	Gly	Ser	Leu
	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
<i>E</i> :															
<i>T</i> :	Asn	Ser	Gln	Trp	Val	Val	Ser	Ala	Ala	His	Pro	CYS	Gly	Val	Thr
<i>CA</i> :	Asn	Gln	Asn	Trp	Val	Val	Thr	Ala	Ala	His	Pro	CYS	Gly	Val	Thr
<i>CB</i> :	Asn	Gln	Asn	Trp	Val	Val	Thr	Ala	Ala	His	Pro	CYS	Gly	Val	Thr
	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
<i>E</i> :															
<i>T</i> :	Gln	Val	Arg	Leu	...	...	Gly	Gln	...	Asp	Asn	Ile	Asn	Val	Val
<i>CA</i> :	Asp	Val	Val	Val	Ala	Gly	Gln	Pro	Asp	Gln	Gly	Ser	Ser	Ser	Gln
<i>CB</i> :	Asp	Val	Val	Val	Ala	Gly	Gln	Pro	Asp	Gln	Gly	Ser	Ser	Ser	Gln
	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
<i>E</i> :															
<i>T</i> :	Asn	Gln	Gln	Pro	Leu	Ser	Ala	Ser	Leu	Ser	Ile	Val	His	Pro	Ser
<i>CA</i> :	...	Ile	Gln	Leu	Leu	Leu	Ile	Ala	Ile	Val	Pro	Leu	Leu	Leu	Leu
<i>CB</i> :	...	Ile	Gln	Leu	Leu	Leu	Ile	Ala	Ile	Val	Pro	Leu	Leu	Leu	Leu
	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105
<i>E</i> :															
<i>T</i> :	Tyr	Asn	Pro	Leu	Thr	Asn	Asn	Asp	Ile	Met	Leu	Leu	Leu	Leu	Leu
<i>CA</i> :	Tyr	Asn	Pro	Leu	Thr	Asn	Asn	Asp	Ile	Met	Leu	Leu	Leu	Leu	Leu
<i>CB</i> :	Tyr	Asn	Pro	Leu	Thr	Asn	Asn	Asp	Ile	Met	Leu	Leu	Leu	Leu	Leu
	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
<i>E</i> :															
<i>T</i> :	Ser	Ala	Ala	Ser	Leu	Asn	Ser	Arg	Val	Ala	Ser	Ile	Ser	Leu	Pro
<i>CA</i> :	Ser	Ala	Ala	Ser	Leu	Asn	Ser	Arg	Val	Ala	Ser	Ile	Ser	Leu	Pro
<i>CB</i> :	Ser	Ala	Ala	Ser	Leu	Asn	Ser	Arg	Val	Ala	Ser	Ile	Ser	Leu	Pro
	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135
<i>E</i> :															
<i>T</i> :	...	Ser	CYS	Ala	Ser	...	...	Ala	Gly	Gln	Thr	CYS	Leu	Ile	Ser
<i>CA</i> :	...	Ser	CYS	Ala	Ser	...	...	Ala	Gly	Gln	Thr	CYS	Leu	Ile	Ser
<i>CB</i> :	...	Ser	CYS	Ala	Ser	...	...	Ala	Gly	Gln	Thr	CYS	Leu	Ile	Ser
	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150
<i>E</i> :															
<i>T</i> :	Gly	Asn	Thr	Leu	Ser	Ser	Gly	Thr	Ser	Tyr	Pro	Asp	Val	Leu	Leu
<i>CA</i> :	Gly	Leu	Thr	Arg	Tyr	Thr	Asn	Ala	Asn	Thr	Pro	Asp	Arg	Leu	Gln
<i>CB</i> :	Gly	Leu	Thr	Arg	Tyr	Thr	Asn	Ala	Asn	Thr	Pro	Asp	Arg	Leu	Gln
	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165
<i>E</i> :															
<i>T</i> :	Leu	Leu	Ala	Pro	Leu	Leu	Ser	Asp	Ser	CYS	Leu	Ser	Ser	Ser	Ser
<i>CA</i> :	Leu	Leu	Ala	Pro	Leu	Leu	Ser	Asp	Ser	CYS	Leu	Ser	Ser	Ser	Ser
<i>CB</i> :	Leu	Leu	Ala	Pro	Leu	Leu	Ser	Asp	Ser	CYS	Leu	Ser	Ser	Ser	Ser
	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180
<i>E</i> :															
<i>T</i> :	Met	Val	CYS	Ala	Gly	...	...	Gly	Asp	Gly	Val	...	...	...	...
<i>CA</i> :	Met	Val	CYS	Ala	Gly	...	...	Gly	Asp	Gly	Val	...	...	...	...
<i>CB</i> :	Met	Val	CYS	Ala	Gly	...	...	Gly	Asp	Gly	Val	...	...	...	...
	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195
<i>E</i> :															
<i>T</i> :	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
<i>CA</i> :	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
<i>CB</i> :	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210
<i>E</i> :															
<i>T</i> :	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
<i>CA</i> :	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
<i>CB</i> :	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225
<i>E</i> :															
<i>T</i> :	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
<i>CA</i> :	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
<i>CB</i> :	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240
<i>E</i> :															
<i>T</i> :	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
<i>CA</i> :	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
<i>CB</i> :	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255
<i>E</i> :															
<i>T</i> :	Val	Ser	Trp	Ile	Leu	Gln	Thr	Ile	Ala	Ser	Asn	...	...	...	...
<i>CA</i> :	Val	Ser	Trp	Ile	Leu	Gln	Thr	Ile	Ala	Ser	Asn	...	...	...	...
<i>CB</i> :	Val	Ser	Trp	Ile	Leu	Gln	Thr	Ile	Ala	Ser	Asn	...	...	...	...

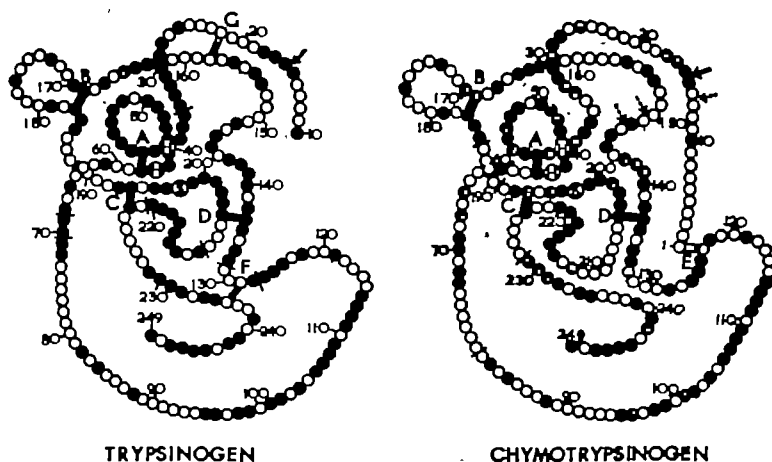


Fig. 1. Disulphide bridges in bovine trypsinogen and chymotrypsinogen A. The shaded circles indicate amino-acid residues which are chemically similar or identical in the two proteins. The disulphide bridges are lettered A-F as in Table 1. H indicates the two histidine residues and S the serine residue of the active centre. The point of activation by trypsin is shown by a full arrow and broken arrows indicate additional chymotryptic splits which form  $\alpha$ -chymotrypsin. The numbering of residues is as Table 1, and deletions are indicated by lines between the circles.

surrounded by the amino-acid sequences shown in Table 1. Six of the half-cystine sequences (around residues 42, 58, 183, 194, 204 and 223) are clearly homologous with trypsin and chymotrypsin, but the sequences around Cys-137 and Cys-169 show little or no homology. Nevertheless, the similarities in sequence are sufficiently striking to suggest that the four disulphide bridges of porcine elastase are homologous with the four bridges which are common to bovine trypsin, chymotrypsin A and chymotrypsin B.

**Comparison of the four sequences.** In order to maximize homologies, a minimum number of arbitrary deletions have been introduced into the sequences shown in Table 1, so that the numbering corresponds to that of some hypothetical evolutionary precursor. Starting at the amino-terminus, we see that the first homology occurs at residues 15-18, the site of tryptic activation of each of the zymogens. Labouesse *et al.*<sup>15</sup> have concluded that the ionization of the amino-terminal Ile-16 affects the catalytic activity of chymotrypsin, so this residue may contribute to the conformation of the active site. The next notable block of similarities occurs between residues 28 and 58, which includes the 'histidine loop' of all the proteins. It is interesting that, although His-57 is present in the active centre of chymotrypsin, the homologies cease abruptly at Cys-58. The stretch of sequence 74-94 contains fewest homologies, but it is relevant to note that in chymotrypsin this sequence contains six lysine residues, regularly spaced, and all the lysine residues of chymotrypsin can be chemically modified without affecting the enzyme activity<sup>16</sup>. Another impressive block of homologies occurs between Tyr-95 and Ser-114, followed by a group of five residues surrounding the Cys 1-123 bridge of chymotrypsin (Fig. 1, E). As previously mentioned, the amino-terminal cysteine-1 is 'deleted' in trypsinogen and we find that serine replaces half-cystine at position 123 in an otherwise homologous sequence. However, a new half-cystine appears close by in trypsin at position 129 which forms a quite different disulphide bridge with Cys-236 near the carboxyl terminus (Fig. 1, F). Thus in the short section of sequence 123-129 trypsin and chymotrypsin form quite different disulphide bridges while elastase has no bridge at all.

The homologous sequence 132-146 includes one of the 'serine knot' bridges (Fig. 1, bridge D) which surround the active centre Ser-198, and is found in all these enzymes. Then follows a stretch (147-180) in which homologies are intermittent but relatively rare. It is in this stretch that the sixth disulphide bridge of trypsin occurs (Fig. 1, bridge G), having no analogue in elastase or chymotrypsin,

and we are at liberty to postulate differences in tertiary structure in this region. However, Cys-169 is part of bridge B which forms a 'methionine loop' in all the enzymes.

The section from Met-181 to Cys-204 includes the active centre serine sequence. Now that we have an extended sequence from porcine elastase we can see that the homologies are even more impressive than could formerly be concluded. Methionine is a relatively rare amino-acid, and since it occurs at position 181 in homologous sequences in all these enzymes, we may suspect that it plays some part in the common catalytic site. We also see homologies between residues 188-193 which are not otherwise apparent. The point is neatly illustrated by observing that Gln-195 is a feature of both elastase and trypsin, and Leu-202 of elastase and chymotrypsin, while Val-203 is common to trypsin and chymotrypsin. These minor differences may be of special evolutionary and possibly enzymatic interest because they occur so

close to the active centre Ser-198; for example, alkylation of Met-195 causes a decreased binding of chymotrypsin substrates without affecting the turn-over rate<sup>17</sup>. For this reason, we might be tempted to look carefully at residues on the other side of the Cys-194 and Cys-204 bridges which form the 'serine knot'. Homologies around Cys-223 and Cys-137 are less exact, and are therefore less likely to form part of the common catalytic site. We also observe that the homologies cease abruptly at Cys-204, and even chymotrypsinogen A and B differ at position 205. We therefore conclude that the 'serine knot' sequence from Met-181 to Cys-204 together with the 'histidine loop' from Pro-28 to Cys-58 are probably components of a common catalytic site in these enzymes.

Regular blocks of similar sequences extend from here on to the end of the chains. The results so far obtained with porcine elastase allow us to say that of the 77 residues of sequence shown in Table 1, 36 (47 per cent) are identical to comparable residues in bovine trypsin or chymotrypsin, or a total of 43 (56 per cent) show chemical similarity. These homologies of sequence and of disulphide bridging between three pancreatic proteolytic enzymes of widely different specificity point both to a common evolutionary ancestor and to common configurations in their catalytic sites.

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## IMPORTANCE OF THE CARBOXYL END OF BRADYKININ AND OTHER PEPTIDES

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THE biological activity of bradykinin as an excitant of smooth muscles can be modified by many kinds of structural alterations in the molecule. The usual finding has been that exchange of one of the amino-acid residues of the hormone for some other reduces but does not destroy the biological action. Thus, exchange of an alanine residue for each residue in turn of the hormone has yielded a series of peptides<sup>1</sup> which still show bradykinin-like actions on smooth muscles, although the potency of all but one of the peptides so obtained is somewhat (and in some cases very considerably) reduced in comparison to bradykinin itself. Many other kinds of structural alteration of the molecule have been examined<sup>1,2</sup>. These investigations show that no one amino-acid residue is essential for biological activity. Indeed, replacements such as alanine for proline in position 3 or glycine for serine in position 6 can be made without changing the biological activity on smooth muscles.

A further feature of the structural specificity of bradykinin has also emerged from these studies. This is that additions can be made to the amino end of the molecule without much loss of biological potency on smooth muscles. Thus, lysylbradykinin (kallidin) and methionyl-lysylbradykinin<sup>1</sup> have almost as much potency as does bradykinin itself, and glycylbradykinin is even more active<sup>3</sup>. Similarly, in our own investigations, acetylbradykinin has shown good activity. It would thus appear that lengthening the molecule at the amino end has only a small effect on potency, at least if the lengthening is not greater than one or two residues.

In contrast to this has been the marked effect of lengthening from the carboxyl end, which is the subject of this article. When just a single amino-acid residue was added to the carboxyl end of bradykinin the potency was markedly reduced.

Although this finding might be of only minor interest if it were restricted to bradykinin alone, it takes on significance when one contemplates the number of biologically active peptides for which the same general phenomenon seems to hold. One begins to ask whether some general principle of importance in understanding the mechanism of action of these important compounds may not reside here. In the cases of angiotensin, gastrin, eledoisin, oxytocin, and even of proteins such as pancreatic ribonuclease, one sees clearly that, just as with bradykinin, the carboxyl end cannot be modified at all, although changes can be made at the amino end (and inside the peptide chain) without serious damage to the biological potency. However, at the outset, an exception must be noted, that is, the case of melanophore-stimulating hormone, for which it is well known<sup>4</sup> that amino-acid residues can be added to the carboxyl end without marked loss of potency. This is clearly shown by the MSH activity of ACTH.

Let us consider, first, the case of bradykinin. The biological activities of the derivatives mentioned at the start of this article have been reviewed elsewhere and tabulated<sup>1</sup> so that the reliability of the generalization made in the first paragraph can be easily verified. The new derivatives of bradykinin, in which amino-acid residues were added at the carboxyl end of the peptide chain, were synthesized by the solid-phase method developed in this laboratory by Merrifield<sup>5</sup>. The tertiary-butyloxycarbonyl (*t*-BOC) derivative of the amino-acid

desired at the carboxyl end of the peptide was esterified to a chloromethylated polystyrene resin. The *t*-BOC protecting group was then removed, as described by Stewart and Woolley<sup>6</sup>, by treatment of the resin with 4 M hydrochloric acid in dry dioxane, and the next amino-acid residue was added to the peptide by means of the dicyclohexylcarbodiimide condensation. The solvent used for this condensation reaction was methylene chloride in all cases except when the *t*-BOC amino-acid residue being added was *t*-BOC-nitroarginine. Because of the low solubility of this amino-acid derivative, the solvent used for that condensation reaction was dimethylformamide. The use of methylene chloride allowed achievement of quantitative yield at each coupling stage with only 2.5 equivalents of each entering *t*-BOC-amino-acid. It was thus to be preferred to the use of dimethylformamide as described by Merrifield in the original synthesis of bradykinin by the solid-phase method.

The completed peptide was liberated from the resin by dry hydrogen bromide in trifluoroacetic acid, and the dinitropeptide so obtained was hydrogenated with the aid of a palladium catalyst supported on barium sulphate. The free peptide was then purified by countercurrent distribution in the solvent system of *l*-butanol and aqueous trifluoroacetic acid.

Each new peptide was obtained as a pure compound which showed a symmetrical peak in countercurrent distribution, a single spot with the expected rate of migration in paper electrophoresis and the expected ratios of amino-acids when hydrolysed and analysed quantitatively according to the method of Moore, Spackman and Stein<sup>7</sup>. The analytical results are summarized in Table 1. The bradykinin-like potency of each peptide was measured with the aid of isolated uterine horns of oestrogenized rats as previously described<sup>8</sup>.

Table 1. NEW ANALOGUES OF BRADYKININ

Compound	Amino-acid ratios found*					
	Gly	Ser	Pro	Phe	Arg	$\Sigma$ †
Bradykinyl glycine	2.00	0.81	2.90	1.96	2.17	1.06
Bradykinyl-L-arginine	1.00	1.08	2.81	1.85	2.00	0.85
Acetylbradykinin	1.00	0.95	3.06	2.14	1.97	3.16

\* Acid hydrolysates were analysed by chromatography (ref. 7).

† Partition coefficient in the countercurrent distribution system *l*-butanol: water: trifluoroacetic acid (60:50:1).

The potencies of several derivatives of bradykinin are shown in Table 2. The derivatives with extra amino-acids at the amino end, namely, lysylbradykinin, glycylbradykinin and methionyllysylbradykinin, were previously known compounds. Acetylbradykinin was synthesized during the course of the present investigation.

Table 2. EFFECT OF ADDED RESIDUES ON BRADYKININ POTENCY

Compound	Activity	Ref.
Bradykinin: Arg-Pro-Gly-Phe-Ser-Pro-Phe-Arg	1	1
Lys-Arg-Pro-Gly-Phe-Ser-Pro-Phe-Arg	1/2 <sup>a</sup>	1
Met-Lys-Arg-Pro-Gly-Phe-Ser-Pro-Phe-Arg	1/3	9
Gly-Arg-Pro-Gly-Phe-Ser-Pro-Phe-Arg	3/2	1
Acetyl-Arg-Pro-Gly-Phe-Ser-Pro-Phe-Arg	1/3	—
Arg-Pro-Gly-Phe-Ser-Pro-Phe-Arg-Gly	1/150	—
Arg-Pro-Gly-Phe-Ser-Pro-Phe-Arg-Arg	1/25	—

The results in Table 2 show that although additions of amino-acid (or acetyl) residues to the amino end of the molecule did not greatly reduce potency, the addition of a single amino-acid residue to the carboxyl end had a marked effect on the potency. An arginine at the carb-

oxyl end did not reduce the activity as much as did an extra glycine, but the effect even of an arginine was marked.

Let us now consider in this light the case of angiotensin. This was perhaps the first case to be seen clearly. Page and Bumpus<sup>18</sup> had observed several years ago that a derivative of angiotensin which was inactive on rat uterus could be found in crude concentrates of angiotensin. This material, angiotensin I, became active when digested with a peptidase which converted it to angiotensin II by removal of a dipeptide from the carboxyl end of angiotensin I. It was thus clear that although angiotensin II was active in causing contraction of the muscle, angiotensin I, which differed from it only in having two amino-acids attached to the carboxyl end, was inactive. Replacement of the C-terminal phenylalanine of angiotensin II by alanine gave an inactive compound. The importance of the structure at the carboxyl end of angiotensin was thus clear. It was also necessary to have it unsubstituted in order to find activity on smooth muscle.

In contrast to this state of affairs, Arakawa *et al.*<sup>11</sup> showed that the amino end of angiotensin need not be free or unaltered. Several residues of *O*-acetyl serine could be added to this end without great loss of potency. Furthermore, angiotensin could be shortened from the amino end by removal of two of the terminal residues without destruction of the biological activity. All these observations showed quite clearly the need for an unnumbered carboxyl end, and the lesser importance of the amino end.

The importance of the carboxyl end of oxytocin has been known for several years. du Vigneaud *et al.*<sup>19</sup> had shown that when the glycine amide portion of the peptide was changed to glycine, and all other parts of the molecule were left undisturbed, all activity was lost. Removal of the entire glycine amide residue or of additional residues from the carboxyl end gave peptides with almost no activity<sup>18</sup>. No derivatives of oxytocin have been made in which the molecule has been lengthened at the carboxyl end, but in the light of present findings with bradykinin, such a manoeuvre would be interesting. Existing results, however, suggest that the carboxyl end must not be modified if high activity is to be retained, not even to the extent of replacing  $-\text{NH}_2$  with  $-\text{OH}$ .

Very recent findings indicate similar results for the two biologically important peptides gastrin<sup>14</sup> and eledoisin<sup>15,16</sup>. The case of gastrin is particularly striking. This heptadecapeptide amide can be shortened from the amino end down to a tetrapeptide amide without marked loss of hormonal activity, but if the carboxyl end is merely changed from the amide to the ester the activity is lost. It will be of great interest to determine whether lengthening the carboxyl end by addition of an amino-acid residue (or more particularly, by addition of an amino-acid amide residue) will also result in inactivation.

The situation with eledoisin is quite similar to that of gastrin. The natural peptide, which is an undecapeptide amide, can be shortened from the amino end down to a hexapeptide amide without great loss of potency, but the intact structure of the carboxyl end seemed to be necessary for biological activity. Exchange of the methionine amide at the carboxyl end of the peptide for the corresponding methionine dimethyl amide destroyed activity, although drastic exchanges of residues could be made in the region of the molecule toward the amino end without significant effect. How little was the importance of electrostatic charge for biological activity could be seen from some of these analogues in which amino-acids with extra ionized groups were replaced by ones with no ionic charge. Thus, the elimination of the lysine residue in position 4, the aspartic acid residue in position 5, or both, caused no serious loss of potency even though great changes had thus been made in the electrostatic charge of the molecule.

Even among the proteins one begins to find evidence of the prime importance of the carboxyl end of the peptide chain. Consider, for example, the case of pancreatic ribonuclease. Potts *et al.*<sup>17</sup> have indicated that the removal of four residues or even of a single residue from the carboxyl end of the enzyme reduced or eliminated its activity. In contrast, the removal of the lysine residue from the amino end of the enzyme and even the addition of polyalanyl groups to all the amino groups<sup>18</sup> did not seem to destroy activity. The results of Finn and Hofmann<sup>18</sup> with the *S*-protein and synthetic portions of the *S*-peptide show that 7 amino-acid residues can be removed from the interior of ribonuclease without complete loss of enzymatic activity.

Additional confirmatory evidence is seen in the cases of insulin and aldolase. When a single amino-acid (asparagine) is removed from the C-terminus of insulin along with the alanine from the C-terminus of the other peptide chain of the protein, the hormonal activity is greatly diminished<sup>20,21</sup>. In the case of aldolase, the ability to bind substrate prior to enzymatic hydrolysis requires the presence of the C-terminal tyrosine residue<sup>22</sup>.

As mentioned near the beginning of this article, MSH and its relative ACTH constitute exceptions to the idea under discussion. Although exceptions are a cause for concern, the considerable number of cases which seem to show the importance of the carboxyl end of peptides has caused us to consider the situation worthy of attention. In connexion with these exceptions, the case of the *S*-peptide of ribonuclease may be noteworthy. Seven amino-acid residues can be removed from the carboxyl end of this peptide before its activity in being able to reconstitute the active enzyme is completely lost. By contrast, as we have just seen, not even a single amino-acid can be removed from the carboxyl end of the entire enzyme without great loss of activity. If MSH were to function biologically (as Hofmann has suggested) by combination with a large 'incomplete' enzyme, just as does the *S*-peptide of ribonuclease, the failure of MSH to conform to the generalization explored in this article might become more understandable.

As yet there seems to be no adequate reason to explain why the carboxyl end of peptides should be of great importance. Woolley *et al.*<sup>23</sup> have suggested that in chymotrypsin the centre which specifically attracts the substrate to the catalytically active site of the enzyme (the so-called specificity centre) is the tyrosine residue at the carboxyl end of one of the peptide chains, but direct proof of this view is lacking.

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## MYXOMATOSIS AND THE RABBIT FLEA

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IN a recent article in *Nature*<sup>1</sup>, P. J. Chapple and N. D. Lewis show that the myxoma virus can survive on the rabbit flea (*Spilopsyllus cuniculi* (Dale)) kept underground without a host for 105 days and still be capable of infecting a rabbit with the disease. This had been assumed without any definite evidence since 1956, when Shanks, Allan and Brown<sup>2</sup> demonstrated that 50 days after the last rabbit in a flea-infested warren had died, other rabbits became infected with myxomatosis when introduced into the warren. It was also known<sup>3</sup> that the myxoma virus remained infective for 220 days on the mouth-parts of mosquitoes which had fed on myxomatous hosts. Furthermore, Allan<sup>4</sup> recorded that unfed rabbit fleas can survive 9 months in a refrigerator at approximately 4°C—an observation corroborated by me<sup>5</sup> for the fed flea—and therefore it was evident that a cold winter passed without the presence of the host would be unlikely to kill off all 'free' fleas in burrows. It is also known that the rabbit flea may survive at least a year on the captive host<sup>4,6</sup>.

The puzzle, therefore, concerning the reappearance of myxomatosis in areas apparently free of the disease centres for periods of more than a year, rather than relatively short periods such as 105 days. In northern Africa it has recently been shown<sup>7</sup> that ticks are among the principal vectors of myxomatosis and it has previously been suggested by several observers that these relatively long-lived vectors might play an unobtrusive part in the spread of the disease in Britain, and possibly account for the reappearance of myxomatosis in areas free of the disease for long periods. About two years ago it was noted<sup>8</sup> that both in northern France (Pont l'Évêque, Normandy) and in England (Ashton, Peterborough), fresh outbreaks of myxomatosis, in areas where the disease has apparently been absent for more than a year, followed quickly on the introduction of flocks of sheep from elsewhere into the areas in question. Allan (personal communication) has made similar observations in Scotland, where the movements of cattle (which also harbour ticks) as well as sheep were involved. In Northamptonshire ticks are not very common on either rabbits or hares; but they frequently parasitize hares and to a lesser extent rabbits in Scotland (from where the sheep at Ashton were obtained) and France, and Allan<sup>6</sup> has recently found that ticks are one of the predominant groups of ectoparasites on the rabbit in Spain (Coto Donaña). Furthermore, Allan and Shanks<sup>9</sup> noted that ticks can transmit the disease in the laboratory. One batch collected in the wild in Scotland was infected with the virus since rabbits injected with these ticks (after grinding up in a pestle) developed myxomatosis<sup>4</sup>. It therefore seems quite possible that both sheep and cattle could pick up infective ticks and thus act as transport hosts for these vectors; information concerning the survival time of the myxoma virus in and on these arthropods would therefore be of considerable interest.

The most perplexing aspect of the epidemiology of myxomatosis in Britain and northern France at the present time is the recent rise of the rabbit-flea index on the hare<sup>10</sup>. Consequently—since the rabbit flea now has an alternative host, if only a transport host—the pertinent question to-day is not how long will the myxoma virus survive on the 'free' vector, separated from the dying or dead myxomatous rabbit, but rather how long will it survive on and in a vector feeding on another host.

In the past the rabbit has been considered the only true host of the rabbit flea in Europe, and the hare regarded merely as an accidental host<sup>11</sup>; but this may have been

a false impression due to the fact that it was a much less favoured host and the flea was present on it in relatively small numbers. Certainly to-day there appears to be a very general infestation of the hare both in northern France<sup>6</sup> and in Britain, and in at least one area at Ashton the rabbit-flea index on the hare rose temporarily, in 1964, above that of the rabbits in the same habitat. There is, however, no proof that the rabbit flea is capable of breeding successfully on the hare, and adapting itself to the different hormone cycle of this host or its surface nest, although the presence on the lactating female of gravid female fleas, and fleas which have been fertilized and have laid eggs<sup>12</sup>, is highly suggestive.

Despite its extreme specialization the rabbit flea is nevertheless capable of considerable adaptation and its habits are not identical throughout its range. LeGac<sup>13</sup> directed attention to the fact that in the forest of Bosen (Var), France, he only found *S. cuniculi* extremely rarely on the body of the host and that it was confined to the burrows of the rabbit. Collado<sup>12</sup> recorded it on the rabbits' ears in Spain, but Allan<sup>6</sup> has recently confirmed that in the summer on the Coto Donaña this species was only present in burrows and not on the body of the host as it is in northern Europe; furthermore, three species of fleas infect the rabbit in Spain. Preliminary experiments with rabbit fleas collected on Herm (Channel Islands) suggest that some populations vary considerably in their response to mammalian hormones. Thus the rabbit flea collected at Ashton responds by maturation to five daily injections into the host (the 8-lb. New Zealand White buck rabbit of 0.01–0.30 mg of hydrocortisone ('Hydrocortisyl'; Roussel Laboratories)<sup>14</sup>, but those collected on Herm require five daily injections of 0.10–0.80 mg to elicit a similar response.

If in fact the rabbit flea is capable of breeding on the hare, the increase in numbers of this host, which has recently been reported in many localities in Britain, would favour a rise in the flea index<sup>14</sup>. Until larval or pupal stages are demonstrated in the 'form' or nest of the hare, it cannot be ruled out that all the fleas found on adult hares (which may number 100 or more per individual) have been acquired by these animals moving about in areas where fleas have abandoned their true hosts dying from myxomatosis. The extraordinary ability of the rabbit flea to attach itself to a passing host when 'free' on pasture has been admirably demonstrated experimentally by Mead-Briggs<sup>15</sup>. The tendency of this flea to transfer from one host to another, not necessarily its own host, if there is contact between two individual animals has also been demonstrated experimentally<sup>16</sup>.

The hare itself at the present time apparently rarely contracts myxomatosis<sup>17</sup>, or if it does so, the disease must generally follow a benign and unobtrusive course in this animal, since it is rarely noted. It seems not impossible, however, that through constant inoculation by infected fleas a strain of myxoma virus adapted to survival in the hare will eventually be evolved. Fleas, therefore, passing on to the hare from infected rabbits will not prove fatal to their host and will survive as potential vectors; it is for this reason that the emphasis has passed from the infective 'free' vector, skulking in burrows or on the ground, to the widely disseminated infective vector feeding on the hare. Some parasites such as the plague bacillus damage the vectors and it was early recognized that many fleas which have ingested *Pasteurella pestis* do not live as long as uninfected fleas<sup>18,19</sup>. So far there

is no evidence that the myxoma virus multiplies in the rabbit flea<sup>1</sup> or that the virus itself is damaging to the flea, but there is evidence that fleas feeding on rabbits with myxomatosis are seriously affected by the course of the disease in the host. Thus the majority of such fleas undergo maturation of their ovaries<sup>14</sup>. This may be due to a hormone imbalance in the rabbit or to the high blood temperatures of the host directly attributable to the disease. In Nature, maturation of the rabbit flea on the normal pregnant host and new-born young is not associated with rises in temperature of the host; nevertheless it has recently been found<sup>6</sup> that a sudden large rise in the ear temperature of rabbits (10°–14° F) will stimulate increased defecation rate in rabbit fleas feeding on them. The increased volume of blood passing through the flea thus automatically increases the amount of corticosteroid hormones ingested by the parasite which in turn can stimulate ovarian development. In any event, the fleas feeding on rabbits suffering from myxomatosis not only display yolk production in the developing oocytes, but also other changes associated with maturation, such as overall increase in the size of the gut, proliferation of the cells lining the gut, hypertrophy of the salivary glands, depletion of the fat body and a somewhat 'ragged' appearance of the internal organs often characteristic of the spent or aged flea. It therefore seems highly probable that infected fleas in Nature which have spent about a week on the diseased host will not survive so long as uninfected fleas on healthy hosts or as Dr. Chapple's fleas<sup>1</sup> which fed for a short period on a rabbit suffering from myxomatosis and were then removed from the host and sunk in tubes in the ground.

The most striking aspect of myxomatosis in Britain to-day is the rapidly changing evolutionary nature of the situation. Both here and in Australia attention has been chiefly centred on the rabbit and the virus. Thus it has been shown that various avirulent strains of the virus have developed in less than a decade<sup>21</sup>, and the rabbit is now suspected of increased resistance, which was the most striking feature of the Australian scene<sup>22</sup>. It was early realized by Sir Christopher Andrewes that the course of myxomatosis in Britain could be modified by the

presence of a successful host-specific vector which was absent in Australia, a situation which would probably favour the survival of a more virulent form of the virus. In this case it would appear probable that the selective pressures on the flea would be simultaneously increased and evolution would inevitably proceed in the direction of poor vectorship. The fact that the rabbit flea responds by maturation on the diseased host, and has also found a widely distributed alternative host—even if it is only a transport host—indicates two lines along which selection could operate. Myxomatosis can therefore be expected to mould the vector as well as the host and the virus. In any event, in this complex and interesting triangle, selection pressures must be exceedingly high and we are thus presented with an unusual opportunity for studying the evolution of a vector.

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## EFFECT OF INHIBITION OF RNA SYNTHESIS ON NEURAL INFORMATION STORAGE

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FOR many years scientists have been intrigued with the problem of how information can be stored within the nervous system. The investigations of Hebb<sup>1</sup> directed attention to the synapse as the site where communication between neurones could be potentially modified by the learning process. This theory recognized behaviour as being determined by the communal response of neurones the random and defined pathways of which interact with each other in a complex fashion. Short-term memory would be explained by short-term changes at the synapse activated by reverberating circuits, and long-term memory by permanent changes at the synapse permanently altering intercellular communication. With the recent advances in molecular biology and the advent of newer biochemical techniques, attention has been directed to nucleic acid macromolecules, specifically RNA, as the site for long-term information storage within the nervous system<sup>2,3</sup>. Each new memory trace would be defined by a new RNA macromolecule which would persist so long as the memory trace persisted. The role of the synapse

and of intercellular communication becomes of lesser significance in this theory, and accordingly, attention is directed to the RNA changes within a single cell, or groups of cells, without considering their effect on one another.

It is clear from the experiments of Hyden<sup>4</sup> and Brattgard<sup>5</sup> that neuronal RNA may change within the central nervous system under a variety of experimental situations. However, it is not clear that such changes in RNA necessitate a theory which defines the engram in terms of a single macromolecule. Dingman and Sporn<sup>6</sup> demonstrated that 8-azaguanine, a nucleic acid antimetabolite, diminished the ability of rats to manoeuvre correctly their way through a new swimming maze, although it had no effect on performance in a previously learned maze. However, it is not clear to what extent normal production and function of RNA were altered by the presence of the 8-azaguanine, and to what extent the demonstrated effect on learning was the result of the new 8-azaguanine-containing RNA species, the lack of normal RNA associated with the learning experience, or some other less-direct effect on cellular metabolism. Chamber-

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lain *et al.*<sup>7</sup> were unable to confirm an effect of 8-azaguanine in rats running a Hebb-Williams maze, although they were able to prolong the latency time for 'fixation' of an asymmetric limb-posturing by administration of 8-azaguanine. In these experiments there are no data to indicate what biochemical effects the antimetabolite had produced. Barondes and Jarvik<sup>8</sup> were able to inhibit the synthesis of mouse brain RNA by intracerebral injection of actinomycin *D* but were unable to affect performance of a learned response.

These experiments using nucleic acid antimetabolites and the data to be presented here using actinomycin *D* to block synthesis of RNA associate changes in RNA with changes in certain aspects of behaviour. The question raised by this article is whether such data unequivocally support the role of RNA as the molecular engram, or whether these data can be interpreted to support the previously accepted 'synaptic theory'.

*O57/BL8* male mice weighing 15–20 g were injected intracerebrally with 20- $\gamma$  (in 20  $\mu$ l.) actinomycin *D* (solubilized with mannitol) and were compared with mice similarly injected with 800- $\gamma$  (in 20  $\mu$ l.) mannitol as described by Barondes and Jarvik<sup>8</sup>. At 18–24 h there were no discernible differences in motor activity either inside or outside the cage, although with the doses used all the animals were dead by 96 h. At 18 h after injection the mice were tested in several different situations. The first task was a novelty activity test in which the mice were placed in a box with a black or white knob in one corner. After 15 min in their cages they were returned to the box where either the same or a novel knob stimulus was presented. The latency of approach and the time in contact with a novel object were recorded and compared with similar measurements recorded when the knob was not changed. In both the actinomycin and the mannitol groups there was a significantly reduced latency until contact with the novel object and a lengthened period of contact with it. There was no difference between the two groups in their performance.

A second group of mice similarly injected with actinomycin *D* or mannitol was tested on the acquisition or retention of skill in a swimming maze. A T-maze was used with the central channel painted grey, one of the arms painted white, and the other black. An escape ladder was always located at the end of the white arm and its position as a right or left turn was altered on a random basis. Mice which were injected, as well as those which were not, required approximately 30–40 trials to reach 80–90 per cent criterion with an error being considered any time a mouse turned away from the ladder. This level of performance could be maintained for longer than one week without additional training. Sixteen animals injected with 20- $\gamma$  actinomycin *D* and 16 animals injected with 800- $\gamma$  mannitol, and tested 18 h post-intracerebral injection, made approximately the same number of errors in the first 10 trials in learning the maze (Table 1A). Similarly, no effect of actinomycin *D* could be demonstrated on the retention of this swimming maze performance, either in errors made or in time necessary to swim the maze (Table 1B).

Table 1

## (A) Acquisition of swimming maze performance

Injection	No. animals	Total trials	Total errors
Mannitol	16	80	46
Actinomycin	16	80	48

Animals were tested 18 h after intracerebral inoculation of 20  $\gamma$  (20  $\mu$ l.) of actinomycin *D* and 800  $\gamma$  (20  $\mu$ l.) of mannitol. They were given 5 trials in the swimming maze, and their performance on trials 6–10 was recorded. The errors on trials 1–5 were also comparable in the two groups.

## (B) Retention of swimming maze performance

Injection	No. animals	Trials	Errors	Time/trial
Mannitol	23	69	14	6 sec
Actinomycin	23	84	17	7 sec

The animals were trained to 90 per cent correct responses by 30–40 trials in groups of 10 trials over 3 days. They were rested 1 day and were inoculated as in (A). They were tested 18 h after injection and approximately 45–48 h after their last trial.

Table 2. LATENCY OF RESPONSE IN JUMPING BOX  
(In sec  $\pm$  S.E.)

Drug	No. animals	1	2	3	4	5
Actinomycin	23	52 $\pm$ 2	25 $\pm$ 4	30 $\pm$ 6	32 $\pm$ 4	24 $\pm$ 4
	Percentage	100	66	57	60	64
Mannitol	23	35 $\pm$ 3	9 $\pm$ 2	7 $\pm$ 1	6 $\pm$ 1	5 $\pm$ 1
	Percentage	100	26	20	17	14

The 51 animals which were tested for retention of swimming maze manoeuvrability were then tested for their ability to jump up 2.5 in. on to a ledge from a grid floor when a shock was passed through the grid. The animals were removed from the box if they had not escaped to the ledge after 60 sec of shock. The trials were spaced 10 min apart. As can be seen in Table 2, there was a definite difference in performance of the actinomycin-injected animal compared with the mannitol-injected animal. This difference in performance was present even in the first trial. Furthermore, the mannitol-injected mice were able to improve their performance so that the latency on the last trial represented 14 per cent of the initial trial, whereas the actinomycin-injected animals had a latency on the fifth trial of 64 per cent of the initial one. This behavioural change was not clearly related to motor weakness because all animals were able to jump and demonstrated no generalized motor defects. It seems more likely to be associated with a defect in the neural interpretation or with a response to the shock applied. Furthermore, when the mice were returned to the swimming maze, accuracy in swimming the maze and the ability to traverse it in less than 10 sec were preserved in the actinomycin as well as in the mannitol-injected animal.

The extent of inhibition of RNA synthesis was evaluated in 10 of the tested *O57/BL8* animals and in 10 *CF* strain white mice, which had not undergone behavioural tests. Orotic acid 6- $^{14}$ C was injected intracerebrally into animals 18 h after receiving 20- $\gamma$  actinomycin *D* or the corresponding mannitol inoculation. Since the incorporation into RNA appeared linear for at least 60 min in control experiments the brains were removed after 45 min, homogenized, and processed in two ways. In one group of animals, RNA was isolated by the hot phenol technique of Scherrer and Darnell<sup>9</sup>; in another group, it was characterized by alkaline hydrolysis of an acid precipitate according to a modification of the technique of Schmidt and Thannhauser<sup>10</sup>. Both techniques demonstrated significant inhibition of RNA synthesis in the actinomycin-injected animals compared with the mannitol-injected ones (Table 3). The degree of inhibition was the same in both strains of mice, and was more marked in the brain stem and sub-cortex than in the cortex.

Although it is probable that these experiments demonstrate an alteration of escape behaviour due to a drug which inhibits RNA synthesis, it is still not possible to make a definitive statement about the role of RNA in

Table 3. INCORPORATION OF ISOTOPES INTO RNA

## (A) Alkali digestion

	Mannitol Specific activity (c.p.m./O.D. at 260)	Actinomycin Specific activity (c.p.m./O.D. at 260)	Inhibition (per cent)
Cortex	326	114	65
Subcortex	345	46	86
Brain stem	380	30	94
Cerebellum	231	61	73

Twelve animals were injected intracerebrally with 2.0  $\mu$ Ci orotic acid 6- $^{14}$ C (0.5 mCi/mole) 18 h after receiving mannitol or actinomycin *D*. Forty-five min later they were killed. The cerebellum was removed, the mid-brain sectioned at the colliculi, and the subcortex scooped out. The tissues were processed by the procedure of Fleck and Munro<sup>11</sup> and the resulting nucleotide solution counted in Bray's solution in a scintillation counter with an overall efficiency of 14 per cent. RNA was estimated at 260 m $\mu$  in a Beckman DU spectrophotometer.

## (B) Phenol extraction

	Mannitol Specific activity (c.p.m./O.D. at 260)	Actinomycin Specific activity (c.p.m./O.D. at 260)	Inhibition (per cent)
Brain	1,190	480	60

Eight animals were injected as in (A) and the entire brain processed according to the method of Scherrer and Darnell (ref. 9). The final RNA solution was counted in Bray's solution with an overall efficiency of 65 per cent.



central nervous system functioning. Of the three tasks tested, performance in only one was affected by the drug. Both acquisition and retention of swimming maze behaviour remained unimpaired and this might seem contrary to RNA playing a part in such tasks. On the other hand, the administration of shock to actinomycin-injected animals definitely interfered with escape behaviour in the jumping box.

Even if it is assumed that the radioactive tracer is a reliable index of RNA synthesis, the demonstration of decreased incorporation of radioactive precursors into RNA associated with an alteration in an animal's response still does not explain what function the RNA serves. RNA is present in all cells, and its synthesis and metabolism are important in cell function. Interference with normal RNA synthesis would then be expected to interfere with cellular metabolism; and one cannot separate injury to RNA as the seat of the memory trace from injury to RNA as a vital constituent in intracellular metabolism which will, in time, affect intercellular communication and the input, storage, or read-out stages of the memory process.

In all the previously described experiments including my own, there seems no way to distinguish between molecules altered in number or quality, and cells so altered. The possibility should seriously be considered that the experiments have been merely chemical ablation studies in which certain groups of cells have been affected which can concentrate the antimetabolite and inhibitor or are especially sensitive to it. Such an interpretation would be suggested by the fact that performance of several behavioural tasks was unaffected and only one behavioural task was altered when 60-70 per cent of RNA synthesis was inhibited. This would also be suggested by the experiments of Flexner *et al.*<sup>11</sup> where interruption of avoidance behaviour was associated with inhibition of protein synthesis by puromycin, and the injection of the drug into the vicinity of the hippocampus and temporal lobe was of paramount importance. Furthermore, in my own experiment, the twisting and writhing movements, staggering, abnormal respiration and rapid loss of responsiveness, which lead to death in 48-96 h after injection of actinomycin D, reflect the disturbed subcortical and brain-stem function. Histological examination of the brains of the experimental and control animals demonstrated injury primarily to neurones with nuclear swelling and loss of morphology throughout the nervous system of the actinomycin-injected animals, especially in the hippocampus, and brain stem (Fig. 1). This finding is in accordance with the greater inhibition of isotope incorporation in the sub-cortex and brain stem than in the cortex itself. However, it does not allow us to decide whether these areas are especially sensitive to the drug or whether they have been merely exposed to a higher concentration.

The unique role of RNA as the engram cannot be deduced solely from experiments which associate increases or decreases in RNA concentration with altered behaviour. That RNA levels vary under different conditions of stimulation is unequivocal, but the question of how such changes are brought about, how long they last, and what functions they may serve are unanswered at present. In the experiments of Hyden, for example, the increased levels and altered base compositions of RNA have been specifically associated with involvement of the RNA as an engram in a 'learning situation'. The probability exists, however, that both in the reported 'balancing' experiment<sup>12</sup> and the more recently published 'transfer of handedness' experiments<sup>13</sup>, the results reflect differences in the extent and pattern of activation of affected cells rather than different situations of 'learning' and 'non-learning'. Our problem is still, then, unresolved. There is no evidence for the synthesis or presumed permanence of an RNA unique to a particular situation. We must still explain how cells may 'interact' and specifically how

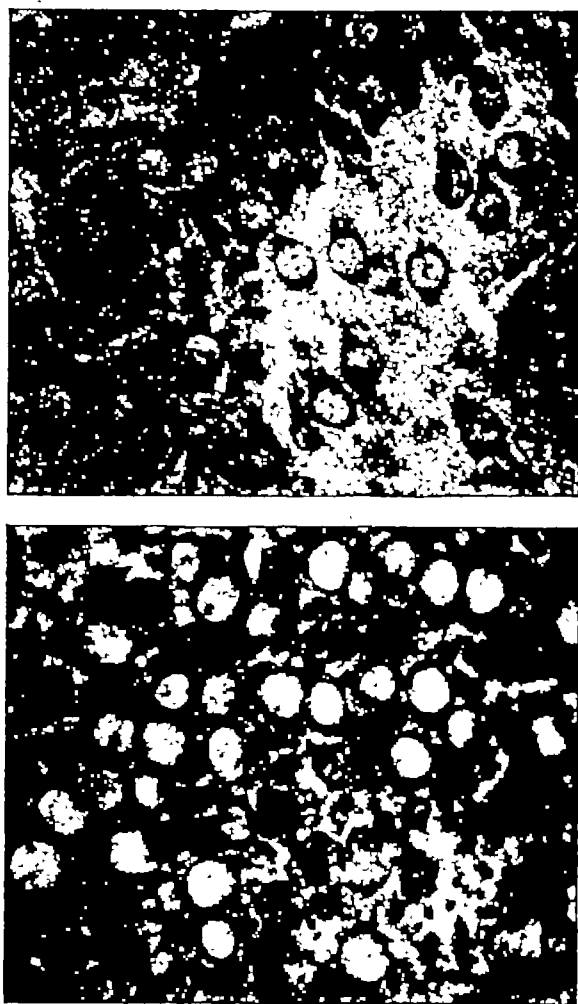


Fig. 1. Methyl green, pyronin stain of hippocampal neurone somas ( $\times 700$ ). (A) 24 h post inoculation of mannitol. (B) 24 h post inoculation of 20  $\gamma$  actinomycin D. The nuclei are swollen with loss of substructure (RNA). Feulgen stain for DNA of similar sections shows only the minimal swelling, but no loss of DNA substructure.

previous experience may facilitate transfer of intercellular information.

In essence, any chemical changes which would affect the synthesis or release of neurotransmitter, its affinity for the post-synaptic receptor, its rate of destruction, the post-synaptic membrane properties, or the nature of the post-synaptic cytoplasm and its ability to propagate a depolarization to the initial axon segment might alter intercellular communication and information transfer. The maintenance of these biochemical changes and of the new pattern or level of intercellular communication need not depend on the indestructibility of any single macromolecule. What would be required is that the new steady state of these biochemical alterations be maintained. Activity could induce or 'derepress' gene expression and lead to transient or sustained alterations in RNA and protein metabolism. This may, in turn, facilitate activity by modifying impulse transmission at a subsequent input or output, and thereby reinforce the biochemical changes which initially produced it. The altered pattern of intercellular communication would not then reside in any permanent macromolecular engram but in the dynamic interaction of a post-synaptic cell with its pattern of pre-synaptic influences. This more effective intercellular communication might depend on the duration of the effective biochemical changes and on the time between an initial and subsequent activation of the pathways involved.

No single component could be omitted for effective operation; and the engram would not be defined as resid-

ing in any single molecule or within any single cell. It would be most adequately defined by the interaction of several neurones, and would be most fruitfully explored by investigating the effect of electrical activity on the biochemical state and the effect of the altered biochemical state on subsequent electrical activity.

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## 19S ANTIBODY PRODUCTION AGAINST SOLUBLE LIPOPOLYSACCHARIDE ANTIGENS BY INDIVIDUAL LYMPHOID CELLS IN VITRO

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RECENT methods<sup>1-3</sup> have greatly facilitated the detection of cellular antibody production *in vitro*. Antibody synthesis by individual cells has been made visible by mixing immune lymphoid cells with the antigen (sheep red cells) in a semi-solid agar medium; after the addition of complement, each antibody-producing cell is surrounded by a clear plaque of lysed red cells against the background of intact cells. The plaque-forming cells (PFC) have been shown to produce large molecular 19S antibodies; the marked increase of 7S antibodies occurring 5-6 days after the antigen stimulation does not result in detectable plaque-formation. On the contrary, the time of increase of 7S antibodies coincides with the decrease of PFC<sup>4</sup>. The negative influence of 7S antibodies on the appearance of the PFC was emphasized by the finding that passively transferred 7S antibodies specifically inhibited the development of the PFC<sup>5</sup>, suggesting that active synthesis of 7S antibodies might inhibit cellular 19S antibody production.

I have made unsuccessful attempts to detect individual antibody-producing cells *in vitro* with red cell antigens from various species (with the exception of cattle erythrocytes, which cross-reacted completely with sheep red cells) and different serum protein antigens adsorbed on to red cells. However, positive results were obtained in the present work with bacterial lipopolysaccharide antigens adsorbed on to sheep red cells. This report is concerned with the immune response against such antigens as revealed by serum antibody production and development of PFC *in vitro*.

The agar plaque-technique was used as described by Jerne<sup>1</sup>. Lymphoid cells to be tested for antibody production *in vitro* were usually taken from the spleen, but in certain experiments they were also taken from lymph nodes, thymus and peripheral blood. 0.1 ml. of the cell suspension was mixed at 45° C with 2 ml. 0.7 per cent agar containing 1 mg DEAE dextran (Pharmacia, Sweden) and  $4 \times 10^6$  sheep red cells coated with the lipopolysaccharide (obtained from *E. coli* 055:B5). The mixture was poured out on to Petri dishes containing a layer of 1.4 per cent agar and incubated for 1 h at 37° C. Thereafter, 2-3 ml. of a 1/10-1/15 dilution of freshly reconstituted lyophilized guinea-pig complement (Sclavo, Italy) was added to each plate and incubation was continued for 30 min at the same temperature. Reading of the plates was facilitated by staining with benzidine as described by Jerne<sup>1</sup>. Three mice were used for each

determination and at least two agar plates were prepared from each cell suspension. A number of different specificity controls were also included. In each experiment non-immune spleen cells were tested against polysaccharide-coated sheep red cells. In addition, specifically immunized lymphoid cells were tested against non-coated red cells, and spleen cells from animals immunized against a non-cross-reacting polysaccharide (from *Salmonella typhosa* 0901) were mixed with red cells sensitized with *coli* polysaccharide. These controls usually showed a low number of PFC (0-100). There was no increase after immunization with the non-cross-reacting polysaccharide, but the values were usually slightly higher against specifically sensitized red cells than against uncoated cells. It seems possible that this background activity is due to the added effect of 'natural' antibody synthesis against both the sheep red cells and the polysaccharide.

The polysaccharides used for coating the sheep red cells were obtained from Difco, Detroit, and were extracted from *E. coli* 055:B5 by the method of Westphal<sup>6</sup>. A solution containing 1 mg polysaccharide per ml. in saline was boiled for 1 h immediately before use and 0.2-1.0 ml. was added to 0.1 ml. packed and washed sheep red cells. The cells were incubated for 30 min at 37° C, washed three times in saline and diluted to a concentration of  $4 \times 10^6$  per ml. Mice were immunized by the intraperitoneal injection of 0.0025-0.1 ml. of isologous red cells coated with lipopolysaccharides or, in a few cases, with a bacterial vaccine.

Simultaneously with the determination of the number of PFC the humoral antibody response of the same animals was examined by haemolytic and agglutination tests. For haemolysis 0.2 ml. serum was serially diluted in BSS and 0.1 ml. of a 2 per cent sheep red cells suspension coated with the *coli* antigen was added to each tube, followed by 0.1 ml. of guinea-pig complement diluted 1/40. The same lot of guinea-pig complement was used in all experiments. The tubes were incubated for 1 h at 37° C and the last tube showing 100 per cent lysis was taken as the end point. Normal serum controls were tested in parallel; they very rarely gave rise to 100 per cent lysis, and only in titres ranging from 1/5 to 1/40. The agglutination test was performed by mixing equal volumes (0.025 ml.) of antibodies serially diluted in 1.5 per cent polyvinyl-pyrrolidone (PVP) and a 2 per cent suspension in BSS of antigen-coated red cells. After

incubation for 1 h at 37°C the tubes were read as described previously<sup>7</sup>.

Immunization of mice of various genotypes with the lipopolysaccharide antigens from *E. coli* resulted in a rapid increase of the number of plaque-forming spleen cells. Already after 24–48 h the number of PFC started to increase exponentially and continued for 3–5 days. After the peak there was a rapid decline, but background level usually was not reached even after 16 days after immunization (Fig. 1). The development of the PFC thus followed the same pattern as previously described for sheep red cell antigens<sup>1,4</sup>. A second injection with antigen-coated isologous mouse red cells after 16 days stimulated an exponential increase of the number of PFC as in the primary response, but the maximum number of PFC was considerably lower (Fig. 1).

The development of PFC against sheep red cells was previously shown to parallel closely the production of 19S serum antibodies, whereas there was an inverse correlation to 7S antibody synthesis<sup>4,8</sup>. Analogous investigations were performed with the polysaccharide antigen. As demonstrated in Fig. 1, the development of haemolytic antibody titres paralleled the increase of the number of PFC during the first 4 days. Thereafter, the number of PFC started to decrease, whereas the haemolytic antibodies continued to increase for 2–5 days and then reached a steady state for 3–5 days. Agglutinating antibodies followed the same pattern. Thus, there was a clear-cut difference between the kinetics of the immune response measured by the appearance of PFC and humoral antibodies, respectively. A discrepancy between the production of PFC and serum antibodies has been observed previously with sheep red cells, but in that case haemolytic and agglutinating antibodies behaved differently; the increase of 7S agglutinins started at the same time as the increase of the number of PFC, whereas haemolytic 19S antibodies paralleled the PFC<sup>4</sup>. The question whether a similar mechanism operated with the polysaccharide antigen at present used was therefore investigated. Sera from mice immunized with polysaccharide were separated into 19S and 7S fractions by 'Sephadex G-200' gel-filtration and the different fractions were tested for haemolytic and agglutinating activity against antigen-coated sheep red cells. It was regularly found that antibodies occurred only in the 19S fraction, both with regard to haemolytic and agglutinating activity (Fig. 2). Even sera from mice immunized four times with the antigen adsorbed on to red cells or incorporated into Freund's complete adjuvant contained only 19S antibodies. The possible contamination of the 19S fraction with 7S antibodies was excluded by treating whole hyperimmune sera with an equal volume of 0.2 M mercaptoethanol for 30 min at 37°C. This treatment selectively inactivates 19S antibodies and it reduced the agglutinin titres of several sera taken at different intervals after immunization from 1/40,000–1/820,000 to less than 1/5. It would appear, therefore,

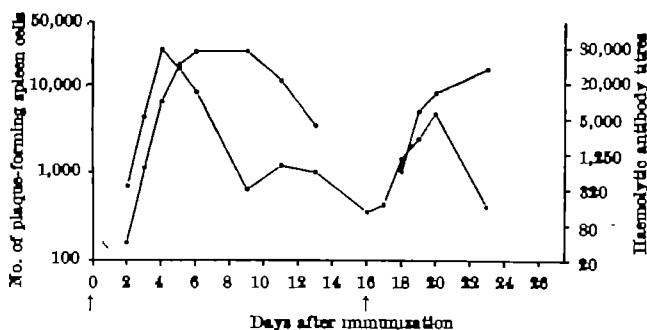


Fig. 1. Development of plaque-forming spleen cells and haemolytic antibodies in A.SW mice after immunization with isologous red cells coated with *E. coli* 065 : B6 lipopolysaccharides. Each point represents the mean value from three mice. The arrows indicate the time of antigen injection. ●, Plaque-forming cells; ○, haemolytic serum titres.

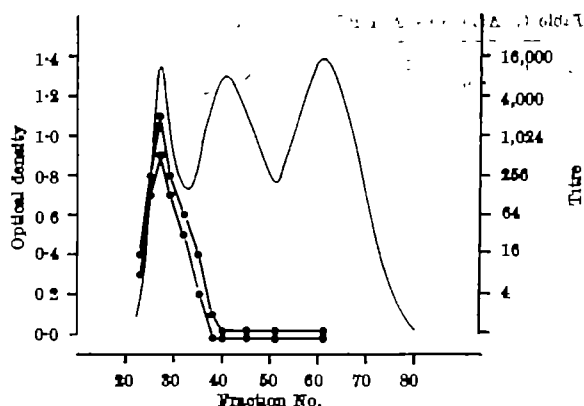


Fig. 2. Results of fractionation of a hyperimmune mouse anti-*E. coli* 065 : B6 serum by 'Sephadex G-200' gel-filtration and subsequent determinations of the haemolytic and agglutinin titres of the various fractions. —, Protein concentration; ○, haemolytic titres; ●, agglutinin titres.

that the polysaccharide antigen used stimulated 19S antibody production exclusively. Thus, the discrepancy between the kinetics of PFC and humoral antibodies in this system cannot be ascribed to the production of 7S antibodies.

The possibility that the prolonged presence of 19S antibodies in the sera of immunized animals was caused by accumulation of previously synthesized antibodies was shown to be improbable by the demonstration that the half-life of passively transferred antibodies was very short (6–10 h). It follows that the antibodies present in the sera of the mice are to a great extent newly synthesized.

A further problem investigated was the question whether antibody production occurred in extra-splenic lymphatic organs. It seems possible that cellular antibody formation occurs predominantly in the spleen during the first 4 days after immunization, but is later transferred to other organs, and this would be responsible for the increasing titres of serum antibodies. Extra-splenic antibody production does not occur to any appreciable extent against sheep red cell antigens, but it is possible that the soluble polysaccharide antigens might disseminate more freely in the body and stimulate antibody production more efficiently in other organs.

Several experiments were performed to test this possibility. Mice were immunized with isologous red cells coated with the polysaccharide antigens, and their spleens, lymph nodes and thymus glands were removed at various intervals and tested for the number of PFC. In addition, lymphoid cells from the peripheral blood were isolated by mixing equal volumes of blood (in isotonic citrate solution) with 3 per cent 'dextran 250' (Pharmacia, Sweden) for 1 h at room temperature. The supernatant, which contained mainly white cells, was washed and used in the agar-plaque technique.

It was constantly found that PFC occurred in extra-splenic lymphatic organs, mostly in the lymph nodes and the peripheral blood but also in the thymus. The number of PFC per million cells in the thymus varied greatly between different animals, but occasionally reached higher values than in the lymph nodes and the peripheral blood. The kinetics of antibody production in these organs, however, paralleled that in the spleen. Thus, the maximum number was found about the same time in all organs (day four as a rule) and thereafter declined rapidly. Usually, significant numbers of PFC were only found at days 4–5 in the thymus, lymph nodes and peripheral blood. Although the number of PFC per 10<sup>6</sup> lymphatic cells was sometimes high in the extra-splenic organs, their absolute amount was insignificant compared to that in the spleen. Since no shift of the antibody-producing cells occurred from the spleen to other lymphatic organs after the peak at days 3–5, it is unlikely that the difference between

Table 1. APPEARANCE OF PFC IN VARIOUS LYMPHATIC ORGANS AFTER IMMUNIZATION WITH COH POLYSACCHARIDE-SENSITIZED ISOLOGOUS RED CELLS

Exp. No.	Strain	Organ	Mean number and range of PFC per 10 <sup>4</sup> cells (days) after.					
			3	4	5	6	7	10
1	A.SW	Spleen	15.0 (9.4-18.5)	506.5 (343.3-848.0)	44.6 (41.9-48.6)			
		Lymph nodes	0.0	25.3 (14.7-31.2)	9.0 (7.6-10.0)			
		Peripheral blood		20.0 (10.0-30.0)	2.8 (1.7-3.3)			
2	A.BY	Spleen	501.0 (180.9-821.0)	516.6 (183.8-1,062.2)	319.3 (78.3-446.3)	63.4 (57.8-68.9)	56.5 (41.7-85.6)	64.6 (14.0-122.6)
		Lymph nodes	10.9 (0.3-19.0)	57.1 (21.4-91.6)	9.6 (2.6-15.4)	10.4 (1.1-19.7)	10.4 (0.0-31.2)	8.3 (0.0-25.0)
		Thymus	1.0 (0.0-2.8)	8.5 (0.0-22.2)	0.1 (0.0-0.2)	1.1 (0.5-3.0)	0.0	0.0
		Peripheral blood	0.0	235.5 (23.3-533.3)	1.5 (0.0-4.5)	0.8 (0.0-2.5)	0.1 (0.0-4.0)	4.1 (0.0-12.5)
3	A.BY	Spleen		256.6 (123.8-419.0)	103.7 (64.0-125.0)	13.5 (1.3-32.7)		
		Lymph nodes		33.5 (4.0-81.8)	7.3 (8.1-14.6)	9.9 (1.6-21.0)		
		Thymus		275.5 (0.0-826.5)	0.4 (0.0-1.4)	1.1 (0.0-2.4)		
		Peripheral blood		41.6 (25.0-75.0)		0.0		
4	A.BY	Spleen	27.0 (13.7-51.8)	234.8 (67.0-537.6)	60.5 (19.1-128.2)	17.1 (11.5-27.1)	16.6 (11.6-21.3)	8.1 (7.4-8.7)
		Lymph nodes	0.1 (0.0-0.3)	0.4 (0.0-0.8)	4.0 (1.3-8.0)	0.0	0.0	0.0
		Thymus	0.0	0.7 (0.0-1.6)	1.4 (0.2-2.2)	0.0	0.0	0.0
		Peripheral blood	0.0	3.5 (0.0-6.3)	40.0 (8.8-68.8)	2.1 (0.0-6.3)	1.0 (0.0-3.1)	0.0

detectable cellular antibody synthesis and serum antibody titres is caused by extra-splenic production.

The foregoing results demonstrate that 19S antibody production against soluble lipopolysaccharide antigens can be detected at the cellular level. The kinetics of the cellular antibody production is similar to that against sheep red cells: a maximum number of PFC is reached after 3-5 days and then rapidly decreases. The humoral antibody response is only of the 19S-type, even after hyperimmunization with antigen incorporated into Freund's complete adjuvant. It develops in parallel with the PFC during the first four days, but then continues to increase and reaches a steady state for 3-5 days before declining, whereas the PFC rapidly disappears after the fourth day. This discrepancy between detectable cellular production of only one type of antibody (19S) and serum antibody titres cannot be ascribed to cumulation of serum antibodies or to extra-splenic antibody synthesis. It is noteworthy, however, that antibody synthesis was observed in the thymus and the peripheral blood. Occasionally the thymus contained a larger fraction of antibody-producing cells than the lymph nodes. A similar observation has also been made by M. Landy (personal communication) in rabbits immunized against polysaccharide antigens. At this stage it is only possible to speculate about the mechanism responsible for the difference in detectable cellular synthesis of antibodies and their presence in the serum. It seems possible that it is caused by changes in some properties of the 19S

antibodies produced, analogous to the shift from 19S to 7S antibodies occurring with other antigens. According to this hypothesis, highly efficient haemolytic 19S antibodies are produced by lymphatic cells during the first 4 days after immunization, which are capable of producing plaques *in vitro*. As a result of a maturation process a new type of antibodies is produced, which is still of the 19S-type, but has a lower haemolytic efficiency. These antibodies would not be detected in the agar-plaque technique, but easily in test-tube methods when mixed with the target cells. The late 19S antibodies would thus have properties similar to 7S antibodies in other immunological systems. Alternative explanations might exist, however, such as a delayed antibody production against new antigenic constituents of the polysaccharide antigen. Such antibodies might be incapable of producing plaques, but efficient in test-tubes.

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## BIOSYNTHESIS OF L-ASCORBIC ACID IN DIFFERENT SUB-CELLULAR FRACTIONS OF PRENATAL AND POSTNATAL RAT LIVERS

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USE of the Hogeboom method<sup>1</sup> for differential centrifugation and isolation of different sub-cellular components from adult rat liver homogenate revealed that D-glucurono reductase and L-gulonolactone, the enzyme systems converting D-glucurono lactone and

L-gulonolactone into L-ascorbic acid, are located almost entirely in the particulate fraction sedimented between 8,500g and 88,700g (refs. 2, 3). This fraction is usually called the microsomal fraction.

Employing the same technique of differential centrifugation<sup>1</sup>, experiments with Sprague-Dawley rats indicate that L-gulonolactone and L-gulonolactone dehydrogenase are

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Table 1. SYNTHESIS OF L-ASCORBIC ACID BY DIFFERENT SUB-CELLULAR FRACTIONS FROM PRENATAL AND POSTNATAL FEMALE RAT LIVER

Condition of rat	Age	Synthesis* of L-ascorbic acid, $\mu\text{g}/\text{mg}$ protein					
		Protein content, mg per g liver		200g fraction		8,000-70,000g fraction	
		200-8,000g fraction	8,000-70,000g fraction	In air	In absence of air	In air	In absence of air
Prenatal†	18 days	10	4	2	6	20	Nil
"	20 days	12	6	2	8	25	Nil
"	21 day (term)	14	8	3	9	—	7
New-born ♀	24 h	20	16	5	9	—	21
Young ♀	15 days	25	20	10	10	—	88

\* Systems and methods employed: total volume of incubation mixture was 2.5 ml. containing 0.25–1.0 g liver equivalent of microsomes, 0.02 M sodium phosphate buffer, pH 7.2, 4 mg of L-gulonolactone, 0.005 M sodium pyrophosphate and 0.001 M KCl; incubated for 1 h at 37° in air. In anaerobic incubation sodium pyrophosphate was omitted, the L-gulonolactone and 2 mg phenazine methosulphate were placed in the side arm of a Thimbley tube. L-Ascorbic acid was estimated by titration with 2,6-dichlorophenol indophenol and the protein was estimated by biuret method after precipitating the protein with trichloroacetic acid†.

† No attempt was made to determine the sex of the foetuses.

entirely absent from the so-called microsomal fraction of liver homogenate obtained from embryonic rats up to 20 days of gestation (Table 1). However, the foetal liver homogenate was found to contain a small amount of activity for the synthesis of ascorbic acid. This activity is located in the fractions sedimented at 200g and between 200g and 8,000g (Table 1). The activity of these fractions did not increase significantly after birth (Fig. 1). On the other hand, the activity of the fraction sedimented between 8,000g and 70,000g which is absent from foetal liver, starts on the 21st day of gestation (term), increases after birth, attaining a maximum on the 14th–15th day, and then falls to values of 50–60  $\mu\text{g}$  ascorbic acid per mg protein (Fig. 1). The further decrease in biosynthetic capacity of liver microsomes from female rats at an age of 35–45 days (Fig. 1), the age of attaining sexual maturity,

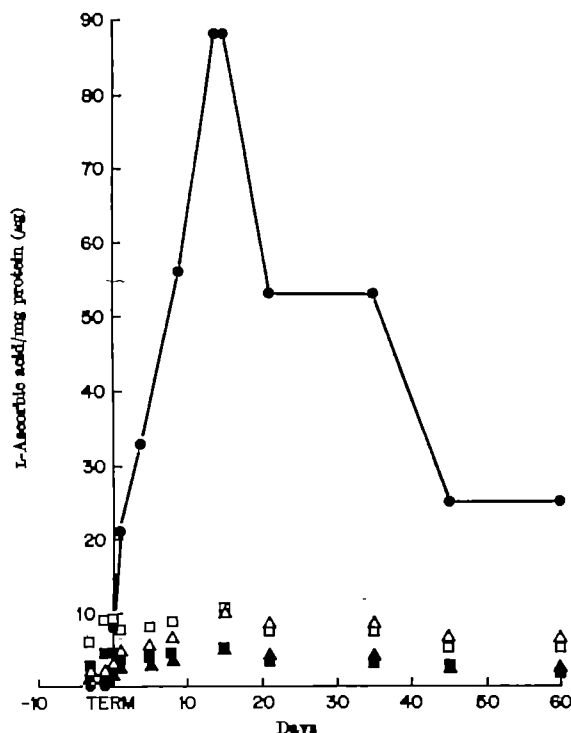


Fig. 1. Synthesis of L-ascorbic acid by different sub-cellular fractions from prenatal and postnatal female Sprague-Dawley rat livers. Conditions were the same as indicated in Table 1. ●, Liver cell fraction sedimented at 8,000–70,000g (synthesis in air); □, liver cell fraction sedimented at 200–8,000g (synthesis in absence of air); ■, liver cell fraction sedimented at 200–8,000g (synthesis in air); ▲, liver cell fraction sedimented at 200g (synthesis in absence of air); △, liver cell fraction sedimented at 200g (synthesis in air). Abscissa: days before and after the 21-day term period for the rat. Ordinate:  $\mu\text{g}$  L-ascorbic per mg protein.

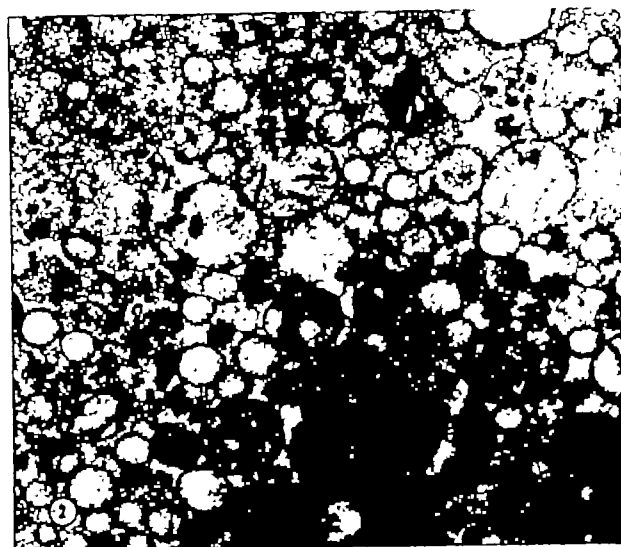


Fig. 2. Thin section of 18-day foetal rat liver fraction sedimented at 200–8,000g. The tissue was ground with a Ten Broeck glass tissue homogenizer employing 0.25 M sucrose. The pellet was fixed with osmic acid and embedded in 'Epon'. Note the unusually large vesicles with attached ribosomes. Fraction contains some mitochondria. ( $\times 55,000$ )

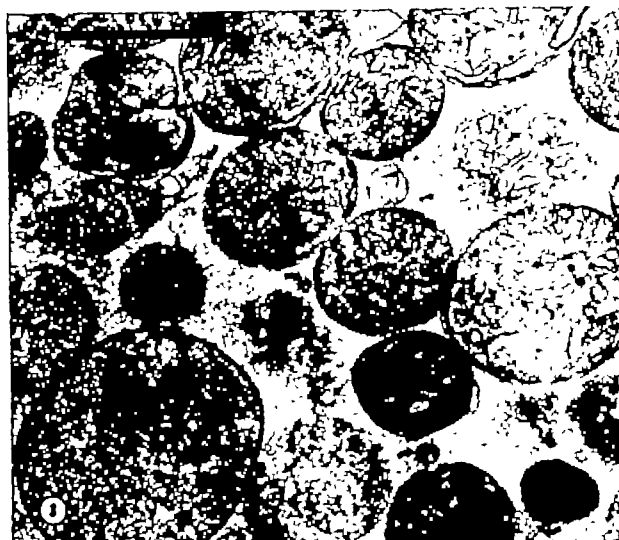


Fig. 3. Thin section of 14-day-old rat liver fraction sedimented at 200–8,000g. The tissue was treated as in Fig. 2. Mitochondria is the essential component with slight microsomal (arrows) contamination. ( $\times 60,000$ )

is not observed with the microsomes from male rats. Similar results are obtained when the enzymatic activity is expressed per g of liver instead of per mg microsomal protein.

It would appear from Table 1 and Fig. 1 that, in the foetal liver homogenate, the ascorbic acid synthesizing enzymes are located in the so-called nuclear and mitochondrial fractions. However, when the so-called mitochondrial fraction from foetal rat liver homogenate, sedimented between 200g and 8,000g, was examined with the electron microscope, the fraction was found to be composed of 90–95 per cent microsomes, the rest being mitochondria and other cell organelles (Fig. 2). On the other hand, the same 200–8,000g particulate fraction from a 14-day-old or an adult rat liver homogenate was found to be composed of almost pure mitochondria with slight microsomal contamination (Fig. 3). In the foetal liver homogenate, the fraction sedimented between 8,000g and 70,000g has been tentatively identified on the basis of morphology as free ribosomes and some smooth vesicles (Fig. 4). The comparable fraction from a 14-day-old or an adult rat liver homogenate contained a larger proportion of smooth vesicles (Fig. 5).

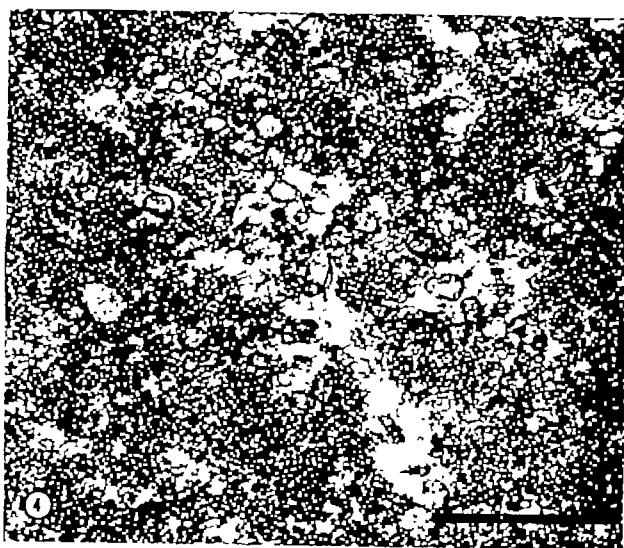


Fig. 4 Thin section of foetal rat liver fraction sedimented at 8,000-70,000g. The tissue was treated as in Fig. 2. The major portion of the fraction consists of what have been tentatively identified as free ribosomes with some relatively large smooth vesicles intermixed. ( $\times 70,000$ )

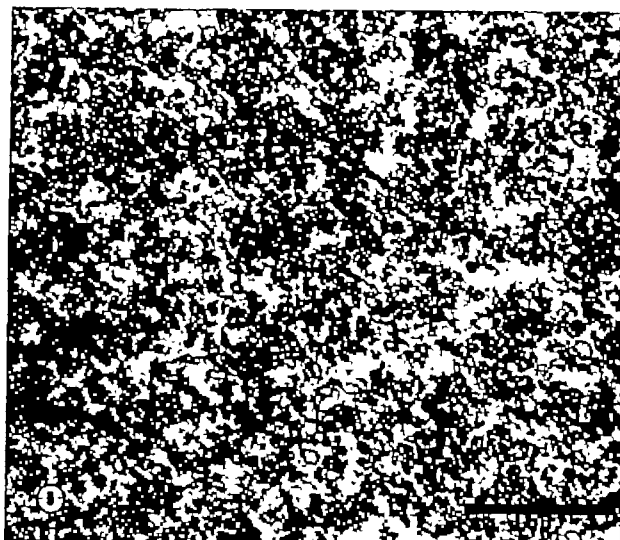


Fig. 5 Thin section of 14-day-old rat liver sedimented at 8,000-70,000g. The tissue was treated as in Fig. 2. The distribution of free ribosomes and vesicles is about equal. A few fragments of smooth tubular endoplasmic reticulum can be observed. ( $\times 55,000$ )

The results indicate that the liver microsomes from embryonic rat are apparently different from those of young or adult rats being sedimented at a much lower centrifugal force and having much less enzymatic activity

for synthesizing L-ascorbic acid. As appears from the electronmicrographs (Figs. 2 and 5), the microsomal vesicles, as well as the ribosomal granules from embryonic liver, sedimented under the same conditions are strikingly larger than those from an adult liver. The larger vesicle size and the ribosomes being attached would perhaps explain why the embryonic microsomes sedimented at a much lower centrifugal force. However, differences in chemical composition of the particulates, as well as differences in the physical properties, may also account for sedimentation at a lower centrifugal force. Further work is needed to explore this and to determine how and when the transition of the foetal liver microsomes to adult liver microsomes occurs. Whether similar transition also occurs in microsomes from other organs has yet to be learnt. The few mitochondria present in the embryonic rat liver proliferate rapidly after birth. The phenomena of mitochondrial proliferation and the transition of foetal microsomes to adult microsomes may not be merely coincidental and may merit further investigation.

It should be mentioned that Peters *et al.*<sup>4</sup> observed a morphological ergastoplasmic differentiation between prenatal and neonatal mouse liver. A smooth tubular form of endoplasmic reticulum was found to appear at birth which was completely absent from a foetal liver of 18 days gestation. In confirmation of their finding, Fig. 2 shows that the microsomes from foetal rat liver are all of rough or granular type and none of them is smooth or agranular. On the other hand, the microsomes from an adult rat liver are known to be composed of both rough and smooth types of vesicles (ref. 5 and Fig. 5). However, it is not known at present whether the ascorbic acid synthesizing enzymes are associated especially with the smooth types of endoplasmic reticulum which are absent from the foetal liver.

Under the electron microscope, the nuclear fractions from both prenatal and postnatal, and the mitochondrial fraction from postnatal, liver homogenates were found to contain some microsomes (Fig. 3). Hence, in confirmation of the previous findings<sup>1</sup> it may be stated that the enzymatic activities of these two fractions are due to contamination by microsomes. The low biosynthetic capacity of the embryonic microsomes is not due to the presence of some inhibitor, since addition of embryonic microsomes to adult microsomes did not inhibit synthesis by the latter.

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## CHROMOSOME DAMAGE IN HUMAN DIPLOID CELLS FOLLOWING ACTIVATION OF LYSOSOMAL ENZYMES

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CHROMOSOME or chromatid aberrations are known to follow exposure of cells to X-radiation, ultra-violet radiation, virus infections and a variety of chemical compounds differing widely in structure<sup>1</sup>. It has been generally accepted that such aberrations are due to direct physical or chemical attack on chromosomal material,

probably DNA. However, several observations have led us to investigate another possibility, that scission of chromatids could be produced by enzyme action and might therefore result from activation of hydrolytic enzymes in the living cell. In particular, it seemed possible that deoxyribonuclease (DNase) or other enzymes

liberated from the cytoplasmic organelles known as lysosomes might be able to enter the nucleus and produce structural alterations in chromosomes.

To test this hypothesis a method of selective activation of lysosomal enzymes developed in our laboratory<sup>3</sup> has been used. The method depends on the fact that living cells take up certain dyes or other compounds and concentrate them in lysosomes. When the cells are exposed to light of wave-length absorbed by the chromophore, photo-oxidative changes in the membranes of the lysosomes are produced. Light of these relatively long wave-lengths is not injurious to cells except where the photosensitizing agent is present. Hence damage to lysosomes, with release of the enzymes they contain, can be achieved without direct damage to the nucleus or other cytoplasmic constituents. This method of activating lysosomal enzymes is much more specific than the use of detergents, enzymes or antibodies. If the concentration of dye or time of illumination is sufficient, the cells are killed<sup>3</sup>, but lower doses produce other interesting effects including the chromosome aberrations now reported.

**Cell cultures.** The human diploid cell strain, WI-38, isolated by Hayflick from human embryonic lung, was obtained from Mr. J. P. Jacobs. Medium for cell growth consisted of Eagle's basal medium (in Hanks's balanced salt solution) containing 8 per cent calf serum and 0.05 per cent bicarbonate, with 200 units penicillin and 100 µg streptomycin per ml. The cells were dispersed and propagated in 4-oz. bottles using the methods described by Hayflick and Moorhead<sup>4</sup>. Repeated tests of the cell strain for bacterial or mycoplasma contamination were negative, and routine examination of the chromosomes of these cells in our laboratory have revealed no abnormalities.

**Photosensitizing agents.** Neutral red, vital and fluorochrome, was obtained from G. T. Gurr, London. Acridine orange was purchased from E. Gurr, London. These samples contained several components and purification was carried out by silica-gel chromatography using three solvent systems, namely, butanol: ammonia, butanol: acetic acid and pyridine, isoamyl alcohol: ammonia. Samples of anthracene and acridine highly purified by zone-melting were obtained from the National Chemical Laboratory.

**Photosensitizing technique.** Twenty-four h after sub-culturing the cells, photosensitizing agents were added to the medium in concentrations varying from 1 in  $10^4$  to 1 in  $2 \times 10^5$  (w/v). The dyes were soluble in the medium, but anthracene and acridine were dissolved in serum as previously described<sup>4</sup> and added to the medium. The cells were incubated at 37°C for 1 h, after which the medium was replaced with fresh medium without the photosensitizing agent and the cells were incubated for a further 0.5 h. This was done to avoid immediately lethal damage to cell membranes which occurs if the photosensitizing agent is present in the medium at the time of illumination. Cells were illuminated with a high-intensity tungsten or low-pressure mercury discharge source with appropriate filters. Cultures were 5 in. from the tungsten source (Philips 'No. 2 Photolite'); this system with a 'Cinemoid 21' filter (Strand Electric Co.) was used for neutral red and No. 17 filter for acridine orange. With acridine and anthracene the mercury vapour source (4 Philips 'TL AD'05 tubes) was used with glass and No. 25 filters 4 in. from the culture. Cells were illuminated for 0.5 or 1 h at room temperature, with heat-absorbing filters and fans to prevent significant rise in temperature. Controls were kept at room temperature for the same time or were illuminated without any photosensitizing agent. All cultures were then incubated at room temperature for a further 24 h before chromosomes were examined.

**Chromosome preparations.** These were made by a modification of the technique of Moorhead and Nowell<sup>5</sup>. Cultures were usually treated for 3 h with 'Colcemid'; some comparisons of break frequencies with and without 'Colcemid' showed no significant differences. The cells were fixed in

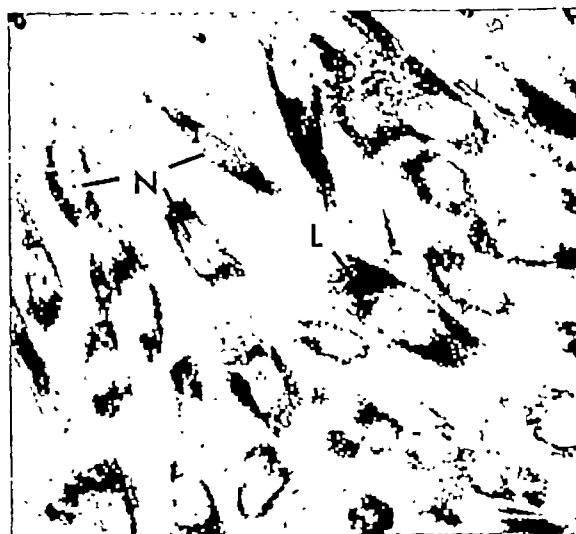


Fig. 1. WI-38 cells exposed to neutral red (1 in 30,000) for 1 h, showing uptake of dye in lysosomes (L). Nuclei (N) are unstained. ( $\times c. 740$ )

acetic alcohol, spread on slides over a low flame, hydrolysed and stained with Giemsa. The slides were coded and counted blind. In each preparation under the low power of the microscope 50 or more well-spread metaphases were chosen for detailed examination at high magnification. Broken cells with scattered chromosomes were rejected. A cell with one or more definite breaks was recorded as positive. Obscured or indefinite breaks, secondary constrictions or unstained gaps were recorded as negative. The final result shows the number of cells with one or more breaks as a percentage of the total number of cells examined. Chromatid and chromosome breaks were not differentiated; the latter were occasionally observed, but much less frequently than chromatid breaks. Occasional cells showed chromatid exchanges and 'triradial' configurations (Fig. 3).

The intracellular distribution of the photosensitizing agents was examined by direct or fluorescence microscopy. As previously described<sup>6</sup> neutral red (Fig. 1), anthracene and other polybenzenoid hydrocarbons and acridine are seen only in lysosomes, whereas acridine orange fluoresces in both the nucleus and lysosomes. Even at concentrations of neutral red, anthracene or acridine a hundred times higher than those used for photosensitization no nuclear uptake is seen unless the cells are damaged. It therefore seems certain that the primary photosensitizing effects of these substances are on lysosomes. Acridine orange was used for comparison with similar experiments previously published on plants<sup>7,8</sup>, which will be discussed here. In this case direct photosensitization damage to the nucleus is possible.

The results of two representative experiments are summarized in Table 1. The number of breaks is clearly higher in cells photosensitized with any of the agents used. The results in Table 1 show that the photosensitizing agent does not produce breaks in the absence of light, and previous observations<sup>9,10</sup> have shown that oxygen must be present for killing cells and for structural alterations in chromosomes to occur.

Three other experiments gave similar results, with statistically significant differences between treated and control cells, although somewhat higher percentages of breaks were found in two sets of controls in which the cells used were at a later passage level, 32 or 33.

Although the experiments were undertaken for different reasons, our results confirm for animal cells the observations previously made by Kihlman<sup>11</sup>, and Dubinin *et al.*<sup>12</sup> in plants. These authors found that by treating broad bean or onion root-tips with low concentrations of acridine orange, rivanol, methylene blue or toluidine blue, and illuminating them with visible light, structural chromo-



Table 1. CHROMOSOME DAMAGE IN WI-38 HUMAN DIPLOID CELLS AT PASSAGES 25 AND 27, AFTER PHOTODIMERIZATION

Photo-sensitizing agent	Concentration (w/v)	Mitoses examined	Mitoses with chromatid breaks	% cells with breaks	Probability of difference from control (%)
Control	—	50	2	4	—
Neutral red	1 in $10^4$	74	9*	12.2	—
Neutral red (no illumination)	1 in $10^4$	50	2	4	—
Acridine	1 in $10^4$	50	10*	20	1
Acridine orange	1 in $2 \times 10^4$	50	4	8	—
Anthracene	1 in $10^4$	50	11	22	1
Control	—	50	1	2	—
Neutral red	1 in $10^4$	58	6	10.3	—
Acridine orange	1 in $10^4$	48	15†	31.3	1

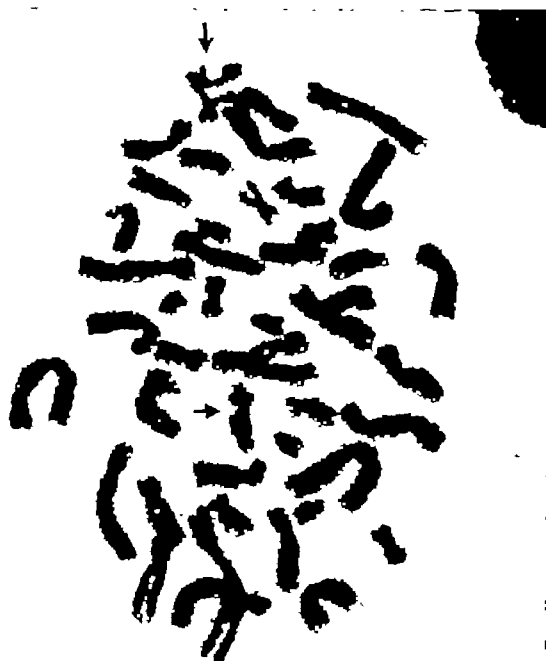
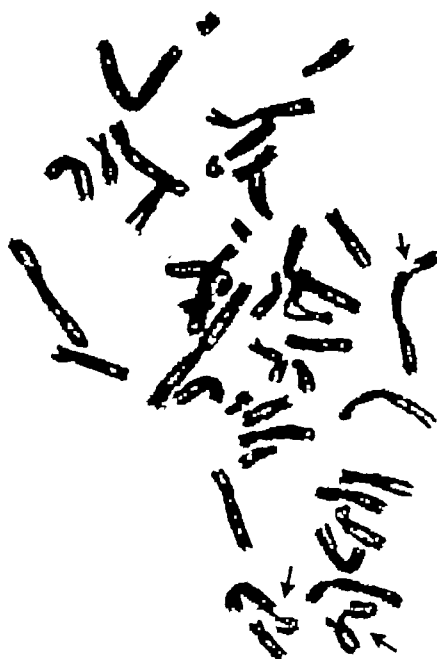
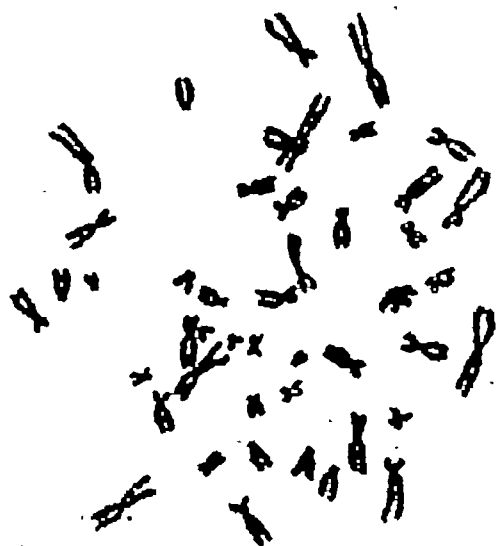
\* Numerous breaks seen in some cells.

† Three triradial configurations present.

some changes could be produced. It was concluded that these treatments damaged nuclei directly, which is possible in the case of acridines but not with methylene blue or toluidine blue. We have observed that in both *Vicia* root-tip cells and mammalian cells methylene blue and toluidine blue are concentrated in cytoplasmic organelles which in the case of mammalian cells can be definitely identified as lysosomes, as Brachet some time ago suggested<sup>8</sup>. The same cytoplasmic organelles in plant cells fluoresce flame red after uptake of acridine orange at concentrations about 1 in  $10^4$  while nuclei fluoresce green, and the fluorescing cytoplasmic organelles may be equivalent to those in plants showing particulate staining for acid phosphatase<sup>10</sup>. From these observations it seems likely that plant cells also have lysosomes, so that our interpretation of chromatid breakage could apply to plant as well as to mammalian cells.

The experimental results suggest that a cytoplasmic event, namely damage to lysosomes, can lead to structural alterations in chromosomes.

The most likely interpretation seems to be that DNase released from lysosomes can break the DNA which is the backbone of the uncoiled interphase chromatid. This enzyme very efficiently fragments lampbrush chromosomes<sup>11</sup> and chromatin from sea-urchin sperm<sup>12</sup>, and produces visible changes in parts of the polytene chromosomes in living *Drosophila* cells<sup>13</sup>. Other enzymes, includ-

Fig. 3. Chromosomes from a culture of WI-38 cells at passage 33 treated with neutral red (1 in  $10^4$ ). Arrows indicate a chromatid break and a triradial configuration.  $\times 1,875$ Fig. 4. Chromosomes from a culture of WI-38 cells at passage 25, treated with acridine orange ( $2$  in  $10^5$ ). Arrows indicate chromatid breaks.  $\times 1,320$ Fig. 2. Chromosomes from a control culture of WI-38 cells at passage 27.  $\times 1,375$ 

ing proteases and RNase, do not break chromosomes. In fact, lysosomal DNase has a remarkable property: it appears from the reaction kinetics to break both strands of a double-helix of DNA at the same time<sup>14</sup>. Simultaneous scission of both strands produced in this way would be much more difficult to repair than breaks in one of the two strands, as is characteristically produced by a single hit with pancreatic DNase<sup>14</sup>. Hence one or a very small

number of scissions by lysosomal DNase might give rise to breaks later visible in the coiled mitotic chromatids.

There are two main difficulties about postulating that lysosomal DNase produces chromatid breaks. The first is that the enzyme would have to enter the nucleus. There is evidence that this could happen: thus DNA-dependent RNA polymerase activity in isolated nuclei is immediately stopped by addition of DNase<sup>18</sup>. Moreover, shortly after infection with herpes simplex virus there is often considerable breakdown of host cell DNA<sup>19</sup>, which is apparently due to activation of lysosomal DNase<sup>17</sup>. It therefore appears that nucleases can enter the nucleus and act on its contents. The second objection is that lysosomal DNase has an acidic pH optimum, but the enzyme has, in fact, appreciable nuclease and phosphodiesterase activity at neutral pH<sup>18</sup>. Experiments with purified lysosomal DNase and inhibitors are planned and these, it is hoped, will provide more direct evidence for or against the hypothesis that this enzyme is involved in the production of chromatid breaks.

Another possible objection to our experiments is that free radicals formed after photon absorption in the lysosomes might be able to diffuse to the nucleus and damage its contents directly. This seems highly improbable. The concentrations of the photosensitizing agents used were very low ( $<10^{-8}$  M) and those of free radicals generated would be lower still. It is known that in the presence of protein and other reactive materials the life-times of such free radicals are exceedingly short<sup>9,10</sup>. Calculations show that the quantum efficiency of chromosome breakage is approximately the same whether the photosensitizer is present in the lysosomes only or in the lysosomes and nucleus.

The results of cytogenetical investigations are consistent with the view that a number of chemicals, including the standard alkylating agents and maleic hydrazide, produce effects similar to those described in this article, perhaps by a similar mechanism. Discussing the results of experiments with maleic hydrazide, Evans and Scott<sup>20</sup> conclude that probably breakage of both old and new strands of DNA in a chromatid occurs during the period of DNA synthesis. At this time the DNA, being fully extended for replication, would be particularly vulnerable to enzymatic attack, and the lysosomal enzyme could break both old and new strands of the helix at the same time. Experiments with synchronized cells have been started to ascertain at what stage of the replication cycle chromosomes are most sensitive to photosensitization damage. We do not imply that the only way in which chromatids are broken is enzymatic attack: clearly direct interactions with DNA or interference with repair are also likely to occur. But enzyme activation could represent an important contributory mechanism in chromosome damage caused by chemicals and radiations, the significance of which has so far been overlooked.

These results have a bearing on the suggestion made previously<sup>4</sup> that lysosomal damage might play a part in carcinogenesis. This suggestion was based on the observation that carcinogenic hydrocarbons are concentrated in lysosomes and can induce permeability changes in their

membranes. Likewise, viruses, ultra-violet and X-radiation and various chemical carcinogens and co-carcinogens are able to bring about release of enzymes from lysosomes. The results of our experiments suggest that the lysosomal enzymes can react with and produce lasting changes in genetic material. Some such change—not necessarily chromosomal—might transform a normal cell into a malignant cell. Since so many diverse agents can produce cancer it seems likely that some common reaction in cells is involved, and lysosomal enzyme activation seems at least as plausible an explanation as alternatives that have been much discussed, namely, protein binding and deletion by carcinogenic chemicals<sup>21</sup> or direct binding carcinogenic chemicals to nucleic acid<sup>22</sup>. Apart from the fact that the lysosomal concept has more generality, evidence has already been presented that activation of lysosomal enzymes can release cells from mitotic inhibition<sup>23</sup>. This view will be elaborated with more detail and further experimental evidence elsewhere; but clearly if such release from mitotic inhibition could be made permanent by elimination of the control mechanism, genetic or otherwise, malignancy might well ensue.

Chromosome aberrations in mammalian cells have now been seen after infections with a number of viruses<sup>4</sup>. Most of these viruses multiply in the nucleus and might damage its constituents directly. However, some viruses giving rise to chromosomal aberrations, such as yellow fever virus<sup>24</sup>, have no known phase of development in the nucleus. Nevertheless they do damage lysosomes (as shown by plaque formation) and chromosome breaks might therefore be produced by the mechanism suggested in this article.

We thank Dr. P. J. Walker for a pure sample of acridine orange, Dr. H. J. Evans for his advice, Mrs. P. Bradbury for the statistical analyses, and Mr. J. Clark and Mr. F. Wanless for the photomicrographs.

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## IRRIGATION OF PLANTS WITH ATMOSPHERIC WATER WITHIN THE DESERT

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THE desert soils are suitable, so far as the climate and the quality of the soil are concerned, for the growth of many forest tree species which can occupy most of the area but the expansion of which is prevented

solely by the lack of water. This shortage has given us the idea of exploiting water from dew and mist of irrigation of forest plants, and of concentrating the little rainwater available into the immediate area of the roots.

There are many species of xerophytes which can be utilized for afforestation even in desert regions where only 100–200 mm of rain fall annually, if they are irrigated for the first 2 or 3 years after planting. This conclusion is based on the results of afforestation in the Negev Desert over the past 20 years, and now woods, avenues and windbreaks of tamarisk, eucalyptus and Aleppo pine lend a touch of green to the grey, arid desert vista.

By exploitation of water from dew and mist, which is actually greatest in the desert, and by concentration of the rainwater in the root region, as is described in this article, it is possible to-day to plant endemic and domesticated xerophytes woody species at every place where such sources of water are available.

**Experimental areas.** The desert vegetation of Israel is defined phytogeographically as Saharo-Sindian, and many Saharan species are represented, such as *Acacia* sp., *Tamarix* sp., *Retama* sp., etc.

The investigation recorded here was limited to the sub-tropical zone of Israel and to those desert regions possessing the following characteristics: (1) A total annual rainfall of 25–200 mm (Beer-sheva-Eilat), limited to the cooler season. (2) The rainfall is limited to 8–10 days in dry years and 20–40 days in normal years. During the rest of the year, the sky is clear and the number of hours of sunlight is close to a world maximum. (3) Extreme annual fluctuations in the rainfall and frequency of drought years. (4) Scarcity of streams and rivers, apart from a gravitational flow from the mountains into the depressions

fluctuations were not only influenced by thermodynamic factors, but also by soil structure and topography (hills). (3) More dew is attracted by plants grown in light soil than in clay soil. (4) That measured in plantations was about half that in nearby exposed areas.

**Experimental Methods.** The water from the dew and mist was collected in exposed areas.

In order to ascertain the quantity of dew and mist falling nightly in different parts of the country and during different seasons, the apparatus illustrated in Fig. 1 was assembled. It was based on a calculation of the amount of water from dew and mist as a function of area. The apparatus was fitted up in the form of an inverted V with the ratio of height to width = 0.5 : 1.0. Each face was 2.5 m<sup>2</sup> in area and was covered with a sheet of polyethylene, 0.1 mm thick. Beside the shorter posts, at the edge of the gutter, were 2 polyethylene bottles into which the water was led through a pipe from a gutter. This 'roof' was supported on two pairs of posts of differing height, in the range of 40–60 cm, so as to form a slope which would lead the water into the bottles from the gutters. Polyethylene was chosen as an inactive water collector as it is non-hygroscopic and becomes cool at night. The flow into the bottles began when the sheet was sufficiently moistened, this preventing loss by evaporation. Apart from this means of measuring the water collected on the upper surface of the sheet, reversal of the 'roof' enabled the measurement of that forming on the underside, thus giving an overall estimate of the dew.

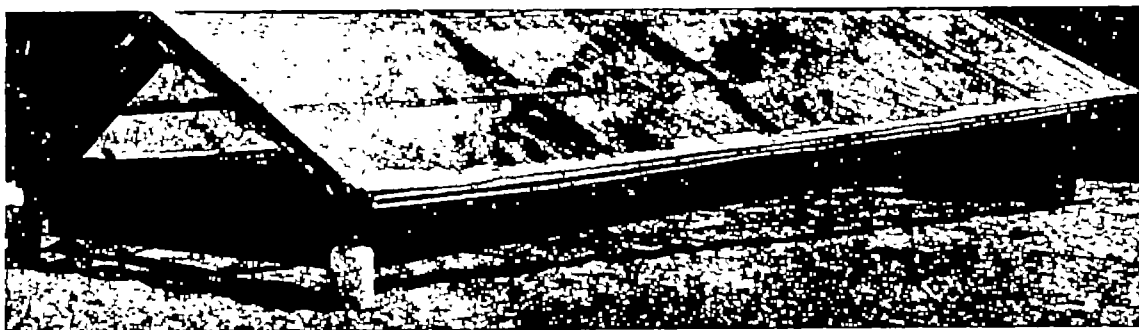


Fig. 1. Measurement of the water from dew and mist as a function of area. The apparatus is covered with polyethylene

and plains. This flow ceases after a few days as the water reaches the Dead Sea and the Red Sea. (5) An almost complete absence of sweet water suitable for drinking or for the use of plants.

Although there are local variations, there is a general distribution in the number of dewy nights<sup>1</sup> as follows: (1) An increase in the number of nights with dew and its quantity going southwards in the country; an annual variation between 5 mm—Dead Sea region and 150 mm—north Western Desert. (2) There are, in fact, the greater number of dewy nights during the hottest and driest months. (3) In the Western Desert, where the rainfall is lowest, the number of nights with dew reaches 250 annually (Gvuloth), 140 of these with 'heavy' dew. (4) Along the Mediterranean coast, in the Central Hula Valley and in the west-central part of the Valley of Israel the dew is heavy. (5) There are about 160 nights with dew in the hills with fluctuations depending on height and exposure; there is least on the eastern slopes.

Gilead and Rosenan<sup>2</sup> measured the number of nights with dew and the quantity collected at 80 meteorological stations in the country at the same time, over a period of 10 years, by means of Duvdevani's apparatus<sup>3</sup>, and reached the following conclusions: (1) The number of nights with dew is more or less constant, and the fluctuations are similar to those found in the rainfall. (2) There was no constancy between the various stations, and the

For irrigation of plants, polyethylene sheets, 1.5–2.0 m<sup>2</sup> in area, were arranged at a slope of 25–30 per cent (Fig. 2) to facilitate flowing of the water and its transportation to the plant-pit. Plastic ducts were attached to the lower ends of the sheets. To create the necessary slope the soil was ridged or, alternatively, the sheets were placed on wooden board.

**Amount of 'Flowing' Dew and Mist per Unit Area.** Some nights were completely dewless; on others, although drops condensed on the sheet, the amount was insufficient to lead to a flow through the gutters into the bottles. The flow of water from dew and mist into the measuring bottles of the apparatus begins when the sheet has become appreciably covered with drops. Table 1 indicates the 'flowing' water collected per square metre of polyethylene. This flow differed in 3 places belonging to different climatic regions (Revivim—desert, Eshtaol—sub-tropical hills, and Rehovot—semi-arid plain), with most at Revivim and least at Rehovot.

The apparatus was arranged with its two planes facing north and south, respectively, and here, also, differences emerged. Table 2 shows the greater flow from the north face at Revivim and Rehovot, and from south face at Eshtaol. In the summer of 1964, however, the southern aspect gave a greater flow at Rehovot.

**Irrigation of Forest Saplings with Atmospheric Water.** In 1962, 8–10-month-old saplings of the following species

were planted at Rehovot and in the Judean Hills (Hulda): *Pinus halepensis*, *P. brutia*, *Cupressus horizontalis*, *Eucalyptus gomphocephala*. *Tamarix aphylla* seedlings were planted in dunes south of Beersheba. Near each plant was arranged a plastic sheet, 1.3 m<sup>2</sup> in area as described previously. The dew was led to the plant-pit through a plastic gutter.

The experiments were repeated in the summer of 1963 and, apart from those saplings planted in the rainy season, others, of the same species, were also planted in August, when the conditions (heat and drought) are unsatisfactory for survival. One group was provided with atmospheric water by means of the plastic sheets, while the other was planted nearby without such aid. While the former survived until the winter rains, the latter had dried up by the beginning of September.

**Concentration of Rainwater by Means of Plastic Sheets.** The same plastic sheets which had proved useful for the collection of dew and mist were also used for the collection of rainwater. Table 3 shows the slight and unreliable rainfall in the desert over the past 10 years with fluctuations from 82 to 177 mm at Revivim, and from 100 to 255 mm at Gvuloth. These figures also include a number of light showers, of 0.5–1 mm, which only wet the upper 1–2 cm of the soil. This moisture evaporates within a few days, owing to the strong sunshine between the showers even during the rainy season. By concentrating this rainfall, its effectiveness can be increased. For example, a sheet of 5 m<sup>2</sup> area supplying a pit of 1 m<sup>2</sup> area may provide 5 times as much water as when water falls on a similar pit which is not provided with a sheet and, consequently, a 5-fold percolation. In fact, the percolation depends on the physical properties of the soil, especially its permeability, which is greatest in dunes, and least in loess.

**Discussion.** The collection of atmospheric water and the trapping of rainwater, by means of hygroscopic material (polyethylene or polyvinyl chloride), increase the sylvicultural potentialities of desert and semi-arid regions.

It was found that 20–30 l. of atmospheric water flowing into the plant-pit from each square metre of the polyethylene roof was sufficient for the continued existence and for some growth of the tree xerophytes used in this work, during the first summer after planting. It is possible, also, to plant woody xerophytes, such as Aleppo pine, *Tamarix* sp., and *Eucalyptus* sp. and others, even in midsummer (July–August) when the amount of dew

Table 3 YEARLY RAINFALL (MM) AND NUMBER OF RAINY DAYS AT TWO LOCATIONS

Year	Location	
	Revivim	Gvulot
1953/54	113.2 (27)	—
1954/55	92.0 (19)	148.8 (19)
1955/56	130.3 (36)	177.0 (39)
1956/57	177.5 (34)	224.5 (43)
1957/58	32.4 (22)	100.3 (25)
1958/59	120.5 (32)	142.3 (22)
1959/60	44.1 (16)	—
1960/61	104.4 (32)	111.5 (8)
1961/62	71.8 (26)	162.1 (24)
1962/63	32.8 (8)	48.9 (20)
1963/64	164.9 (33)	248.7 (38)

reaches maximum, and they can be maintained without wilting so long as they are provided with moisture from dew and mist until the advent of the rains. In such places as the deserts of Chile and Peru, for example, this is even more important, since the mists may reach an annual value of 300–400 mm, while the actual rainfall does not exceed 40 mm.



Fig. 2 Irrigation of pine, tamarisk and eucalyptus plants with atmospheric water. At the edge of each polyethylene sheet is a gutter fitted with a small plastic pipe which leads the water into the plant-pit.

Table 1. MONTHLY AMOUNT OF 'FLOWING' DEW AND MIST (CC) PER SQUARE METRE OF POLYETHYLENE IN 1964

Month	Location		
	Rehovot	Revivim	Bahtal
July	1,148	1,608	1,222
August	1,648	2,653	2,048
September	876	1,643	1,555
October	2,056	2,631	2,684
November	862	1,270	968

Table 2. 'FLOWING' DEW AND MIST (PER CENT) FROM THE NORTHERN AND SOUTHERN SLOPES OF THE INSTRUMENT IN 1963

Month	Rehovot		Location		Revivim	
	North	South	North	South	North	South
August	50.41	49.59	55.8	54.2	60.7	39.3
September	54.72	45.28	47.9	52.1	64.5	35.5
October	51.58	48.42	47.9	52.1	50.9	49.1
November	61.18	38.82	49.1	50.9	61.5	38.5
December	60.64	39.36	—	—	50.52	49.48

The arbori-sylviculture possibilities in the desert may also be increased by the concentration of the little rainwater available in the region of the root. Here, also, the efficiency depends on the area of the sheet, and it can be adapted to the species. If, for example, a certain region would allow the growth of any forest tree species so far as the soil and climate are concerned, but the annual rainfall is only 200 mm instead of the 1,000 mm necessary, it is possible to increase the rainwater available by leading the run-off from a 5 m<sup>2</sup> sheet into a plant-pit of 1 m<sup>2</sup> area.

The xerophytes which are being used to-day in afforestation in Israel can be planted in any place where there is sufficient dew and mist to cause a flow into the plant-pit; this method overcomes the need for transportation of water by tankers or the installation of irrigation systems. This collection of rainwater also serves to wash out the salts which have become concentrated in the upper soil layer, especially due to the low chlorine content of atmospheric water.

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# REGULATION OF PROTEIN AND NUCLEIC ACID SYNTHESIS BY GIBBERELLIN DURING LEAF SENESCENCE

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**D**URING senescence in leaves, yellowing and loss of chlorophyll occur together with a progressive decline in the rates of synthesis of protein and nucleic acid. These changes can be retarded by the addition of certain plant growth regulators. Thus when excised leaf disks of *Xanthium* or *Nicotiana*<sup>1,2</sup> are supplied with a synthetic kinin or when those of *Prunus*<sup>3</sup> are treated with an auxin the incorporation of radioactive precursors into protein and RNA is maintained or even enhanced and senescence of the disks is retarded, although no other additional substrates are supplied. These observations have led to the proposal<sup>4</sup> that senescence in leaf cells is associated with the hormonal regulation of protein and nucleic acid synthesis and that both auxins and kinins retard senescence by maintaining the DNA as a functional template for DNA-dependent synthesis of RNA.

The hormonal content of leaves declines as they grow older. Where retardation of senescence can be brought about by the addition of synthetic auxin or kinetin it seems most probable that senescence is causally related to a limiting concentration of an endogenous auxin or kinin.

Evidently the hormone needed to retard senescence is not the same for all species, for an auxin will not retard leaf senescence in *Xanthium*<sup>5</sup> and kinetin is ineffective when supplied to *Prunus*<sup>3</sup>. So far, however, there is little information concerning the regulation of biochemical processes of senescence by another major group of endogenous hormones, the gibberellins.

Although it has been observed that gibberellin (GA) delays autumnal yellowing in some deciduous trees<sup>6</sup> and to some extent increases longevity of leaves of sugar beet<sup>7</sup>, no analyses of the physiological or biochemical effects have been made.

These aspects have now been studied in a wide range of species and it is clear that senescence can be effectively retarded in leaves of *Taraxacum officinale* by either gibberellin A3 or by kinetin. The effects of GA appear to be species specific, for in 17 other plants studied, no retardation of leaf senescence was observed. Some of the experiments on GA regulation of senescence processes in *Taraxacum* are now reported.

Mature leaves were gathered from wild plants, and disks punched from interveinal portions of the blades. Matched disks were placed in Petri dishes on filter paper moistened with equal volumes of either water, or an aqueous solution of GA. The dishes were maintained at high humidity and incubated in darkness at 25° C. After four days the chlorophyll was extracted with hot 80 per cent ethanol and the optical density of the solutions measured at 665 mμ, the absorption peak of chlorophyll *a*. The values for the optical densities of the chlorophyll extracts from disks treated in various concentrations of GA are plotted in Fig. 1. The chlorophyll content of the control disks drops to less than half during the experimental period whereas in the disks treated with GA most of the chlorophyll is retained. GA concentrations

from 10 to 50 mg/l. appear to be optimal and 25 mg/l. has regularly been used in the biochemical studies.

With other species the addition of auxins or kinins<sup>1-4</sup> during senescence not only delays the loss of green colour in the leaves but also retards the fall in levels of total protein and RNA. The following experiment, based on the method described, was designed to ascertain whether GA maintains the protein and RNA levels in *Taraxacum*. Duplicate sets of disks cut at the start of the experiment, and disks that had been treated with water or GA for 4 days, were macerated and extracted, first in 80 per cent ethanol, then with 5 per cent TCA at 0° C, and finally with absolute ethanol and ethanol:ether (3:1 v/v). For protein determinations, the residue was subjected to N NaOH at 100° for 4.5 min to solubilize the protein, and an aliquot of the solution assayed by the biuret method. RNA was estimated in separate sets of disks. The residue remaining from the final extraction with ethanol:ether was hydrolysed with 0.3 N KOH for 18 h, the DNA precipitated by acidification to pH 2.0 with HClO<sub>4</sub>, and the RNA determined in the supernatant fraction by the orcinol reaction.

It is seen from Table 1 that after 4 days the contents of chlorophyll, protein and RNA in the disks that received only water have fallen considerably below the original level, while in those treated with GA this fall has been arrested, though not wholly prevented.

Table 1. CHLOROPHYLL, PROTEIN AND RIBONUCLEIC ACID CONTENT IN LEAF DISKS OF *Taraxacum officinale*, AFTER TREATMENT WITH 25 MG/L. GIBBERELLIN (GA) OR WATER

Sample	Treatment	Chlorophyll optical density at 665 mμ	Protein (mg)	RNA (μg)
0 day	—	98	4.22	440
4 day	GA	73	3.06	353
4 day	H <sub>2</sub> O	43	2.11	304
S.E.		3.2	0.12	19.6

To establish whether GA retards the fall in protein level, by preventing the breakdown of already existing protein, or by maintaining its synthesis, the incorporation of <sup>14</sup>C-leucine into protein was determined after disks had been pretreated with either water or GA for 1 day.

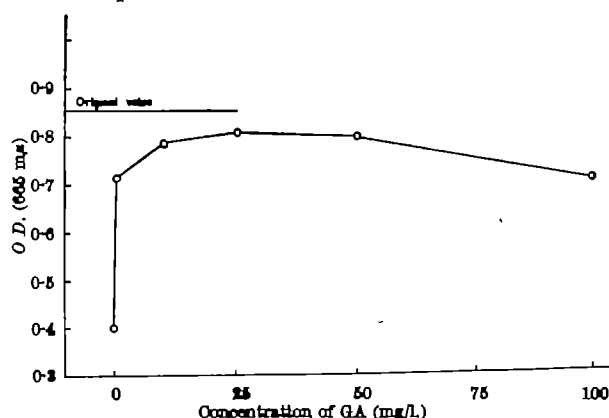


Fig. 1. Retardation of chlorophyll degradation in leaf disks of *Taraxacum officinale* by gibberellin (GA). Chlorophyll content expressed as optical densities (O.D.) of 80 per cent ethanol extracts at 0 and 4 days

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A smaller disk was then excised from each original disk, so that all the disks presented freshly cut edges, and they were then floated on 1 ml. of aqueous medium containing 1.5  $\mu$ c. of  $^{14}$ C-leucine and 40,000 units of penicillin. After 3 h the disks were quickly rinsed and the protein extracted.

An aliquot of the protein solution was transferred to a planchet and the radioactivity was determined. These values were compared with those for the incorporation of  $^{14}$ C-leucine by comparable sets of leaf disks cut directly from the same leaves.

It is evident (Table 2) that after one day both the incorporation of  $^{14}$ C-leucine into protein and the specific activity of the protein in the disks supplied only with water is less than the initial values, indicating a lower rate of protein synthesis after one day. In the disks receiving GA, however, the synthesis of protein after one day is not only maintained but also enhanced, and the specific activity is higher than that on the initial day.

It is clear from another experiment (Table 3) that the synthesis of RNA is also stimulated by the addition of GA. The incorporation of  $^{14}$ C-adenine into RNA and the specific activity of the RNA are both significantly enhanced by pretreatment with GA for only 1.5 h. If actinomycin D is included in the medium before and during treatment with GA, the total incorporation of the  $^{14}$ C-adenine added later is reduced and the stimulation due to GA is abolished. Other experiments have

Table 2. INCORPORATION OF  $^{14}$ C-LEUCINE INTO PROTEIN IN LEAF DISKS FROM *Taraxacum officinale* FOLLOWING TREATMENT WITH GIBBERELLIN (GA)

Sample	Treatment	Protein mg	Total counts/min	Protein specific activity
0 day	—	4.81	13,075	3,025
1 day	H <sub>2</sub> O	4.06	10,275	2,525
	GA	4.56	14,650	3,200
S.E.		0.13		60.4

Disks pretreated with either gibberellin (25 mg/l.) or water for 1 day and incubated with  $^{14}$ C-leucine (U) (10.7 mc./mM) for 3 h.

Table 3. EFFECT OF GIBBERELLIN (GA) ON THE INCORPORATION OF  $^{14}$ C-ADENINE INTO RNA IN THE PRESENCE OR ABSENCE OF ACTINOMYCIN D (AM) IN LEAF DISKS OF *Taraxacum officinale*

Treatment	RNA $\mu$ g	Total counts/min	RNA specific activity
Am + GA	451	1,576	3.48
Am + H <sub>2</sub> O	440	1,512	3.44
GA	446	2,800	6.28
H <sub>2</sub> O	446	2,240	5.01
S.E.	21.4		0.15

Disks were pretreated in either H<sub>2</sub>O or Am (50  $\mu$ g/ml.) for 1.5 h at 25°. GA (to give 31 mg/l.) or H<sub>2</sub>O was then added to one set of disks from each pretreatment and incubation continued for 1.5 h. Adenine-8- $^{14}$ C sulphate (31.3 mc./m.mole) was then added to each treatment (to give final concentrations of  $^{14}$ C-adenine 2  $\mu$ c./ml., GA 25 mg/l. and Am 40  $\mu$ g/ml.) and incubation continued for 3 h.

shown that when this concentration of actinomycin D is added after the treatment with GA, the GA stimulation of RNA synthesis is fully maintained.

These findings indicate that the retardation of leaf senescence by gibberellin in *Taraxacum* is like that of auxin<sup>4</sup> and kinetin<sup>5</sup> retardation in other species, closely linked to a regulation of protein and RNA synthesis. These findings also support the contention that hormonal retardation of leaf senescence is associated with the maintenance of the DNA as a functional template for DNA-dependent synthesis of RNA. A fuller account will be published elsewhere.

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## PLANT GROWTH RESPONSE IN AN ELECTROKINETIC FIELD

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IT has been shown previously that plants grown in an electrostatic field of sufficient magnitude have reduced ability to grow, as compared with corresponding control plants which did not experience such potential gradients<sup>1</sup>. In the investigation to be reported here, similar, but more severe, plant-leaf damage has been observed in yellow bush beans grown in a continuous 60-c/s alternating electric field. The extent of plant and leaf damage has been observed to be directly related to the magnitude of the electric potential gradient.

These experiments were conducted in a specially designed environmental chamber. The chamber was constructed of glass-panelled aluminium, air vented, and lit artificially with a bank of 100-watt incandescent lamps arranged to produce near daylight intensities at the soil surface in the experimental plot. Day length in all experiments was extended to 12 h, and soil moisture was rigidly maintained at  $12 \pm 1$  per cent by weight. A sand-humus-soil composite was used as the soil base. Chamber temperature and soil temperature were closely checked throughout all experiments to ensure identical thermal variations for all experimental data obtained. High-voltage power was continuously supplied during the entire 11-day period by a high-voltage a.c. testing transformer. The high-voltage experimental plot, which was placed in the chamber, was constructed entirely of polystyrene and epoxy-coated wood. The anode or upper electrode was made of an aluminium screen mesh; and

the cathode or soil base-plate was solid sheet aluminium which covered the base of the 300-in.<sup>2</sup> plot. This design was identical to that used in my previous work<sup>2</sup>.

Yellow bush beans were planted as seed directly into the field plot. The duration of each experimental run, determined by preliminary investigation, was established as 11 days. This period allowed the seeds to germinate, and the plants to attain heights just below the anode height of 12 in. above the soil surface. At least 25 seeds were planted, at 1-in. spacing, in each experimental run. The germination in these experiments is shown in Table 1, where electric field strength is represented as relative potential gradient in the manner established in previous investigations<sup>3</sup>. It should be apparent from Table 1 that germination is unaffected by an electrokinetic field as contrasted with supposed differences observed by Blackman *et al.*<sup>4</sup> for seeds germinated in a d.c. field.

Plant growth response, however, was very definitely affected by the electrokinetic field. Fig. 1 illustrates rather consistent plant growth retardation as a function of the relative potential gradient expressed in kV/m. The

Table 1. YELLOW BUSH BEAN GERMINATION IN AN ELECTROKINETIC FIELD

Relative potential gradient (reference)	Germination (%)
20 kV/m	89
40 kV/m	96
60 kV/m	78
80 kV/m	96
Control	90

\* Average of two replications.

growth retardation trend established in Fig. 1 is somewhat amplified by a dry weight analysis of plant leaves as shown graphically in Fig. 2. In Fig. 2, it is observed that leaf growth is significantly stimulated in the range 10–50 kV/m, while leaf yield on a dry weight basis decreases significantly at higher field gradient magnitudes.

Comparing the results of this research with previous results for grasses grown in an electrostatic field<sup>1</sup> suggests that the mechanism of destruction is similar, but plant damage is generally not a consistent phenomenon as illustrated in Fig. 2. It would seem, therefore, that the basic mechanism which initiates leaf damage in plants grown in an electrokinetic field is a type of field ionization action, as suggested earlier for grasses grown in an electrostatic field<sup>1</sup>. Such a field action is not associated with

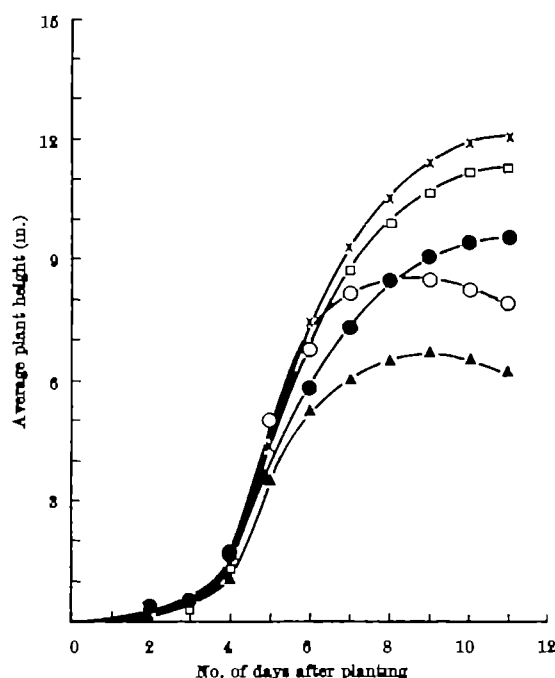


Fig. 1. Plant growth-response curves for yellow bush beans grown in an electrokinetic field of various potential gradients. x, Control, □, 20 kV/m, ●, 40 kV/m, ○, 60 kV/m, ▲, 80 kV/m.

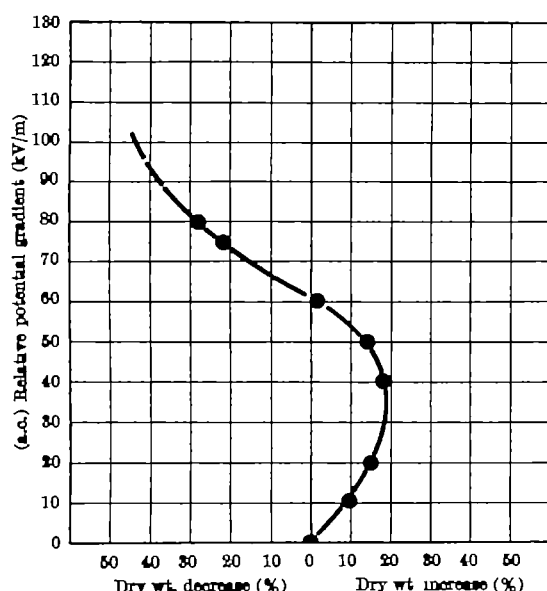


Fig. 2. Yellow bush bean leaf response based on an average of two dry weight comparisons of activated leaves at each electrokinetic potential gradient condition, and control leaves of an experimental run at the beginning and the end of the activated series.

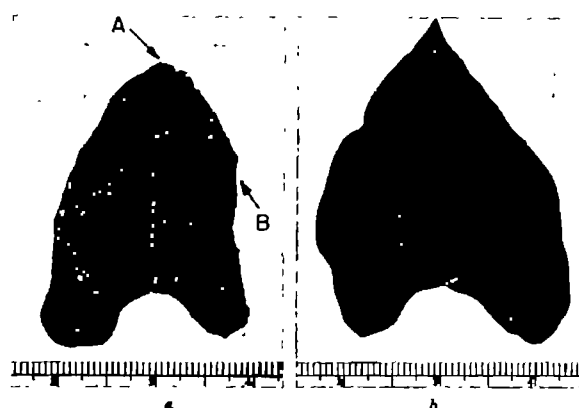


Fig. 3. Photographs of typical yellow bush bean leaves, (a) activated plant leaf at an electrokinetic field gradient of 60 kV/m. Cell damage and tissue rupture are shown at the tip (A), and edge shrinkage caused by tissue dehydration due to the electrokinetic field action as shown at (B); (b) control leaf.

polarization of inorganic radicals in the plant biosystems, but rather a direct elastic rupture of epidermal cells by a type of molecular field fatigue. Such a process would be considerably amplified by an alternating field as compared with direct field action; and this has essentially been shown in the results presented in Fig. 1. However, it is apparent from Fig. 2 that the growth response based on leaf weight is affected by the potential gradient of the field.

Further proof of epidermal rupture by field action is presented in the photographs of Fig. 3. Leaf-tip and edge damage of a typical bush bean leaf exposed to a relative potential gradient of 50 kV/m is shown in Fig. 3 (a), and compared with an equally typical control (unexposed) leaf in Fig. 3 (b). Tissue disruption, elastic shrinkage, and leaf dehydration are unmistakable in (a).

These results provide evidence that the enzyme activity detected in plants grown in an electrostatic field<sup>1</sup> is associated with plant metabolic and respiratory acceleration, resulting from the direct epidermal destruction by the electric field action. Thus, following the initial exposure of leaf tissue and internal systems to electrokinetic field action, it is possible that more complete leaf damage results from the over-stimulation of respiratory enzymes, which are automatically concentrated in the leaf tissue by the metabolic recovery mechanisms. Similar cell damage by enzyme overstimulation has been observed for simple yeast cells by Schulke<sup>2</sup>.

The results presented in Fig. 2 can perhaps be interpreted in terms of such an enzyme stimulation. That is, for potential gradients of a moderate level (10–50 kV/m) leaf damage due to tissue rupture is not of a sufficient level to cause total leaf destruction. Respiratory stimulation is probably at a level where the plant is actually growing at a high rate. As the field strength is raised, the over-stimulation becomes 'toxic' to the leaf metabolism as suggested for grass plants grown in an electrostatic field<sup>1</sup>. This enzyme toxicity, combined with increased epidermal damage due to direct field evaporation of leaf tissue molecules, produces decreased yields, and eventual plant destruction. This phenomenon would seem to indicate that growth stimulation is possible in an electrokinetic field if the potential gradient is properly adjusted to eliminate severe leaf damage.

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# LETTERS TO THE EDITOR

## ASTROPHYSICS

### Equatorial Angle Distribution between the Electrons captured in the Outer Van Allen Radiation Belt

FROM the measurements registered by *Explorer VI* in the outer Van Allen radiation belt, C. Y. Fan, P. Mayer and J. A. Simpson<sup>1</sup> succeeded in determining the number of particles which, in their longitudinal motion between two magnetic mirrors, cross the equator with a given pitch angle. In the polar diagram (number of particles versus equatorial pitch angle,  $\chi_e$ ) obtained by those authors a maximum exists for the equatorial pitch  $\chi_e = 35^\circ$ . This maximum has a sort of dynamical stability, because it is restored after a magnetic storm. In fact the authors observed a perturbation of the magnetic field, which shifted the maximum from  $35^\circ$  to  $48^\circ$ , but the value  $\chi_e = 35^\circ$  was resumed in spite of the fact that the number of electrons with a given velocity  $v$  was changed by a factor about 3.8.

The trajectories corresponding to  $\chi_e = 35^\circ$  possess some properties worthy of note. Considering the geomagnetic field as produced by a dipole, and using Alfvén's perturbation theory<sup>2</sup>, we can write the following equation for the angle  $\chi$  between the velocity  $v$  and the magnetic field  $H$ , as a function of the magnetic latitude  $\varphi$ :

$$\cotg \chi = \frac{v_{\parallel}}{v_{\perp}} = \sqrt{\frac{H_i}{H} - 1} = \sqrt{\frac{\eta_i}{\eta} - 1}$$

where

$$\eta = \frac{\sqrt{1 + 3 \sin^2 \varphi}}{\cos^2 \varphi}$$

The index  $i$  is related to the quantities calculated for the mirror point. Taking  $\varphi = 0$ , we find a relation between the equatorial pitch  $\chi_e$  and the latitude  $\varphi_i$  at the mirror point:

$$\cotg \chi_e = \sqrt{\eta_i - 1}$$

Moreover, the inclination  $\psi$  of  $H$  on the equator and the latitude  $\varphi$  are related by the formula:

$$\tan \psi = 1/3 [\cotg \varphi - 2 \tan \varphi]$$

The guiding centre of the particle performs its motion as a material point bound to a line of force of the geomagnetic field and is reflected at the mirror points. When  $\chi_e = 35^\circ$ , according to the formulae (Figs. 1 and 2), the mirror points lie near the magnetic latitude

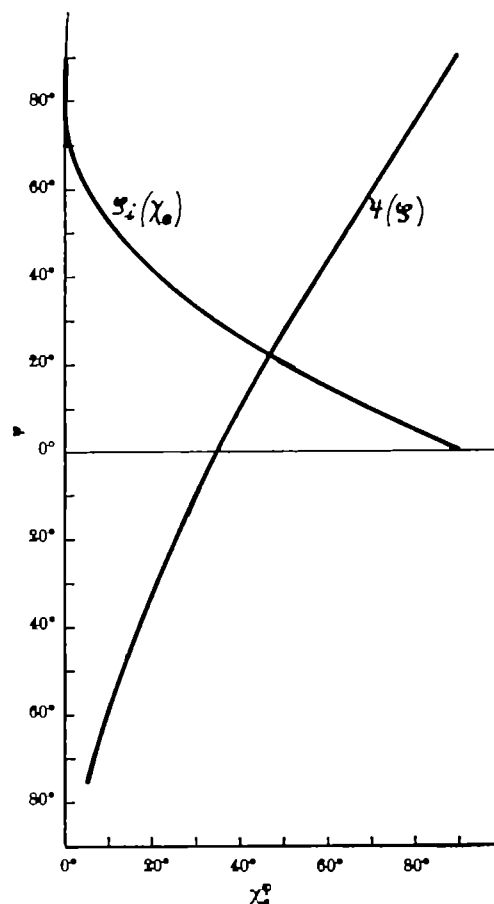


Fig. 2

$\varphi = 35^\circ$ , where the magnetic field is parallel to the magnetic equator and where the dipole equivalent to the gyrating particle is perpendicular to the terrestrial dipole. So the axis of the gyrating particle oscillates at  $180^\circ$  in a half longitudinal period. Moreover, the mirror points coincide with the points of the line of force where the distance from the magnetic equator attains its maximum.

The latter circumstance suggests the existence of a repulsing force from the equator; but the question is not

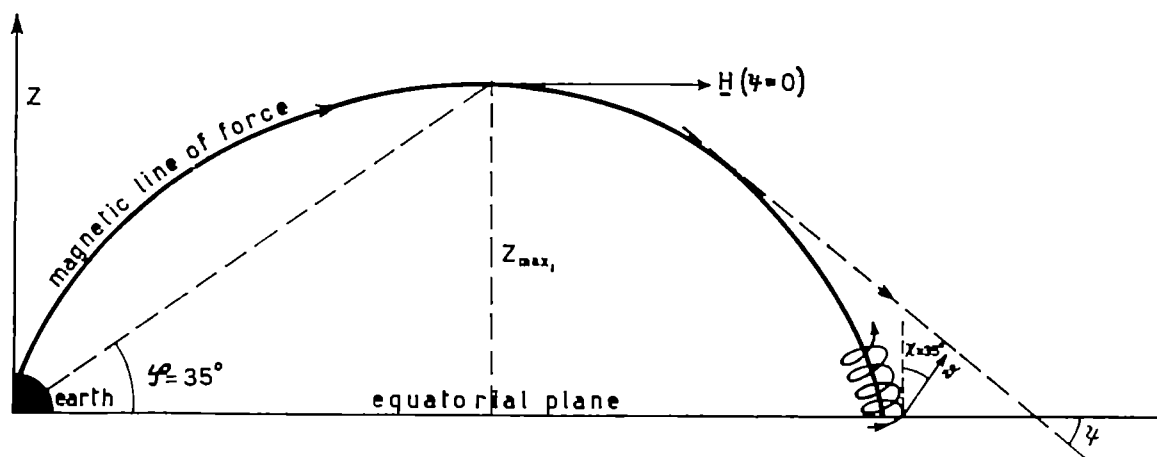


Fig. 1

easy to deal with from the dynamical point of view. The quasi- (but not exact) coincidence could be a result of the approximation, both theoretical and experimental, or perhaps an essential feature of the phenomenon.

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### Polarization Effects on the Profile of the H $\beta$ Line in the Light from the Star $\gamma$ Ursa Majoris

DURING February 1965 we observed some absorption lines in the spectra of several stars in orthogonal planes of polarization. The measurements were made with a photoelectric grating spectrometer<sup>1</sup> to which a rotatable 'Polaroid' had been added after the exit slit. The instrument was used at both the Newtonian and Cassegrain foci of the 48-in. telescope of the University of Padua's Observatory at Asiago, Italy.

Differential polarization effects across the absorption lines were sought by looking for departures from the instrumental polarization curve as the spectrometer was scanned.

Ohman<sup>2</sup> has shown that, in the case of rapidly rotating early-type stars, variations of polarization might appear across the absorption lines, and that, for a simple model, the degree of polarization might attain 0.8 per cent at the centre of the profile.

Considerations of the photometric accuracy required to measure an effect of this size make photographic spectrometry unlikely to succeed. However, calculations showed that polarizations of this order might just be detected by the instrument mentioned here, using a 1-10-sec integration time, provided the stars were brighter than 6th magnitude.

Observations of five stars were made at the Newtonian focus, the Cassegrain not being normally available for auxiliary instruments. As a result of a preliminary analysis, it seemed that  $\gamma$  (A0 ve, 2.5) and  $\eta$  (B3, 1.9) Ursa Majoris were worthy of further investigation. We were extremely fortunate in having the Cassegrain focus made available to us and decided to observe  $\gamma$  Ursa Majoris, it being the more suitably placed star at the time of observation.

Of the ten 1-Å resolution scans taken of the H $\beta$  line of this star, six were taken with the 'Polaroid' transmission axis perpendicular to the exit slit and four with it parallel to the slit. Ratios were formed of the corresponding ordinates of these two sets. The smooth change of this ratio with wave-length represents the variation of the overall instrumental polarization. However, in the neighbourhood of the H $\beta$  absorption, there are marked deviations from this smooth curve. It can be shown that if  $R(\lambda)$  represents the ratio of corresponding ordinates from orthogonal scans at wave-length  $\lambda$ , and  $R_*(\lambda)$  the ratio which it is assumed would have been obtained in the absence of source polarization (that is, interpolated values from the smooth curve), then:

$$P(\lambda) = \frac{R(\lambda) - R_*(\lambda)}{R(\lambda) + R_*(\lambda)}$$

where  $P(\lambda) = p(\lambda) \cos 2\varphi(\lambda)$ ,  $p(\lambda)$  being the degree of polarization and  $\varphi(\lambda)$  the angle formed by the direction of the polarized component of the light from the source and the transmission axis of the 'Polaroid'. The value of  $p(\lambda)$  can only be separated from  $P(\lambda)$  if the absolute instrumental polarization is known. Values of  $P(\lambda)$  were evaluated at 1.5-Å intervals across the line. Both the H $\beta$  profile and  $P(\lambda)$  are shown in Fig. 1. The values of

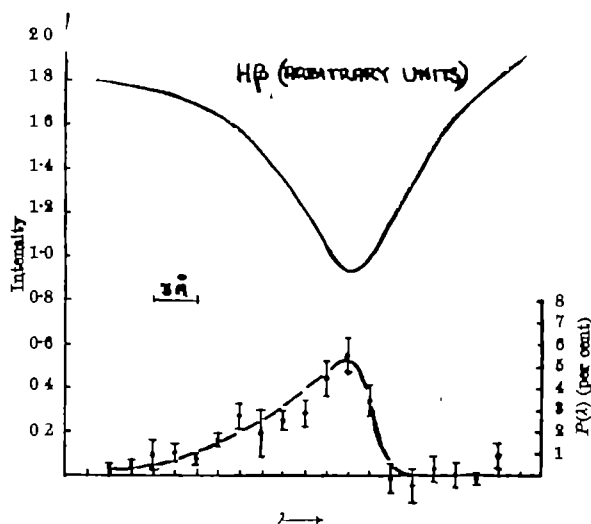


Fig. 1

$P(\lambda)$  are the means of the data and corresponding standard deviations are shown by the bars.

We conclude that:

(1) A differential polarization effect has been observed in the region of the H $\beta$  line in the light from  $\gamma$  Ursa Majoris. Since  $p(\lambda) > P(\lambda)$ , the differential polarization across the line must be at least 5 per cent. The magnitude of the effect is surprisingly large if the processes suggested by Ohman<sup>2</sup> are occurring. A variation of polarization of this magnitude should just be detectable by photographic spectrometry and, in this connexion, it is interesting to note that Ohman<sup>2</sup> found 'faint' polarization effects in the H $\gamma$  line of  $\beta$  Lyrae.

(2) The angle  $\varphi(\lambda)$  must have been different from  $\pi/4$  since the effect was observed. If  $\varphi(\lambda)$  is independent of  $\lambda$  over this region, then the form of  $p(\lambda)$  and the line profile exhibit the same asymmetry.

We thank Prof. J. Ring for facilities in his department and Prof. L. Rosino and the staff of the University of Padua's Observatory for their co-operation. This work was supported by the Department of Scientific and Industrial Research.

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## PHYSICS

### Narrow Spectral Emission from a Passively Q-spoiled Neodymium-glass Laser

THE spectral behaviour of the Nd-glass laser has recently attracted much attention. Normally the output of the Nd-glass laser consists of a broad band of wave-lengths (approximately 50 Å wide) composed of hundreds of lines centred about 1.06  $\mu$  (see Fig. 1A). This structure has been described in detail by Snitzer<sup>1</sup>. Time-resolved spectra<sup>2,3</sup> and investigations of the pumping energy dependence of the output band-width<sup>4</sup> all may be interpreted on the basis of inhomogeneous behaviour of the Nd<sup>3+</sup> emission. The purpose of this communication is to report the efficient generation of a single narrow spectral line from a giant pulsed Nd<sup>3+</sup> glass laser by passive Q-spoiling.

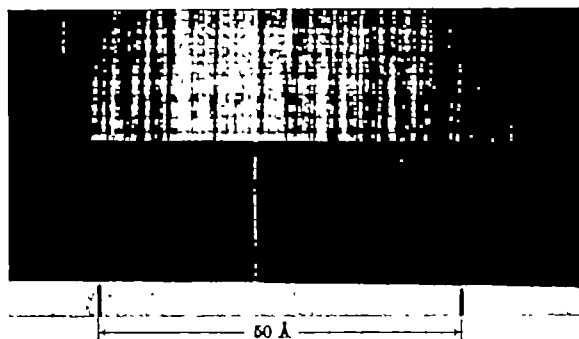


Fig. 1. Spectral emission of  $\text{Nd}^{3+}$ -doped glass. A, Conventional emission; B, passively Q-spoiled emission. Wave-length increases to the left. The line-width in this figure is slit-limited.

The experiments were carried out with a  $\frac{3}{8}$  in.  $\times$  4 in. Corning glass rod. Q-spoiling was accomplished using a reversibly bleachable polymethine dye absorber<sup>4</sup>. In conventional non-Q-spoiled laser operation at energy inputs approximately 10 times threshold, outputs of 5 joules were obtained. Introduction of the Q-spoiling dye resulted in 0.5 joule (50 MW peak power) giant pulses of 10 nsec duration with remarkably narrow spectral width of 0.02 Å. Energies were measured calorimetrically and, in order to ensure that only a single giant pulse was obtained, each shot was monitored with a fast-rise Korad KD1 photodetector.

The spectra of conventional laser emission and passively Q-spoiled laser emission as observed with a 3.4 metre Jarrell-Ash spectrometer are shown in Figs. 1A and 1B. A Fabry-Perot interferometer was used for line-width measurements. The wave-length of the single narrow line obtained with each giant pulse varied between 10,600 Å and 10,624 Å. However, the line-width as measured with the Fabry-Perot interferometer was consistently observed to be  $< 0.02$  Å.

This result is noteworthy, in that the results of previous workers<sup>1-3</sup>, which may be interpreted on the basis of inhomogeneous behaviour of the  $\text{Nd}^{3+}$  emission, need to be contrasted with the present results which imply homogeneous behaviour to the extent that cross-relaxation within the band exists with characteristic time less than  $10^{-8}$  sec. We have observed similarly narrow spectral output from ruby lasers Q-spoiled with bleachable dye<sup>4</sup>, bleachable glass, or semi-conductor mirrors.

We thank O. R. Duncan for aid with the spectroscopic measurements and H. Kimura for preparing the Q-spoiling dye.

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### Thiometallates of the Group-eight Metals

Rhodium III, palladium II, iridium III and platinum IV form simple thiometallates. There is some evidence that iron II, cobalt II, nickel II, ruthenium and osmium (both possibly IV) also form such compounds. Palladium, platinum and iridium also form polythiometallates.

Several thiometallates are listed in the literature<sup>1-3</sup> for the group-eight metals:  $\text{Na}_2\text{PdIVS}_4$ ,  $(\text{NH}_4)_2\text{PdS}_{11}$ ,  $\frac{1}{2}\text{H}_2\text{O}$ ,

$(\text{NH}_4)_2\text{PtS}_{11} \cdot 2\text{H}_2\text{O}$  and  $(\text{NH}_4)_2\text{IrS}_{11}$ . Thiometallates based on the simpler metallates are also known for many adjacent transition metals, for example,  $\text{Na}_2\text{VS}_4$ ,  $\text{Na}_2\text{MoS}_4$ ,  $\text{Na}_2\text{CuS}_4$ , etc., but very little else is known about them except the conditions for the precipitation of sulphides in chemical analysis<sup>4,5</sup>. If the common chloro-acids of ruthenium, osmium, rhodium, iridium, palladium, and platinum are dissolved in sodium carbonate or hydroxide, treated with hydrogen sulphide, and the solution filtered, yellow, brown, or red solutions are formed which on acidification deposit the following sulphides:  $\text{RuS}_2$  at pH 9.6,  $\text{OsS}_2$  at pH 0,  $\text{Rh}_2\text{S}_3$  at pH 0,  $\text{Ir}_2\text{S}_3$  at pH -0.2,  $\text{PdS}$  at pH 0, and  $\text{PtS}$  at pH 0. On partial acidification, sodium thionitrate gives a deep red solution at pH 4, which might be the free acid.

Hofman and Hochstetler and Lebedinaki *et al.* (refs. 2-5) used ammonium polysulphide saturated in sulphur when preparing their compounds. If less thionated ammonium polysulphide is used, similar solutions are obtained which give brown solids such as  $(\text{NH}_4)_x\text{PtS}_z$ , where  $x$  is 9.1, 9.6, 10.0, 12.1 or 13.8. Acidification of such solutions gives hydrogen sulphide, the normal metal sulphide, and free sulphur. Palladium and iridium were similar, but due to insufficient material only the platinum compounds were fully characterized. Similar experiments were carried out using sodium hydrosulphide. As this compound tends to oxidize to polysulphides, etc., in air, it was prepared under oil, and crystallized as shining white needles, which were redissolved to make a saturated solution. When this was reacted with sodium hexahydroxyplatinate IV or chloroplatinate IV, hydrogen sulphide was evolved. The resultant golden brown solutions on evaporation at room temperature in vacuum gave brown solids analysing as  $\text{Na}_2\text{PtS}_4$ : found 29.35 per cent S, theory 28.6 per cent. Due to the small quantity of material available, only rough analyses could be made for platinum, but these indicated a three to one sulphur to platinum atomic ratio. Sodium hexachloroiridate III and tetrachloropalladate II gave similar deep red solutions, but without evolution of hydrogen sulphide. On vacuum evaporation, these gave small quantities of brown powders. The palladium complex was much more soluble than the other two. Unfortunately these powders could not be purified without partial decomposition to sulphides, as indicated by analyses for metal above that for thiometallate, and low sulphur figures. However, approximately stoichiometric preparations indicate that the complexes are probably  $\text{Na}_2\text{Ir}(\text{SH})_6$  and  $\text{Na}_2\text{Pd}(\text{SH})_4$ , analogous to the hydroxy compounds. The difference from platinum is in the lack of hydrogen sulphide evolution.

It is evident from the foregoing results that the multi-sulphur compounds are formed by replacement of other anionic ligands by polysulphide groups as was postulated by Sidgwick<sup>6</sup>. Even when freshly made, the corresponding metal sulphides are very difficult to dissolve in sodium hydrosulphide, to form the thiometallates. It therefore seems probable that these salts are made by direct substitution rather than by precipitation and resolution.

If iron II or III, or cobalt II, or nickel II carbonate complexes are saturated with hydrogen sulphide, very unstable simple thiometallate solutions can be made, but they are too unstable to be isolated as solids. As both the iron valences give bluish solutions of the same hue, and both give iron II salts on acidification, they are presumably both two-valent. The cobalt and nickel solutions were both golden brown. No cobalt III solution could be made; cobalt III carbonates gave only the two-valent sulphide. Attempts to prepare thiocobaltate II by the action of sodium hydrosulphide on sodium cobaltate II gave cobalt sulphide, and a pale yellow solution containing only traces of cobalt, which may even have been colloidal sulphide. It is interesting to note that iron has the least stable thiometallate, and cobalt the most stable for these three elements. This is reflected in the relative

instability of the ruthenium compound, and the stability of that formed by iridium.

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### True Stress to Fracture and Hyper-velocity Crater Depth

SERIOUS discrepancies and considerable variations have been observed in the numerous empirical, semi-empirical, and theoretical equations which have been proposed to describe craters formed in metal targets as a result of hyper-velocity impact<sup>1</sup>. These inconsistencies in the mathematical models can be attributed to a paucity of precise engineering data and to a basic disagreement about the mechanism of impact. One group of experimenters has attempted to relate the extent of hyper-velocity damage to the mechanical properties of the impacted target; another group has stressed the hydrodynamic model of cratering.

We have observed that the hyper-velocity impact force per crater surface area can be correlated to the true stress to fracture of the target material with the equation  $d = \left(\frac{mv^2}{4\pi s}\right)^{1/3}$ , where  $d$  is the depth of the hemispherical crater,  $m$  and  $v$  the projectile impact mass and velocity, respectively, and  $s$  the true stress to fracture. The applicability of the correlation of impact force with this mechanical property is shown in Table 1 for the impact condition of both similar and dissimilar materials. A representative sampling from 207 experimental points is given. Six types of aluminium alloys and oxygen-free high-conductivity copper were used as target materials. Spherical projectiles of glass, nylon, aluminium, copper and lead<sup>2,3</sup> with a mass range from 0.047 to 1.265 g were accelerated in vacuum with a light gas to impact velocities in the range from 3.66 to 9.35 km/sec. True stress to fracture data were obtained by the experimental method devised by J. Nunes and F. R. Larson<sup>4</sup>.

It must be stated that the proposed equation for crater formation, involving the true stress to fracture of the target material, permits the prediction of the crater depth, as observed from the excellent concordance between experimental and computed values summarized in Table 1. We recognize that other forms of energy dissipation are

operative: radiation, lip formation, highly strained volume element surrounding the crater, etc. In addition, the true stress to fracture is an average value since it is a parameter sensitive to such factors as composition, grain size, grain orientation, inclusions, cracks, etc. We selected this parameter, however, since 'true stress' denotes the actual stress corresponding to the force and area measured at the same time.

The proposed model appears to be valid for ductile aluminium which is a body-centred cubic material. Under the imposed experimental conditions the shear stress applied is considerably greater than the target shear strength and the target reacts as if there were a time delay for plastic deformation. This time delay should be longer than the period of time for crater formation. The material flows not hydrodynamically but according to metallurgical properties, without strain hardening; that is, once the material flows it will continue until the energy in the material has decreased below a critical, or threshold, level or until the time delay for strain hardening is attained. The process of flow and fracture ceases when the energy in the target has decreased below the energy under the true stress-strain curve obtained with strain at normal strain rates.

We are in the process of further testing the proposed cratering model by impacting an aluminium alloy at room temperature, where it behaves in a ductile fashion (20 per cent reduction in area), and at a temperature of -196° C, where it behaves in a brittle fashion (4 per cent reduction in area). In addition, we are impacting a face-centred cubic target, which should be more strain-rate sensitive than the present group of targets.

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### GEOPHYSICS

#### Power Spectrum of Sporadic-E at Wilkes and Byrd Stations, Antarctica

Chivers and Hargreaves<sup>1</sup> have reported the occurrence of quasi-periodic variations in the time series of high-frequency radiowave absorption at high-latitude conjugate locations. The absorption is of the type known as 'auroral zone absorption' (Type II) which is thought to result from ionization in the upper D-region produced by keV energy electrons dumped from, or freshly accelerated in, the outer Van Allen region of the magnetosphere. We report here periodic variations of the ionospheric sporadic-E layer in Antarctica with similar frequency and latitude

Table 1. SUMMARY OF IMPACT CRATER DATA

Projectile mass (g)	Impact velocity (km/sec)	Material		Crater dimensions (cm)			Target true stress to fracture (dynes/cm <sup>2</sup> × 10 <sup>8</sup> )
				Observed		Calculated hemisphere	
		Projectile	Target	Depth	Radius		
0.047	9.14	Al	Al 1100- <i>F</i>	1.04	0.91	1.066	2.654 × 10 <sup>8</sup>
0.158	8.92	Al	Al 1100- <i>F</i>	1.83	1.43	1.556	2.654
0.378	6.87	Al	Al 1100- <i>F</i>	1.96	1.66	1.749	2.654
1.265	5.72	Al	Al 1100- <i>F</i>	2.56	2.27	2.316	2.654
0.047	9.25	Al	Al 2014- <i>T6</i>	0.75	0.73	0.878	4.723
0.158	7.97	Al	Al 2014- <i>T6</i>	1.09	1.07	1.191	4.723
0.378	6.88	Al	Al 2014- <i>T6</i>	1.20	1.35	1.359	4.723
0.376	6.84	Al	Al 2017- <i>T4</i>	1.25	1.25	1.315	6.187
0.075	4.64	Glass	Al 6061- <i>T6</i>	0.661	0.572	0.664	4.399
0.299	5.43	Nylon	Al 6061- <i>T6</i>	1.016	1.060	1.168	4.399
0.800	4.64	Al	Al 6061- <i>T6</i>	1.321	1.324	1.461	4.399
0.376	6.46	Al	Al 7075-0	1.344	1.151	1.366	3.041
0.376	6.14	Al	Al 7075- <i>T6</i>	1.130	1.171	1.209	6.384
0.5084	5.96	Cu	Cu	1.29	1.27	1.288	6.660
0.6101	5.76	Pb	Cu	1.47	1.27	1.343	6.660

characteristics. These results may have considerable importance in suggesting a theory of high-latitude sporadic-*E*.

The sporadic-*E* critical frequency ( $f_oE_s$ ) has been scaled at 15-min intervals from the original ionogram film taken at the Antarctic stations, Wilkes and Byrd. Each sample consists of five consecutive days and was selected on the basis of visual inspection of the hourly tabulated values to insure that a fairly continuous time series of occurrence existed. The samples were chosen from local winter (July and August 1957 and 1958) in order to reduce the possibility of spurious results introduced by diurnal variation of *D*-region absorption or the regular

*E*-layer. In addition to computing the hanned spectral estimate for each sample by the method of Blackman and Tukey<sup>3</sup>, spectral estimates were computed from the mean of the autocorrelation functions of five samples at each station. We present in Fig. 1 the results for single five-day samples at Byrd and at Wilkes; the five-sample-average spectral estimates possess the same characteristics. Error bars indicate the 90 per cent confidence interval.

In addition to a low-frequency peak resulting from the diurnal modulation, statistically significant high-frequency spectral peaks are noted in each sample. The principal (and secondary) periods are: Byrd, 2.2 h (1.1 h); Wilkes, 1.7 h (2.4 h). No significant variation was noted above 1 cycle per h and the data are not presented. In view of the rather large amplitude of the principal (2.2 h) peak at Byrd, the secondary peak may simply be a harmonic. This may not be said of the spectrum at Wilkes.

We suggest that these periodicities are real and that they may be similar to the absorption variations noted by Chivers and Hargreaves. Without entering into a detailed discussion of the relative merits of various production mechanisms, we suggest consideration of the magnetospheric time-of-flight spectrometer effect<sup>4</sup>. An impulsive source of keV energy electrons, which is localized in longitude, will produce distant, quasi-periodic, ionospheric effects (on the same *L*-shell<sup>4</sup>) which may be characterized by the longitudinal magnetospheric drift time of a mean bombarding energy. Such a mechanism would also result in quasi-periodic behaviour of auroral zone absorption.

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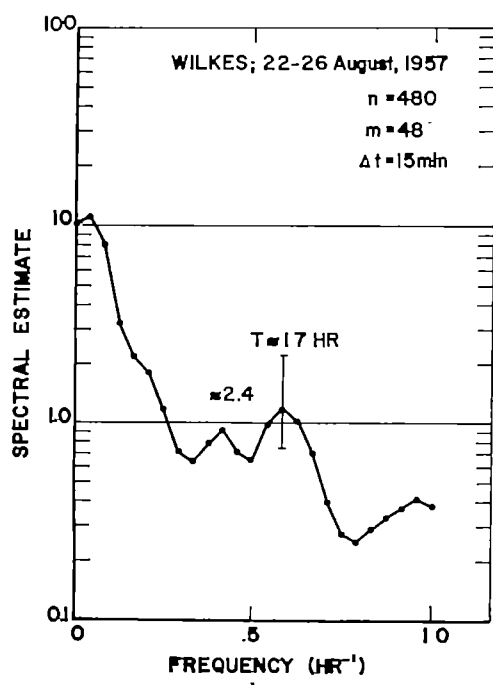
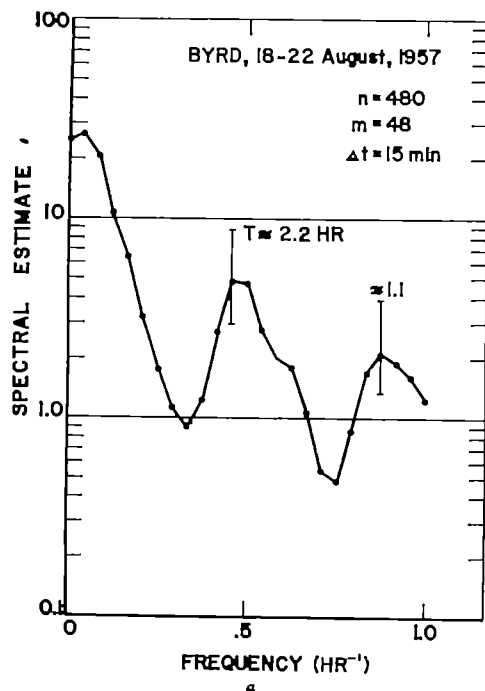


Fig. 1. Spectral estimate of the time series of sporadic-*E* critical frequency for five consecutive days at (a) Byrd and (b) Wilkes during August 1957.  $n$  is the total number of data points,  $m$  is the maximum lag, and  $\Delta t$ , the data interval, is also the lagging interval.

### Attempted Synthesis of the Epigenetic and Syngenetic Theories of Secondary Ore-body Formation

At the present time there are two rival theories of the formation of many copper deposits, and many other secondary ore-bodies are described as anomalous<sup>1</sup>. Mohr has suggested that a synthesis of these two theories might one day be possible<sup>2</sup>.

From the point of view of geochemical transportation of heavy metals, there are two kinds of rivers: those that tend to develop a pH of greater than seven, and those that do not. The latter are relatively well known, and have familiar chemical reactions. The former are complicated in that most heavy metals must be complexed as anions in order to remain in solution. While such ligands as fluoride, tartrate, glycinate, and other organic acids derived from vegetation may sometimes form such soluble complex metallates, by far the commonest such ligand is the carbonate ion itself. Many such carbonate-complexes are described in the early literature, and while the stability often decreases with falling pH, some, such as carbonatocobalt II, can only be prepared in the presence of bicarbonate ions. In almost every case, solutions only just alkaline will not carry large quantities of heavy metals; they will, however, carry such metals. Table 1 lists the various ways that metals may exist in carbonate waters, and Table 2 shows the effect of carbonate concentration on the transportation and stability of copper and other metals as carbonate complexes. Examination of alkaline Ethiopian rivers shows that they



first two being metabolized by plants and soil organisms. In confirmation that carbonic acid will form sodium bicarbonate, samples of basalt from the Entoto ridge in Addis Ababa were leached with saturated carbon dioxide water (mineral or soda water), and the pH measured regularly; after about one month, the pH had changed from about 4 to 8, and the presence of bicarbonate was confirmed by titration. The residues left from the basalt closely resemble the common black clays of Ethiopia. In control experiments using pure water and aqueous sodium chloride, the final pH was slightly above 7. Sodium carbonate can be formed from the bicarbonate, possibly to a small extent by a continuation of the above sodium exchange process, but definitely by contact with hot igneous rocks as at the hot springs along the Ethiopian Rift (those around Lake Shalla actually emit carbon dioxide along with boiling water containing dissolved sodium carbonate and bicarbonate). Vegetation can also form carbonate by metabolizing carbon dioxide. Algae grown in typical Ethiopian river and lake waters containing sodium bicarbonate can raise the pH to well over 9, and one strain (isolated from Green Lake, Debra Zeit, alias Bischoftu, by R. B. Baxter) reached pH 12 when guarded against atmospheric carbon dioxide. Not all igneous rocks give rise to alkaline water, nor are all the rivers rising on basalt and similar basic rocks alkaline. So far a few acidic rivers have been found in Ethiopia, but these have all been associated with pockets of limestone, or marshes where the water is shielded from the underlying basalt by clay. Even these rivers contain some sodium bicarbonate, and may change to alkaline in their less accessible lower reaches.

Basic igneous rocks are often closely associated with sulphide rocks\*. This is not surprising if the denser basalts and sulphides are associated with the deeper layers of the Earth's structure. From the foregoing it is evident that alkaline waters will produce 'anomalous' ore-bodies (see Mohr<sup>2</sup> for a discussion of two cases) and that such waters are associated with certain types of volcanic activity. It is therefore probable that both volcanic and weathering action are necessary to form these ore-bodies. The volcanic activity is necessary to form the basalts and other basic rocks which later weather to make alkaline carbonate waters, and also to bring up the primaevial sulphides, etc., which are later leached and if necessary oxidized by the alkaline waters. Subsequent deposition by hydrolysis, evaporation or precipitation from these carbonate-complexes leads to secondary ore-body formation. Thus both syngenetic and epigenetic processes are essential to the formation of these deposits.

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### An O<sup>+</sup> Layer at 1,200 km

THE determination of the ionic composition of the topside ionosphere by means of the ion energy spectrometer on *Ariel I* (1962 *omicron*) and the analysis of some of the data have already been reported<sup>1</sup>. Recently, we have analysed data obtained over the early summer of 1962 during days 117–181.

The broad features follow and amplify the diurnal and latitude variation already indicated and will be reported in greater detail elsewhere. The purpose of this com-

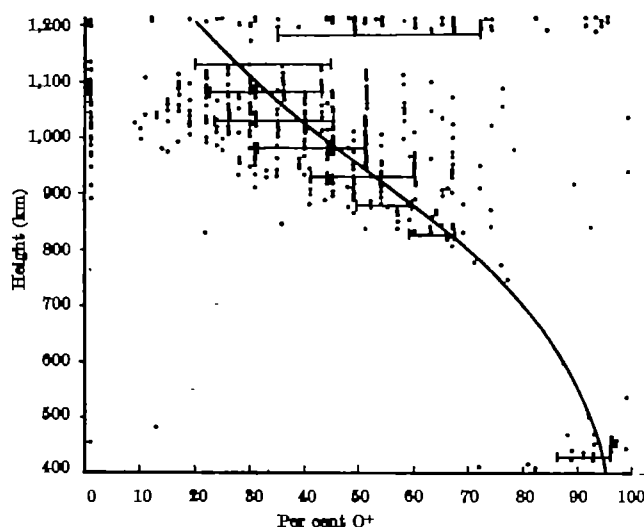


Fig. 1. Observations of the percentage of O<sup>+</sup> in the ionosphere as a function of altitude. The horizontal lines connect the quantiles of the distributions of the observations when divided into 50-km height intervals. The curve shows the composition calculated on the assumptions of diffusive equilibrium and equal concentrations of O<sup>+</sup> and He<sup>+</sup> at 950 km.

munication, however, is to direct attention to an unforeseen effect.

Fig. 1 shows the percentage of oxygen ions as a function of altitude in the afternoon between the hours 1200 and 1700, during which O<sup>+</sup> and He<sup>+</sup> are predominant and the relative concentrations are only changing slowly with time. The points apply to geographic latitudes in the range 10°–40° N. It will be noticed that a region of increased percentage concentration of O<sup>+</sup> appears in the afternoon at great heights. The raw data have been carefully examined to ensure that this effect is not introduced by systematic variation of other parameters with satellite altitude, and while the data will not allow a complete separation of effects due to latitude season and local time and possible instrumental effects due to attitude (it is known that the sensitivity of the spectrometer is attitude dependent), there is no hint either from theory or experiment that the effect can be accounted for in any other way than a genuine departure from hydrostatic equilibrium.

Similar experiments on the satellite *S.48* (1964 *SLA*) and on the forthcoming satellite *S.30A* (*D.M.E.-A.*) may be expected to provide further data. In the meantime, we wish to direct attention to the geophysical significance of the data we have.

Hanson<sup>2</sup> has shown that at heights above about 800 km the ion temperature will be close to the electron temperature. The curve in Fig. 1 is the theoretical percentage of O<sup>+</sup> calculated under the following assumptions—diffusive separation of He<sup>+</sup> and O<sup>+</sup> under hydrostatic equilibrium; ion temperature 2,000° K;  $g$  (constant) = 750 km/sec; 50 per cent O<sup>+</sup> at 950 km.

These assumptions give a scale height for the O<sup>+</sup>/He<sup>+</sup> ratio of 184 km which is probably an upper limit for the greater altitudes and is certainly too large for the lower altitudes in view of the lower ion temperature there and the greater value of  $g$ . Even with this upper limit for the likely scale height, about 90 per cent of the points above 1,150 km are well above the theoretical value, and it is clear that a theoretical composition curve based on hydrostatic equilibrium cannot be made to fit the data.

It is, of course, not possible to obtain a vertical profile of the ion mass spectrum from a single satellite pass. Instead, reliance must be placed on the slow change of orbit parameters. The large scatter of the points is an indication of the considerable variability of the composition of the topside ionosphere from day to day, but the trend is unmistakable in spite of the scatter.



If we consider how a relatively high concentration of  $O^+$  could be maintained at 1,200 km it is clear we have a dynamic situation in which the ionization as a whole must be rather closely in hydrostatic equilibrium in the gravitational and electric field; but a departure from a static equilibrium distribution of diffusive separation may exist as a result of an opposing flow of helium and oxygen ions. Ion-ion collisions ensure that the temperature of the two ion species cannot be very different and that the relevant diffusion coefficient is much smaller than for the ions in the neutral gas.

A mere thermal expansion of an isothermal ionosphere in hydrostatic equilibrium, due to the diurnal heating, would not involve the flow of one ion species through another. The departure from hydrostatic equilibrium, therefore, requires that the net production of ions be able to maintain the concentration gradient. Either the production of  $O^+$  at great heights or the production of  $He^+$  near the  $F$ -region maximum would give an effect of the sign observed; but the latter is ruled out on the ground that it would require a much larger percentage of  $He^+$  near 400 km than at night, which is the opposite to what is observed. It seems, therefore, that we must postulate a substantial production of  $O^+$  at great heights.

The day-time thermal expansion of the atmosphere results in the concentration of neutral oxygen changing from its night-time value at great heights by a very large factor, so that at 850 km its concentration in the afternoon is about equal to that of helium. Oxygen may, therefore, be expected to play a much more important part in ionization production in the afternoon. The results suggest that helium ions diffuse upwards through an opposing falling stream of oxygen ions. A calculation shows the ion-ion relative velocity of the stream to be about  $0.1 \text{ km sec}^{-1}$ . The downward flux of oxygen ions at 950 km is about  $2 \times 10^7 \text{ cm}^{-2} \text{ sec}^{-1}$ , and the ionosphere above this height has a scale height of about 400 km leading to an oxygen ion loss rate of  $0.5 \text{ cm}^{-2}$ .

The photo-ionization rate alone is insufficient to maintain this loss, and is too small to build up this  $O^+$  concentration in the preceding hours of daylight.

The reaction  $O + H^+ \rightleftharpoons O^+ + H$  is in a state of thermal unbalance since  $T_e \sim 2T_{\text{gas}}$ , which may favour the forward direction, but even then its rate seems inadequate. A further possible source of  $O^+$  production might be energetic streams of electrons<sup>1</sup>, but here again the fluxes so far observed seem too small.

It is possible that a combination of these factors is sufficient to account for the  $O^+$  production, especially in view of the rather large uncertainties in many of the quantities and parameters needed to evaluate the rates.

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## GEOLOGY

### Geology of Part of the Southern North Sea

WORK on the geology of the floor of the southern North Sea, under a Department of Scientific and Industrial Research grant to the University of Hull, is now in its second year. It has been carried out with a chartered 110-ft. Diesel trawler, using a 10-cwt. gravity corer for sampling, and a continuous reflexion profiler for recording structures in the rocks below the sea bed and the thickness of superficial deposits. The profiler consists of a 1,000 W-sec Edgerton boomer and a directional hydrophone and amplifier of the type developed by the National Institute of Oceanography<sup>1</sup>. It gives a penetration of about 900 ft. below the sea-floor in favourable circumstances.

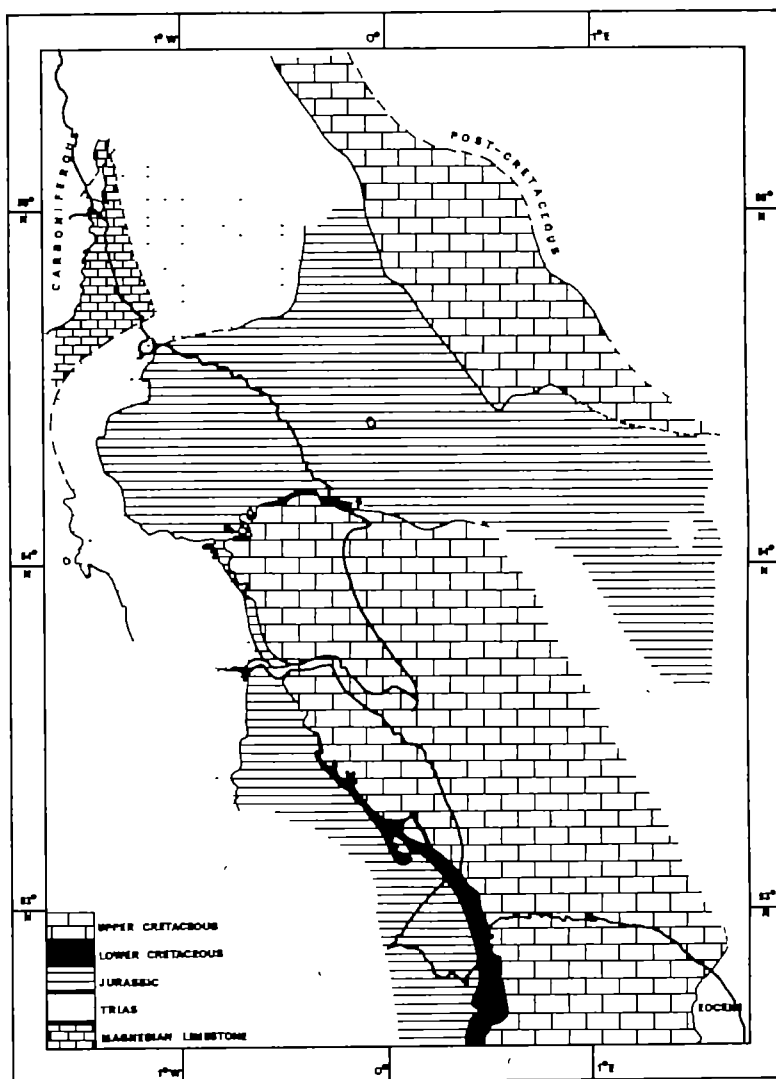


Fig. 1

The extent of the outcrops of pre-Quaternary systems proved so far is shown in the map (Fig. 1). All the outcrops have been proved by sampling, while many details of the boundaries have been taken from continuous reflexion traverses. The outcrop of the Magnesian Limestone offshore is based on the continuous reflexion profiling and offshore borings by the National Coal Board<sup>2,3</sup>; we have not worked in this area. Land outcrops have been simplified from the map of the Geological Survey, and the western limit of the Eocene in Norfolk is taken from

Downing<sup>4</sup>. The outcrop shaded as Trias includes all rocks between the Magnesian Limestone and the Jurassic.

South of Flamborough Head the pre-Quaternary rocks are covered by an almost unbroken mantle of Pleistocene deposits, but they can be sampled in the 'pits' where erosion has breached the Pleistocene cover. Chalk has been proved in the Inner Silver Pit, the channel leading into the Wash, and Codling Hole (the deep trench on the western side of Outer Dowsing Shoal).

Between Flamborough Head and the mouth of the Tyne Mesozoic rocks are extensively exposed on the sea-floor, and have been sampled at intervals of a few miles. The detailed stratigraphy and structure of this area is being worked out. The Jurassic rocks off north-east Yorkshire are gently folded, with dips up to about 4° and a prevailing strike north-north-west. A dome at 54° 22' N., 0° 7' W., is responsible for a small inlier of Trias. The Jurassic rocks appear to extend eastwards towards the Outer Silver Pit. In the western part of this pit at 54° 08' N., 1° 26' E., is another outcrop of Trias known to fishermen as the Red House.

The outcrop of Chalk north-east of the Yorkshire coast has been sampled in numerous places along its edge; north-eastwards it passes under Pleistocene and superficial deposits. An outcrop of post-Cretaceous rocks beneath these deposits has been inferred from continuous reflexion records.

Pleistocene rocks in the area consist mainly of boulder clay attributed to the Last Glaciation. In the Inner Silver Pit at about 53° 27' N., 0° 41' E., marine silts of probable Pleistocene age have been sampled. They are believed to underlie the boulder clay<sup>5</sup>.

Superficial deposits of any thickness are of restricted occurrence. An accumulation of sand, up to 70 ft. thick, extends from the south-western part of the Dogger Bank to within 15 miles of Flamborough Head. There are many sand waves in this area<sup>6</sup>. North of this area much of the Chalk and the post-Cretaceous rocks are covered by superficial deposits which have not been studied in detail. South of latitude 53° 35' N. are a number of sand banks. Outside the areas just mentioned superficial deposits are generally thin, often no more than a few inches thick. Isolated sand waves and groups of sand waves occur locally.

The Inner Silver Pit is a closed basin, eroded through Last Glaciation boulder clay into Pleistocene marine silts and clays and Chalk. It appears to be of geologically recent origin. It cannot be a submerged river valley and has possibly been cut by tidal scour<sup>7</sup>.

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### Differentiation of Alkali Basaltic Magma

In recent years the attention of petrologists has been directed towards understanding the behaviour of basaltic magma in the light of experimental data. While observational and experimental results have progressively enriched our ideas on the reaction principles in sub-alkaline basic magma, "the possible and necessary reactions" in alkali basaltic magma series have been overlooked<sup>1</sup>. The broad petrographic differences between tholeiitic and alkali basaltic series obviously reflect the possible deviations in the reaction processes in the two magma types. The nature and cause of such deviations in reaction

relations will be discussed elsewhere. The normal course of fractionation of alkali basaltic magma leads to the development of an undersaturated alkalic liquid where the undersaturation is inherited from the parent magma. Such late liquids are normally more alkaline than similar products from tholeiitic magma as the mafic phases in alkali basaltic magma invite greater amounts of calcium at the expense of plagioclase, causing a relative enrichment of soda in the residue. Accordingly the derivative of the alkali basaltic magma descends down to the lower quadrilateral in Petrogeny's Residua system<sup>2</sup>, though the exact degree of undersaturation will largely depend on the bulk composition of the initial parent magma and the compositions of the separated mafic phases. Even if the liquid comes down just below the feldspar join in the said system, fractionation of alkali feldspar leads to a decrease of silica:alkalis ratio in the liquid and thus its undersaturation is augmented.

The association of acid derivatives with undersaturated alkaline basic rocks, however, poses an interesting problem. Such co-existence of members with opposite characters has been interpreted by invoking various mechanisms<sup>3,4</sup>, some of them conjectural. It is relevant to note that the promising postulation of Bowen<sup>5</sup>, bearing on this problem, did not receive adequate support either in the field or in the laboratory. It is known now from experimental studies that the liquids on the feldspar join have their vapours slightly oversaturated in silica<sup>6</sup>. Further, some alkali feldspars are shown to have excess silica in their constitution<sup>7</sup>. Obviously, loss of hydrous vapour during fractionation or separation of silica-rich alkali feldspar will have a significant role in moulding the compositional variation of the liquid and causing an association of feldspathoidal rocks with those having excess silica.

An alternative trend of differentiation of the alkali basaltic magma seems to be a promising mechanism for developing acid derivative out of the undersaturated parent magma and thus bringing chemically divergent members together in space and time. This alternative course of diversification of alkalic basic magma will be manifested when aluminium is present in considerable amounts in the constitution of the separated mafic phases, favoured by high water pressure<sup>8</sup>. As aluminium is accommodated in the structures of the mafic silicates (by diadochic replacement of Si) at the expense of plagioclase—the remaining liquid is enriched in silica and depleted in alumina. Such a mechanism having experimental support<sup>1</sup> may ultimately produce a peralkaline siliceous liquid. The present discussion, however, does not overlook the possibility that assimilative reaction of feldspathic liquid with carbonates may cause an association of rock types having diverse characters. The reactions observed in the crucible<sup>9</sup> are envisaged as having occurred in Nature.

In Koraput, Orissa (Eastern India), successive emplacement of alkali gabbro-calcalkali syenite-perthite syenite-nepheline syenite, is a manifestation of crystallization differentiation of alkali basaltic magma in a plutonic environment<sup>10</sup>. These co-magmatic rocks represent another instance of the normal trend of differentiation of alkali basaltic magma where feldspathoidal syenite is found to be the ultimate derivative. Besides the above members in the suite there occurs a feldspar pegmatitic granite (alsakite) solely confined within the gabbro. The oversaturated rock containing small amounts of aegerine-augite occurs in irregular pockets and appears to have formed *in situ* within the gabbro. Detailed petrological investigation of the whole suite establishes the fact that the acid member does not lie directly on the liquid line of descent. There is also no field or laboratory evidence to suggest the origin of the granite by fusion of pelitic country rocks.

A remarkable mineralogical characteristic of alkali gabbro—the host rock for granite—is the profuse development of a brown sodicalcic amphibole<sup>11</sup> which, like others

of its type<sup>1</sup>, is undersaturated in character. Development of such amphibole at the expense of the plagioclase constituents was favoured by high water pressure<sup>11</sup>. The crystallization of such undersaturated amphibole in profusion played a significant part in moulding the composition of the residual liquid in a closed system. Because of the increasing degree of substitution of Si by Al in the structures of the mafic phases there was an accumulation of the former element and depletion of the latter in the residual liquid. Thus ultimately a peralkaline acidic liquid of moderately high temperature was formed interstitially and it developed local irregular pockets of quartz-poor hypersolvus granite within the alkali gabbro.

It should be pointed out that the trend of differentiation of alkaline basic magma will be greatly influenced by the relative distribution of Al and Si in the crystals and the liquid. If Si/Al ratio in the mafic crystals is higher than that in the magma, the liquid becomes depleted in Si relative to Al. A reversed relation favoured by high water pressure will cause the development of a slightly oversaturated liquid. Fractional crystallization of alkali feldspar in the two cases will respectively lead to further undersaturation and oversaturation of the residual magma. It is stressed that the distribution factor in a large measure controls the trend of differentiation of alkali basaltic magma and this in its turn is influenced by the prevalent water pressure. Fluctuations of water pressure may cause further complexity in the evolution of the magmas.

I thank Prof. A. E. Ringwood for advice.

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### Isotopic Composition of Strontium in a Variety of Rocks from Réunion Island

THE island of Réunion is situated some 375 miles to the east of Madagascar in the Indian Ocean. The isotopic composition of strontium (Table 1) has been determined in a suite of rocks ranging from basalt to quartz-bearing syenites. The investigation was undertaken to see whether or not variations in the isotopic composition of strontium were present in a wide range of rock types situated in an oceanic environment.

In the samples analysed no significant differences were observed although the rocks had differing Rb/Sr ratios. This is in general agreement with results obtained from Hawaii<sup>1</sup> and suggests that all the Réunion rocks could be derived from an area in the mantle below Réunion having a constant Rb/Sr ratio. The lack of any significant varia-

tion in the isotopic composition of strontium is of particular interest in the case of the potassium rich (5.12 per cent K<sub>2</sub>O) quartz syenite and indicates that this quartz-rich rock has been formed from an environment having an Rb/Sr ratio similar to that of the basic and ultra-basic rocks present on Réunion.

Four analyses of the Massachusetts Institute of Technology Elmer Amend shelf strontium standard circulated by Prof. Hurley gave an <sup>87</sup>Sr/<sup>86</sup>Sr ratio of 0.7075 ± 0.002, which is slightly lower than that obtained by other laboratories. In order to compare these results with those obtained by other laboratories they should be increased by between 0.001 and 0.002. An error of ± 0.002 is placed on the measured <sup>87</sup>Sr/<sup>86</sup>Sr ratios and is a combination of instrument error and other errors, the major one of which is considered to be a function of the chemical purity of the strontium when placed on the filament. If the full value of inter-laboratory comparison is to be obtained by comparison with a strontium standard, this should consist of a rock powder rather than a pure strontium salt.

The results recorded here form part of a general survey of strontium isotopes, Rb/Sr, and K/Rb ratios of oceanic rocks, the full details of which will be published shortly. I thank Dr. B. G. Upton for providing the Réunion specimens.

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### METALLURGY

#### A Strengthening Effect of High Hydrostatic Pressure on Grain Boundary Walls of a Polycrystalline Zinc

DURING investigations of dislocation behaviour in a polycrystalline zinc a number of interesting facts were found. (All experiments were performed at room temperature and specimens 6 mm<sup>2</sup> × 12 mm were machined from a commercially pure zinc cast of the composition shown in Table 1. Specimens were annealed at 350° C for 5 h and furnace-cooled to a grain size of American Society for Testing Materials micro-grain size No. 00 (except for one shown in Fig. 1 which was not annealed). The polishing and etching reagents for revealing grain boundaries and dislocations were similar to those used by J. J. Gilman<sup>1</sup>).

(1) Twin formation and the movement of grain and twin boundaries besides crystallographic glide are responsible for a large plastic flow of this metal during uniaxial stressing, creep, relaxation and fatigue testings, as is shown in Fig. 1.

(2) By the application of high hydrostatic pressure of the order of thousands of atmospheres, dislocations line up along grain boundaries. Twin formation is suppressed to a great extent and grain boundary movement is completely blocked. However, multiplication and movement of dislocations are still persistent and cause a plastic strain of the order of one ten-thousandth. Fig. 2 shows the build-up of dislocation walls along grain boundaries and dislocation line-ups in crystals with the application of hydrostatic pressure.

(3) Once the aforementioned structure of dislocations is formed the zinc specimen is greatly strengthened: the result of uniaxial compression tests showed that the stiffness was increased by 20 per cent when a specimen was pressure-treated at 3,000 atm. for 1 h. When an initial

Table 1. ISOTOPIC COMPOSITION OF STRONTIUM IN ROCKS FROM RÉUNION

Sample No.	Rock type	Sample type	<sup>87</sup> Sr/ <sup>86</sup> Sr*	<sup>87</sup> Sr/ <sup>86</sup> Sr
RE. 232	Olivine scoria	Whole rock	0.7037	0.1201
RE. 114	Oceanite	Whole rock	0.7038	0.1189
		Olivine	0.7037	0.1196
RE. 106	Mugearite	Whole rock	0.7036	0.1203
		Plagioclase	0.7029	0.1200
RE. 243	Mugearite	Whole rock	0.7031	0.1193
RE. 87	Feldsparphyric basalt	Whole rock	0.7035	0.1206
		Feldspar	0.7038	0.1225
		Whole rock	0.7050	0.1206
RE. 16	Quartz syenite	Feldspar	0.7040	0.1190
		Average	0.7037	0.1201

\* Normalized to 0.1194.

Table 1. COMPOSITION

Lead	Cadmium	Iron	Tin	Zinc
0.0008%	0.098%	10 p.p.m.	<1 p.p.m.	Balance

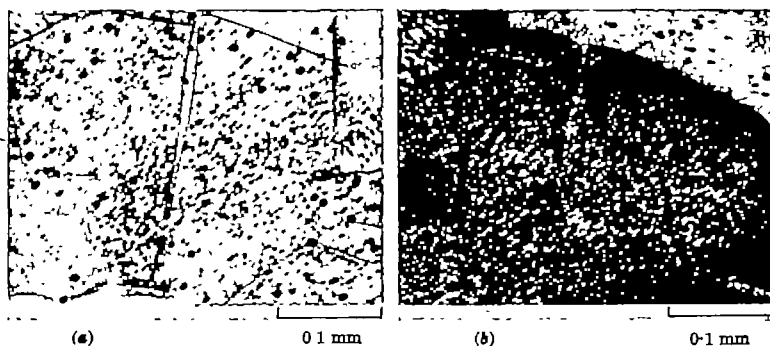


Fig. 1. The same grain (a) before and (b) after a 1.7 per cent plastic strain. The multiplication of dislocation etch pits and the movement of grain boundaries are observed

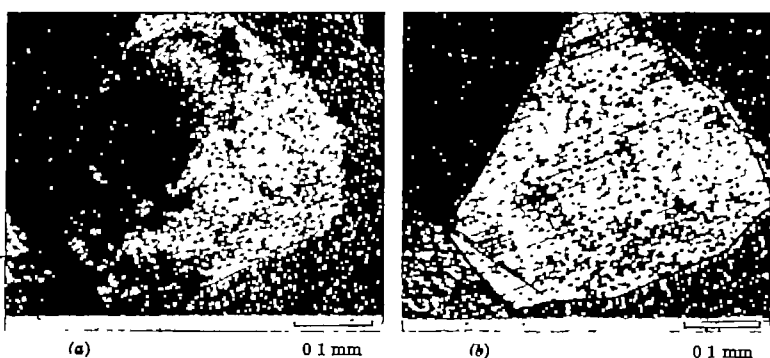


Fig. 2. The same grain (a) before the application of hydrostatic pressure, and (b) after 3,000 atm. hydrostatic pressure had been applied for 10 min

compressive load of 70 kg was applied at a cross-head speed of 0.5 mm/min and the cross-head held still for several hours, the relaxation of load was observed to reduce to 2.3 kg for a pressure-treated specimen from 14.2 kg for a non-treated one.

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## ENGINEERING

### Ultrasonic Grinding of Natural Graphite

FINE grinding of graphite is usually carried out in a mortar or in a ball mill. The reduction of the particle size proceeds rather slowly due to the lubricating action of the grain surfaces, and the ground crystals contain various kinds of lattice imperfections including stacking faults—the extent of the imperfections being subject to the period and method of grinding. Recently, Bacon<sup>1</sup> and others<sup>2-4</sup> studied this phenomenon, both using an electron microscope and X-rays. I myself carried out some experiments on similar lines with Madagascar graphite ground in a cell excited with ultrasonic waves. The original powder was 50–65 mesh graphite crushed in a mortar. The slurry containing the powder was treated with 20-ke/s, 150 V amp ultrasonic radiation in a cylindrical cell 26 mm in diameter. The slurry entered the cell through a pipe attached on the face of the cylinder and flowed out through another pipe attached on the opposite side, and it was circulated slowly with a gear pump. The ultrasonic power was supplied through the top of the cell which was the end plane of the oscillator horn. The height of the cell was adjustable. In the range of height from 1 to 10 mm the change of

grinding speed was not marked. The following media were used: Pure water, 1 per cent aqueous solution of tannic acid or detergent, glycerine, paraffin oil and dense bromoform (specific gravity 2.8). The bromoform seemed to have superior grinding powers, but was troublesome in extended use due to its corrosiveness and irritating vapour. The glycerine was found to be unsuitable for the purpose. Aqueous solution of tannic acid or detergent wetted the graphite particles, and the powder was uniformly dispersed after several passes through the cell. Crushing then proceeded continuously, although pure water did not wet it and the agglomerated powder floated on it for a long time without becoming dispersed in it. When ultrasonic vibration was applied in an open cell containing the slurry, for example by simply inserting the horn end in a beaker, the graphite particles were expelled from the horn and floated on the liquor surface even in water containing tannic acid or detergent. Paraffin oil was inferior to the aqueous solutions.

Under an optical microscope ultrasonically ground particles revealed flat glancing faces often exhibiting clear twin boundaries. The surfaces of particles ground in a mortar with a pestle were commonly rougher, showing close fringes of laminated steps on the basal plane. Apparently, ultrasonic treatment de-laminates each particle and removes surface roughness. Fig. 1 is a micrograph of the graphite particles obtained with an electron microscope operated at 60 kV. The particles had been ground in the cell for 50 h in 1 per cent tannic acid liquor. In the photograph, flake-like particles are seen gathering side by side, partially overlapping each other and transparent to the electron beam even in particles as large as 1  $\mu$ . Several black rod-like grains are presumably similar flakes orienting flat surfaces parallel to the electron beam. Comparing this with the electron micrographs published by Walker and Seeley<sup>5</sup>, it is clear that these particles are flakes de-laminated more uniformly than particles ground in a ball mill, and suitable for lubrication or surface coating.

Successive stages of grinding were examined with the powder camera (24 cm diameter) using CoK $\alpha$  radiation and the samples placed in cellulose tubes 0.8 mm in diameter. Fig. 2 shows the intensity curves of the diffraction patterns in a region in which the intensity is reduced to the true intensity from the microphotometer curves. The



Fig. 1

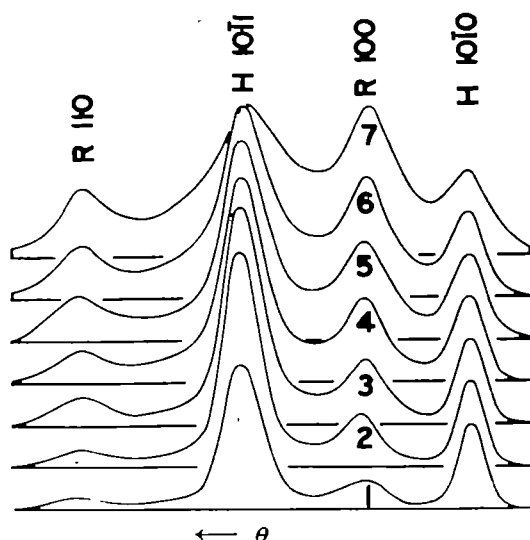


Fig. 2

lowest curve 1 is the original 50-mesh graphite, and curves 2, 3, 4, 5, 6 and 7 are the particles subjected to the ultrasonic grinding for, respectively, 1, 1.5, 3, 6, 12 and 50 h in 1 per cent tannic acid liquor. In Fig. 1 the lines (1010) are seen to be the same height. The rhombohedral lines (100) and (110) increase progressively with the grinding period as already reported by Bacon and others. A marked feature of the present patterns is the great increase in intensity of the rhombohedral lines although the line widths are relatively narrow. In the case of ball-mill grinding the (110) line almost disappeared, covered by the diffused hexagonal line (1011) when the intensity of the line (100) increased to the extent of the curves as shown in Fig. 2. In the present pattern the line appears clearly separated from its neighbours, and the present treatment has been found to produce the modification in a more perfect state.

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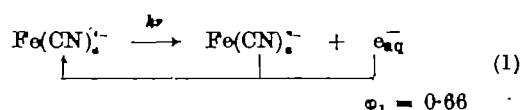
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## CHEMISTRY

### Primary Processes of Photo-oxidation of Aqueous Fe(II) Species

SOME years ago an investigation of the species produced in the photo-oxidation of aqueous reducing anions and cations was begun in these laboratories<sup>1</sup>. More recently, by using  $N_2O$  and other specific electron scavengers, it was shown that the primary process is one of electron detachment for  $I^-$  (ref. 2),  $OH^-$ ,  $Br^-$ ,  $Cl^-$  and  $SO_4^{2-}$  (ref. 3), and that the hydrated electron,  $e_{aq}^-$ , which is so formed has relative reactivities similar to those of the electron produced on absorption by water of ionizing radiations and is influenced by ionic strength in predictable ways. We have obtained similar results for the electron photo-detachment at 2537 Å from  $Fe^{2+}$ , but certain solutions containing  $Fe(ON)_4^{2-}$  show unusual effects which have caused us to re-examine this system carefully<sup>4</sup>.

Briefly, the phenomena observed on illumination of neutral or alkaline solutions of  $K_4Fe(CN)_6$  are explicable in terms of the primary act (1):



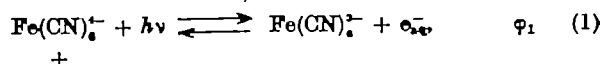
with geminate recombination of  $e_{aq}^-$  and  $Fe(CN)_6^{3-}$  which can be suppressed by sufficient concentration of an electron scavenging solute. If a hydrogen atom scavenger (isopropanol) is used, no hydrogen atoms are detected. If two solutes,  $S_1$  and  $S_2$ , are used, values of  $k(e_{aq}^- + S_1)/k(e_{aq}^- + S_2)$  agree with those obtained for the radiation chemically produced electron and are influenced by  $\mu$  in the expected manner, for example,  $k(e_{aq}^- + NO_2^-)/k(e_{aq}^- + N_2O) = 0.79 \pm 0.08$  (photochemical) and 0.72 (calculated from radiation chemical data<sup>5</sup>) when  $\mu = 0.04$  and  $t_1 = 6 \times 10^{-9}$  sec.

However,  $\log_{10} k(e_{aq}^- + NO_2^-)$  for the photochemical case increases proportionately to  $(2.3 \pm 0.4) \mu^{\frac{1}{2}}/(1 + \mu^{\frac{1}{2}})$ , whereas in the radiation chemical case the dependence on  $\mu$  is much less marked. This result can be understood if it is assumed that  $e_{aq}^-$  reacts with an  $NO_2^-$  ion while still within the relaxed ion atmosphere of the ferrocyanide ion from which it was ejected. Application of the theory of ionic reactions to this model predicts a linear dependence of  $\log_{10} k(e_{aq}^- + NO_2^-)$  on  $2.5 \mu^{\frac{1}{2}}/(1 + \mu^{\frac{1}{2}})$  under conditions where simplifying assumptions are possible.

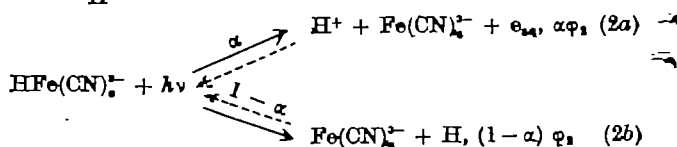
When one of the solutes is  $H^+$  (that is, acidified solutions) different results are obtained. Thus, the rate constant ratios are normal for, say, solutions of  $La_4(Fe(CN)_6)_3$ ,  $k(e_{aq}^- + H^+)/k(e_{aq}^- + N_2O) = 1.55 \pm 0.1$ , but are abnormal for solutions of  $K_4Fe(CN)_6$ ,  $(\log_{10} (k(e_{aq}^- + H^+)/k(e_{aq}^- + N_2O)))$  computed from the dependence of  $\varphi(N_2)$  on the ratio of bulk concentrations of  $[H^+]$  to  $[N_2O]$  is equal to  $0.25 (\pm 0.1) + 2.8 (\pm 0.5) \mu^{\frac{1}{2}}/(1 + \mu^{\frac{1}{2}})$  when  $[H^+] \ll [Fe(CN)_6^{4-}]$ .

Moreover, if the hydrogen atom scavenger  $i$ -PrOH is present in addition to or instead of  $N_2O$ , the apparent quantum yield of the primary act identified as the limiting value of  $\varphi(N_2) + \varphi(H_2)$  at high scavenger concentration is less than 0.66 and diminishes with increasing acid concentration although such hydrogen atoms as are formed react at the expected relative rates with  $Fe(CN)_6^{4-}$ ,  $NO_2^-$  and  $i$ -PrOH.

These anomalies are only explicable in terms of the simultaneous primary acts (1)–(2) (dotted arrow denotes geminate recombination):

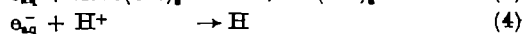
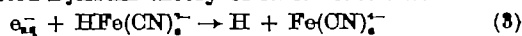


+



for which  $\varphi_2 \leq \varphi_1$  and  $\varphi_1 = 0.66$ .

The equilibrium constant  $[HFe(CN)_6^{3-}]/[H^+][Fe(CN)_6^{3-}] = 2.24 \times 10^4 M^{-1}$  and so conditions can be devised under which, although more than 95 per cent of the  $H^+$  is associated with  $Fe(CN)_6^{3-}$  as  $HFe(CN)_6^{3-}$ , about 75 per cent of the light is absorbed by the unprotonated ferrocyanide ion. The competition between  $N_2O$  and  $HFe(CN)_6^{3-}$  for  $e_{aq}^-$  emerging largely from process (1) can be investigated, and it is shown that  $k_2 = 0.1 k_1$  and that the effect of ionic strength on  $k_2$  is that expected from the Brønsted-Bjerrum theory of ionic reactions:



It follows from this mechanism that, with sufficient  $i$ -PrOH present to scavenge all the hydrogen atoms, increasing  $[H^+]$  should cause  $\varphi(H_2)$  first to increase as reaction (3) competes with the geminate reverse reaction (–1) and then to decrease as the primary act changes progressively from (1) to (2). This too has been observed.

Full details of this and related investigations are in the course of publication<sup>4</sup>.

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### Action of Atomic Hydrogen on Ferri-Cytochrome c in Aqueous Solution

Atomic hydrogen is one of the simplest chemical reagents. Its structure can be described in exact theoretical terms. In aqueous solution it interacts only relatively weakly with the solvent. It is neutral and thus not influenced by ionic effects in its reaction with specific substrates. Therefore its reactions are of interest from the point of view of fundamental chemical kinetics in aqueous solutions. Recently, a method has been established for the investigation of the quantitative aspects of the reactivity of atomic hydrogen with various substrates dissolved in aqueous solutions<sup>1</sup>. Using a high-frequency electrode-less discharge to dissociate pure hydrogen gas into hydrogen-atoms and passing these under carefully controlled conditions into aqueous solution, it was shown that the reactive species is identical in its reactions with hydrogen-atoms produced by ionizing radiations or by ultra-violet light<sup>2</sup>. Therefore these reactions serve to elucidate also, independently, some aspects of the mechanism of the action of ionizing radiations or ultra-violet light on such systems. In this method, however, hydrogen atoms are the only reactive species present so that their specific action may be examined separately. The reactions of simple substrates (inorganic ions<sup>1,3</sup>; their inorganic complexes<sup>4</sup>; simple organic solutes<sup>5</sup>) were used to establish details of the reactivity of hydrogen atoms. The work was extended to enzymes (chymotrypsin<sup>6</sup>, trypsin<sup>7</sup>, ribonuclease<sup>8</sup>) to elucidate the action on biologically interesting substrates.

In the work recorded here we investigated the action of atomic hydrogen on aqueous solutions of ferri-cytochrome c. On one hand, this enables us to compare the reactivity of atomic hydrogen with other substrates containing a simple inorganic ion, such as the ferric ion<sup>3</sup>. However, the present case involves a protein associated with the complexed iron ion. On the other hand, the reactions of cytochrome c could be compared with the reactions of atomic hydrogen with various amino-acids<sup>9</sup> and with various proteins which do not contain an inorganic ion associated with the protein molecule<sup>4</sup>. In this way, the special features involved in the reactions of atomic hydrogen with cytochrome c could be elucidated.

Experimental details of the treatment with hydrogen-atoms were described previously<sup>1,3</sup>. The cytochrome c used was prepared by the method of Margolias<sup>10</sup> and only fraction I was used. Dose rates were determined using the ferri-cyanide method for the determination of the atomic hydrogen yield<sup>11,12</sup>.

All dose-rates in the work described here were  $2 \times 10^{-4}$  mole l.<sup>-1</sup> sec<sup>-1</sup>. 25 ml. of solution was used in the reaction vessel for each experiment. Control experiments were carried out, passing hydrogen gas only without discharge in order to ascertain the effect of bubbling alone on the stability of cytochrome c. No changes were observed due to bubbling only. In the actual experiments after passing hydrogen gas without discharge in order to clear the solution of oxygen, the discharge was lit and the atomic

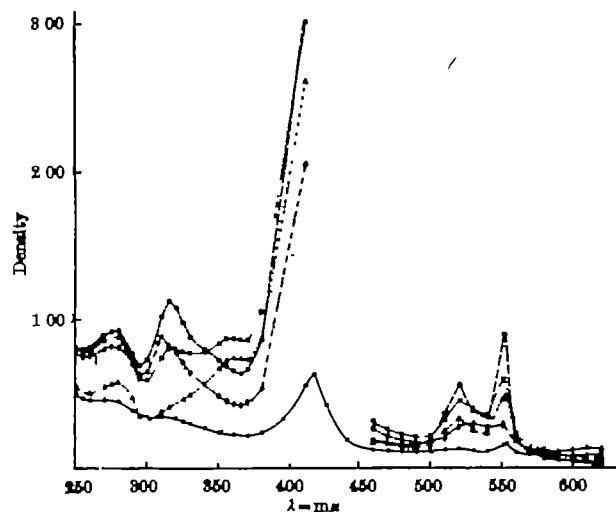


Fig. 1. Absorption spectrum of  $3 \times 10^{-4}$  M ferri-cytochrome-c solutions at pH 7, after hydrogen atom treatment for:  $\Delta$ —0;  $\Delta$ — $\cdot$ — $\cdot$ —20;  $\Delta$ — $\cdot$ — $\cdot$ —60;  $\Delta$ — $\cdot$ — $\cdot$ —1,800 sec. Spectra taken under  $H_2$  atmosphere

hydrogen passed for the specified times. After treatment, samples could be withdrawn into a spectrophotometric cell under hydrogen pressure without air being admitted. The reproducibility of our results was of the order of  $\pm 10$  per cent for this series of experiments. The time of hydrogen atom passage was varied between 10 sec and 30 min through solutions containing 0.85 to  $3 \times 10^{-3}$  M of cytochrome c at pH 7 (maintained by 0.05 M phosphate buffer). The spectra obtained showed that at low doses only such spectroscopic changes occurred as were consistent with a simple reduction of ferri-cytochrome c. The ferri-cytochrome c formed in solutions treated up to this stage remained non-autoxidizable. When hydrogen atoms were continued to be passed into solutions in which ferri-cytochrome c was already reduced to ferro-cytochrome c, further far-reaching changes in the absorption spectrum were observed (Fig. 1), including the disappearance of the maximum at 315 mμ and a very considerable decrease of the optical density at 415, 520 and 550 mμ. The ferro-cytochrome c is now autoxidizable. When hydrogen atoms are continued to be passed, for example, for 90 min, further bonds are opened and free iron may be detected. The dependence of the secondary processes on the initial concentration of ferri-cytochrome c showed that the secondary processes occur only when ferri-cytochrome c is no longer present. So long as the ferri form is present, hydrogen atoms react preferentially with it. From the initial rates of the reaction (assuming that initially atomic hydrogen is used up for the reduction of ferri- to ferro-cytochrome c only) the rate constant of the reaction between atomic hydrogen and ferri-cytochrome c was calculated according to the procedure of Navon and Stein<sup>13</sup>. The value of  $k = 2 \times 10^9$  l. mole<sup>-1</sup> sec<sup>-1</sup>. In radiation chemical experiments, a value of the order of  $1 \times 10^9$  l. mole<sup>-1</sup> sec<sup>-1</sup> was obtained<sup>14</sup>. In Table 1, the rate constant now obtained is compared with the rate constants of other ferric complexes obtained with atomic hydrogen using the same experimental technique<sup>15</sup>. It will be seen that the rate constant of the reaction of atomic hydrogen with ferri-cytochrome c is similar to that of the fastest reactions of ferric ion complexes and is among the fastest measured for hydrogen atom reactions with various substrates<sup>15</sup>. The presence of protein thus

Table 1. REACTION RATE CONSTANTS (CALCULATED FOR 25° C, REF. 25) IN AQUEOUS SOLUTION OF HYDROGEN ATOMS WITH FERRI-CYTOCHROME-C AND OTHER FERRIC COMPLEXES

(For details see refs. 25 and 26)				
Substrate:	$Fe^{3+}$ cyt c	$Fe^{3+}$ (o-pho)	$Fe^{3+}$ (dipy)	$Fe^{3+}$ (ON <sup>-</sup> )
$k$ , l. mole <sup>-1</sup> sec <sup>-1</sup>	$2 \times 10^9$	$2.9 \times 10^9$	$2.2 \times 10^9$	$1.5 \times 10^9$
	$Fe^{3+}$ Cl <sup>-</sup>	$Fe^{3+}$ (H <sub>2</sub> O) <sub>6</sub>		$Fe^{3+}$ OH <sup>-</sup>
	$4.8 \times 10^8$	$< 4 \times 10^8$		$2.3 \times 10^9$

Table 2. EFFECT OF pH ON THE REACTION RATE CONSTANT OF HYDROGEN ATOMS WITH FERRI-CYTOCHROME c

pH	7.0	6.0	5.0	2.5
$k/k_{\text{H}}$	1	0.66	0.58	0.14

does not slow down the reaction. The value now obtained may also be compared with the rate constant of ferri-cytochrome c with solvated electrons,  $e_{\text{aq}}^-$ , for which the same value as for the reaction<sup>6</sup> of ferri-cyanide ion with  $e_{\text{aq}}^-$ ,  $k = 3 \times 10^9$  l. mole<sup>-1</sup> sec<sup>-1</sup>, was found<sup>7</sup>. It will be seen that for ferri-cyanide and for ferri-cytochrome c, the rates of reduction with hydrogen atoms and with solvated electrons are very similar.

The dependence of the rate constant of the reaction of atomic hydrogen with ferri-cytochrome c on changes in the pH is shown in Table 2. The rate constant decreases on going from pH 7 to pH 2.5. The oxidation reduction potential increases and thus  $\Delta G$  of this reaction becomes more negative on going from pH 7 to pH 2.5 (Fig. 2). Therefore, the usual correlation between  $\Delta G$  of the reaction and the rate constant is not observed. For the reaction of ferri-cyanide with ferro-cytochrome c and ferro-haemoglobin respectively, Sutin and Christman<sup>8</sup> pointed out the lack of correlation between  $\Delta G$  and the rate constants. In our case, such behaviour may be attributed to configurational changes which have to take place during the reaction. In the present case, it is known that a pH dependent change in the configuration of the protein in its reduced form is observed at about pH 2.5. The 'crevice' in the amino-acid structure leading to the haem is open in both the ferri and the ferro form above pH 2.5, but is closed in the ferro form at and below pH 2.5. This configurational change, which is necessary in order to carry out the reduction process as pH 2.5 is approached, may slow down the reaction velocity.

It is interesting to compare the behaviour of ferri-cytochrome c with other proteins. It was found that enzyme proteins, for example trypsin and chymotrypsin which do not contain a metal prosthetic group but contain S—S linkages, are inactivated by reactions with atomic hydrogen which results in the opening of S—S bonds<sup>9,10</sup>.

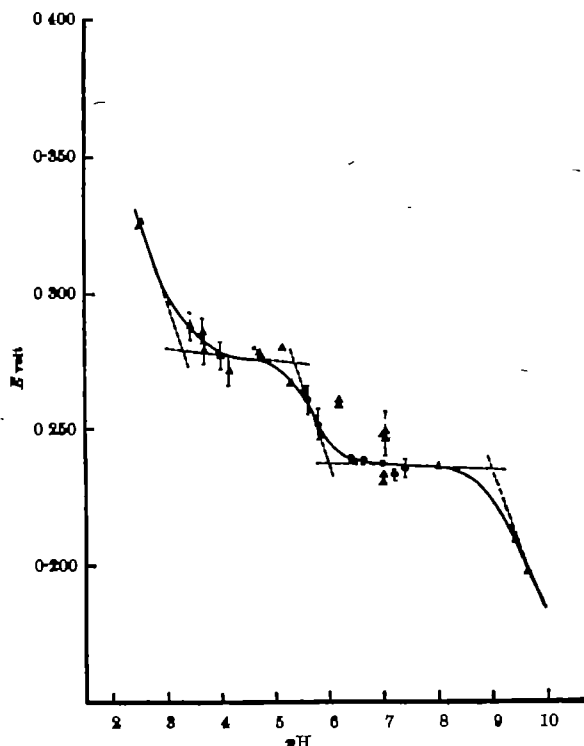


Fig. 2. Dependence of  $E^\circ$  on the pH for aqueous solutions of fraction I of cytochrome c. Points ▲ determined by potentiometric, ○ by spectrophotometric, measurements. For details see N. Frohwirth, Ph.D. thesis, Jerusalem (1962).

Similar observations were made in the case of ribonuclease<sup>11</sup>. The rate constant of the reaction of hydrogen atoms with S—S links in trypsin is similar to that of hydrogen with ferri-cytochrome c. Cystine reacts with hydrogen atoms with a similar rate constant<sup>12</sup>. Unlike hydrolytic enzymes, cytochrome c does not contain any disulphide linkages, only thioether ones<sup>13</sup>. Its tertiary structure is probably maintained through links formed in hydrophobic areas within the protein structure.

It appears that were S—S links present near the active groups of cytochrome c, an oxidation reduction enzyme, they might have competed, as easily reducible groups, with the proper functioning of the enzyme. This work with hydrogen atoms indicates that the absence of S—S bonds in cytochrome c and the necessity to maintain protein structure by means other than in hydrolytic enzymes may be necessitated by such functional considerations.

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### Magnetic Resonance Investigation of the Raney Nickel Catalyst

PREVIOUSLY<sup>1,2</sup>, one of us reported that the Raney nickel catalyst lost its activity through thermal treatment and that this might reasonably be related to the elimination of the crystal distortion in this catalyst. The structural change in the Raney-nickel catalyst by thermal treatment with the electron spin resonance technique has now been investigated.

Two samples of the catalyst, sample A being active in the hydrogenation reaction and B less active, were prepared by developing a nickel-aluminium alloy, the nickel content of which was 49 per cent, by the usual method<sup>3</sup>, then treating them at 70° and 300° C, respectively, in a vacuum (0.001 mm mercury) for 2 h and sealing them in a quartz tube with a 1-mm inner diameter.

The specific magnetization which was obtained at room temperature by the Faraday-type magnetic balance with an applied field of 3,000 oersteds was 0.22 and 0.51 for samples A and B, respectively. These values of the specific magnetization are expressed as  $\sigma/\sigma_0$ , where  $\sigma$  is the specific magnetization of the sample, and  $\sigma_0$  is that of nickel metal.

The increase in the magnetization on heating at 300° C may be caused by the evolution of hydrogen in the catalyst<sup>4</sup>.

The resonance spectra of the samples were taken at room temperature with a modulation field of 100 kc/s and



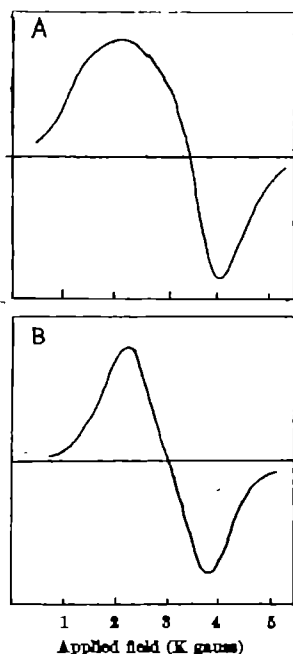


Fig. 1. A, Electron spin resonance signal from sample 4. The  $g$ -factor is 2.09; B, from sample B. The  $g$ -factor is 2.22

a microwave frequency of 9,403 mc/s on Nippon Denshi 3BX type electron spin resonance spectrometer. These spectra are shown in Fig. 1 A and B.

It is seen from these spectra that the signal of sample A is asymmetrical and broad while that of B, the activity of which was reduced by treating at 300° C, is symmetrical. These phenomena observed in sample A are accounted for by anisotropy of the  $g$ -factor in its microcrystals.

The measured  $g$ -factor on sample B, 2.22, agrees with that obtained for nickel by other workers<sup>1</sup>.

The results presented here suggest that the lattice imperfections in the active catalyst are diminished by the thermal treatment. The decline of the catalytic activity may be closely related to the diminution of the lattice imperfections in microcrystals of Raney nickel catalyst.

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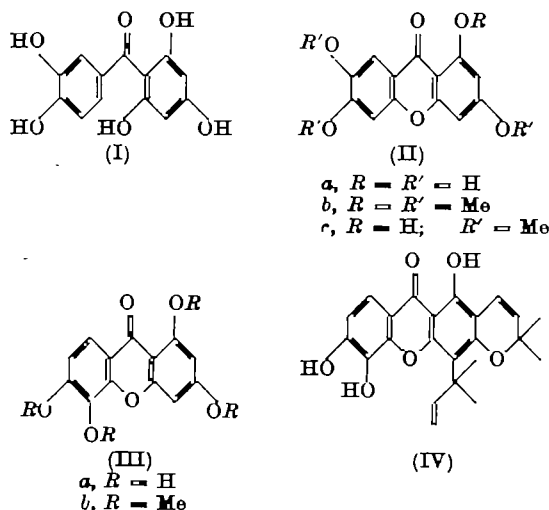
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### Presence of 1,3,6,7-Tetrahydroxyxanthone in Maclurin from *Chlorophora tinctoria* (L) Gaud. (*Morus tinctoria* L) (Moraceae)

DURING the course of our work on Guttiferae we required maclurin (I). Examination of commercial maclurin extracted from *Morus tinctoria*\* (Moraceae) (we thank the Aldrich Chemical Co. for disclosing their source of maclurin) showed a small amount of impurity which was identified as 1,3,6,7-tetrahydroxyxanthone (IIa) by comparison with an authentic sample using paper chromatography (Whatman No. 1; 60 per cent aqueous acetic acid;  $R_F$  0.70). 1,3,5,6-Tetrahydroxyxanthone (IIIa) has a similar  $R_F$  value but a different colour under ultra-violet light, and the structure of the

impurity was therefore confirmed by methylation with dimethyl sulphate. The resulting 1,3,6,7-tetramethoxyxanthone (IIb) was identified by thin-layer chromatography (silica gel by Stahl; benzene-chloroform, 3:7;  $R_F$  0.35) because under these conditions the isomeric 1,3,5,6-tetramethoxyxanthone (IIIb) has a higher  $R_F$  (0.42). Authentic xanthones were prepared by unambiguous synthesis<sup>1</sup>.



Although 1,3,6,7-tetrahydroxyxanthone (IIa) has not been previously detected in *Morus tinctoria* its presence is not surprising now that we have shown that its formation from maclurin (I) occurs *in vitro* by phenol oxidative coupling. Thus irradiation of a 1 per cent boiling ethanolic solution of maclurin with ultra-violet light (Hanovia medium pressure lamp) in a stream of oxygen for 30 h gave 1,3,6,7-tetrahydroxyxanthone (45 per cent) (IIa) which was isolated after methylation with diazomethane as the insoluble sodium salt of 1-hydroxy-3,6,7-trimethoxyxanthone (IIc) (ref. 2). 1,3,5,6-Tetrahydroxyxanthone (IIIa), which can also be theoretically formed from maclurin, was not detected under these conditions although macluraxanthone (IV) with this oxygen substitution pattern has been obtained from *Maclura pomifera*<sup>3</sup>.

At this stage we cannot preclude the possibility that 1,3,6,7-tetrahydroxyxanthone may have been formed from maclurin during extraction and isolation from *Morus tinctoria*.

We thank the Department of Scientific and Industrial Research for a grant to purchase equipment, and the Royal College of Advanced Technology, Salford, and the Plastics Institute for a grant to one of us (A. J.).

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\* A further synonym of the plant is *Maclura tinctoria* (L) D. Don ex Steud. However, *Chlorophora tinctoria* is now regarded as the correct name. We thank P. St. J. Edwards for help on this nomenclature.

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### A Chemical Model for Electrolytic Oxidation of Iodate

DURING an investigation of the electrolytic production of periodate from iodate, the oxidation of iodate was expressed mathematically as a chemical reaction in which gaseous oxygen formation competed with the desired

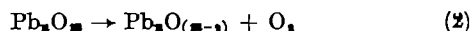
reaction for the available supply of a common precursor material<sup>1</sup>. In writing the equations, liberties, which could be mathematically rationalized, were taken with the classical forms of the rate equation, but these liberties resulted in equations that could only approximate real counterparts. In particular, it was stated that "sometimes, in the early stages of the oxidation, the reaction proceeds at a very slow rate. This is attributed to an initial lack of catalytic activity at the surface . . .". The question has always remained, whether or not the classical equations, which were modified mainly to facilitate solution, would not better express the total behaviour of an operating cell. Another intriguing question has been the identity and quantitative behaviour of the precursor material. Now the set of classical equations has been solved on an analogue computer and insight has been gained into the electrode processes which could occur.

The set of classical equations investigated was as follows:

$$\begin{aligned} y' &= K_1 z (1 - y) \\ x' &= K_2 x^2 \\ x' + y' + z' &= \frac{i}{2F} \end{aligned} \quad (1)$$

where  $\frac{i}{2F}$  is the rate of atomic oxygen production,  $x$  is the amount of gaseous oxygen,  $y$  is the amount of periodate,  $z$  is the amount of the oxygen precursor to both  $x$  and  $y$ ,  $K_1$  and  $K_2$  are the specific reaction rates for periodate and oxygen, respectively. Primes refer to the time rates for the variables.

This set argues that the oxygen production rate is bimolecular, as it would be if free radical oxygen were the precursor. Several comparisons for data fit were made between this assumption and the previous assumption<sup>1</sup> that the oxygen rate follows a monomolecular law, which could occur if the oxygen precursor were an oxide of lead so that a reaction such as:



could occur. In such a case, the periodate oxidation could still be monomolecular with respect to iodate by a reaction such as:



It is realized that the true reactions may be much more complicated and involve several possible chemical entities, such as  $\text{H}_2\text{O}_2$ ,  $\text{Pb}(\text{IO}_3)_2$ , and  $\text{Pb}(\text{IO}_4)_2$ , at the anode interface. At the present state of technology in measuring the instantaneous compounds of lead, knowledge about them must be primarily non-specific and arrived at by inference.

Unfortunately, the computer data gave no information regarding molecularity. The two curves were so similar in shape that either could be fitted to the chemical data within experimental error. The only effect was to change the ratio  $K_1/K_2$ , as one might expect from examining the equations. Since the precise reactions are not known, the possibility of determining the absolute value of either of the specific rates seems remote.

A fault with the simplification previously made to derive the approximate model was the omission of  $z'$  from the material balance equation. If  $z'$  is truly zero, then  $z$  could be a constant and the oxygen production equation would be unnecessary. The solution then degenerates into a simple first-order equation in  $y$ .

Under certain conditions and over a restricted range the computer investigation disclosed that  $z$  is approximately linear in time and that then the shapes of the approximate and classic curves are the same within experimental error for the chemical data.

Comparison of the classical and approximate models is shown in Fig. 1.

Where the chemical data showed an appreciable 'induction' period, the classical model fitted the total range, whereas the approximate model fitted only the later portion. In some cases, however, these data did not show an

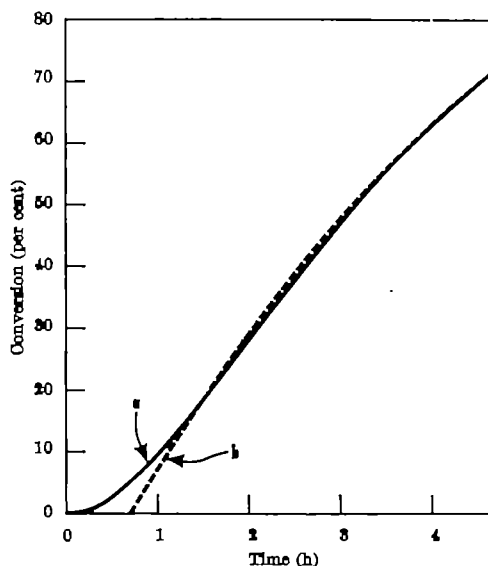


Fig. 1. Difference between classical (a) and approximate (b) models in early stages of oxidation

induction period. An attempt was made to force the classical equation toward the approximation by making  $K_2$  large relative to  $i/2F$ . The curve shape did not yield readily to this attempt.

The second attempt to explain the lack of induction in some data was to assume a third parameter in the classical equation. By making the initial value of  $z$  equal to about half the final value, the induction period was lost. This adjustment gave good fits for a wide range of data, from an inefficient cell to a most efficient one, and for those either showing or not showing an induction period. Some typical curves of  $y$  and  $z$  are given in Figs. 2 and 3. The parameters for several runs are given in Table 1, where the 'efficiency factor'  $K_1/K_2$  is compared with the approximate values.

Table 1. COMPARISON OF EFFICIENCY FACTORS IN APPROXIMATE AND CLASSICAL MODELS

Cell	Current (amp)	$i/2F$ % Theoretical conversion/h	Efficiency factor, $K_1/K_2$ Approximate	Classical ( $K_1 = i/2F$ )
A	30	24.7	4.3	5.3
A	65	25.7	3.1	3.1
A	100	41.1	2.6	3.1
B	65	25.7	5.8	7.0
O*	65	25.7	2.8	2.3

\* Anode area 3.7 times as great; flow rate, lower.

The value for  $K_2$  did not alter the early shape of the curve appreciably. To prevent the value of  $z$  from exceeding the computer voltage tolerance,  $K_2$  was taken to be equal to  $i/2F$ . At infinite time,  $x'$  approaches  $i/2F$ .

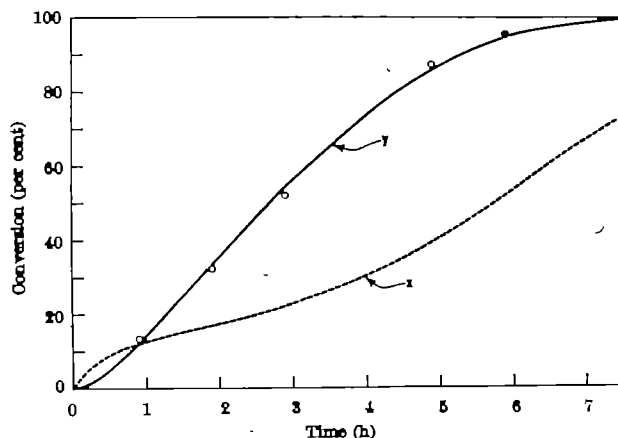


Fig. 2. Classical curves with an appreciable induction period

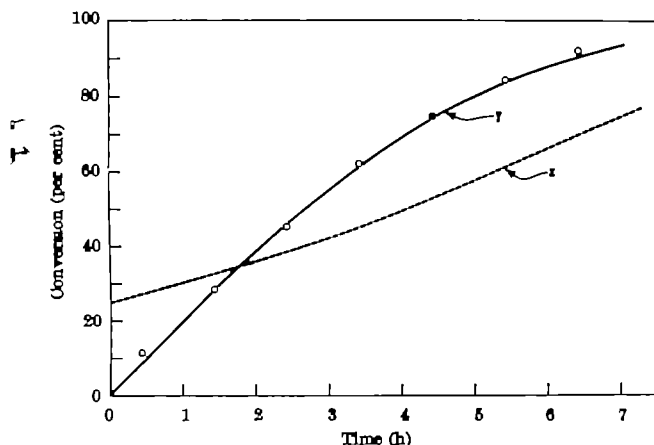


Fig. 8. Classical curves without an induction period

Thus,  $z$  is always equal to or less than unity, which was scaled to be the maximum computer voltage.

In the equations,  $K_s$  turns out to be equal to  $1/(s_{\max})^2 \cdot i/2F$ . Consequently  $K_s$  is not so much a specific rate as a measure of the oxide-holding capacity of the anode relative to the amount of current applied. It is therefore somewhat dependent on current density, as an analysis of the results in Table 1 shows.

These investigations demonstrate that the approximate model has good empirical justification over most of the data range and that the classical model, as formulated, has some flaws. Considering the greater complexity that would be involved in using the classical model, the approximate one is suggested for engineering calculations.

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## BIPHYSICS

### Advantages resulting from the Addition of a Versatile Multichannel Analyser to an Electron Spin Resonance Spectrometer

A MULTICHANNEL analyser has been added to our electron spin resonance spectrometer to be used as a computer of average transients<sup>1</sup>. This communication describes the elements of the system and some of the important advantages that a versatile analyser can provide. Briefly, the principle of the computer of average transients is that successive sweeps of a spectrum are added in a multichannel memory system, so that the signal, coherent with each sweep, adds linearly in the memory, while the noise, which is random, adds in quadrature. This results in an improvement of signal-to-noise ratio after  $N$  sweeps of  $\sqrt{N}$ .

The analyser system used here consists of a clock pulse generator, 400 channel analyser and a magnetic tape calculator (Intertechnique types HC40, SA40B and RG28 respectively) and a high-gain d.c. amplifier (Rochaz type A1388). Modifications were carried out by the manufacturers at our request to suit our spectrometer which include the high-gain d.c. amplifier placed between the spectrometer and the analyser, and a biasing circuit placed before the analogue-to-digital converter, so that any negative voltages that might arise from the signal can be offset. The manufacturers themselves have introduced a useful variant of the standard analyser for

computer of average transients applications by using a pulse train of 10 times the frequency of the address advance, which is used to open the 'gate' of the analogue-to-digital converter ten times in each channel, thus sampling the signal ten times. In this manner  $N$  sweeps of the spectrum represent  $10 \times N$  samples of this spectrum. The signal-to-noise ratio then can be considered to be improved by a factor of  $\sqrt{10N}$ .

Since the analogue-to-digital converter in its most sensitive setting quantizes voltages in increments of 10 mV, and because our spectrometer<sup>1,2</sup> was constructed to drive a 10-mV recorder, the high-gain d.c. amplifier was necessary in order to see signals which are less than 10 mV in amplitude. This amplifier has a variable gain in steps of times 20, 50, 100, 200, 500, 1,000.

The sweep of the multichannel memory is synchronized with the sweep of the magnetic field which provides the electron spin resonance spectrum, by a 'start' pulse circuit which is triggered when the voltage across the magnetic field sweep coils reaches a pre-set value. It is planned to modify this to provide a steady magnetic field value for each channel, by driving the magnet sweep coils from a staircase wave-form obtained from analyser channel address register, via a suitable current amplifier.

We have found that, with our system, the optimum results are obtained with a sweep of 100 gauss in 4-8 sec. We have so far limited operations to 100 sweeps, giving an improvement in signal-to-noise ratio of approximately 30. At the moment this is quite adequate. Such a sweep time allows one also to retain a small time constant in the spectrometer, thus eliminating some noise before the analyser. It was found that our sweep generator (or 'start' pulse circuit) could be triggered by the 50 c/s mains frequency ripple, and if the dwell time in any channel is less than 10 msec, the half-period of the mains, an undesirable addition of 50 c/s pick-up occurs with each sweep. This can be eliminated by careful choice of channel frequency or total number of channels used.

The improvement of  $\sqrt{10N}$  is not considered to be one which can be increased indefinitely. It is limited by two factors, the frequency stability and the magnetic field stability of the electron spin resonance system. If these are poor then the lines in a spectrum are not quite coherent for each sweep and consequently they will be averaged out. For wide lines these instabilities are relatively unimportant. For a line 20 gauss wide, however, using 4-sec sweeps, if one assumes bad drifts of field and frequency of, say, 1.0 gauss/h and 1.0 Mc/s/h, and that the maximum tolerable shift of the line is 0.2 of its width, then we calculate that the maximum number of sweeps possible is  $2.7 \times 10^3$ , which limits the signal-to-noise improvement to 164.

In addition to the improvement in sensitivity, the other advantages found with this system are the operations which are possible using the magnetic tape calculator. First, by storing a 'background' spectrum, for example the spectrum from an empty sample cell, any contribution resulting from this can be subtracted later from the spectrum of interest taken with a sample inside the cell.

Secondly, the sample spectrum when recorded on tape can be integrated and the integral recorded, which can, in turn, be integrated again. In this way first derivative spectra can be integrated twice to provide information about the number of spins contained in the sample. There are more steps involved in this operation, however, which is conducted in the following manner:

(1) A straight line,  $f_1 = a$ , is obtained on the analyser by storing the same number of counts in each channel, a facility which is provided on the analyser. This straight line is recorded on tape.

(2) The straight line is played back into the analyser in the integration mode, where the contents of each channel represents the total of all channels before it. The line  $f_2 = ax + b$  is thus obtained and stored on tape.

(3) A background spectrum is taken and stored on tape (Fig. 1a).

(4) Now the sample spectrum is taken and the background is subtracted from it, leaving in the memory a curve  $f_s = \frac{d\chi''}{dx} + c$ , in which  $x$  is proportional to  $H$  and  $c$

is a constant. This is recorded on tape (Fig. 1b).

(5) The curve  $f_s$  is now integrated to give  $\int f_s dx = \chi'' + cx + d$ . From this is subtracted  $f_s$  multiplied by a suitable constant to leave  $f_a = \chi'' + e$ . This represents the true absorption curve superimposed on a base-line of height  $e$  (Fig. 1c).

(6) Again this is recorded and integrated to give  $\int f_a dx = \int \chi'' dx + ex + f$ ;  $f_a$  is subtracted again multiplied by a suitable constant to leave  $f_b = \int \chi'' dx + g$ . Now  $\int \chi'' dx$  is proportional to the number of spins and  $g$  is merely a constant (Fig. 1d).

The process is calibrated using a sample containing a known number of spins. Although this appears to be a long process, in fact each recording or playing back operation takes only 4 sec. More time is spent in obtaining the spectra. The errors involved in the usual method of manual integrations of pen recording traces have been found to be as high as 10 per cent; with this procedure described here they are reduced to about 1 per cent.

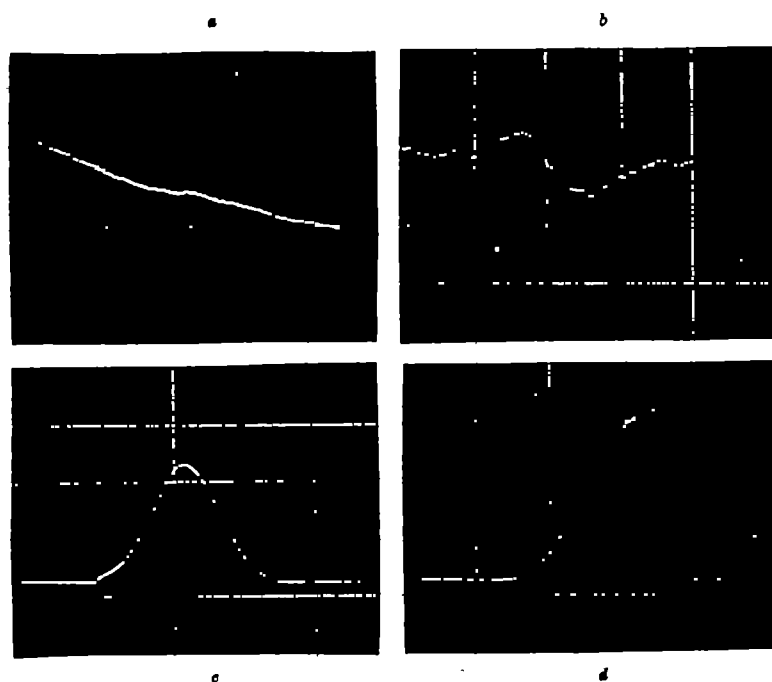


Fig. 1

One further point about the integration is that the integration procedure tends to introduce a further apparent reduction of noise, but one which depends on the channel number. This arises from the fact that, in channel 400, the addition of the contents of all 399 channels before it results in the noise ratio of channel 400 to channel 1 being improved by  $\sqrt{400}$ , while at channel 10 an improvement of only  $\sqrt{10}$  results.

In addition to the integration facility one can also differentiate a spectrum by subtraction of the same spectra shifted relative to each other by a number of channels small relative to the line width. As is well known this is particularly useful for the measurement of  $g$  values since the second derivative will show an antinode at the value of  $g$ . The analyser is provided with markers so that the exact channel number of an antinode can be determined. In this way, by calibration against a standard sample, an accuracy of 1 in 10,000 in  $g$  can be obtained, the accuracy depending mainly on magnet stability.

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## BIOCHEMISTRY

### Changes in Concentration of Polyamines in the Developing Mouse Brain

SPERMIDINE and spermine have recently attracted much interest because of their physical affinity to nucleic acid<sup>1-3</sup> and stimulating effect on cell proliferation<sup>4-6</sup>. These amines occur in high concentrations in actively proliferating tissues such as human semen<sup>7</sup>, growing chick embryo<sup>8</sup>, and germinating plant seeds<sup>9</sup>. These tissues contain a high concentration of deoxyribonucleic acid (DNA). Rama<sup>8</sup>

has recently found high concentrations of polyamines in the chick embryo which later decreased during incubation. We<sup>10</sup> have described the localization of spermine in the cell nucleus using nucleated and non-nucleated blood cells. These findings suggest the importance of polyamines in the growth process of plant and animal tissues.

Among the animal tissues, it is of advantage to use brain tissues for the study of a substance during growth since each component of the tissue, such as cell body, neuronal fibre, and myelin sheath, grows in different phases of the development and since the concentrations of substance which associate with each cellular component change in parallel with their cytological features.

In the present study the concentrations of spermidine and spermine were determined in brain and, for comparison, in liver, at various stages of development in the mouse (*ddO* strain) according to the method reported previously<sup>11</sup>. Although this method was devised originally for the determination of the amines in nervous tissue, examination of the specificity and recovery rate proved the method to be applicable to the determination of the amines in the mouse liver. The concentration of DNA in the brain was determined in parallel according to Schmidt

and Thanhauser<sup>12</sup>.

A high concentration of spermidine found in the foetal mouse brain decreased during the first 28 days after birth. The concentration of spermine in the brain remained at a high level until the twenty-first day after birth and fell during the following 7 days (Fig. 1). It is known from histological investigations of the rat brain that during the 20 days after birth it is characterized by cell division and the increase in size of individual cells and especially by the extension of axons and dendritic connexions<sup>13</sup>. In general, the cause of development of the mouse brain has been shown to be almost the same as that of the rat<sup>14,15</sup>. Later, the concentration of both amines remained at lower constant levels until the adult stage was reached (Fig. 1).

As shown by early experiments in other species<sup>16-18</sup>, the concentration of DNA in the mouse brain was highest in the pre-natal stage, dropped abruptly during the first 7 days after birth, and decreased slowly from then to the adult level (Fig. 2). These changes are similar to those of

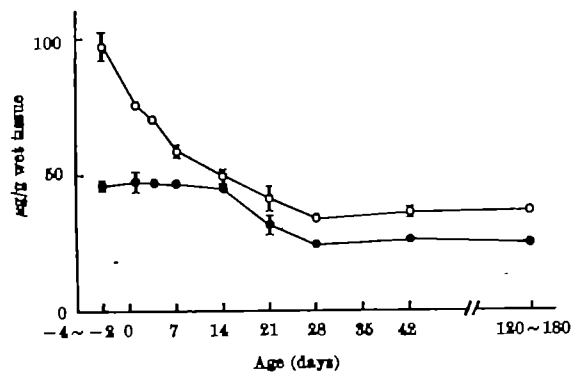


Fig. 1. Changes in the concentrations of spermidine and spermine of mouse brain during maturation. ○, The concentration of spermidine; ●, the concentration of spermine. Each spot is the average of three experiments. Vertical lines indicate standard deviations. Spots without vertical line have S.D. < 2.0 µg/g.

the polyamines, but changes in DNA concentration preceded those in the concentration of polyamines.

In the liver, of which the cells are known to reach maturation at the early pre-natal period and to continue relatively constant proliferation thereafter, no change in the concentration of the amines was observed during the early period after birth, in contrast to the marked changes in the brain. Gradual and slight decrease of spermidine and increase of spermine were observed (Fig. 3).

In summary, the concentration of polyamines in the mouse brain was shown to be higher in the pre-natal and early post-natal life than in adult. This trend was more apparent if the concentrations were calculated on a dry

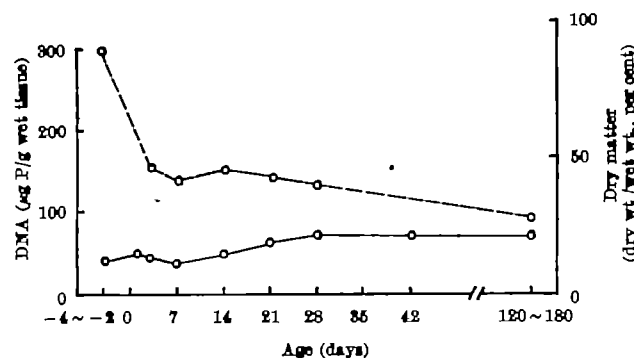


Fig. 2. Changes in the concentration of DNA and in the proportion of dry matter of mouse brain at different ages. ○, concentration of DNA as microgram DNA phosphorus per g of wet tissue; ●, proportion of dry matter as percentage of wet tissue.

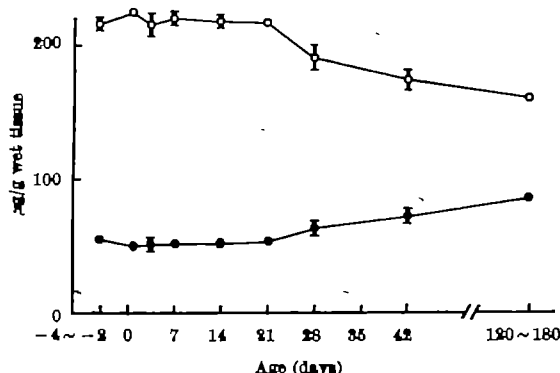


Fig. 3. Changes in the concentrations of spermidine and spermine of mouse liver during maturation. ○, The concentration of spermidine; ●, the concentration of spermine. Each spot is the average of three experiments. Vertical lines indicate standard deviations. Spots without vertical line have S.D. < 6.0 µg/g.

weight basis, since the water content in the brain decreases during development (Fig. 2). A high concentration of spermidine and spermine of the brain during the proliferation stage followed by a drop in concentration after this stage is considered to provide further evidence of their biological function in cell growth. Most substances and enzyme activities in the brain increase during development, in contrast to polyamines and DNA.

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## Isolation and Identification of 2-Aminoethylphosphonic Acid from Bovine Brain

In the course of isolating unidentified amino-compounds from bovine brain<sup>1-3</sup>, a substance was isolated from the neutral amino-acid fraction of the trichloroacetic acid of the brain, and was identified as 2-aminoethylphosphonic acid from the results of elementary analysis, infra-red spectrography and chromatography. The occurrence of 2-aminoethylphosphonic acid in protozoa<sup>4,5</sup> and in the sea anemone<sup>6</sup> has been reported both in free and combined form; but its presence in the higher animals has not been reported in either form until now.

Seventy-five bovine brains, weighing 28.5 kg, were extracted with trichloroacetic acid and desalted<sup>1</sup>. The pooled and concentrated extract (800 ml.) was separated into the acidic, neutral, and basic fraction by the methods described for the isolation of  $\beta$ -aminoisobutyric acid from urine<sup>1</sup>. The neutral fraction was concentrated to about 800 ml. *in vacuo*, filtered, and was applied to a 6 cm  $\times$  7 cm column of 'Amberlite IR-120',  $\times$  8 (100-200 mesh, pyridine form). The resin was washed with 800 ml. of water and the substances were eluted with 1 N pyridine. The first 800 ml. of the eluate was discarded, and the following 2,300 ml. was collected, concentrated to 150 ml. and filtered. The filtrate was acidified to pH 2.0 by the addition of formic acid and the solution was charged on to a 5.3 cm  $\times$  137 cm column of 'Amberlite OG-120',  $\times$  8 (200-400 mesh), previously buffered with a mixture of pyridine-formic acid-water (1:9:190, v/v), pH 2. The same buffer was used for elution, and the emergence of compounds was followed by examining aliquots of the eluate by paper chromatography. Two unidentified ninhydrin-positive substances emerged in the eluate between 1,760 ml. and 2,880 ml. This portion of the eluate was concentrated to a small volume, and was further purified by chromatography on a 2 cm  $\times$  30 cm

Table 1. PAPER CHROMATOGRAPHIC AND ELECTROPHORETIC PROPERTIES OF 2-AMINOETHYLPHOSPHONIC ACID AND THE RELATED COMPOUNDS

	R <sub>F</sub> values with solvent systems				Migration distances (cm) toward anode with buffers	
	Pyridine-acetone-3 N ammonia (60:30:25, v/v)	Isopropanol-formic acid-water (8:1:1, v/v)	n-Butanol-acetic acid-water (4:1:1, v/v)	Phenol-water (8:2, v/v)	Pyridine-acetic acid-water (1:10:180, v/v) pH 5.4 100 V/cm, 1 h	Formic acid-acetic acid-water (3:8:56, v/v) pH 1.8 40 V/cm, 30 min
Isolated compound	0.03	0.23	0.24	0.35	1.1	4.1
2-Aminoethylphosphonic acid	0.03	0.23	0.24	0.35	1.1	4.1
1-Aminoethylphosphonic acid	0.02	0.26	0.30	0.34	0.7	3.6
2-Aminoethylphosphate	0.02	0.17	0.22	0.35	0.8	3.9

column of 'Dowex 1', × 10 (200–400 mesh), equilibrated with a mixture of 2,4-lutidine-acetic acid-water (10.7:1.5:488, v/v), pH 7.0. Substances were eluted from the column by 140 ml. of the same buffer followed by a mixture of 2,4-lutidine-acetic acid-water (10.7:0.3:489, v/v), pH 7.7. One of the unidentified substances emerged in the eluate between 51 ml. and 171 ml. The eluate was evaporated to dryness *in vacuo*. The residue was crystallized from 1 ml. of water, 1 ml. of ethanol and 0.5 ml. of acetone to obtain 18 mg of needles, m.p. 255° C (decomp.), raised to 270° C (decomp.) on recrystallization. Elementary analysis gave: C, 19.41; H, 6.57; N, 11.12; C<sub>5</sub>H<sub>10</sub>NPO<sub>3</sub> requires C, 19.21; H, 6.44; N, 11.20. Phosphorus was detected by wet ashing.

On hydrolysis of a portion of the crystal with 6 N hydrochloric acid at 105° C for 15 h, neither change in the chromatographic behaviour of this substance nor the liberation of inorganic phosphate was observed. Stability of the compound to acid hydrolysis suggested the presence of a covalent linkage between C and P. In paper chromatography<sup>1,2</sup> and electrophoresis<sup>1,2</sup> this substance migrated to a position close to that of 2-aminoethyl phosphate. These data were compatible with those of either 1-aminoethylphosphonic acid or 2-aminoethylphosphonic acid. On comparison with synthetic compounds the isolated substance behaved like the latter in paper chromatography with four different solvent systems and in paper electrophoresis under two different conditions, but on neither occasion like the former (Table 1). The infra-red spectrum of the isolated substance was identical with that of the β-crystal<sup>6</sup> of synthetic 2-aminoethylphosphonic acid.

2-Aminoethylphosphonic acid has a C-P linkage, the occurrence of which has not been reported in the body of vertebrates. Little information about the enzymatic formation and degradation of the linkage is available. Rosenberg<sup>3</sup> showed that there was an exchange of 2-aminoethylphosphonic acid between the protozoa, *Tetrahymena pyriformis*, and the surrounding medium. Horiguchi and Kandatsu<sup>12</sup> found recently that oral or intraperitoneal administration of radioactive 2-aminoethylphosphonic acid to rat resulted in the accumulation of this compound in the liver, kidney, spleen, and many other tissues. As *Tetrahymena* exists in the bovine rumen symbiotically, there is a possibility that 2-aminoethylphosphonic acid isolated from bovine brains is derived from the protozoa in the rumen. Considering the recovery of the substance during isolation, the concentration of free 2-aminoethylphosphonic acid in bovine brain is calculated to be about 2 μg/g wet tissue.

The combined forms of 2-aminoethylphosphonic acid with glycerol<sup>4</sup> and with ceramide<sup>11</sup> have been demonstrated in protozoa and in sea anemones, respectively. As brain is abundant in phospholipid, this tissue was expected to contain a combined form of the phosphonic acid. In our preliminary experiments, however, the concentration of 2-aminoethylphosphonic acid in the hydrolysate of the lipid fraction (chloroform-methanol soluble component) of bovine brain was found to be less than 1 μg/g wet tissue, if present.

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Bioassay of Pituitary Gonadotropins

DURING the course of purification of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from horse pituitary glands<sup>1</sup> the following method was used to determine the degree of separation of FSH and LH at two purification steps. A pH-5 soluble gonadotropic fraction and a purified FSH preparation from the third zone-electrophoresis were compared as to their effect on the increase in the weight of ovaries as a measure of FSH and ventral prostate as a measure of LH in hypophysectomized rats (Holtzman Co., Madison, Wisconsin). A dose-response relationship was developed between the ovarian weights obtained by different doses of the pH-5 soluble fraction. The doses of the FSH fraction from the third zone-electrophoresis were selected so as to induce essentially the same ovarian response as was obtained with various doses of the pH-5 soluble fraction. The average weights of the ventral prostate produced by these doses were used as a measure of the LH content of the two fractions. The results are presented in Fig. 1 and Table 1.

The slope of the dose-response relationship for LH decreased significantly<sup>2</sup> from 0.13 for the pH-5 soluble gonadotropic fraction to 0.07 for the FSH from the third zone-electrophoresis while the slope for the dose-response relationship for FSH of the same preparations increased from 0.43 to 0.63. The slope ratio of FSH to LH for pH-5 soluble gonadotropins increased from 3:1 to 9:1 for FSH preparation after the third zone-electrophoresis. These observations suggest that significant separation of FSH and LH was achieved and further suggest that FSH and LH are separable and different entities in the horse pituitary gland. It will be of interest to learn whether

Table 1. HORMONAL ACTIVITY OF HORSE PITUITARY GONADOTROPIC FRACTIONS IN HYPOPHYSECTOMIZED RATS

Preparation	Protein mg/g fresh tissue	Slope FSH	(b) LH	Slope ratio FSH: LH
pH 5 Soluble gonadotropin	11.6	0.43	0.13	3:1
Third zone electrophoresis FSH	0.44	0.63	0.07	9:1

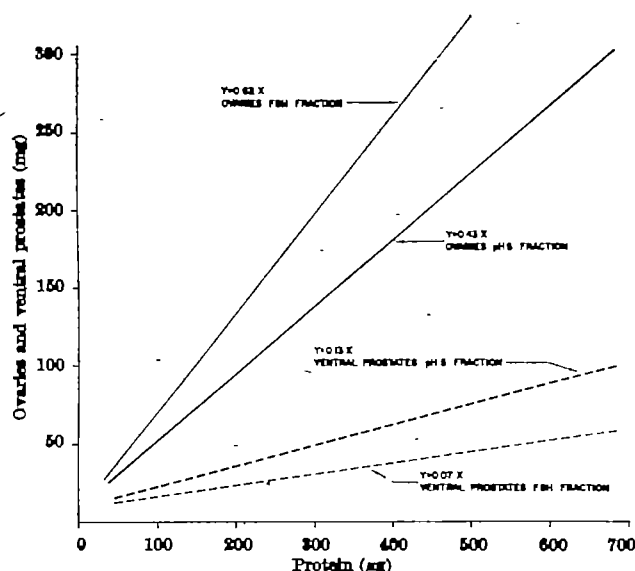


Fig. 1. Graphs showing results of the bioassay in hypophysectomized rats of the pH 5 soluble fraction of horse pituitary glands and the FSH preparation obtained from this fraction after the third zone electrophoresis.

this observation can be used in following the separation of two substances with overlapping biological activity.

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## Effect of Human Growth Hormone on Insulin Basic Protein Complex

It is generally agreed that there are two forms of insulin circulating in human blood plasma: (i) the immunologically inactive form bound to a basic protein<sup>1-3</sup>; (ii) the immunologically active free form<sup>4</sup>.

In our *in vitro* studies of the binding reactions between <sup>125</sup>I-bovine insulin (Radiochemical Centre, Amersham) and a commercial insulin antibody preparation (Burroughs Wellcome) the effect of other hormones on these binding reactions was observed.

The method used to indicate <sup>125</sup>I-insulin-antibody binding was similar to that described by Meade and

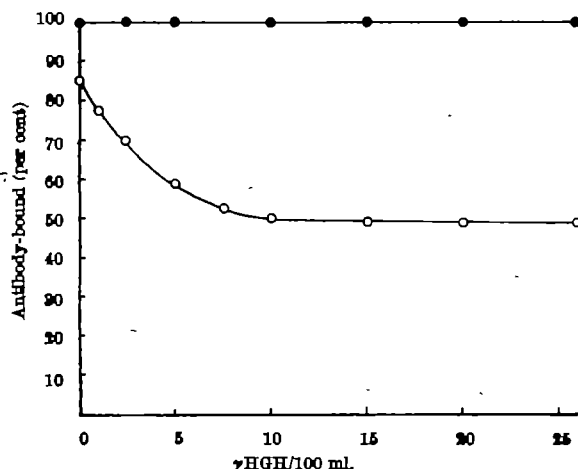


Fig. 1. Effect of added HGH on <sup>125</sup>I-insulin-antibody binding. O, Percentage bound in serum; ●, percentage bound in 3.95 per cent human serum albumin.

Kiltgaard<sup>5,6</sup>, who used an anion exchange resin to separate the free <sup>125</sup>I-insulin from the insulin bound to that antibody, this giving a good index of the bound-to-free ratio of <sup>125</sup>I-insulin.

It was found, on adding human growth hormone (HGH) (U.S. National Institutes of Health) to insulin antibody in the presence of human fasting serum, that the proportion of <sup>125</sup>I-insulin bound to antibody decreased, with increase in the amount of HGH added. When repeated with antibody in 3.95 per cent human serum albumin, no change occurred (Fig. 1).

It was postulated that HGH displaced endogenous insulin from the insulin-basic protein complex, and made it available for antibody binding. This theory was supported when it was found that <sup>125</sup>I-HGH<sup>7</sup> could be partly extracted from serum by the cation exchange resin 'Dowex 50' (Na cycle) but not from 3.95 per cent albumin. This indicated that some HGH is bound to a basic protein in human blood plasma.

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## Phenolic and Indole Amines in the Urine of Schizophrenics

The hypothesis that schizophrenia may be caused by a metabolic abnormality of phenolic or indolic amines has gained supporting experimental results by some research groups, but conflicting results by others. This confused state was reviewed by Kety<sup>1</sup>, and the contradictory results could be explained by various factors related to different environmental and physical conditions which are secondary to the psychosis. An attempt was made to examine in this laboratory the excretion of various phenolic and indole amines using the subjects under careful control of environmental factors.

Seven schizophrenic patients having 5-7 year courses of the disease and eight normal subjects who were on the same diet were selected. Routine clinical examinations revealed no physical abnormalities in both groups of subjects. Medications were withdrawn for more than a month before the collection of urine and both patients and controls were engaged in the same degree of physical work. Twenty-four hour urine specimens of schizophrenics were collected under strict supervision for 3 days, but collection of a full 24-h urine failed frequently because of pathological behaviour of the patients. Thirteen specimens of complete 24-h urine could be collected. Urine was collected on the second day from normal subjects. Urine was kept in a refrigerator during the 24-h collection and then kept at -20° C. Quantitation of amino nitrogen, urea, creatinine, creatine and uric acid of these specimens showed that there were no pathological changes in the amounts of these nitrogen compounds in the urine of both groups.

Paper chromatograms of phenolic amines were prepared according to the method of Kakimoto and Armstrong<sup>2</sup>. Diazotized *p*-nitroaniline was used for the detection of phenolic amines. Paper chromatograms of indole amines were prepared in the same manner as phenolic amines and rendered visible with the *p*-dimethylaminobenzaldehyde reagent. Compounds listed in Table 1 were detected



Table 1. PHENOLIC AND INDOLE AMINES AND THE FREQUENCY OF THEIR APPEARANCE IN URINE SPECIMENS OF SCHIZOPHRENICS AND NORMALS

Compounds	Frequency	
	Schizophrenics	Normals
No. of urine specimens examined	18	8
Normetanephrine	13	8
Metanephrine	13	8
p-Tyramine	11	8
m-Tyramine	13	8
Histamine	10	4
3-Methoxytyramine	7	0
p-Hydroxybenzylamine	2	0
p-Sympathol	8	2
Serotonin	13	8
Tryptamine	13	8

in the urine specimens from the normal subjects and patients. Frequency of the appearance of the spots on the paper chromatograms are shown in Table 1. Seven unidentified indole compounds which are not listed in Table 1 were found in several specimens of both groups, but these compounds were not specifically detected in one of the two groups. A trace amount of 3-methoxytyramine was found only in the urine of schizophrenics in this investigation. This difference can, however, not be taken as difference in the metabolism of catecholamines between the patients and normals, because this compound has also been detected in paper chromatograms of urine of normal subjects in a previous investigation<sup>3</sup>, and because this amine is a normal constituent of human urine as a conjugated form. Bufotenin has been claimed to be a constituent of the urine of schizophrenic patients<sup>4</sup>, but no detectable amount of bufotenin was found in the present investigation. Considering the sensitivity of the method used, the amount of bufotenin is less than 20 µg in 24-h urine, if present.

Phenolic acids and indole acids in the urine specimens were also examined by paper chromatography according to the methods of Armstrong *et al.*<sup>4,5</sup>, and no qualitative differences of various acids were observed between the two groups.

In our previous investigation the finding by Friedhoff *et al.*<sup>6</sup> that 3,4-dimethoxyphenylethylamine is found in the urine of schizophrenic patients but not in normal urine has not been supported<sup>7</sup>. In the investigation recorded here other phenolic and indole amines were examined in the urine of schizophrenic patients: no result was obtained which would support a hypothesis of abnormal metabolism of these amines in the disease.

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## PHYSIOLOGY

### Catheptic Activity of the Gastrointestinal Tract, Liver, Spleen and Kidney of the Rat

As reviewed by Fruton<sup>1</sup>, cathepsins are intracellular proteinases with acid pH optima, ubiquitously found in animal tissues. In the mammal, highest concentrations of cathepsins are found in liver, kidney and spleen<sup>2-4</sup>. However, perfused rat liver was found to catabolize only 13–16 per cent of the total albumin broken down in the whole animal<sup>4</sup>.

The rapid turnover of the gastrointestinal tract is well documented. The finding of catheptic activity in rat intestinal mucosa of magnitude comparable to that of rat liver<sup>5</sup> prompted a comparison of catheptic activity in the various segments of the gastrointestinal tract with that of rat liver, spleen and kidney.

Male Sprague-Dawley rats (300–370 gm) were decapitated after a 24-h fast and the livers, kidneys and spleens were washed and homogenized in 0.25 M sucrose. The small intestine was divided into three equal parts; that next to the stomach was arbitrarily called the duodenum, next the jejunum and next the ileum. These segments, as well as the stomach, caecum, and colon, were homogenized in 0.25 M sucrose after careful washing. The proteolytic activity of the homogenates was determined in 0.1 M acetate buffer pH 3.7, with denatured haemoglobin (10 mg/ml) as substrate. Proteolytic activity was measured as free amino-acids expressed in terms of tyrosine equivalents. Proteolytic activity and protein concentrations of the tissues were measured by the colorimetric method of Miller<sup>7</sup>.

Results in Table 1 show the high catheptic activity and proteolytic capacity of the gastrointestinal tract. The figures in the last column give an approximation of the proteolytic capacity of the organs. A more accurate picture could be obtained only by taking into account the turnover rate of the various organs, a consideration which would probably magnify the catabolic capacity of the gastrointestinal tract. Friedberg *et al.*<sup>8</sup>, for example, showed that when <sup>35</sup>S methionine is given to fasted rats, the intestinal mucosa proteins show the highest specific activity of <sup>35</sup>S and also a rapid loss of activity.

Table 1. COMPARATIVE CATHEPTIC ACTIVITY OF THE GASTROINTESTINAL TRACT, LIVER, SPLEEN AND KIDNEY OF THE RAT

Tissue	Average wet wt./rat (g)	Specific activity	Calculated proteolytic
		nanomoles tyrosine released/min/mg protein	capacity haemoglobin (g) hydrolysed/rat/24 h
Stomach	1.60	62.5	10.3
Duodenum	3.68	15.8	4.06
Jejunum	3.66	6.8	7.62
Ileum	3.39	11.8	2.94
Caecum	1.12	7.3	1.36
Colon	2.11	11.5	2.63
Liver	9.53	5.07	17.8
Kidney	2.75	15.1	10.4
Spleen	.69	25	4.75

Values are the averages of 3 experiments, each including 16 rats. The high value for stomach may have been contributed to by pepsinogen activated by the low pH of incubation.

Our data indicate that in any quantitative study of overall catheptic activity or breakdown of plasma proteins, it would be advisable to include the different segments of the gastrointestinal tract.

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### Transauricular Perfusion of the Pituitary Gland in Rats and Mice

So far as we know, intrapituitary injections<sup>1-7</sup> or implants<sup>8-11</sup> have been performed only on adult rats<sup>1-3, 8-11</sup> or on bigger animals such as rabbits<sup>4, 7, 12-14</sup> or dogs<sup>15</sup>. For these experiments stereotaxic methods are usually adop-

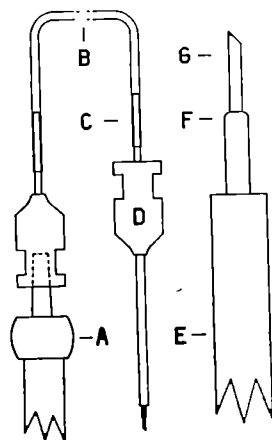


Fig. 1. Apparatus for intrapituitary micro-injections. A, microsyringe with needle inserted; B, vinyl tube; C, connection between the vinyl tube and the needle for micro-injections; D, needle for micro-injections; E, outer sheath; F, middle sheath; G, tip

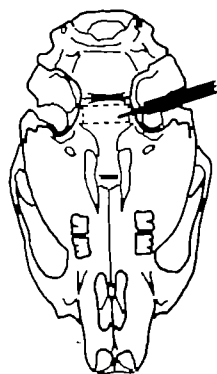


Fig. 2. Drawing of a skull of a 21-day-old rat, showing actual position and size of needle (in solid black) during the perfusion into the hypophysis (outlined area). The outer sheath of the needle rests against the petiotic capsule wall, the middle sheath fits into the hole made by the perforating needle and the point enters the pituitary

ted. In this communication a technique is described which renders it possible to perform injections or perfusions into the pituitary gland of both immature and adult rats and mice. To this purpose a needle has been devised which allows entrance to the hypophyseal tissue by the transauricular approach. Such a route had been already used to perform, with suitable instruments, both pituitary ablation<sup>10,11</sup> and transplantation<sup>12</sup>.

The equipment (Fig. 1) consists of a special needle connected through a vinyl tube to a hypodermic needle mounted on a microsyringe. The special needle is made of three stainless steel tubes of increased length (outer sheath, middle sheath and tip) coaxially fitted and soldered to a socket. These features will determine and maintain the proper position of the needle during the injection (Fig. 2). Another steel tube (outside diameter 1.1 mm) is soldered to the opposite side of the socket for the junction with the vinyl tube. The vinyl tube (inside

diameter 1.0 mm, outside diameter 1.4 mm, 20–25 cm long) acts as a flexible connexion with the needle mounted on the microsyringe. This latter, fixed on the table and mechanically driven, can release fluid at various output rates. This equipment is suitable for microinjections in both rats and mice, diameters and lengths of the free portions of middle sheath and point being changed according to the animal's size (Table 1). The animal to be injected is anaesthetized and held by the operator as previously illustrated<sup>13</sup>. The transauricular route to the hypophyseal capsule is opened by a needle as for hypophysectomy<sup>14</sup>. When the perforating needle is removed, the needle for micro-injections is introduced and gently pushed forward, through the open pathway, until the point penetrates the lateral portion of the pars distalis. The fluid can now be perfused into the hypophysis at a rate of 0.5–2  $\mu$ l./min, according to the size of the animal, for as long as is necessary. When required, the perfusion can be repeated. The needle is extracted at the end of each treatment. If the time elapsed between two treatments is no longer than 10 days, no further opening of the pathway to the hypophysis is needed. After each injection, the animals are given intraperitoneally tetracycline hydrochloride for 3 days at a daily dose of 0.05 mg/g body-weight. The accuracy of procedure can be checked by perfusing, before autopsy, a dye solution under the same conditions followed during the experiment<sup>4</sup>; the presence of a stained pituitary without any trace of dye outside the capsule (positive dye-test) will give evidence of the accuracy of previous treatment(s), since it was observed that the needle always follows the route of the first injection. A pilot experiment on rats and mice is reported. Twenty-four male immature Sprague-Dawley rats were treated for 5 min with saline, delivered at 1.2  $\mu$ l./min. The perfusion was repeated, every second day, for four times. At autopsy, the weight of testes, adrenals and thyroid of the animals with positive dye-test were taken and compared with controls. Forty-five male adult albino Swiss mice were treated only once with saline, given at 0.8  $\mu$ l./min for 5 min. The autopsies were performed 10 days after the treatment, as for the rats.

Table 1. DIMENSIONS OF THE CHANGING PORTIONS OF THE NEEDLES FOR TRANSAURICULAR INTRAPITUITARY MICRO-INJECTIONS IN RATS AND MICE OF DIFFERENT SIZE

Animal	Tail-length (cm)	Middle sheath		Gauge No.	Point Length of the offset portion (mm)
		Outside diameter (mm)	Length of the offset portion (mm)		
Rat*	<10.5	0.8	1.8	26	1.2
	10.5–14.5	0.8	2.0	25	1.5
	>14.5	1.0	2.2	25	1.8
Mouse†	<8.0	0.5	1.1	27	0.9
	>8.0	0.5	1.3	27	1.2

\* Sprague-Dawley strain.  
† Albino-Swiss strain.

The results of the sample experiment on rats and mice are given in Table 2. Single or repeated transauricular intrapituitary injections of saline cause no death, nor significant change in the weight of body, testes, adrenals and thyroid. Gross examination of the pituitary region never showed abscesses or haemorrhages. The number of positive dye-tests was satisfactorily high (rats: 96 per cent, mice: 93 per cent). On the basis of these results the transauricular route appears to furnish a new, safe and reliable

Table 2. RESULTS OF THE SAMPLE EXPERIMENT OF TRANSAURICULAR PITUITARY PERFUSION IN RATS AND MICE

Animals	Treatment	Total volume ( $\mu$ l.) (No. of injections)	No of animals			Average body weight (g)		Average organ weight (mg)		
			At the beginning	Alive after 10 days	With positive dye-test	Initial	Final	Testes	Adrenals	Thyroid
Rats*	None	—	24	24	—	56	80	620 $\pm$ 24.8†	14.0 $\pm$ 0.45	8.8 $\pm$ 0.30
	Saline	24 (4)	24	24	23	56	80	608 $\pm$ 27.7	13.6 $\pm$ 0.30	8.8 $\pm$ 0.23
Mice†	None	—	45	45	—	22	31	204 $\pm$ 4.13	2.96 $\pm$ 0.11	—
	Saline	3 (1)	45	45	42	22	30	204 $\pm$ 4.21	3.33 $\pm$ 0.14	—

\* Sprague-Dawley strain.  
† Albino-Swiss strain  
‡ S.E.

approach for intrapituitary microinjections in rats and mice.

We thank Mr. Ansperto Beretta for his assistance and his help in the construction of the equipment.

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## PHARMACOLOGY

### Valency Investigations of Iron Dextran ('Imferon')

THE parenteral iron preparation 'Imferon', containing 5 per cent iron and 20 per cent dextran, is frequently used intravenously as well as by the intramuscular route for the treatment of anaemia due to iron deficiency.

Several theories have been put forward as to the chemical nature of this iron dextran preparation. These, broadly speaking, fall into two groups, namely, that: (1) ferric iron is chemically bonded to the dextran; (2) ferric iron is present as colloidal ferric hydroxide protected by the dextran. Recent investigations<sup>1</sup> using gel-filtration, ultrafiltration and ultracentrifugation have lent support to the theory that the iron is present as a ferric-dextran complex.

A striking feature of iron dextran is its very low intravenous acute toxicity ( $LD_{50}$ , 1,000 mg Fe/kg)<sup>2</sup>. This is probably a reflexion of a low ionic iron content. From *in vitro* haemolysis investigations of human red cells the ionic iron content was deduced to be about 1/300th of the total iron present<sup>3</sup>.

Polarography and high-voltage electrophoresis have been used to show the presence of very small amounts of loosely bound ferrous iron in 'Imferon' and to obtain an estimate of its concentration. At the same time, the overall results are in general agreement with the concept of a ferric-dextran complex.

The presence of ferrous iron in freshly opened ampoules of 'Imferon' was established by a high-voltage electrophoretic separation on 3 M.M. Whatman filter paper followed by detection with 4:7 diphenyl 1:10 phenanthroline. Separations suitable for quantitative evaluation were obtained using pH 5.6 acetate-acetic acid buffer solution and a potential gradient of 35 V/cm applied for 45 min. Evaluation of the developed ferrous-bathophenanthroline complex was obtained by: (a) planimetric scanning, and (b) elution of the complex with methanol followed by a spectrophotometric determination. The results for freshly opened ampoules of 'Imferon' showed the ferrous iron content to be in the range 50-100 mg/100 ml., whereas those which had been exposed to the atmosphere for several days contained no ferrous iron.

Table 1. HALF-WAVE POTENTIALS OF 'IMFERON', FREE FERROUS AND FERRIC IRON

	First wave ( $Fe^{3+} \rightarrow Fe^{2+}$ ) $E_1 \pm S.O.E. \uparrow$ (V) at 25°	Second wave ( $Fe^{3+} \rightarrow Fe^0$ ) $E_2 \pm S.O.E. \uparrow$ (V) at 25°
'Imferon'	-0.83	-1.39
Ferrous iron	—	-1.38
Ferric iron	+0.04	-1.38

\* 'Imferon', Eli Lilly Pharmaceuticals, Ltd.  
† S.O.E., standard calomel electrode.

The polarography of 'Imferon', free from ferrous iron, gave two distinct waves, as shown in Fig. 1.

The half-wave potentials of these waves together with those of ferrous and ferric iron are given in Table 1.

It is considered that these results indicate complex formation and that the ferric to dextran bonding is quite strong. The reduction of 'Imferon', at the dropping mercury cathode, probably occurs in two stages: (1) ferric-dextran complex  $\rightarrow$  ferrous-dextran complex + e; (2) ferrous-dextran complex  $\rightarrow$  iron + 2e.

Further confirmation is provided by the ratio of the diffusion currents, 1 to 2.02, compared with a theoretical value of 1 to 2.

The slight shift in half-wave potential from -1.38 V for ferrous iron to -1.39 V for ferrous-dextran indicates this is an extremely weak complex. It is probable that all the ferrous iron in 'Imferon' is present as this complex. The view that the ferrous iron is probably present in 'Imferon' as a weak ferrous-dextran complex is supported by the polarography of ferrous iron in the presence of 'Dextran C' (the dextran fraction, of average molecular weight 5,000, used in the manufacture of 'Imferon'). The ferrous iron half-wave potential was again displaced from 1.38 to -1.39 V. This was accompanied by a 20 per cent decrease in the diffusion current, which could not be accounted for by a change in the viscosity of the base electrolyte to which 'Dextran C' had been added.

The increase in the ratio of the diffusion currents in the first and second stages of reduction has been used to estimate the ferrous iron content. In the presence of ferrous iron the ratio is no longer 1:2; values varying from 1:2.09 to 1:2.17 were obtained, which correspond with ferrous iron contents of 50-100 mg/100 ml. These values are in agreement with those obtained by electro-phoresis.

This work has shown that the ferric iron is most probably present in 'Imferon' as a ferric-dextran complex and that ferrous iron is present, probably as an extremely weak ferrous-dextran complex usually in the concentration range 50-100 mg/100 ml.

In the anaesthetized cat, intravenous iron dextran induces hypotension of rapid onset and short duration, similar to that produced by small doses of acetylcholine

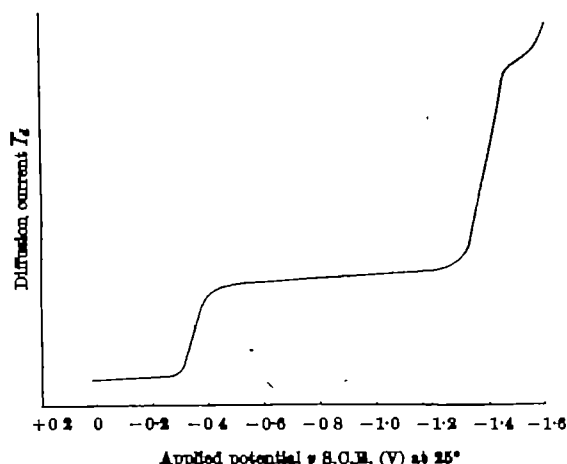


Fig. 1. Polarography of 'Imferon' (1 in 500 dilution in pH 5.6 acetate buffer solution)

or histamine<sup>4</sup>. Moreover, this fall in blood pressure is similar to that produced by ferrous iron. Indeed, there is a rough correlation between the hypotensive response of 'Imferon' and its ferrous iron content. Ferric iron, however, produces a different type of response; for an equivalent dose of ionic iron there is a smaller transient depressor response followed by a more sustained pressor response<sup>5</sup>.

The hypotensive effects of intravenous iron dextran were completely abolished at all dose-levels by reducing the speed of injection to 2.5 ml./min or by preliminary dilution of the iron dextran with isotonic saline.

So far as these observations apply to the clinical use of iron dextran, they emphasize the need for care in administering any form of iron intravenously.

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## HAEMATOLOGY

### Distribution of the $D_i^*$ Factor in Argentine Jews

FROM the initial results of their investigations about Diego antigen<sup>1,2</sup>, Layrisse and Arends supposed that it was a non-Caucasoid factor and related to the Carib Indians. Later studies on 2,600 Caucasoids from varied sources<sup>3,4</sup> corroborated that hypothesis on account of negative findings. Studies made in 1956 on Chinese<sup>5</sup> showed that the  $D_i^*$  gene belonged to Mongoloids rather than to American Indigenous.

A very low incidence in Russian and Polish individuals<sup>6</sup> of Tartarian origin suggests a Mongoloid hybridization on a Caucasoid stock.

Negroids do not carry the  $D_i^*$  gene, and the very low frequency which appears sometimes is attributed to a Mongoloid hybridization<sup>7</sup>.

Negative findings were also reported in South-west Asia including south, east and west India, Punjab and Pathans of Pakistan, Vedas of Ceylon<sup>8</sup> and Arabs of Iran. A low incidence of the antigen in Oraon of east India is attributed to Mongoloid neighbourhood.

About Jews there is an unpublished work<sup>9</sup> made by Gurevitch in Israel on 45 'Black Jews' of Cochin (India), with negative results.

Because of the widespread distribution in *golah*, the migratory life and high intermarriage rate of the Jewish people, I thought that the  $D_i^*$  factor might be detected in some frequency owing to a Mongoloid admixture.

I have analysed 400 specimens from 200 ashkenasim and 200 sephardim. Ethnic, cultural and other characters are described elsewhere<sup>10</sup>.

Blood specimens were taken by venous or digital puncture from unrelated Jews of La Plata, Burzaco and Buenos Aires cities; the donors were native and descendants from European, African and Asiatic origin<sup>11</sup>.

Indirect antiglobulin method with washed red blood cells in a 2 per cent suspension in normal saline and anti-

$D_i^*$  serum kindly provided by Dr. Miguel Layrisse from the Instituto Venezolano de Investigaciones Cientificas (Caracas) was used.

The factor was detected in 6 Jews and it was absent in the other 394 samples. It was absent in the 200 ashkenasim, and the positive findings belong to 3 natives from Esmirna (Turkey) and the other three are Argentine, from which two have both parents born in Esmirna and the last one has the father born in Urla—distant 40 km from Esmirna—and her grandparents both born in Esmirna.

Shown in Tables 1, 2, 3 and 4 are respectively: phenotypic and genic frequencies for Jewish people as a whole; Sephardim and Ashkenasim, independently; Sephardim of Turkey and Sephardim of Esmirna and neighbouring villages.

Table 1. PHENOTYPIC AND GENIC FREQUENCIES OF  $D_i^*$  FACTOR IN JEWISH PEOPLE (400 samples)

Phenotypes (%)	$D_i^*$ (a+)	1.50
	$D_i^*$ (a-)	98.50
Genes (%)	$D_i^*$	0.76
	$D_i$	99.24

Table 2. PHENOTYPIC AND GENIC FREQUENCIES OF  $D_i^*$  FACTOR IN SEPHARDIM AND ASHKENASIM

Sephardim (200 samples)	Phenotypes (%)	Ashkenasim (200 samples)
$D_i^*$ (a+)	3.00	0.00
$D_i^*$ (a-)	97.00	0.00
$D_i^*$	1.51	0.00
$D_i$	98.49	0.00

Table 3. PHENOTYPIC AND GENIC FREQUENCIES OF  $D_i^*$  FACTOR IN SEPHARDIM OF TURKEY (79 samples)

Phenotypes (%)	$D_i^*$ (a+)	7.59
	$D_i^*$ (a-)	92.41
Genes (%)	$D_i^*$	8.87
	$D_i$	91.13

Table 4. PHENOTYPIC AND GENIC FREQUENCIES OF  $D_i^*$  FACTOR IN SEPHARDIM FROM ESMIRNA AND NEIGHBOURING VILLAGES (THERE, MANTIN, URLA, AYDIN) (65 samples)

Phenotypes (%)	$D_i^*$ (a+)	9.67
	$D_i^*$ (a-)	90.33
Genes (%)	$D_i^*$	4.96
	$D_i$	95.04

According to the ABO system, Jewish people do not constitute a homogeneous group such as Armenians or Gipsies.

Studies performed in Israel<sup>12-13</sup> and Syria showed a definite but not wide difference between Ashkenasim and Sephardim. Jews of North Africa have more percentage of A and B than the neighbouring Arabs.

In India there are two communities, 'Black Jews' and 'White Jews' of Cochin, that are physically different. The latter show a high frequency of A because it is a highly endogamic community. 'Black Jews' have a higher proportion of O than the neighbouring low-caste Indians<sup>14</sup>.

Ashkenasim from Israel and Canada have a high frequency of  $R_1$  ( $CD_e$ ) and a higher frequency of  $R_2$  ( $cDE$ ) than North-European populations;  $d$  is lower than Centre- and North-European peoples.

Chown and Gurevitch have reported a high percentage for  $R_2$  ( $cDE$ ) which is of African origin.

Sephardim show a typically high level of  $R_1$  ( $CD_e$ ) and a low level of  $R_2$  ( $cDE$ ), but  $r$  ( $cde$ ) is higher than the Mediterranean values. The frequency of  $R_2$  ( $cDE$ ) is the highest one of any other Mediterranean people.

A Mediterranean and African component is deduced from Rh-Hr tests in Ashkenasim and Sephardim. According to the present findings on Diego factor in Sephardim we conclude that it may be admitted in hybridization with Turks, who belong to a Mongoloid origin.

Sero-anthropological investigations in Jews, including this work, contribute to sustain Mourant's hypothesis<sup>15</sup> that this ancient people resembles its local neighbours to a varying and sometimes a very close degree from a biological point of view.

I thank Drs. Miguel Layrisse and Tulio Arends for their kind co-operation and provision of anti-Di<sup>a</sup> serum.

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### Some Antigen Similarities between Mouse Erythrocytes and Ehrlich Ascites Tumour Cells

It has been demonstrated that rabbit anti-mouse erythrocyte serum is capable of agglutinating ascites tumour cells<sup>1</sup>. Nothing is known, however, about the components of the erythrocyte which are responsible for producing the agglutinin; the present experiment was carried out to clarify this point, and mouse-specific ascites tumour cells and mouse erythrocytes were used.

Three female guinea-pigs were intramuscularly injected four times with 0.2 ml. erythrocyte ghosts of OF1 male mice with Freund's adjuvant every two weeks. Three other animals were treated in the same way with the ghosts of Ehrlich ascites tumour cells (EATC). The erythrocyte ghosts were prepared by Ponder's method<sup>2</sup>, and EATC were subjected to Ponder's procedure after homogenization. Two weeks after the last injection the antisera (the anti-mouse erythrocyte ghost and the anti-EATC ghost) were collected from the animals, and were treated at 56° C for 30 min before use. Almost the same technique was adopted for the agglutination test as was used in the previous investigation<sup>3</sup>. The erythrocytes tested were obtained from various strains of mice, such as A, CF1, C57BL, DDS and dd—though scarcely any difference was observed between the different strains on the titres resulted. The antisera were absorbed with equal volume of each absorbent shown in Table 1. The notation "crushed . . ." means that the absorbents with which the antisera were absorbed were prepared from Ponder's ghosts by pounding them with powdered dry ice using a mortar and pestle, and then washing with saline solution (Fig. 1a).



Fig. 1. Mouse erythrocytes used for the absorption ( $\times 1,000$ ). a, Crushed erythrocyte ghosts; b, erythrocyte ghosts by Ponder's method; c, intact erythrocytes.

Table 1. AGGLUTINATION OF MOUSE ERYTHROCYTES AND EHRLICH ASCITES TUMOUR CELLS WITH VARIOUS ABSORBENTS

Antiserum	Absorbed* with	Maximum titre† giving agglutination of Mouse eryth.‡	HATO§
Anti-mouse eryth. ghost	No absorbent used	512	64
	Normal mouse sera	512	64
	Ascites of tumour-bearing mouse	512	64
	Intact mouse eryth.	Neg†	64
	Ponder's mouse eryth. ghosts	Neg	64
	Crushed mouse eryth. ghosts	Neg	Neg
	Crushed guinea-pig eryth. ghosts	512	64
Anti-HATO ghost	EATC	512	Neg
	No absorbent used	8	512
	Normal mouse sera	8	512
	Ascites of tumour-bearing mouse	8	512
	Intact mouse eryth.	Neg	512
	Ponder's mouse eryth. ghosts	Neg	512
	Crushed mouse eryth. ghosts	Neg	4
Normal guinea-pig serum	Crushed guinea-pig eryth. ghosts	8	512
	EATC	Neg	Neg
No absorbent used		2	Neg

\* Antisera were absorbed with equal volume of each absorbent at 37° C for 60 min.

† Equal volume of antiserum dilution and erythrocytes (2.5 per cent in phosphate buffered saline) or Ehrlich ascites tumour cells (5 per cent) incubated at 37° C for 60 min.

‡ Eryth. erythrocyte.

§ EATC Ehrlich ascites tumour cell.

|| Neg. no agglutination at serum dilution 1:2.

|| Crushed erythrocytes were obtained from Ponder's ghosts by pounding them with powdered dry ice in a mortar.

The observations and the findings are briefly summarized here: (1) The ghosts injected as antigens contain practically no water-soluble component. When the antisera are absorbed with normal mouse sera or ascites of tumour-bearing mouse, neither antiserum loses its agglutinating capacity—indicating that any water-soluble antigen is not concerned in these agglutination reactions.

(2) The anti-erythrocyte ghost serum is capable of agglutinating both the mouse erythrocytes and EATC, indicating that the EATC-agglutinogens are shared with erythrocyte ghosts and the cell surface of EATC. The anti-EATC ghost serum can agglutinate EATC strongly, but erythrocytes very weakly. If anti-erythrocyte serum is absorbed with EATC, its titre against erythrocyte does not decrease. When both anti-erythrocyte ghost and anti-EATC ghost sera are absorbed with intact erythrocytes, their titre against EATC never decreases; one may conclude that the haemagglutinogens are different from the EATC-agglutinogens.

(3) When both antisera are absorbed with intact mouse erythrocytes (Fig. 1c) or with Ponder's ghosts which keep their disk-like shape (Fig. 1b), they retain completely their EATC-agglutinating capacity. On the other hand, they almost lose the capacity when absorbed with crushed mouse erythrocyte ghosts. Both antisera do not, however, lose their agglutinating capacities when absorbed with crushed guinea-pig erythrocyte ghosts. In short, all the foregoing findings indicate that the mouse erythrocyte ghost and EATC contain at least common EATC-agglutinogens, and that the EATC-agglutinogens do not exist on the outer surface of the erythrocyte membrane, but rather seem to exist on the inner surface of the membrane. In other words, the outer surface of the cell membrane of EATC and the inner surface of the cell membrane of mouse erythrocyte closely resemble each other so far as their antigenicities are concerned.

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## HISTOCHEMISTRY

## Retention of Nitrogenous Material in Unfixed Sections during Incubation for Histochemical Demonstration of Enzymes

THE dilemma of enzyme histochemistry is that if the tissue is not fixed, to avoid denaturation, the enzymes may dissolve out of the section into the incubation medium but, conversely, if the tissue is fixed to stop such loss of soluble enzymes, the fixation will inhibit activity and cause generalized denaturation. Thus, for most enzymes, it is argued that too much of the 'soluble' or lyo-form of otherwise bound enzymes<sup>1</sup> will be lost from unfixed sections during incubation in an aqueous medium; consequently it is considered advisable to fix the tissue prior to such incubation<sup>1-3</sup>. Although it is usually conceded that the histochemistry of dehydrogenases depends on the use of unfixed sections, some<sup>4</sup> have argued that even these should be studied in fixed tissue. Consequently it follows that the scientific basis of enzyme histochemistry must remain uncertain as long as a considerable loss of the activity to be investigated must be expected, whether this is caused by solution into the medium from unfixed sections, or from inhibition of activity produced by the preparative procedures.

This problem has been accentuated by the development of quantitative methods of evaluating enzyme activity in histochemical systems<sup>5</sup>. The full value of such studies will depend on the use of unfixed and well-preserved sections. On the other hand, Jones<sup>6</sup> has emphasized that, with his procedures, as much as 70 per cent of the 'fixed nitrogen' of his sections was lost in the first 5 min of incubation for succinic dehydrogenase activity, irrespective of the thickness of the sections used. Similarly, in their work on pentose-shunt dehydrogenases in unfixed sections, Gahan and Kalina<sup>7</sup> lost the whole content of these enzymes into the incubation medium after only 1 min. This was especially serious since an elevated level of activity of these enzymes seems to be a characteristic feature of invasive growth of cancer<sup>8</sup> and their loss into the incubation medium would render their histochemical study either impossible or meaningless. This problem has therefore been investigated in sections of rat liver, as was used by Jones<sup>6</sup> and by Gahan and Kalina<sup>7</sup>.

Female albino Wistar rats were killed with nitrogen. Pieces of liver about 0.5 cm<sup>3</sup> were plunged into hexane (B.D.H. 'free from aromatic hydrocarbons') maintained at about -70° C by an outer bath of solid carbon dioxide and alcohol. After not more than 1 min, the tissues were transferred, with chilled forceps, to a dry, corked tube, previously equilibrated against solid carbon dioxide in a Dewar flask, and stored in solid carbon dioxide for not longer than 2 days. Sections were cut at 8, 14 and 20  $\mu$  in a cryostat at about -30° C; the haft of the knife was cooled further by the presence of carbon dioxide ice packed around it. The sections were picked off the knife on to slides which were at the ambient temperature of the laboratory, and they were stored in a Dewar flask containing solid carbon dioxide until used, namely, for up to about 2 h. They were exposed to the air only long enough to dry them of obvious moisture before they were immersed in the relevant incubation medium at 37° C in microcells<sup>9</sup>.

The media contained 0.15 per cent neotetrazolium chloride (Sigma Chem. Co., U.S.A.); NADP (1 mg/ml.); glucose-6-phosphate sodium salt (1 mg/ml.) or 6-phosphogluconate trisodium salt (0.5 mg/ml.); 0.01 M potassium cyanide; 0.08 M calcium chloride in 0.05 M glycylglycine buffer at a pH of 7.6 for the former, and 8.4 for the latter, dehydrogenase. To these were added either 0, 10, 15 or 20 per cent w/v of polyvinyl alcohol (PVA). This substance was obtained from Bush, Beach and Segner Bayley, Ltd., London; the grade used was 'Polyviol M05/140':

this designation indicates that it has a 'medium' molecular weight, possibly of the order of 30,000, a viscosity of about 5 centipoises at 20° C when dissolved as a 4 per cent solution, and a saponification number of 140, indicating a low acetyl content. Sufficient activity for measurement was obtained after incubating for 20 min. The formazan produced by the dehydrogenation was eluted with 10 per cent *n*-heptanol in tetrachloroethane<sup>9</sup> and measured spectrophotometrically; the nitrogen content of the section was determined after digestion with 72 per cent perchloric acid (AnalaR grade)<sup>10</sup>. The area of the sections was calculated by planimetry of their image, projected by means of a photographic enlarger.

Table 1. THE EFFECT OF DIFFERENT CONCENTRATIONS OF PVA IN THE INCUBATION MEDIUM IN RETAINING NITROGENOUS MATERIAL

Section thickness ( $\mu$ )	Concentration of PVA (%)	mg N/unit area of section after incubating for		Amount of nitrogen (%) retained after incubating for	
		(a) Glucose-6-phosphate dehydrogenase	(b) 6-phosphogluconate dehydrogenase	(a)	(b)
8	0	12.5*	10.0 $\pm$ 1.2	56	45
8	10	18.2 $\pm$ 2.0	18.9 $\pm$ 2.6	82	86
8	15	26.4 $\pm$ 0.8	23.7 $\pm$ 4.6	120	108
8	20	23.4 $\pm$ 2.8	18.5 $\pm$ 1.4	100	88
8	No incubation	22.2 $\pm$ 3.6			
14	0	21.1 $\pm$ 3.9	16.5 $\pm$ 0.6	62	48
14	10	22.9 $\pm$ 1.4	22.7 $\pm$ 2.8	67	67
14	15	29.1 $\pm$ 3.5	32.6 $\pm$ 1.8	85	96
14	20	34.2 $\pm$ 4.4	35.2 $\pm$ 2.3	100	103
14	No incubation	34.1 $\pm$ 0.1†			
20	0	23.5 $\pm$ 7.7	24.0 $\pm$ 4.0	54	55
20	10	26.8 $\pm$ 2.5	30.1 $\pm$ 0.6	61	69
20	15	38.7 $\pm$ 0.5	34.1 $\pm$ 3.7	88	78
20	20	43.9 $\pm$ 3.5	48.5 $\pm$ 4.0	100	110
20	No incubation	43.8 $\pm$ 0.4			

\* In this experiment, only one value was obtained.

† These values exclude one section which was obviously of twice the normal thickness.

The results indicated that with 20 per cent PVA in the incubation medium no nitrogenous material was lost from the sections, irrespective of thickness. Reduction of the concentration to 15 per cent gave nearly complete retention, the excessive results with the 8  $\mu$  sections being due, probably, to the insensitivity of the method at such low concentrations of nitrogen. With the procedures used for preparing and incubating the sections, the loss of nitrogen was never greater than 50 per cent, even in the absence of PVA, while the addition of this inert molecule gave strikingly improved retention of the nitrogenous matter of all thicknesses of sections studied.

It seemed reasonable to make a direct test of whether the dehydrogenases were being lost from these sections into the incubation medium in the microcells, as was found by Gahan and Kalina<sup>7</sup>. It could have been argued<sup>7</sup> that the deposition of formazan found in the sections incubated even in the presence of 20 per cent PVA could have been produced by loss of the enzyme into the medium, where it generated NADPH, which then acted as substrate for tissue-bound diaphorase, or 'NADPH-neotetrazolium reductase'. To investigate this, sections of all three thicknesses were incubated for 5 min in the relevant incubation media lacking substrate and coenzyme to allow leakage of enzyme. They were then transferred to another microcell which contained fresh, complete media, and incubated for 20 min. When 10 per cent PVA was used the reduction in enzyme activity produced by such pre-incubation was just apparent; when 15 per cent PVA was used, no significant difference could be seen between sections which had been so treated and those which had been incubated normally. This was in marked contrast to the findings of Gahan and Kalina<sup>7</sup>, who lost all of both these dehydrogenases into the medium after 1 min; consequently no staining would have been expected when these sections, having been pre-incubated without substrate or coenzyme for 5 min, were transferred to a complete medium. This difference in resistance to pre-incubation, with different concentrations of PVA, correlated well with the retention of nitrogenous material as detected by chemical estimation of the nitrogenous matter.

Thus it seems possible that the presence of sufficient polyvinyl alcohol in the incubation medium may allow all the nitrogenous matter of the sections, and even 'soluble' enzymes, to be retained during the histochemical reactions for enzymes. This is possible without the use of chemical fixatives which will inactivate or denature the enzymes. Polyvinyl alcohol has been shown to be very inert cytochemically<sup>11</sup>; its effect seems to be comparable to that of polyvinylpyrrolidone in biochemical studies on isolated mitochondria<sup>12</sup>.

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## PATHOLOGY

### Possible Relationship between Aortic Acid Mucopolysaccharides and Species-susceptibility to Experimental Atherosclerosis

DIFFERENCES between species in susceptibility to experimental atherosclerosis is one of the most stimulating problems of atherogenesis. Animal species less susceptible to cholesterol-induced atherosclerosis showed a greater lipolytic activity of the arterial wall<sup>1-3</sup> and a higher alpha-beta serum lipoprotein ratio<sup>4,5</sup> than susceptible ones. Moreover, the thyroid gland was found to be more active in the former group than in the latter<sup>6-8</sup>.

On the other hand, very scarce subendothelial ground substance was observed in normal guinea-pigs (a slightly susceptible species)<sup>9</sup>, whereas the reverse was observed in the intimal layer of the rabbit, which is notoriously highly susceptible<sup>11</sup>.

On the basis of the foregoing observations, the serum lipid pattern and the acid mucopolysaccharide (MPS) content of the aorta were studied in ten fasting healthy animals, of both sexes, of the following species: mouse, rat, hamster, guinea-pig, monkey, pig, dog, rabbit, pigeon and chicken. A battery of histological tests was used, since no one method is considered to be reliable by itself for the demonstration of acid MPS. Material which was metachromatic with Toluidine Blue and Azur A (the former on fixed and the latter on unfixed sections), and which gave a positive reaction with Alcian Blue and Hale's stain, modified by Rinehart and Abul-Hay, was considered to be acid MPS.

Our serum lipid findings did not show any significant correlation with specific differences in susceptibility to cholesterol-induced atherosclerosis. Almost similar alpha-beta serum lipoprotein ratios were found both in resistant (rat, hamster) and in susceptible species (pigeon, chicken). It might therefore be suggested that the efficiency of serum lipid homeostasis might be more significant in rela-

tion to the different susceptibilities. This homeostasis, indeed, is very efficient in the dog, the hamster, and the rat<sup>1,7,12-14</sup>, where a sustained hyperlipaemia cannot be induced by feeding a hypercholesterolic diet, whereas it is much lower in the rabbit and in the chicken, where serum cholesterol concentration can be increased by such a dietary manipulation, 30-fold and 12-fold, respectively<sup>17</sup>.

It is interesting that we have constantly found a parallel between the amount of acid MPS and the reported different species-susceptibility to cholesterol-induced atherosclerosis. Aortas of rat, hamster and mouse were found to be almost completely devoid of acid MPS, whereas aortas of chicken, pigeon and rabbit showed large quantities of acid MPS, intermediate levels being observed in guinea-pig, monkey, dog and pig.

Our histochemical findings, in agreement with the reported affinity between plasma lipoproteins and acid MPS of the arterial wall<sup>15-18</sup>, suggest that lipid deposition may more readily occur in arterial walls particularly rich in acid MPS. This may constitute at least a partial explanation of the species differences in susceptibility to experimental atherosclerosis.

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### Comparison of the Physicochemical Properties of a Urinary Pigment of Human and Bovine Origin

A GENETICALLY determined syndrome has been described in the Holstein-Friesian strain of cattle which causes the calf to die in hypoglycaemia soon after birth. Affected calves of this strain are carried as many as 90 days beyond the normal 280 days of gestation. The genetic defect appears when the calf is homozygous for the trait. Both the heterozygous cow and bull are capable



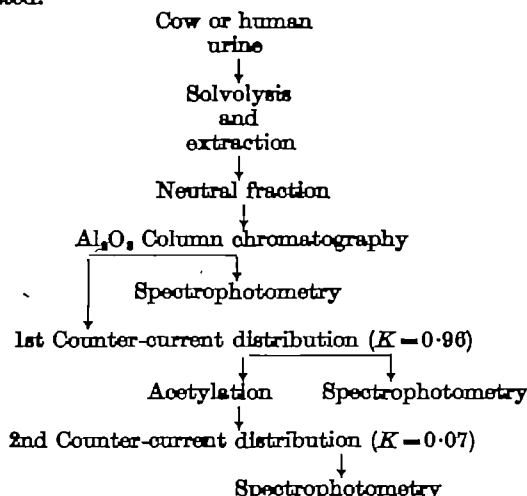
of generating a normal progeny if crossed with a non-affected animal; and the cow is capable of normal-length pregnancy if it does not carry a homozygous, affected calf. Holm<sup>1</sup> has reported that during an affected pregnancy the urine of these cows lacks a magenta pigment that can normally be detected in organic solvent extracts of hydrolysates of urines of non-affected cows. These findings are reported to be consistent enough to be used for the screening of the pregnant cows to detect those that carry an affected foetus.

Our experience in processing human urines for steroid hormone estimation is that a superficially similar pigment is present in human urines. A study was therefore undertaken to compare the physicochemical properties of the pigments of human and bovine origin.

Urines from four normal, non-pregnant Holstein-Friesian cows were obtained through the courtesy of Dr. Holm. Human pooled urine was used for comparison. All four of the bovine samples and a sample of pooled human urine of identical volume (400 ml.) were hydrolysed by solvolysis with sulphuric acid and ether<sup>2</sup>. The ether extracts were washed with 0.1 N NaOH in water, and evaporated to dryness. The neutral extracts were chromatographed on neutral alumina using increasing concentrations (0.02, 0.2, and 2.0 per cent) of ethanol in benzene as eluant. Both human and bovine magenta pigments were eluted at the same ethanol concentration (2 per cent). The chromatographically purified pigments were scanned in a Beckman 'DU' spectrophotometer. Both human and bovine pigments had closely similar spectra in the range of 4000–6000 Å, and a single absorption peak in the visible range with a maximum absorption at 5320 Å.

A 100 transfer counter-current distribution was run on the chromatographically purified materials using benzene/hexane/methanol/water (35:65:70:30) as the solvent system. The *K* calculated according to Craig *et al.*<sup>3</sup> was identical, *K* = 0.96, for pigments from humans and cows.

After counter-current distribution there was a shift in the absorbency maximum of both pigments to 5440 Å. The pigments were then acetylated in pyridine using <sup>14</sup>C-labelled acetic anhydride. Excess acetic anhydride was removed by extraction with ether and water. There was no appreciable change of colour after acetylation, and the maximum absorbency remained at 5400 Å. The derivatives were then re-run in 100 transfer counter-current. Both derivatives behaved identically, showing a new *K*, *K* = 0.07. After the second counter-current, demonstration of radioactivity in the pigments showed that they were acetylated.



While a statement about the identity of the human and bovine pigment must await their chemical characterization, the evidence so far obtained strongly suggests that they are identical.

Holm had previously reported that the pigment was recoverable from bovine urine after enzymatic hydrolysis with beta-glucuronidase. In preliminary work, we investigated the method of hydrolysis and extraction that would produce optimum yields. Three methods were compared using three aliquots of human urine from the same pool: (1) solvolysis with 2 N H<sub>2</sub>SO<sub>4</sub> using ether as the organic phase; (2) addition of concentrated HCl up to 15 per cent concentration, followed by 15 min reflux; (3) enzymatic hydrolysis with beta-glucuronidase ('Ketodase', Warner-Chilcott). The activity of the enzyme preparation was previously assayed, using the method of Talalay *et al.*<sup>4</sup>. All preparations were extracted, and the extracts were dried and taken up in benzene for chromatography on Al<sub>2</sub>O<sub>3</sub>. The eluted magenta pigments were then dissolved in a constant volume of absolute ethanol and their absorbencies were compared in a Beckman 'DU' spectrophotometer at 5320 Å. The highest yield was obtained from the H<sub>2</sub>SO<sub>4</sub>-ether solvolysis method. Using this figure as 100, HCl hydrolysis resulted in a recovery of 75 per cent, and enzymatic hydrolysis gave only 15 per cent recovery. We interpret these results to mean that in humans the pigment is not conjugated as a glucuronide to any great extent. As glucuronide conjugation in cattle has been reported by Holm, there is probably a species difference in this respect. It is conceivable that if the genetically determined condition of cattle has a parallel in human species, estimation of this pigment in urine may be of use in the detection of such a condition. Studies are in progress to assess the range of normal excretion of the pigment in man.

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## IMMUNOLOGY

### Serological Relationship between Adenovirus Haemagglutinins of Group 2

HETEROLOGOUS haemagglutination-inhibition (HI) antibody responses have recently been reported<sup>1</sup> to occur in volunteers inoculated with adenovirus type 26 or type 27. A significantly increased frequency of type 13 responses was observed among men inoculated with type 26 as well as high frequencies of reciprocal responses between types 26 and 27. These findings were interpreted as indicating the existence of antigenic relationships among these types. Heterologous responses to 17 other types also occurred; however, these were less frequent and were distributed in an apparently random fashion, so that additional antigenic relationships between individual serotypes could not be identified.

The various serotypes of adenovirus have been classified by Rosen according to the haemagglutination (HA) reactions of virus suspensions with rhesus erythrocytes and with rat erythrocytes<sup>2</sup>. The agglutinating serotypes are classified as follows: Group 1 includes serotypes that agglutinate rhesus erythrocytes only (types 3, 7, 11, 14, 16, 20, 21, 25, 28); Group 2, serotypes which completely agglutinate rat erythrocytes (types 8, 9, 10, 13, 15, 17, 19,

22, 23, 24, 26, 27, 29, 30); Group 3, serotypes which partially agglutinate rat erythrocytes (1, 2, 4, 5, 6). In this communication, the serotypes of the inocula used and of the antibody responses observed in the aforementioned investigation will be grouped according to the HA classification.

Details of the inoculation procedure and antibody determinations have been presented previously<sup>4</sup>. Briefly, seventeen volunteers were infected with adenovirus type 26 and fifteen volunteers with type 27; both these types are classified in HA group 2. Sera were collected prior to inoculation and 3-4 weeks later, and HI antibody determined<sup>4</sup>. A rise in antibody was considered significant if the titre increased from <1:10 to 1:10 or greater, or when any initially measurable titre increased at least fourfold.

In Table 1 are shown the frequencies of rises in antibody according to serotype, presence or absence of pre-existing antibody, and type of adenovirus inoculated. In Table 2 are shown the same data reclassified according to HA group and presence or absence of pre-existing antibody. The results in the two groups of volunteers were combined since both the inocula belong to HA group 2 and since the patterns of responses were quite similar. Excluded are homologous responses and heterologous responses previously interpreted as showing an antigenic relationship. Among paired sera in which pre-existing antibody was absent the percentage of responses directed against serotypes of HA group 2 was significantly greater than that of the responses to serotypes of HA group 1 ( $P < 0.01$ ) but were not significantly greater than the percentage directed against serotypes of group 3 ( $P = 0.07$ ). Among paired sera in which pre-existing antibody was present the percentage response was significantly greater in the serotypes of group 2 than in those of group 1, and in those of group 3 ( $P = 0.03$ ,  $P < 0.01$ , respectively). Comparison of the percentage response against group 2 serotypes with that against groups 1 and 3 combined shows that the former was significantly greater ( $P < 0.01$ ) regardless of the presence or absence of pre-existing antibody.

Table 1. FREQUENCY OF RISES IN ADENOVIRUS HAEMAGGLUTINATION INHIBITION (HI) ANTIBODY TITRES FOLLOWING INOCULATION WITH TWO TYPES OF ADENOVIRUS ACCORDING TO TYPE AND STATUS OF PRE-EXISTING ANTIBODY

Adenovirus serotype	Adenovirus 26 inoculation				Adenovirus 27 inoculation			
	Pre-existing HI antibody Absent		Present		Pre-existing HI antibody Absent		Present	
	No. tests	No. rises	No. tests	No. rises	No. tests	No. rises	No. tests	No. rises
1	14	0	3	0	11	0	4	0
2	15	2	2	0	15	2	0	0
3	15	0	2	0	15	1	0	0
4	16	0	1	0	10	0	5	0
5	13	4	4	0	10	0	5	0
6	17	2	0	0	15	0	0	0
7	14	0	3	0	15	1	0	0
8	15	3	2	0	13	1	2	1
9	14	2	3	1	13	3	3	2
10	16	2	1	1	12	3	3	1
11	16	0	1	0	15	0	0	0
13	17	7	0	0	13	1	2	1
14	15	1	2	0	14	0	1	0
15	16	0	1	0	15	1	0	0
16	17	0	0	0	15	0	0	0
17	15	4	2	0	13	2	2	1
19	16	2	1	1	15	2	0	0
20	17	0	0	0	15	0	0	0
21	17	0	0	0	15	1	0	0
22	16	2	1	1	15	0	0	0
23	17	4	0	0	14	1	1	0
24	15	3	2	1	15	1	0	0
25	17	17	0	0	14	0	1	1
27	12	8	5	5	15	13	0	0

Table 2. COMPARISON OF FREQUENCY OF HETEROLOGOUS HAEMAGGLUTINATION-INHIBITION ANTIBODY RISES PRE-EXISTING HI ANTIBODY

HA group	No. tests	Absent			Present		
		No. tests	No. rises	%	No. tests	No. rises	%
1	215	4	2	$\chi^2 = 20.8$	9	0	0
2	254	37	14	$P < 0.01$	24	10	42
3	135	10	7	$\chi^2 = 3.2$	0	0	0
				$P = 0.07$			$\chi^2 = 10.2$
							$P < 0.01$
1, 3	351	14	4	$\chi^2 = 18.0$	33	0	0
2	254	37	14	$P < 0.01$	24	10	42
							$\chi^2 = 18.0$
							$P < 0.01$

In summary, volunteers inoculated with adenovirus classified in HA group 2 exhibited heterotypic rises predominantly to serotypes also classified in group 2, which suggests that haemagglutinins of serotypes comprising the HA group may be serologically related. In this connexion, it should be noted that soluble haemagglutinins of HA group 2 as well as complement-fixing antigens and virus particles have similar chromatographic elution characteristics<sup>4</sup>. Moreover, using rabbit antisera in HI tests, Rosen has reported that heterotypic reactions occur to serotypes within HA groups rather than to viruses in different HA groups<sup>4</sup>.

In another investigation, volunteers were immunized with a soluble antigen prepared from a suspension of adenovirus belonging to HA group 3 (type 1). Preliminary results indicated a greater frequency of HI antibody rises against serotypes of group 3 than against serotypes of groups 1 and 2 (ref. 6).

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## BIOLOGY

### Sex Determination and Genic Balance of *Ophryotrocha puerilis*, a Hermaphrodite Polychaete Worm

PURE male and female individuals were obtained from protandrous hermaphrodite strains of *Ophryotrocha puerilis* through selection for the prolongation of the male phase and for the anticipation of the female phase. Both the Atlantic and the Mediterranean sub-species of *O. puerilis* show polygenic sex determination<sup>1</sup>.

Observations have been made until recently on live specimens by looking for the appearance of oocytes which marks the beginning of the female phase. Pure males are characterized by the absence of any externally detectable oocyte during the whole life-cycle and pure females by the appearance of oocytes at the beginning of sexual maturity.

The normal gonadal differentiation has recently been studied through cytological investigation on individuals collected from natural populations or belonging to freshly established cultures of *Ophryotrocha*<sup>1</sup>. A firm basis for comparison was provided in this way for research on the gonadal differentiation of individuals belonging to lines which were selected either for the prolongation of the male phase (the so-called arrhenogenous lines) or for the anticipation of the female phase (the so-called thalygenous lines).

Marked deviations from the typical development of the female phase gonads—consisting mainly in a scarce differentiation between oocytes and nurse cells—have been observed in individuals from the fourth generation of arrhenogenous lines. A vacuolization of the cytoplasm in the primary and secondary spermatocytes, a peculiar

stickiness of the spermatids and irregularly shaped sperm-like structures are observed on the other hand in the young stages of the individuals from the fourth or fifth generations of thelygenous lines. Selection against male or female factors results, therefore, in deviation from the normal male or female differentiation during the stages that correspond to the male or female phases of unselected individuals. Unchecked proliferation of undifferentiated germ cells—which may fill out coelomic cavities of the worms—also often takes place during such stages, as shown in Fig. 1. The selection against the factors for either sex phase accounts for most of such results although the irregular production of undifferentiated germ cells in the place of normally built gonads still needs closer investigation.

Unexpected alterations of the maturation of the sex cells were observed, on the other hand, also in the phases that were favoured by artificial selection. Individuals belonging to the fourth or the fifth generation of arrhenogenous lines show, namely, some unmistakable alterations of the male sex cells which consist in the frequent appearance of piconotic nuclei and in the stickiness of the spermatids. Corresponding alterations of oogenesis—which consist in the formation of dwarf nurse cells and in altered spatial relationships between such cells and oocytes—are also observed in the fourth and fifth generations from thelygenous lines (Fig. 2).

It can be concluded, therefore, that selection against the female-determining factors and against the male-determining factors affects the sex phase against which selection is carried on, and also the sex phase that is apparently favoured by selection. Prolonged selection for either sex alters in other words the balance among sex factors in such a way as to alter the expression of both sexes.

Classic research work showed that given thresholds of sex balance must be reached in *Lymantria*, in *Drosophila* and in other organisms in order to obtain normal male or female phenotypes. It seems that the normal expression of the male and of the female phase can only be obtained in the hermaphrodite Polychaete *Ophryotrocha* within given limits of balance between male- and female-determining factors. Such limits appear, however, to be not sharply defined because such balance is not conditioned in *Ophryotrocha puerilis* by the presence of major genes or gene blocks which are located in the sex chromosomes of gonochorist species. The present results point, on the other hand, to the conclusion that factors of the complementary sex are necessary for the normal expression of either sex; which can be regarded as an extension of the classic principle of the genetic bipotency of the sexes.

The foregoing mechanism of sex balance reduces the fitness of the sex homozygotes and it functions as a

homeostatic mechanism which reduces sex variability in natural populations of *Ophryotrocha puerilis*.

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### Red Water Bloom of a Dinoflagellate in Hillsborough Bay, Florida

WATERS of Tampa Bay, Florida, are regularly sampled for plankton in connexion with studies of the Florida red-tide organism (*Gymnodinium brevis*, Davis) and estuarine ecology. On May 5, 1964, various division stages and chain formations of an armoured dinoflagellate, *Ceratium furca* (Ehrenberg), were detected in the surface waters of Hillsborough Bay, an upper arm of Tampa Bay. The water samples were tinted brown and contained an average count of  $2.294 \times 10^4$  cells of *C. furca* per litre. Two weeks later, during an aerial survey of Tampa Bay, two staff biologists noticed patches of blood-red water in the upper portion of Hillsborough Bay. On May 25, during a special cruise in Hillsborough Bay, water in the entire upper portion of the Bay was discoloured with brownish and reddish patches. The most intense discoloration was noticed in areas protected from the wind. No mortality of fish or any other organism was observed in the field. Blue crabs, *Callinectes sapidus* Rathbun, and scaled sardines, *Barongula pensacolas* Goode and Bean, were actively swimming through the patches of blood-red water without showing ill effects.

All organisms were enumerated in the living state by counting techniques previously reported<sup>1</sup>. Before counting the organisms, two-litre Erlenmeyer flasks containing the samples were placed under a fluorescent light in the laboratory to concentrate phototropic organisms. *C. furca* concentrated in the meniscus of the flasks 15–30 min after exposure to the light, giving it a brownish-red tint. [In red water samples, *C. furca* was noted in very high concentrations, probably the highest on record for this dinoflagellate. The density of cells varied from  $2.24 \times 10^4$  to  $14.29 \times 10^4$  cells per litre.

Microflagellates, 4–7  $\mu$  in diameter, were also abundantly represented in the water samples. Furthermore, they were numerous in non-discoloured water from the same general area, suggesting that they played no part in the red discoloration. Some samples containing *C. furca* and these flagellates also yielded very small numbers of *Polykrikos* sp., *Prorocentrum miconis* Ehrenberg, *Gonyaulax* sp., *Coscinodiscus centralis* Ehrenberg, *Rhizosolenia setigera* Brightwell, *Biddulphia sinensis* Greville, and veliger-type larvae. These organisms were rare and did not contribute significantly to the intense colour.

A third cruise was made to the area on June 1. At this time the discoloured water contained blooms of *Scletonema costatum* (Greville) Cleve in addition to blooms of *C. furca*. The discoloration was much less pronounced than in blooms consisting principally of *C. furca*. Patches of blood-red water were absent, but the water retained a reddish hue. Concentrations of *C. furca* varied from  $3.1 \times 10^4$  to  $5.57 \times 10^4$  cells per litre. The cell density of *S. costatum* ranged from  $4.9 \times 10^3$  to  $1.45 \times 10^4$  cell chains per litre. By June 12, discoloured water had disappeared from the area

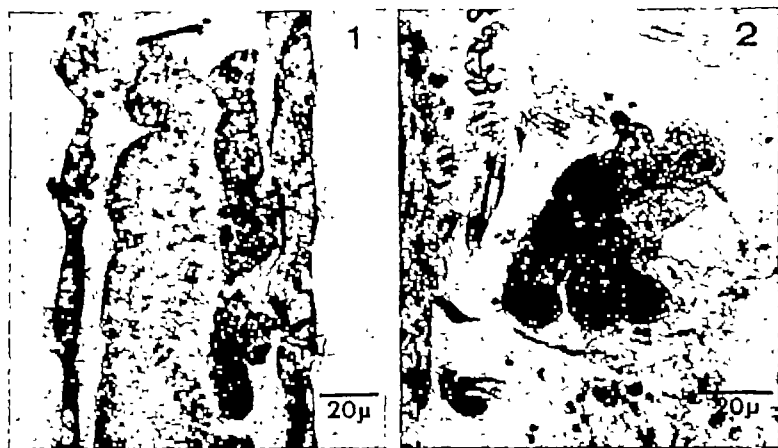


Fig. 1

Fig. 2

and only very low concentrations of *O. furca* and *S. costatum* were present in the samples. The area was inspected again on June 22, and no discoloration was noted.

A laboratory experiment was conducted to test light absorbency as an indicator of the degree of water coloration by *O. furca* cells. Aliquots from a bloom containing  $14.3 \times 10^6$  cells per litre were serially diluted from 100 per cent to 5 per cent by volume in 5 per cent increments. The diluted aliquots were then shaken slightly to distribute the organisms. Spectrophotometer cells of one-centimetre path length were filled with the dilution, and the absorbency measured at 400 m $\mu$ . The pattern of the observed absorbencies agreed with the volume dilutions. A maximum of 0.180 was noted for the 100 per cent volume and 0.018 for the 10 per cent volume.

To test the toxicity of a *Ceratium furca* bloom on fish, several specimens of *Lagodon rhomboides* (Linnaeus), 95–112 mm (FL), *Paralichthys albigutta* Jordan and Gilbert, 104–5 mm (FL), and a specimen of *Chaetodipterus faber* (Broussonet), 125 mm (FL), were selected. These fish were kept for 36 h in an aerated 15-gal. tank containing high concentrations ( $14.29 \times 10^6$  cells per litre) of this dinoflagellate. The fish remained active and behaved normally throughout the experiment. After the experiment, all *O. furca* were deliberately killed. As the dead dinoflagellates settled to the bottom of the tank, the water regained its normal transparency.

The observations presented indicate that unusually dense concentrations of *O. furca* were responsible for the blood-red discoloration, and that there was no toxic effect on fish. The possibility that discoloration was also caused by the bloom of *S. costatum* cells is remote since the samples taken on May 25 from blood-red patches of water contained only blooms of *O. furca*. In water samples from areas of *O. furca* and *S. costatum* blooms, the intensity of discoloration was less than in water containing only *O. furca* blooms. *S. costatum* is one of the most abundant diatoms in Hillsborough Bay. Each year, blooms are observed throughout the winter and into the spring months. No water discoloration has been associated with *S. costatum* blooms.

The association of *O. furca* blooms and water discoloration in Florida is reported here for the first time. One of the more frequently observed sea-water discolorations along the south-western coast of Florida is commonly known as 'red tide'. This is a natural fish-killing phenomenon and is always associated with discoloured water containing dense populations of *Gymnodinium breve*, a naked dinoflagellate<sup>1</sup>. The discoloration of water during the outbreaks of the Florida red tide may vary between brown, red and green. Another discoloration of biological origin in Tampa Bay and off the west coast of Florida was reported by Hutton<sup>2</sup>. He did not mention the colour of the water, which he associated with blooms of *Gymnodinium splendens* Lebour. He described the discoloration in offshore waters as streaks of reddish-brown water, approximately one mile in width. The water contained  $3.5 \times 10^4$  specimens of *Acartia tonsa* Dana per litre. *Labidocera aestiva* was also present in Hutton's samples but in smaller numbers than *A. tonsa*. Hutton observed no damage of fish in connexion with the blooms of *G. splendens*, *A. tonsa* or *L. aestiva*.

A blue-green alga, *Skujella* (*Trichodesmium*) *thiebauti* De Toni, is associated with brownish discoloration in coastal waters of south-western Florida. Fish kills caused by this alga have not been reported from the area.

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## Relationship between the Ovary and Oviduct in the Domestic Hen

DURING the course of experiments designed to investigate the relationship between ovulation and nesting behaviour in the domestic hen, a number of hens were operated on to form a fistula in the magnum. While the technique of this operation has been described elsewhere<sup>1</sup>, certain observations on these hens, cogent to the problem of egg production and reproduction in general, are given here.

In each of four birds the fistula was so made that any ovum entering the infundibulum and descending in the oviduct would be shed externally through the fistula on the ventral abdomen. The fistula was covered with cheese cloth, secured around the bird's back by safety pins. Each bird was kept in an individual pen with a number of trapnests at her disposal.

In general, ovulation in this species is followed by nesting behaviour, the form of which depends on the environment. In the pens in which these birds were kept, a normal hen first exhibits some restlessness, then 'examines' several nests for a variable time, possibly returning to feeding and other activities between 'nest examination'; finally, she enters a trapnest and settles into a sitting position. In the operated birds, a proportion of nestings, that is, 'nest examination', entry and sitting on the nest, were unaccompanied by shedding of an ovum through the fistula. These nestings not preceded by ovum shedding occurred during the winter months shortly before the birds went off lay or when they were coming back into lay. At these times the combs and wattles indicated that the ovary was active, and the birds were generally in a laying condition; but examination through the fistula showed that the oviduct was in a shrunken state. Thus, it appeared that the ovary and oviduct were out of phase, and this suggested that internal laying had occurred during these periods. At post-mortem each bird examined had a fully active ovary with many mature follicles and a mass of yolk material was present in the body cavity. The oviducts were clearly incapable of dealing with the shed ova. This anomaly is not likely to have been due to the surgery, for nesting without oviposition has been found to increase at similar periods in normal birds, the egg-laying and nesting records of which have been closely observed (Fig. 1).

These observations appear to indicate that two separate mechanisms are involved, one for the control of ovarian activity and the other for the control and maturation of the oviduct. In cats, Michael<sup>2</sup> has shown a comparable

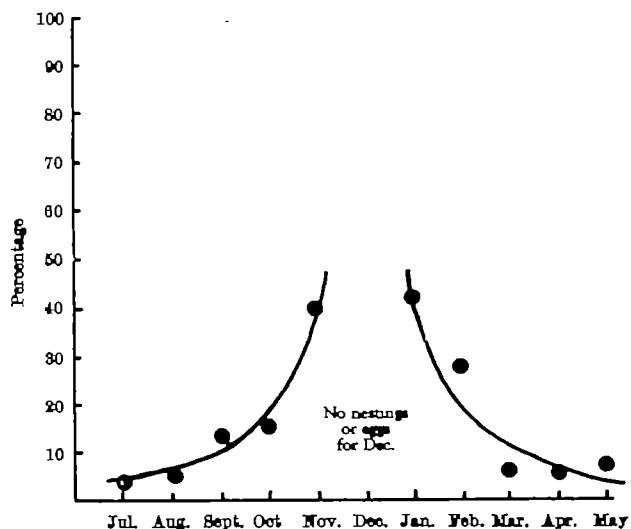


Fig. 1. Nestings without oviposition as a percentage of total nestings; that is, percentage loss of possible eggs due to the oviduct not engulging the shed ovum.

separation between oestrous behaviour and vaginal and uterine state. Young *et al.*<sup>2</sup>, working with guinea-pigs, also found ovarian activity and oestrous behaviour to be imperfectly correlated, and, furthermore, their results show that the relationships between the three measures of ovarian activity were also variable. It is very probable that during the laying season of a bird a number of potential eggs are lost through lack of co-ordination between the ovary and oviduct.

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### Floral Meristem-organizing Gradient in Tobacco Stems

THE existence of a gradient reflecting the ability to form flower buds, along the stems of tobacco plants (var. 'Wisconsin 38'), has been previously reported<sup>1</sup>. Internode fragments, cultured on a simple medium devoid of growth substances, produce a callus which reflects this gradient in terms of its tendency to organize. Fragments isolated from the basal part of the stem produce only callus, those from the vegetative mid-part of the stem may produce 2 or 3 vegetative buds<sup>2</sup>, whereas those isolated from the flowering part of the stem form calluses producing 10 to 20 flowering buds<sup>3</sup>. The higher the fragments are cut, the greater the ratio of flower to vegetative buds per callus.

The gradient thus observed on internode fragments cultured *in vitro* probably exists in the intact flowering plant. The problem is to provoke a tissue proliferation *in vivo*, such that it bears newly formed organs, which would enable one to determine whether organ formation reflects such a gradient. The fact that tumours induced by certain strains of *Agrobacterium tumefaciens* can organize<sup>4-6</sup> might prove useful in uncovering the existence of a gradient *in vivo*.



Fig. 1. Floral organogenesis on crown-gall tumour developed on *Nicotiana tabacum*, 'Wisconsin 38'

*Agrobacterium tumefaciens* (Strain T37) was injected into the internodes of flowering *Nicotiana tabacum* plants (var. 'Wisconsin 38'). At the inoculation sites, tumours developed and organized, the nature of the organization depending on the level of the stem at which the injection was made. On the flowering part of the stem, the tumours were small, and bore organs some of which produced flower-buds (Fig. 1). Just underneath the inflorescence the tumours developed small stems with leaves and then flower-buds. Lower still, teratologic organoids were observed, and further towards the base of the stem only unorganized tumours were obtained. A flowering gradient was thus observed, and the gradient apparently exists in the intact plant as well as in isolated fragments.

Both *in vivo* and *in vitro*, the tendency for teratomatous tissue of tobacco to organize depends on the hormonal milieu in which such tissue is growing, particularly in respect to auxin and gibberellin<sup>6</sup>. For normal tobacco callus, the kinins also appear to play a critical part in bud organization<sup>7</sup>. The gradient of organogenesis described in these preliminary experiments is therefore presumably due to a gradient of hormone concentrations inside the plant, but whether the flowering of the organs formed on the inflorescence-axis is entirely a hormonally controlled process cannot be decided until floral organogenesis can be experimentally induced with specific hormones. I thank Dr. Manigault for kindly supplying the bacterial strain.

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### Influence of Ioxynil on the Photo-reduction of Endogenous Plastoquinone by Isolated Chloroplasts of *Vicia faba* L.

ALTHOUGH plastoquinone (a 2,3-dimethyl *p*-benzoquinone, with a C<sub>4</sub> side chain at C<sub>6</sub>) was first isolated in 1946 by Kofler from alfalfa grass, it did not excite much interest until Crane<sup>1</sup> extracted the compound from the chloroplasts of green leaves. Plastoquinone (PQ) was first implicated as a participant in photosynthetic electron transport systems by Bishop<sup>2</sup>. Recently, Redfearn *et al.*<sup>3</sup> have shown plastoquinone to be an intermediary electron carrier in the photosynthetic system, being a part of the light-dependent water-splitting mechanism as outlined by Duyssens *et al.*<sup>4</sup>. We have recently reported<sup>5</sup> that ioxynil (4-hydroxy-3,5-diiodobenzonitrile)<sup>6</sup> inhibits photophosphorylation and the accompanying reduction of pyridine nucleotide by chloroplasts. The results reported have been improved in recent months to give 50 per cent inhibition of both processes at a concentration of ioxynil of  $5 \times 10^{-4}$  M. In an attempt to elucidate the action of ioxynil on the photosynthetic electron transport system, the ability of chloroplasts to photo-reduce endogenous plastoquinone was investigated.

Chloroplasts were isolated and washed as previously described<sup>5</sup>. The technique involving the measurement of plastoquinone was that described by Redfearn<sup>3</sup> in which the steady-state oxidation-reduction levels are measured. Replicate tubes contained 0.05 M Na/K phosphate buffer pH 7.0 containing 0.01 M potassium chloride, and chloroplasts equivalent to 1 mg chlorophyll/ml. in a final volume of 3.0 ml. Other chemicals were added as required. The tubes were illuminated from both sides by two

250-W photoflood bulbs, using water filters to eliminate over-heating. After 5 min, a solution of pyrogallol in methanol ( $-20^{\circ}\text{C}$ ) was added to prevent re-oxidation of the reduced plastoquinone formed, during the extraction procedure. The tubes were removed from the light, and an equal volume of  $40^{\circ}\text{--}60^{\circ}$  petroleum ether was added. The plastoquinone was extracted by partitioning the petroleum ether layer with 95 per cent methanol. The petrol layer was evaporated to dryness under vacuum and the residue dissolved in 3 ml. of pure ethanol. The spectrum of the extract was determined on a Unicam S.P. 800 recording spectrophotometer between 220–310 m $\mu$ , and the value of  $\Delta E$  at 255 m $\mu$ , after reduction with a few grains of  $\text{NaBH}_4$ , was taken as a measure of the amount of plastoquinone in the oxidized form.

*o*-Phenanthroline, a known inhibitor of the Hill reaction, was used as a reference in comparing the activity of ioxynil in inhibiting the photo-reduction of plastoquinone. The results are shown in Table 1.

Table 1		
Additions	Incubation	% PQox
None	Dark	87.5
None	Light	75.0
Ioxynil	Dark	86.2
Ioxynil	Light	87.5
<i>o</i> -Phenanthroline	Dark	95.8
<i>o</i> -Phenanthroline	Light	Photo-oxidation

The final concentrations of ioxynil and *o*-phenanthroline were  $5 \times 10^{-4}$  M.

From Table 1 it can be seen that 12 per cent of the plastoquinone present becomes reduced on illumination, while no similar reduction was observed when the mixture was incubated with either ioxynil or *o*-phenanthroline. Indeed, a photo-oxidation was observed in several instances with *o*-phenanthroline. This observation has also been noted by other workers<sup>7</sup>. Since plastoquinone is considered to be the acceptor of electrons from the chlorophyll *b* mediated reaction involving the splitting of a water molecule, both *o*-phenanthroline and ioxynil appear to inhibit this reaction. The net result is that plastoquinone remains in the oxidized form, instead of being reduced.

Table 2			
Additions	Incubation	% PQox	
None	Dark	87.5	88.5
None	Light	75.0	68.5
Ioxynil	Dark	87.5	91.0
Ioxynil and ascorbate and DOPIP	Light	87.5	80.0
<i>o</i> -Phenanthroline	Dark	Photo-oxidation	95.8
<i>o</i> -Phenanthroline and ascorbate and DOPIP	Light	56.2	50.0
Ascorbate and DOPIP	Light	43.8	45.9

The final concentrations of ioxynil, ascorbate, 2,6-dichlorophenolindophenol and *o*-phenanthroline were  $5 \times 10^{-4}$  M,  $1.6 \times 10^{-3}$  M,  $8 \times 10^{-4}$  M and  $5 \times 10^{-4}$  M respectively. The chlorophyll concentrations in the chloroplast suspension used were: (A) 1.01 mg/ml; (B) 1.16 mg/ml.

The effect of adding ascorbic acid/dichlorophenolindophenol (DOPIP) was investigated in an attempt to by-pass this apparent site of inhibition. The results are shown in Table 2.

The effect of ascorbate on the system is to generate extra reducing equivalents. Since there is a vast photo-reduction of plastoquinone in the light in the presence of ascorbate, compared with the control, there must be untapped plastoquinone present which is not reduced under normal conditions. This reduction in the presence of ascorbate is only slightly inhibited by *o*-phenanthroline, indicating that the electrons donated by ascorbate are by-passing the site of inhibition. This situation, however, does not occur in the presence of ioxynil under the same conditions.

It has been suggested by Witt *et al.*<sup>8</sup> and by Trebst<sup>9</sup> that there may be more than one site involving plastoquinone in the photosynthetic electron transport chain. Our implication is that, this being the case, it would appear that ioxynil has a blocking action at more than one site of plastoquinone, since there is no enhanced reduction of plastoquinone in the presence of ascorbate. This may be

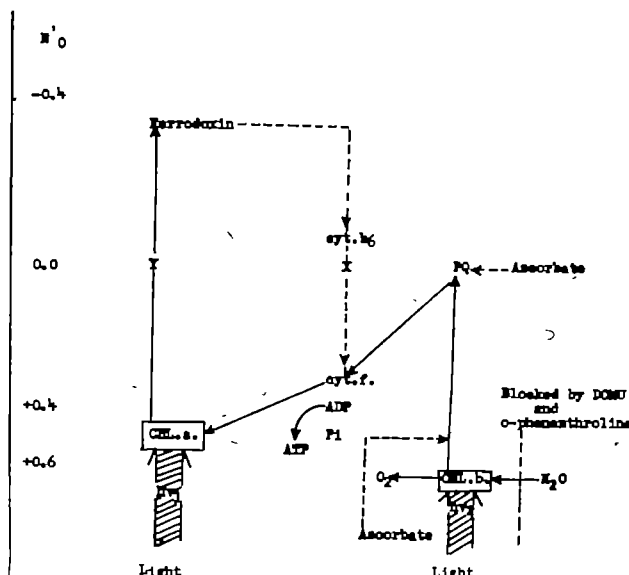


Fig. 1

shown in Fig. 1, set out on the basis of redox potentials, according to Arnon<sup>10</sup>.

In the light of these results, ioxynil, apart from inhibiting the reaction  $h\nu^2$  as does *o*-phenanthroline and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DOMU), also has a blocking action at some other part of the chain. It may be the case that plastoquinone is also located at those parts of the chain indicated by X and Y in Fig. 1. If this is so, then this may be the explanation for the enhanced reduction of plastoquinone by ascorbate in the light, and also the added sites of action of ioxynil. A second site for plastoquinone has been suggested by Redfearn for this enhanced reduction of plastoquinone in the presence of ascorbate. The results described would seem to confirm this suggestion. This being so, plastoquinone could presumably function at X or Y, two points along the cyclic phosphorylation pathway, which we already know to be inhibited by ioxynil.

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## MICROBIOLOGY

### Post-irradiation Recovery of *Escherichia coli*

RADIATION sensitivity of *Escherichia coli* strains is influenced by conditions for growth prior to and after radiation exposure. Stapleton and Engel<sup>1</sup> have shown that *E. coli* B/r (CSH) grown in a buffered-peptone medium developed higher X-ray resistance. The cells grown in rich medium, however, might have become more exacting in their nutritional requirements and their recovery was limited on basal media<sup>2</sup>. At the same time, the recovery of ultra-violet or X-irradiated cells was higher when they were subjected to sub-optimal growth conditions<sup>3-5</sup>.

It can, therefore, be assumed that the cells grown in rich medium may withstand radiation injury better than those grown in basal medium; but the latter may possess greater capacity for recovery. To test this hypothesis, *E. coli* strain B was grown in buffered-peptone medium<sup>1</sup> and in Davis's glucose-salts medium<sup>2</sup>. After irradiation, the survivors were further subjected to cold starvation in phosphate buffer at 7° C for 14 days. The effects of such stresses were measured by the loss of colony-forming ability.

*E. coli* B was grown in peptone broth for 18 hours at 37° C on a shaker. This corresponded to the terminal logarithmic phase of the culture. In glucose-salts medium, 21 h were required to reach the same phase. Both cultures were centrifuged and washed twice with 0.067 M phosphate buffer. The washed cells were then resuspended in the same buffer to give approximately 10<sup>8</sup> cells/ml. by adjusting the optical density to a standard value. Aliquots of these suspensions were irradiated with γ-rays to receive 10 krad from a cobalt-60 source (dose rate: 63.3 krad/h). Irradiation was carried out in air with the temperature maintained at 18° C to minimize cold sensitization<sup>3</sup>. This resulted in 99 per cent inactivation of the initial population.

Both irradiated and non-irradiated cell suspensions were further diluted in buffer to give approximately 1,000 cells/ml. and stored at a sub-growth temperature of 7° C (ref. 8). Daily samples were drawn from this suspension for 14 days and the viable counts were determined by simultaneously plating 0.1 ml. portions on solidified peptone agar and glucose-salts agar. Platings were made in triplicate from two identical experiments and the standard error was calculated from total of 6 plates each. All plates were incubated at 30° C (ref. 9) for 24–48 h. No additional colonies developed after this period.

The buffer suspended *E. coli* B was fairly stable at 7° C. The cells grown in buffered peptone retained approximately 60 per cent of their initial viability after 14 days while cells grown in glucose-salts remained 40 per cent viable after the same period (Tables 1 and 2). The cold starvation, therefore, appears to exert only a mild stress to healthy cells.

The cells were grown in buffered peptone medium for at least 2 weeks before they were used as inocula. Despite

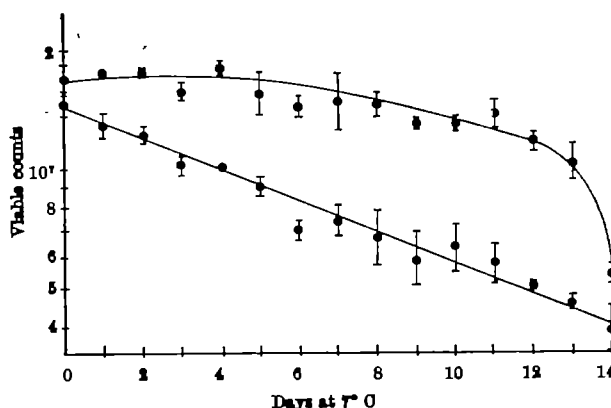


Fig. 1. 10 krad γ-irradiation surviving *E. coli* B grown in buffered peptone broth and held in 0.067 M phosphate buffer at 7° C. ●, Plated on peptone agar; ○, plated on glucose salts agar

this, the peptone-grown cells formed nearly equal number of colonies on glucose-salts agar (Table 1). This indicates that the peptone-grown population was free of auxotrophs.

During cold starvation, the peptone-grown cells increased in number for the first 2–3 days. *E. coli* is known to carry on metabolism at 0° C at a much reduced rate<sup>10</sup>. Although no normal growth was expected to occur below 8° C, the reduced rate of endogenous metabolism may have been able to complete some division initiated before gathering the cells. Since no similar phenomenon was encountered with cells grown in glucose-salts, such delayed division might have been carried out by metabolites accumulated during growth in the richer medium.

The radiation survivors behaved differently. A steady decrease of viable cells was noted during cold starvation when peptone-grown cells were enumerated on the same medium (Fig. 1). The same cells plated on glucose-salts agar did not show this steady decline. Viable counts on glucose-salts agar were always higher than on peptone agar. The viable counts on glucose-salts agar remained relatively constant for about 10 days, while the peptone agar counts declined rapidly, and approached the same level as those on peptone agar on the fourteenth day.

Since the cold starvation was not sufficiently strenuous to normal cells, the rapid decline of viability among the radiation survivors on peptone agar may be due to a continuous loss of sub-lethally injured cells from the population. If such damage was on the metabolic potential as was indicated with freezing<sup>11</sup>, phenol treatment<sup>12</sup> and with X-ray reported by Stapleton *et al.*<sup>3</sup>, the recovery on peptone agar should have been superior to glucose-salts.

Alper and Gillies<sup>3</sup>, Stapleton *et al.*<sup>4</sup> and Gillies and Alper<sup>5</sup>, however, have shown that the sub-optimal recovery conditions were conducive for a greater recovery of irradiated *E. coli*. The slower rate of growth in glucose-salts medium then may have been responsible even for the recovery of peptone-grown cells. The lag period in peptone broth was approximately 40 min, while 4 h were needed before turbidity could be detected in glucose-salts broth. Although the lag period on solid recovery medium could not be measured, similar difference in the length of this period would be expected. It is conceivable that peptone-grown cells should have required even a longer lag period on glucose-salts. Such prolonged recovery period, therefore, might have enabled the damaged cells to recover even if it required an adaptation to a minimal medium. Such adaptation could have been possible because the peptone-grown population was free of auxotrophs. The nature of such injury is being further investigated.

The cells grown in glucose-salts, after irradiation, could be recovered equally well on either peptone or glucose-salts agar (Fig. 2). The more rapid viability loss of the non-irradiated population during cold starvation indicates, however, that the glucose-salts-grown cells possessed lesser

Table 1. NON-IRRADIATED *E. coli* B GROWN IN BUFFERED-PEPTONE BROTH AND HELD IN 0.067 M PHOSPHATE BUFFER AT 7° C

Days at 7° C	Plated on buffered-peptone agar Viable count (×10 <sup>8</sup> )	% of max.	Plated on glucose-salts agar Viable count (×10 <sup>8</sup> )	% of max.
0	1.07 ± 0.02	72.7	1.02 ± 0.10	69.3
1	1.20 ± 0.11	81.6	1.22 ± 0.25	91.1
2	1.26 ± 0.21	85.7	1.47 ± 0.06*	100.0
3	0.96 ± 0.23	65.3	1.23 ± 0.07	82.9
4	1.23 ± 0.25	83.9	1.29 ± 0.15	87.7
5	1.14 ± 0.04	77.5	1.22 ± 0.19	86.7
6	0.90 ± 0.07	67.3	1.14 ± 0.07	77.5
7	0.99 ± 0.37	67.3	1.14 ± 0.16	77.5
8	0.96 ± 0.06	65.3	1.30 ± 0.10	88.4
9	1.12 ± 0.17	76.1	1.22 ± 0.06	82.9
10	1.03 ± 0.10	70.0	1.16 ± 0.11	78.3
11	0.63 ± 0.07	56.4	1.21 ± 0.06	80.1
12	1.00 ± 0.02	68.0	1.17 ± 0.06	79.5
13	0.74 ± 0.02	50.3	1.19 ± 0.04	80.9
14	0.93 ± 0.02	63.3	0.89 ± 0.06	60.5

\* The maximum count obtainable from identically treated samples.

Table 2. NON-IRRADIATED *E. coli* B GROWN IN GLUCOSE-SALTS BROTH AND HELD IN 0.067 M PHOSPHATE BUFFER AT 7° C

Days at 7° C	Plated on buffered-peptone agar Viable count (×10 <sup>8</sup> )	% of max.	Plated on glucose-salts agar Viable count (×10 <sup>8</sup> )	% of max.
0	1.45 ± 0.10	96.7	1.50 ± 0.13*	100.0
1	1.43 ± 0.04	94.6	1.23 ± 0.12	88.6
2	1.24 ± 0.10	82.6	1.23 ± 0.10	92.0
3	1.16 ± 0.06	77.3	1.06 ± 0.06	70.6
4	1.06 ± 0.06	70.6	1.22 ± 0.08	81.3
5	1.21 ± 0.07	80.6	0.97 ± 0.06	64.6
6	1.03 ± 0.12	68.0	0.91 ± 0.07	60.6
7	0.94 ± 0.20	62.6	0.81 ± 0.06	54.0
8	0.87 ± 0.19	58.0	0.75 ± 0.06	50.0
9	0.93 ± 0.13	61.3	0.77 ± 0.08	51.3
10	0.88 ± 0.07	54.6	0.73 ± 0.12	48.4
11	0.71 ± 0.08	47.3	0.64 ± 0.07	42.3
12	0.78 ± 0.20	52.0	0.62 ± 0.06	41.3
13	0.66 ± 0.06	43.3	0.61 ± 0.06	40.7
14	0.57 ± 0.06	38.0	0.57 ± 0.06	38.0

\* The maximum count obtainable from identically treated samples.



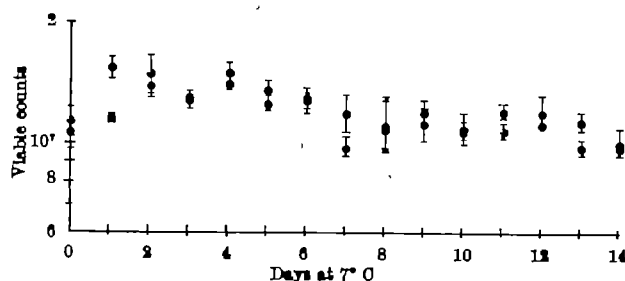


Fig. 2. 10 krad  $\gamma$ -irradiation surviving *E. coli* B grown in glucose-salts broth and held in 0.067 M phosphate buffer at 7° C. ●, Plated on peptone agar; ○, plated on glucose-salts agar.

survival potential under this stress. The cells grown in glucose-salts were more sensitive to radiation than those grown in peptone (Table 3). The relative stability of irradiated cells during cold starvation then might have been due to such population containing a lesser number of sub-lethally injured cells. If we assume that the rapid growth following injurious treatments has resulted in forced recovery, cells grown in glucose-salts might have been able to compensate for such stress by having diversified enzymatic machinery.

Table 3. PERCENTAGE INITIAL SURVIVAL OF *E. coli* B AS INFLUENCED BY PRE-IRRADIATION GROWTH MEDIA AND BY POST-IRRADIATION\* RECOVERY MEDIA

Growth media (broth)	Recovery media (agar)	Glucose-salts
Buffered-peptone	1.36 $\pm$ 0.13	1.63 $\pm$ 0.23
Glucose-salts	0.73 $\pm$ 0.07	0.63 $\pm$ 0.09

\* Irradiated in 0.067 M phosphate buffer.

It appears that sub-lethally injured *E. coli* require a slow recovery process which is not compatible with the rigour demanded of optimum growth. Such injured state could be reversed even after prolonged starvation at lower temperature. The nature of this injury, nevertheless, does not appear to be an impaired metabolic capacity.

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## VIROLOGY

### Immune Adherence to Tissue Culture

HAEMADSORPTION is often used to detect virus infections of tissue cultures. This technique is not, however, of universal application because the phenomenon cannot always be elicited even in cases where the virus is known to cause haemagglutination.

Another type of agglutination of primate erythrocytes by micro-organisms and other antigens can be demonstrated if both a specific antiserum for the antigen and complement are included in the mixture. This phenomenon has been called by Nelson<sup>1</sup> 'immune adherence'.

During attempts to cultivate several group B arboviruses in chick embryo tissue cultures growing on poly-

Table 1. IMMUNE ADHERENCE TO TISSUE CULTURE

Mixtures	Adherence (positive or negative) to chick embryo lung cells growing on polythene
1 Human O RBC + antiserum + O'	+
2 Human O RBC + antiserum	—
3 Human O RBC + O'	—
4 'Non-primate' RBC + antiserum + O'	—

\* Sheep, rabbit, guinea-pig, chicken or goose erythrocytes.

thene<sup>2,3</sup>, I was unable to observe any cytopathic effect and there was no adsorption of goose erythrocytes in the pH range used for the demonstration of haemagglutination by these viruses. I was thus led to consider immune adherence. To discover whether this was practicable, an antiserum for chick embryo cells was prepared by the intramuscular injection into guinea-pigs of a suspension of chick chorio-allantois. 0.1 ml. of a mixture of washed human O erythrocytes in physiological saline, the antiserum inactivated at 56° C for 0.5 h, and guinea-pig complement (O'), was allowed to sediment for 0.5 h at 37° C on to cultures of chick embryo lung cells growing on disks of polythene. The cultures were then washed in a beaker of saline, mounted in 5 per cent glycerol in saline on a microscope slide and examined with phase contrast illumination. The erythrocytes were firmly adherent to the cell sheet. Adherence occurred at room temperature or at 37° C but not at 4° C. The optimum concentration of erythrocytes was found to be 0.5 per cent, and by titration, the optimum amounts of antiserum and of complement were chosen to be four times the minimum amounts required to allow immune adherence. Table 1 shows the effects of omitting or altering components of the mixtures. The phenomenon is not a complement-dependent mixed aggregation<sup>4</sup> because adhesion still occurs after both the antiserum and the complement have been absorbed with human O erythrocytes. To demonstrate immune adherence to virus-infected tissue cultures, immune sera to a neurotropic strain of yellow fever virus were prepared in guinea-pigs by the intranasal inoculation of a mouse brain suspension of live virus. Two weeks after an inapparent infection, the guinea-pigs were bled. Chick lung cultures growing in 'Medium 199' on disks of polythene were infected with yellow fever virus: the disks were examined after 24 h. Mixtures of human O erythrocytes, inactivated immune serum and guinea-pig complement were allowed to sediment on to the disks and immune adherence occurred (Fig. 1). No adherence was observed to disks of uninfected lung cultures nor to infected cultures with any of the mixtures numbered 2, 3, 4 in Table 1. The sensitivity of this method of virus assay requiring only 24 h was of the same order as titration by inoculation of suckling mice. Similar results

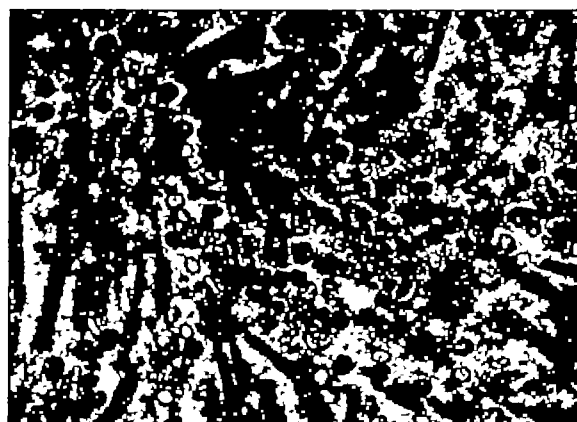


Fig. 1. Immune adherence of human O erythrocytes after infection of a tissue culture of chick lung with a neurotropic strain of yellow fever virus. ( $\times$  c. 840)

were obtained with two other arboviruses using in each case the specific antiserum.

Apart from the use of immune adherence in applications analogous to the example described or in cases where the virus does not cause haemagglutination, the same technique may prove useful for the detection of new antigens in cells transformed by oncogenic viruses. Immune adherence to tissue cultures might also lead to the discovery of new viruses. Material for culture could be obtained from an individual in the acute stage of a virus infection and specific immune serum would be provided later by the same individual in convalescence.

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## CYTOLOGY

### Electron Microscopic Observations of Human Chromosomes Isolated by Micrurgy

THE elucidation of the structure of the human chromosome is of basic biological importance. A more detailed understanding of the mechanisms involved in utilization, duplication, and transfer of genetic information requires a concept of the physical manner in which this information exists during these three processes. To this end a number of models of chromosome structure have been proposed. These models have been based largely on light microscopic observations and electron micrographs of fragments of chromosomes of various plants and animals<sup>1,2</sup>. So far, none of these models has been universally accepted as being representative of the human chromosome.

Published electron micrographs of mammalian mitotic chromosome structure have shown filaments of certain thicknesses, but so far they have failed to reveal credible reproducible configurations at the various levels of structure in any given chromosome<sup>3-5</sup>. I believe that the problem lies in the difficulty of fixing and retaining geometrical relationships of structures composed of widely different chemical entities such as DNA and protein. In an effort to pass gently from the physiological state to the dry preparation for electron microscopic observation, the following variation of the technique of McClendon<sup>6</sup> has been devised.

Chang liver cells *in vitro* are pre-treated with colchicine and mounted in an open-ended chamber for micrurgical procedures. The chamber is filled with a physiological chemically defined medium into which the chromosomes are to be isolated. A cell in metaphase is located and an incision is made in its cytoplasmic membrane. During the following 2 min or so the mitotic apparatus slips out of the cell through the incision. Next the tips of two microneedles are carefully inserted into the isolated mitotic apparatus in an effort to grasp the spindle. The microneedles are then gently pulled away from each other just enough to separate the chromosomes along lengths of the spindle which is then attached to the cover-slip. During this process several chromosomes are inadvertently damaged or destroyed by being stretched or smeared on to the cover-slip. However, many of the other chromosomes are readily identifiable as belonging to one of the various groups of the human set<sup>7</sup> and show neither damage nor any non-chromosomal material (contamination) which can be seen with the light microscope (Fig. 1). At the next step, which is fixation, any of a variety of fixatives may be used. At this time, any protein in the medium around the chromosome may precipitate and care must be exercised



Fig. 1. Phase contrast micrograph of human chromosomes isolated from Chang liver cell by micrurgy. The background was printed to medium grey to show the lack of non-chromosomal material in this fixed preparation.

to remove as much as possible without damaging the chromosomes. Finally, the points of attachment of the spindle to the coverslips are loosened and segments of spindle with attached chromosomes are placed on a 'Formvar'-coated grid, at which time the fixative may be removed and replaced by a solution which undergoes minimal change of pH and salt concentration as the preparation is dried in air. At this time the preparation is ready for examination with the electron microscope.

Human chromosomes prepared in this fashion and examined with the electron microscope show considerable structural detail. Although there appear to be structural variations along the length of at least some chromatids and between some chromosomes, preliminary analysis of several chromosomes suggests the following generalizations.

The centromere has been clearly seen as a quasitriangular structure about 7000 Å across its largest dimension (Fig. 2). Occasionally the centromere may appear heart-

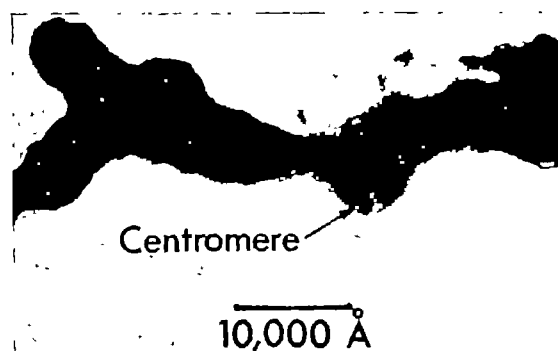


Fig. 2. The centromere of this chromosome is clearly seen. It also appears obvious that at least some of the chromosome fibrils extend through the region occupied by this centromere.

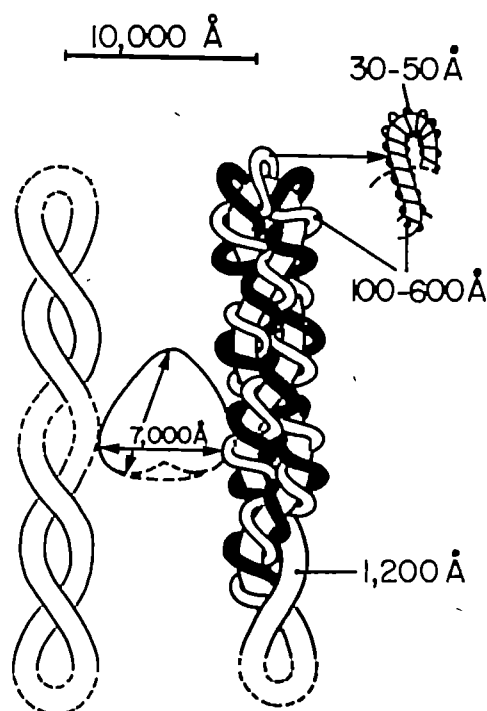


Fig. 3. A schematic representation of general features of human mitotic metaphase chromosomes

shaped and the indentation is interpreted as part of the early stages of division (Fig. 3).

From the region of the centromere there appears to extend through the length of each chromatid a rather large (about 1200 Å thick) filament having little electron density. At the telomere it appears that this filament bends back on itself and courses back through the length of the chromatid in such a way that there appear to be two such filaments loosely twined about one another (Fig. 3).

Around each of these segments of about 1200 Å there appear to be other filaments of various sizes ranging from about 100 Å through about 600 Å thickness. These filaments have moderate electron density, and at least some of them appear in turn to be surrounded by 30-50 Å thick filaments which have relatively great electron density.

Secondary constrictions and satellites have been clearly recognized as such in some electron micrographs, but too few have been seen to permit statements as to the precise nature of their structure.

Although these results are to be regarded as preliminary, they are based on chromosomes fixed and/or dried in acetic acid, ethanol, methanol, formaldehyde, glutaraldehyde, chromium trioxide, lanthanum nitrate, potassium molybdate and phosphotungstic acid.

Chromosomes in cells treated with hypotonic solutions followed by fixation with acetic acid and squashing fail to reveal recognizable units of structure. Instead, such chromosomes are seen as amorphous densities overlain by clumps of spherules, as are similarly prepared chromosomes in Fig. 9 of Barnicot and Huxley<sup>4</sup>. Some of the other fixatives and drying solutions have yielded results which are compatible with half chromatids composed of a hierarchy of pairings of smaller into larger and larger fibres as reported by Osgood<sup>5</sup> and by Cole and Langley<sup>6</sup>. However, numerous preparations which appear to be in a better state of preservation of structure show distinct patterns of smaller coils winding around the larger coils as in Fig. 3.

Investigations are now being made to construct complete models of specific chromosomes and to compare chemically, enzymatically, and microscopically induced dissociations of structure from otherwise intact chromosomes of diploid human cells.

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## GENETICS

### Inbreeding and Genetic Loads in Irradiated Experimental Populations of *Drosophila melanogaster*

Morton, Crow and Muller<sup>1</sup> suggested that measurements of the inbreeding depression can be used to estimate the magnitude of the genetic loads which populations carry as well as to differentiate between the mutational and balanced components of such loads. Several studies designed to investigate this problem have been done in *Drosophila*<sup>2-4</sup> and in *Tribolium*<sup>5</sup>. The validity of the technique proposed by Morton, Crow and Muller has been questioned (see Levene<sup>6</sup> and Dobzhansky<sup>7</sup> for references). Inbreeding investigations, nevertheless, remain useful to the examination of genetic loads in populations, although the possibilities of discrimination between the mutational and balanced components have become remote. This communication summarizes the results obtained from investigations aimed at comparing the magnitudes of the genetic loads in experimental populations of *Drosophila melanogaster*. These populations have been exposed to X-irradiation (to the males) for a number of generations and then maintained without further irradiation for varying periods of time. Their radiation histories are outlined in Table 1.

The populations (except the control) had been subjected to a cumulative X-radiation exposure of 120,000 r, although at different rates. The egg-adult viability in these populations, after the initial rise for 3-4 generations following the cessation of the irradiation, has remained at a level between 70 and 75 per cent, below the level in the control population (87-90 per cent). This situation has persisted for more than 57 generations in VI-ER-20, 47 generations in LV-ER-80 and 35 generations in II-EGR-60.

The experiments investigating the effects of inbreeding on viability were carried out over a period of four consecutive contemporaneous generations, 70-73 in the control, 46-49 in VI-ER-20, 36-39 in IV-ER-30 and 23-26 in II-EGR-60. In all populations except the control, these represent the number of generations of relaxation from irradiation. The technique adopted was the same as that used by Malogolowkin *et al.*<sup>4</sup>. A total of 140 single-pair matings were set up per population. From each of the single-pair progenies, virgin females and males were collected and three types of crosses were set up as follows: (1) unrelated—between groups of 10 females from one culture and 10 males from a different

Table 1. CHARACTERISTICS OF THE EXPERIMENTAL POPULATIONS

Population	Treatment	Started
C-1	Control, no radiation	Sept., 1962
VI-ER-20	Males received 6,000 r. per generation for 20 generations	July, 1963
IV-ER-30	Males received 4,000 r. per generation for 30 generations	Dec., 1963
II-EGR-60	Males received 2,000 r. per generation for 60 generations	June, 1964

culture; (2) brother-sister matings—between groups of 10 females and 10 males from the same culture; (3) half-sib matings—a single male was crossed to a female from one culture and two days later the same male was crossed to a female from a different culture, and from the progenies groups of 10 females and males were taken and intercrossed. The inbreeding coefficients in the three kinds of matings are respectively  $F = 0$ , 0.25 and 0.125.

The egg-adult viability was studied in the progenies of the foregoing three types of mating. The flies were placed in 8-in. glass vials with spoons containing cornmeal-molasses-agar medium darkened with an admixture of charcoal for easy visibility of the eggs. Two samples of 50 eggs each were taken from each group of parents and were allowed to develop (in regular half-pint culture bottles containing cream of wheat-molasses medium) at 25° C. Complete counts of the adults hatching in the cultures were made.

The numbers of cultures with different percentages of survival from egg to adult stage are summarized in Table 2. It is obvious that the viability in the irradiated populations is lower than in the control. More interesting, however, is the distribution of the viabilities over a short range (and hence small variance). This is not unexpected, since all the populations were maintained under conditions of relaxed natural selection (for details, see Sankaranarayanan<sup>10,11</sup>). The *Drosophila* populations utilized for inbreeding studies so far were obtained either from Nature<sup>1-4</sup> or from population cages<sup>5,6</sup>, where natural selection is presumably more intense.

The mean viabilities observed on the inbred and outbred progenies are shown in Table 3. As expected, the outbred progenies ( $F = 0$ ) are the most viable, followed by the progenies of half-sib matings ( $F = 0.125$ ), and full-sib matings ( $F = 0.25$ ). In the control, the reduction in the viability due to inbreeding is relatively small. In the other three populations the inbreeding depression is greater, but not as great as observed in the studies on natural populations of *D. pseudo-obscura*<sup>1,2</sup> and *D. willistoni*<sup>3</sup>. In addition, the relative reduction in viability due to inbreeding is of the same order of magnitude in spite of the fact that these populations have been relaxed from irradiation for different numbers of generations. These data compare very well with those obtained by Torroja<sup>4,6</sup> for experimental populations of *D. pseudo-obscura*.

The  $A$  and  $B$  statistics and the  $B/A$  ratios and their standard errors are also presented in Table 3. These values have been calculated from the data following the method of Malogolowkin *et al.*<sup>4</sup>. The  $A$  value is a measure of the mortality in outbred progenies ( $F = 0$ ) and this mortality is due to a combination of genetic and environmental causes. The  $B$  value measures the additional mortality due to complete inbreeding.

Table 2. NUMBERS OF CULTURES WITH DIFFERENT PERCENTAGES OF SURVIVAL FROM EGG TO ADULT STAGE

Population	F	45	49	53	57	61	65	69	73	77	81	85	89	93	N
C-1	0	..	..	..	..	..	..	..	..	..	20	62	36	2	130
	0.125	..	..	..	..	..	..	..	..	7	52	61	12	..	132
	0.25	..	..	..	..	..	..	..	..	11	82	41	3	..	137
VI-NR-20	0	..	..	..	..	10	33	55	31	7	..	..	..	..	126
	0.125	..	..	..	6	6	27	49	23	1	2	1	..	..	129
	0.25	..	2	15	16	40	27	24	9	1	..	..	..	..	124
IV-NR-30	0	..	..	..	..	2	17	71	28	11	1	..	..	..	130
	0.125	..	1	4	20	22	25	27	8	..	..	..	..	..	127
	0.25	..	3	81	53	42	18	7	..	..	..	..	..	..	124
II-EGR-60	0	..	..	..	..	1	20	54	29	10	..	..	..	..	124
	0.125	..	1	2	18	26	45	22	6	..	..	..	..	..	122
	0.25	2	6	23	40	42	18	5	..	..	..	..	..	..	126

Table 3. PERCENTAGES OF EGGS DEVELOPING TO THE ADULT STAGE IN OUTBRED AND INBRED PROGENIES AND ESTIMATES OF  $A$ ,  $B$  AND  $B/A$  VALUES

		C-1	VI-NR-20	IV-NR-30	II-EGR-60
Max to adult viability					
$F = 0$		87.23 ± 0.26	74.18 ± 0.22	71.52 ± 0.28	71.81 ± 0.20
$F = 0.125$		85.00 ± 0.24	69.56 ± 0.20	66.03 ± 0.46	66.04 ± 0.20
$F = 0.25$		83.42 ± 0.20	64.07 ± 0.48	60.81 ± 0.42	59.66 ± 0.42
$A$		0.1870 ± 0.003	0.2970 ± 0.004	0.3263 ± 0.004	0.3287 ± 0.004
$B$		0.1810 ± 0.015	0.5720 ± 0.033	0.6921 ± 0.031	0.7165 ± 0.022
$B/A$		1.22 ± 0.13	1.92 ± 0.13	2.06 ± 0.11	2.12 ± 0.11

The  $A$  value is small in the control and is larger in the populations with radiation histories. The  $B$  value is, again, small in the control and is much larger in the other populations. Among the irradiated populations VI-NR-20, which had the greatest number of generations of relaxation from irradiation (and hence was exposed to the action of normalizing natural selection for a greater length of time), has the lowest  $B$  value, followed by IV-NR-30 and II-EGR-60. The differences, although not significant, are in the expected direction. It is interesting to point out that the  $B$  values are somewhat smaller than those calculated for natural populations<sup>4,6</sup>.

The  $B/A$  ratios are small, ranging from 1.32 for the control to about 2 in the other populations. This does not necessarily mean that the genetic loads in these populations are predominantly balanced. This is unlikely in the radiated populations which have been under induced mutation pressure for several generations and then relaxed without irradiation for a period between 30 and 57 generations. It is possible that under the selection conditions in which these populations were maintained, numerous induced mutants affecting viability and which are semidominant still persist. The consequence of this will be an inflated  $A$  value resulting in a small  $B/A$ . This, coupled with the questionable validity of the  $B/A$  ratio to determine the genetic structure of the populations, makes the utility of the  $B/A$  statistic quite dubious. Dobzhansky *et al.*<sup>3</sup>, Malogolowkin *et al.*<sup>4</sup> and Levene *et al.*<sup>7</sup> came to the same conclusion.

$A$ ,  $B$  and  $B/A$  values calculated taking into consideration only two of the three examined viability values are not significantly different from those obtained using all three viability values. This indicates that there are no significant departures from linearity within the range of the  $F$ 's studied.

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## PSYCHIATRY

### Object-naming by Dysphasic Patients

In previous communications<sup>1,2</sup> two of us reported that with normal subjects the time taken to name an object is inversely proportional to the logarithm of the frequency of the name-word in the language, as estimated by a standard word-count<sup>3</sup>. These experiments were originally undertaken to clarify the incapacity to name objects shown by individuals who have suffered some forms of brain injury, and the study has accordingly now been extended to subjects of this kind. The technique has necessarily been simplified and now comprises manual display of cards bearing the 26 pictures of objects origin-

ally used, and timing with a stop-watch (1/100 sec) in place of the optical projection and timing by voice-key and pen-recorder in the experiment previously reported.

The subjects were all men with penetrating gunshot head-wounds, the majority incurred in the Normandy campaign of 1944. Their ages ranged from 37 to 61, with a mean age of 44.5 years. The total number of cases tested was 102, but of these, 24 had bilateral lesions and these have been excluded for the present purpose as have those 4 who were not unquestionably right-handed. Fig. 1 shows the frequency-latency relationship for the remainder divided into the relevant lesion groups, having similar mean ages and verbal intelligence quotients. Also shown are results for the university group used in the previous experiment together with those for a hospital control group (patients of age and verbal level similar to the brain injured but with no ascertained neurological or psychiatric disorder). In the case of the dysphasics those results have been rejected in which, following presentation of the stimulus, two or more verbal interjections were made before the correct response. Once such an individual becomes involved in circumlocutory speech; the further period thus expended is somewhat random in amount.

With regard to Fig. 1, a linear relationship was found to subsist between mean latencies for the 26 objects and the logarithm of their frequencies, the coefficients of correlation ranging from  $-0.80$  to  $-0.87$  ( $P < 0.001$ ). Analysis of variance confirmed the goodness of fit of these five linear regressions at  $P < 0.001$ . Analysis of variance showed the latencies for all sub-groups to differ from each other at  $P < 0.01$  to  $P < 0.001$ , with the exception of the university subjects and the hospital control group, where the difference failed to reach significance.

A comparison of regression coefficients showed that the slopes of the three brain-damaged groups differed significantly from the hospital controls ( $P < 0.01$  to  $P < 0.001$ ), and the dysphasics versus left-hemisphere group without dysphasia at  $P < 0.05$ . The difference between slopes for the dysphasics as compared with the right-sided group, and the left- and right-hemisphere groups without dysphasia failed to reach significance, as did the slopes for the university subjects and hospital controls.

We draw the following conclusions from these results.

(1) Lower verbal intelligence and greater age bring about a trend toward increased latency compared with findings for a university population. (2) Any form of brain lesion

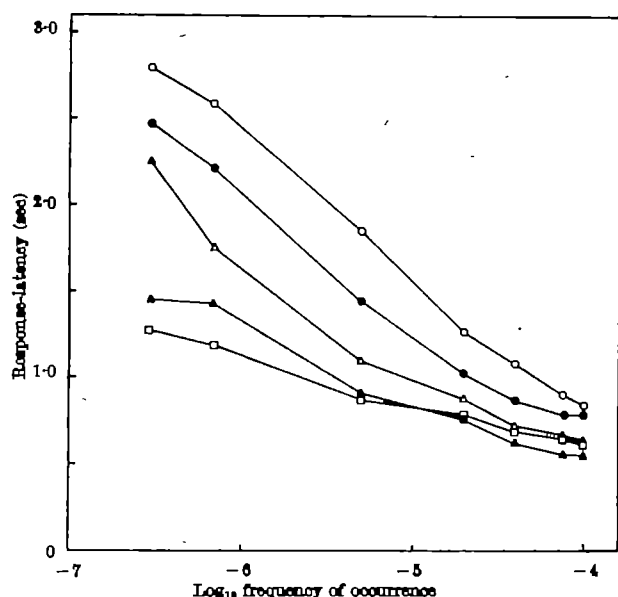


Fig. 1. Response-latency in naming objects versus  $\log_{10}$  frequency of occurrence of the name in English for brain-damaged patients with and without dysphasia, hospital controls and university subjects. O, Dysphasia; ●, right hemisphere not dysphasic; Δ, left hemisphere not dysphasic; ▲, hospital control group; □, university subjects

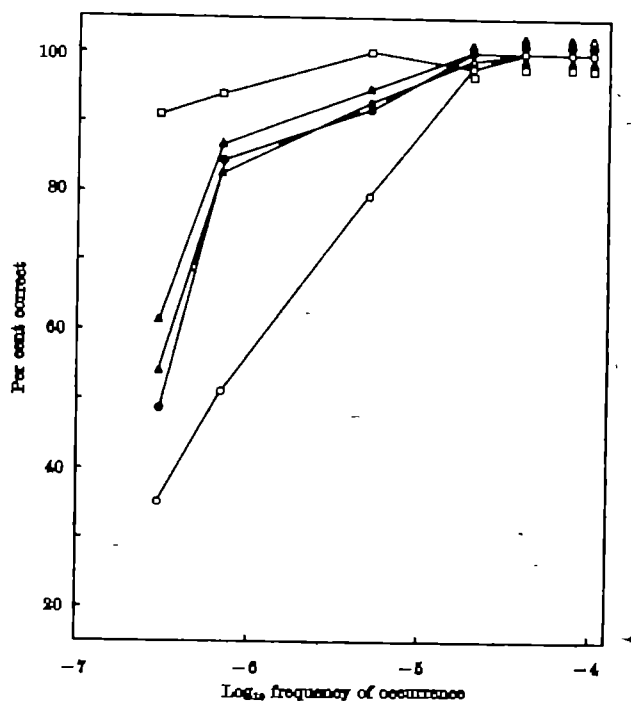


Fig. 2. Percentage of subjects correctly naming objects versus  $\log_{10}$  frequency of occurrence of the name in English. O, Dysphasia; ●, right hemisphere not dysphasic; Δ, left hemisphere not dysphasic; ▲, hospital control group; □, university subjects

results in a further and significant increase. (3) Right-sided lesions exercise a larger effect in this connexion than those left-sided ones which produce no speech disorder. (4) Lesions productive of speech-disorder, as assessed by clinical test, show the greatest increase in the time required to name objects.

Attention has been directed by Paterson and Zangwill<sup>4</sup>, and Milner<sup>5</sup> (reviewed by Piercy<sup>6</sup>) to the disturbing effects of some right-hemisphere lesions on visual perception. This could account for the differences between right and left non-dysphasic patients, but further analysis of the localization data, now in progress, is required to clarify this.

Fig. 2 shows the results, for the same groups, in terms of percentage correct naming against log frequency of the word-names. This affords clear evidence that the detection of slight, or residual, speech-disorder by a naming test requires the use of objects (or pictures of them) which are of a certain order of rarity, as assessed by their name-words. With objects the names of which have a frequency of above 10 per million, there is virtually no difference in the number correctly named (98 versus 100 per cent), while above 80 per million, in this case, no failures at all occur (even if such a difference may appear with the more refined technique of latency-measurement). A marked difference in correct responses appears only with objects the names of which have a frequency below  $10^{-5}$ .

We thank Dr. W. Ritchie Russell for the facilities granted us, and for permission to study patients under his care.

FREDA B. NEWCOMBE

Department of Neurology, Churchill Hospital.

R. C. OLDFIELD

A. WINGFIELD

Medical Research Council Psycholinguistics Research Unit,  
Institute of Experimental Psychology,  
University of Oxford.

<sup>1</sup> Oldfield, R. C., and Wingfield, A., *Nature*, **208**, 1081 (1964).

<sup>2</sup> Oldfield, R. C., and Wingfield, A., *Quart. J. Exp. Psychol.* (in the press).

<sup>3</sup> Thorndike, M. L., and Lorge, I., *The Teacher's Word Book of 30,000 Words* (Columbia University, New York, 1944).

<sup>4</sup> Paterson, A., and Zangwill, O. L., *Brain*, **67**, 331 (1944).

<sup>5</sup> Milner, B., *Proc. Assoc. Res. Nerv. Dis.*, **35**, 244 (1958).

<sup>6</sup> Piercy, M., *Brit. J. Psychol.*, **118**, 310 (1964).

## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, September 13—Friday, September 17

INSTITUTE OF ELECTRONICAL ENGINEERS (at Savoy Place, London, W.C.2)  
—International Conference on "The Microwave Behaviour of Ferrimagnetics and Plasmas".

Tuesday, September 14

UNIVERSITY OF LONDON (in the Meyershall Lecture Theatre, Westminster Medical School, Horseferry Road, London, S.W.1), at 5.15 p.m.—Prof. D. J. Hanahan (University of Washington): "Observations on Lipid-Protein Involvement in Human Erythrocyte Membranes".\*

Wednesday, September 15

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. J. W. Pearson and Mr. K. R. Williams: "Hydrocarbon Fuel Cells".

Thursday, September 16

OIL AND COLOUR CHEMISTS' ASSOCIATION (in the Small Physics Lecture Theatre, Imperial College of Science and Technology, London, S.W.7), at 7 p.m.—Mr. G. R. Pye: "The Decoration of Plastics".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

POST-DOCTORAL FELLOW (cell biologist or biochemist) IN THE DEPARTMENT OF ZOOLOGY, to work with Dr. L. G. H. Bell on problems associated with cell surface function—The Deputy Secretary, The University, Southampton (September 14).

RESEARCH FELLOW (preferably with a special interest in the solution of partial differential equations) IN NUMERICAL ANALYSIS; an ASSOCIATE RESEARCH FELLOW to work in the field of industrial statistics or operational research; and a RESEARCH ASSISTANT to work, under guidance, on the analysis and appraisal of technical literature with particular reference to process control—The Staff Officer, College of Advanced Technology, Gosta Green, Birmingham, 4, quoting Ref. 432/2 (September 14).

ASSISTANT LECTURER IN ZOOLOGY—The Registrar, The University, Manchester, 13, quoting Ref. 165/65/NA (September 15).

POST-DOCTORAL RESEARCH ASSISTANT (S.R.C.) for research on low-temperature organic chemistry—The Registrar, The University, Bristol, 2 (September 15).

TECHNICIANS IN THE DEPARTMENT OF BOTANY for general duties and to assist with research in plant physiology—The Registrar, University College of Wales, Aberystwyth (September 15).

RESEARCH-MICROSCOPE TECHNICIAN (preferably with previous experience in electron-microscopy) IN THE DEPARTMENT OF APPLIED PHYSICS, to assist with the operation and maintenance (day-to-day only) of the Department's JEOL-7 electron-microscope—The Secretary, Welsh College of Advanced Technology, Cathays Park, Cardiff (September 15).

DEMONSTRATOR OR TEACHING FELLOW IN THE DEPARTMENT OF PHYSICAL CHEMISTRY—The Registrar, The University, Leeds, 2 (September 20).

RESEARCH FELLOW (graduate in medical or biological science and preferably some postgraduate training in laboratory methods) IN THE DEPARTMENT OF MATERIA MEDICA AND THERAPEUTICS, to work as a member of an established research team investigating the hormonal disturbances associated with thyroid disease—The Secretary, The University, Aberdeen (September 20).

LECTURER (with a good honours degree, some experience of teaching and/or research, and preferably special qualifications or experience in fluid mechanics and gas turbine engineering) IN MECHANICAL ENGINEERING—The Registrar, King's College (University of London), Strand, London, W.C.2 (September 21).

SENIOR LECTURER (honours graduate with good teaching experience) IN CROP HUSBANDRY—The Principal, Essex Institute of Agriculture, Writtle, Chelmsford, Essex (September 23).

ASSISTANT IN APPLIED PSYCHOLOGY—The Secretary, University College, Cork, Republic of Ireland (September 24).

SCIENTIFIC OFFICER (with a first- or good second-class honours degree in geology and preferably a knowledge of X-ray crystallography) IN THE DEPARTMENT OF PEDOLOGY, for work concerned with optical, mineralogical and petrological studies in soils—The Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen (September 24).

D.S.I.R. SENIOR RESEARCH ASSISTANT to work with Dr. J. B. Horderby on a programme of structure determination in liquid metals and alloys using neutron techniques—The Registrar, The University, Sheffield, 10 (September 25).

DEMONSTRATOR IN AGRICULTURE (Agronomy) to assist with experimental work and for general University duties—The Registrar, The University, Leeds, 2 (September 27).

SENIOR LECTURER or LECTURER (preferably with wide experience of West African conditions) IN THE DEPARTMENT OF AGRICULTURAL CHEMISTRY AND SOILS, University of Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (September 27).

POST-DOCTORAL FELLOW (with experience in plasma physics, microwave techniques or electro-magnetic theory and use of a computer) in a plasma physics group which is studying the scattering of microwaves by various plasma configurations—The Registrar, University College of Wales, Aberystwyth (September 28).

CHAIR OF NUCLEAR PHYSICS—The Secretary, University of Lancaster, Bailrigg House, Lancaster (September 30).

CHAIR OF PHARMACEUTICAL TECHNOLOGY—The Registrar, University of Strathclyde, Glasgow, C.1 (September 30).

LECTURER IN CIVIL ENGINEERING at the University of Adelaide, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 30).

PROFESSOR OF ZOOLOGY (Pleasantburg); and a SENIOR LECTURER IN ZOOLOGY (Pleasantburg), University of Natal, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, September 30).

READER or SENIOR LECTURER (medical) IN THE DEPARTMENT OF PHARMACOLOGY—The Registrar, The University, Manchester 13, quoting Ref. 171/65 (September 30).

RESEARCH ASSISTANT (with a Ph.D. degree in chemistry) to Dr. J. S. Whitehurst, to work on steroids—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devon (September 30).

RESEARCH STUDENT (suitably qualified electronic or electrical engineer or physicist interested in research in the field of digital frequency modulation) IN THE DEPARTMENT OF ELECTRONICS—Prof. G. D. Sims, University of Southampton, Southampton (September 30).

SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER (with an honours degree and basic training in microbiology, together with research experience and preferably a Ph.D.) to carry out fundamental research on food micro-organisms particularly the bacteria associated with the spoilage of eggs and egg products—The Secretary, Low Temperature Research Station, A.R.C., Downing Street, Cambridge (September 30).

SENIOR LECTURER or LECTURER (with extensive experience in digital computing) IN THE FIELD OF COMPUTING at Monash University—The Registrar, Monash University, P.O. Box 92, Clayton, Victoria, Australia; or The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 30).

SENIOR RESEARCH OFFICER (Dental) (with previous experience in research and preferably a higher degree) IN THE INSTITUTE OF DENTAL RESEARCH—The Secretary, United Dental Hospital of Sydney, 2 Chalmers Street, Sydney, New South Wales, Australia (September 30).

BIOCHEMIST (Senior Research Officer) to organize and develop a new Biochemistry Laboratory in the Clinical Sciences Building, Royal Melbourne Hospital, IN THE UNIVERSITY DEPARTMENT OF PSYCHIATRY—Prof. B. M. Davies, Department of Psychiatry, University of Melbourne, Parkville, M.2, Victoria, Australia (October 1).

SENIOR LECTURER (with special interest in physical oceanography, including dynamic oceanography and/or marine geology) IN OCEANOGRAPHY; and a LECTURER (preferably with a special interest in oceanography) IN BIOCHEMISTRY—The Registrar, University College of Swansea, Singleton Park, Swansea (October 1).

BIOCHEMIST or CHEMIST (Ph.D. or equivalent experience) IN THE DEPARTMENT OF BIOCHEMISTRY to participate in a research programme on naturally occurring sulphate esters—Prof. K. S. Dodgson, Department of Biochemistry, University College of South Wales and Monmouthshire, St. Andrew's Place, Cardiff (October 2).

LECTURER (with qualifications and a special interest in the teaching of mathematics or chemistry) IN EDUCATION—The Registrar, The University, Sheffield (October 2).

COMPUTER MANAGER (with previous experience with large electronic computers) for the KDF9 installation in the Electronic Computing Laboratory—The Registrar, The University, Leeds, 2 (October 3).

RESEARCH FELLOW (graduate in physics or physical chemistry) IN PHYSICAL CHEMISTRY to experiment on the electronic properties of organic vapours—The Registrar, University of Queensland, Brisbane, Australia (October 14).

ASSISTANT EXPERIMENTAL OFFICER (graduate in a biological science (pure or applied) with an interest in field work) IN THE NEMATOLOGY DEPARTMENT to assist in work on nematodes injurious to cereals and their control—The Secretary, Rothamsted Experimental Station, Harpenden, Herts, quoting Ref. 1063/80 (October 15).

SENIOR LECTURER IN ELECTROMAGNETISM IN THE DEPARTMENT OF ELECTRONIC AND ELECTRICAL ENGINEERING—The Assistant Registrar (Science), The University of Birmingham, Birmingham 15 (October 15).

CHAIR OF BIOLOGY (in the field of Plant Physiology) AT THE UNIVERSITY OF SYDNEY, AUSTRALIA—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 25).

CHAIR OF THERMODYNAMICS AND FLUID MECHANICS (a second chair in the Department of Mechanical Engineering)—The Secretary, The Queen's University of Belfast, Belfast, Northern Ireland (October 30).

LECTURER (with a Ph.D. degree or equivalent qualifications) IN THE SCHOOL OF CHEMISTRY, University of New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 30).

ARNOLD YILDHAM and MARY RAINE MEDICAL RESEARCH FELLOWS (medical or science graduates) in arteriosclerosis and allied diseases in the Faculty of Medicine, University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 31).

CHAIR OF MATHEMATICS (R.A.A.F. Academy), University of Melbourne, Australia—The Registrar, University of Melbourne, Melbourne, Australia; or The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (November 1).

ASSISTANT FORENSIC SCIENTIST (with a B.Sc. degree including physics or chemistry or botany (or similar professional qualification) with some research experience, and preferably a knowledge of photography) with the British South Africa Police in Salisbury, Rhodesia—The Police Recruiting Officer, Rhodesia House, Strand, London, W.C.2.

BIOLOGY GRADUATES to train as Cyto-technologists IN THE CYTOLOGY SECTION OF THE PATHOLOGY DEPARTMENT—The Hospital Secretary, Christie Hospital and Holt Radium Institute, Manchester, 20.

ENTOMOLOGIST (national of the United Kingdom or the Republic of Ireland, with a degree in natural science, preferably with second-class honours, of which zoology should be a principal subject; some postgraduate experience is desirable) in Malawi, to supervise phytosanitary services including exports and imports; to carry out systematic work in the museum; to identify pests and advise on control measures; and to carry out some entomological research—The Appointments Officer, Ministry of Overseas Development, Room 301, Strand House, Stag Place, London, S.W.1, quoting Ref. RO 218/184/06.

GRADUATE RESEARCH OFFICER (preferably with experience in medical statistics, biometry or genetics) IN STATISTICS IN THE OXFORD RECORD LINKAGE STUDY AND UNIT OF CLINICAL EPIDEMIOLOGY—The Medical Director, Oxford Record Linkage Study, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford.

LABORATORY TECHNICIANS, Grades III and IV, in the DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY for work connected with chemistry, biochemistry, or physiology—The Assistant Bursar (Personnel), University of Reading, Reading, Berkshire.

LECTURER IN BOTANY; a LECTURER IN CHEMISTRY; and a LECTURER IN PHYSICS at the University of Basutoland, Buthaburane Protectorate and Swaziland—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1.

LECTURER (with a special interest in spectroscopy, and preferably a research degree and able to teach physical chemistry to honours degree standard) IN PHYSICAL CHEMISTRY—The Registrar, Regional College of Technology, Leicester.

MICROBIOLOGISTS and/or ORGANIC CHEMISTS to study antibiotic-producing and similar fermentations in an intensive programme combining academic

interest with practical importance—Dr. J. D. Pu'Lock, Department of Chemistry, The University, Manchester, 13.

POST-DOCTORAL FELLOW IN THE CHEMICAL LABORATORY for work on synthetic organic chemistry—The Assistant Registrar (Establishment), University of Sussex, Stanmer House, Stanmer, Brighton, Sussex.

PRINCIPAL BIOCHEMIST to have responsibility in association with the Consultant Chemical Pathologist for the chemical pathology services at Paddington General and St. Charles Hospitals—The Group Secretary, Paddington General Hospital, London, W.9.

PROFESSOR OF MECHANICAL ENGINEERING—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 42/AF.

RESEARCH ASSISTANT IN THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY to work on problems relating to egg shell structure and strength—The Assistant Bursar (Personnel), University of Reading, Reading, Berkshire.

RESEARCH FELLOW (chemist or physicist with research experience) for work on high pressure chemistry—Dr. B. S. Bradley, School of Chemistry, The University, Leeds, 2.

RESEARCH OFFICER (Biologist) (national of the United Kingdom or the Republic of Ireland, with a first- or second-class honours degree in biology, and preferably postgraduate experience, particularly in pest control work, ecology or physiology) with the East African Common Services Organization, to study all aspects of the control of aquatic snails which are the intermediate hosts of trematode diseases, particularly by chemicals—The Appointments Officer, Ministry of Overseas Development, Room 301, Mland House, Stag Place, London, S.W.1, quoting Ref. RO 213/514/06.

SCIENCE GRADUATE FOR A CLINICAL INVESTIGATION UNIT, to work in medical laboratory research in association with the Department of Therapeutics, University of St. Andrews—The Medical Superintendent, Maryfield Hospital, Dundee.

SENIOR RESEARCH ASSOCIATE (Post-Doctoral) IN THE SCHOOL OF CHEMICAL SCIENCES for work in the preparation and spectroscopic study of diamagnetic aromatic molecules or co-ordination compounds—Prof. S. F. Mason, School of Chemical Sciences, University of East Anglia, Wilberforce Road, Norwich, NOR 77H.

SENIOR RESEARCH FELLOW (suitably qualified graduate) IN THE DEPARTMENT OF PRODUCTION AND INDUSTRIAL ADMINISTRATION to be concerned with the development of a high precision 3-co-ordinate measuring machine incorporating an interferometric laser system—The Registrar, The College of Aeronautics, Cranfield, Bedford.

SENIOR TECHNICIAN (with experience in the use of an electron microscope, preferably Siemens Elmiskop I, and familiarity with biological preparatory methods) for electron microscopy in the HISTOLOGY DEPARTMENT—The Registrar, The University, Liverpool 3, quoting Ref. 242/N.

SCIENTIFIC GLASSBLOWER (fully trained and experienced)—Dr. A. J. Croft, Garendon Laboratory, University of Oxford.

TECHNICAL ASSISTANT (preferably with some knowledge of biology or histological techniques) in the ELECTRON MICROSCOPE UNIT—Prof. H. J. J. Blackwood, Royal Dental Hospital, 32 Leicester Square, London, W.C.2.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

Society for the Promotion of Nature Reserves. *Advances in British Nature Conservation*. By B. M. Nicholson. (Preprint from the Society's Handbook for 1965.) Pp. 16. (London: Society for the Promotion of Nature Reserves, 1964.)

Bulletin of the British Museum (Natural History). Entomology. Vol. 17, No. 1: Delphacidae from Australia and New Zealand (Homoptera: Fulgoroidea). By R. G. Fennah. Pp. 1-59. (London: British Museum (Natural History), 1965.) 22s. 6d.

Technical Composition: a Service to Science. Pp. 15. (London: Spottiswoode, Ballantyne and Co., Ltd., 1965.)

Ministry of Technology. *Ergonomics for Industry No. 8: Thermal Comfort in Industry*. By Dr. R. H. Fox. Pp. 20. (London: H.M. Stationery Office, 1965. Obtainable from Warren Spring Laboratory, Stevenage, Herts.) Gratis.

The National Council for the Unmarried Mother and her Child. *Annual Report April 1964-March 1965*. Pp. 52. (London: The National Council for the Unmarried Mother and her Child, 1965.)

Patent Office. *Periodical Publications in the Patent Office Library: List of Current Titles*. Third edition. Pp. v+436. (London: H.M. Stationery Office, 1965.) 28s. net.

British Museum (Natural History). *Instructions for Collectors*. No. 10: *Plants*. Sixth edition. Pp. 72. (London: British Museum (Natural History), 1965.) 3s.

The Grower's Digest of Current Research, Vol. 2, covering Publications Issued Between July 1963 and July 1964. Compiled by R. T. Peard. Pp. 88. (London: Grower Publications, Ltd., 1965.) 20s. net.

Ministry of Overseas Development. *Report of the Anti-Locust Research Centre, 1st April 1961 to 31st December 1964*. Pp. v+49+8 plates. (London: H.M. Stationery Office, 1965.) 5s. 6d. net.

### Other Countries

The Carlsberg Foundation's Oceanographical Expedition Round the World 1928-30 and previous "Dana" Expeditions. "Dana" Report No. 65: *Ostracoda-Mysidocopa*. Part II: *Cypridiniformes-Ruditiermatidae, Sarsellidae and Asteroidea*. By Erik M. Poulsen. Pp. 484. (Copenhagen: Andr. Fred. Hest and Son, 1965.) 145 D kr.

Deutscher Wetterdienst. *Deutsches Meteorologisches Jahrbuch 1961*. Pp. xxxv+221. (Offenbach, a.M.: Deutscher Wetterdienst, 1963.)

National Museum of Canada. Bulletin No. 197 (Anthropological Series No. 65). *Songs of the Newfoundland Outports*. Collected and edited by Kenneth Peacock. Vol. 1: Pp. xxv+1-234. Vol. 2: Pp. xli+325-644. Vol. 3: Pp. xli+645-1036. (Ottawa: Queen's Printer, 1965.) 15 dollars the three volumes.

Records of the Dominion Museum, Wellington. Vol. 5, No. 11 (7 April 1965): *New Sea-Occumbers (Holothuroidea) from New Zealand Waters*. By David L. Paxon. Pp. 75-82. Vol. 5, No. 12 (7 April 1965): *The Mesocostal Groove in Ziphidae, with special reference to its closure by Ossification in Mesopoda-Osteoda*. By Charles McOmnn. Pp. 82-88. (Wellington: Dominion Museum, 1965.)

Estacion Experimental del Zaidin, Granada. *Memoria (1963-1964)*, Número 2. Pp. H+61. (Granada: Estacion Experimental del Zaidin, 1965.)

World Health Organization. *Technical Report Series, No. 209: Specifications for the Identity and Purity of Food Additives and Their Toxicological Evaluation*. Food Colours and some Antimicrobials and Anti-oxidants—Eighth Report of the Joint FAO/WHO expert Committee on Food Additives. Pp. 25. (Geneva: World Health Organization; London: H.M. Stationery Office, 1965.) 2 Sw. francs; 2s. 6d.; 0.60 dollars.

Smithsonian Contributions to Astrophysics. Vol. 8, No. 5: *On the Luminous Efficiency of Meteors*. By Franco Vernani. Pp. 141-173. 20 cents. Vol. 8, No. 7: *Meteor Geomagnetic Effects*. By Sydney Chapman and Attila A. Aschour. Pp. 181-197. 20 cents. (Washington, D.C.: Government Printing Office, 1965.)

Annuaire 1965 du Génie Atomique. (Publié par l'Association des Ingénieurs en Génie Atomique.) Pp. 127. (Paris: l'Association des Ingénieurs en Génie Atomique, 1965.)

Bulletin of the Museum of Comparative Zoology, Harvard University. Vol. 123, No. 1: *Gular Musculature in Delphinids*. By Barbara Lawrence and William M. Schvill. Pp. 1-65. Vol. 123, No. 2: *A Revision of the Genus Rhabdophrys in the Americas (Hymenoptera, Bethyridae)*. By Howard M. Evans. Pp. 67-151+7 plates. Vol. 123, No. 3: *Rhabdophrys serpentina, a New Rhabdophrys Pterygophorid from the Texas Lower Permian*. By John Newland Chase. Pp. 153-223+5 plates. (Cambridge, Mass.: Museum of Comparative Zoology, Harvard University, 1965.)

The Use of Citation Data in Writing the History of Science. Pp. v+76. (Philadelphia: Institute for Scientific Information, 1964.)

The Weizmann Institute of Science. *Scientific Activities 1964*. Pp. iii+284. (Rehovoth, Israel: The Weizmann Institute of Science, 1965.)

Food and Agriculture Organization of the United Nations. *FAO Agricultural Development Paper No. 80: Methods of Farm Management Investigations for Improving Farm Productivity*. Prepared by W. Y. Yang. Revised edition. Pp. xli+253. (Rome: Food and Agriculture Organization of the United Nations; London: H.M. Stationery Office, 1965.) 17s. 6d.; \$5.50 dollars.

Australia: Commonwealth Scientific and Industrial Research Organization. *The Radiophysics Laboratory, Sydney, 1963-1964*. Pp. 29. (Sydney: The Radiophysics Laboratory, C.S.I.R.O., 1965.)

Museum of Comparative Zoology, Harvard University. *Breviora*. No. 218 (May 7, 1965): *New Frogs of the Genus *Craugastor* (Anura) from the Solomon Islands*. By Walter O. Brown. Pp. 16 (2 plates). No. 219 (May 7, 1965): *The Early Evolution of the Heliconiinae*. By H. Barrclough Fell. Pp. 17. No. 220 (May 7, 1965): *A New Species of *Eleutherodactylus* from Guadeloupe, West Indies*. By John D. Lynch. Pp. 7. No. 221 (May 7, 1965): *New Melanesian Ants (Hymenoptera: Formicidae) of Zoogeographic Significance*. By Robert W. Taylor. Pp. 11. No. 222 (May 23, 1965): *The Genus *Leptotyphlops* in the West Indies, with Description of a New Species from Hispaniola (Serpentes, Leptotyphlopidae)*. By Richard Thomas. Pp. 12. No. 223 (May 23, 1965): *A New Subspecies of *Crotalus ophiomaculatus* (Serpentes: Colubridae) from the Island of Grenada*. By Allen H. Greer. Pp. 6. (Cambridge, Mass.: Museum of Comparative Zoology, Harvard University, 1965.)

The Yearbook of the National Institute of Sciences of India 1964. Pp. 133. (New Delhi: National Institute of Sciences of India, 1965.)

Unesco. *World Guide to Science Information and Documentation Services*. Pp. 211. (Paris: United Nations Educational, Scientific and Cultural Organization, 1965.)

Canada: Department of Mines and Technical Surveys. *Geological Survey of Canada. Bulletin 103: Late Upper Jurassic and Early Lower Cretaceous Fossil Zones of the Canadian Western Cordillera*. British Columbia. Pp. x+70+22 plates. 4.50 dollars. Paper 65-9: *Salt Beds, South-western Ontario*. By B. V. Sanford. Pp. 7. 75 cents. (Ottawa: Queen's Printer, 1965.)

Food and Agriculture Organization of the United Nations. *FAO Agricultural Studies No. 64: Vascular Plants and the Liver Fluke*. By R. L. Taylor. Pp. xxi+224. (Rome: Food and Agriculture Organization of the United Nations; London: H.M. Stationery Office, 1964.) 15s.; 3 dollars.

Australia: Commonwealth Scientific and Industrial Research Organization. *CSIRO Divisions and Sections, 1965*. Pp. 23. (East Melbourne: Commonwealth Scientific and Industrial Research Organization, 1965.)

New Zealand Forest Service: Forest Research Institute. *Forestry Research Notes*. No. 39: *The Lowry Humpy-Cell Process for Preservation of Radiata Pine Timber—Treating and Kiln-Drying Trials*. By J. A. Kilmunth. Pp. 15. (Wellington: Government Printer, 1965.)

Zoology Publications from Victoria University of Wellington. No. 88 (21 May 1965): *Zooplankton of Wellington Harbour, New Zealand*. By R. G. Wear. Pp. 31. (Wellington: Government Printer, 1965.)

British Guiana. *Report on the Geological Survey Department for the year 1962*. Pp. 82+2 plates. (Georgetown: Geological Survey Department, 1964.)

Organization for Economic Co-operation and Development. *European Nuclear Energy Agency. Second Report on the Activities of the Eurochemic Company, 1962-1964*. (European Company for the Chemical Processing of Irradiated Fuels) Pp. viii+533. (Paris: Organization for Economic Co-operation and Development, European Nuclear Energy Agency, 1965.)

Canada: Department of Mines and Technical Surveys. *Geological Survey of Canada. Map 6-1965: Oil and Gas Fields, South-Western Ontario*. (Ottawa: Director, Geological Survey of Canada, 1965.)

5th Inter-American Symposium on the Peaceful Application of Nuclear Energy. (Symposium sponsored by the Inter-American Nuclear Energy Commission and the Government of Chile, with the co-operation of the U.S. Atomic Energy Commission, at Valparaiso, Chile, March 9-13, 1964.) Pp. vii+260. (Washington, D.C.: Pan American Union, 1965.) 2.50 dollars.

Memories of the Mount Stromboli Observatory. No. 17: *Catalogue of Open Clusters South of -45° Declination*. By A. R. Hong. Pp. 17. (Osteria: The Australian National University, 1965.)

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## THE PLIGHT OF LIBRARY SERVICES IN BRITAIN

IN his presidential address to the Library Association at Harrogate in May, Sir Frank Francis denied that at present Britain had any library system as such. He did, however, comment on such recent developments as the coming into force of the Public Libraries and Museums Act, with its provisions for a more comprehensive and far-reaching public library service and for establishing a Libraries Advisory Council, the creation in the Department of Education and Science of a national organization for scientific and technological information and the proposals of the Library Association for a national bibliographical advisory council and a national bibliographical centre. While Britain's libraries may present a sort of pattern, they have grown up in numerous ways and there is no formal relation between the various groups. Even such arrangements as exist are really only attempts to overcome obstacles to the supply of information caused by Britain's unorganized provision of libraries.

Sir Frank put his finger unerringly on the vital weakness, and it is this which needs to be tackled first. Indeed, unless we can advance considerably towards the development of a national system, it is unlikely that realistic and sound decisions regarding provision for the libraries of the universities and other institutions of higher learning will be taken when the Parry Committee reports. Again, sound as in principle is the creation of the new Office for Scientific and Technological Information, it represents a piecemeal approach which can endanger much more vital elements in a national system. The new organization is comparatively unimportant compared with the Patent Office Library and the new National Reference Library for Science and Invention which is to succeed it. From the scientific and technical point of view, the latter Library, which is the essential counterpart to the National Lending Library for Science and Technology at Boston Spa, is a first priority in any national system, but no Government has yet given the scheme the whole-hearted and urgent support that the national interest demands. While the new organization set up by the Department of Education and Science may in time provide much information which will be invaluable in formulating national policy in the whole field, its present importance is trivial compared with bringing into effective service the National Reference Library for Science and Invention.

It is in the light of this situation that the numerous proposals for developments in the field of libraries and information services are best appraised at the present time. Sir Frank Francis himself emphasizes that the biggest problems are centred in the learned libraries and in the adequacy of the library and information services at the highly specialized level. Since it is proposed, however, that the new National Reference Library for Science and Invention should form part of the British Museum, it was strange that Sir Frank made no reference to the delays in starting the new building or even to the urgency of establishing such a library on a much more adequate scale than even the Patent Office Library which it is to replace. Nor, in discussing the part that inter-library lending should play in Britain's library economy and the most satisfactory basis on which to plan such lending, did he consider the relation between the new National Reference Library for Science and Invention and

the National Lending Library for Science and Technology. Since the declared policy of the latter is to concentrate on literature of the past fifty years, there is an obvious gap if the needs of the inventor as well as of the historian of science or technology are to be served reasonably. This cannot be shrugged off merely by defending the sound practice of the British Museum Library that the visiting scholar should be assured that a very high proportion of his requirements can be met on the spot.

There is a difficulty here which must be met by considered policy and some reasonable arrangements regarding holdings of older scientific and technical literature, either for reference or for loan of the originals or of photo- or micro-copies. It is one of the problems which a national system can (and should) solve, and it is also one of those which the division of Government responsibility for information services between two Ministries, as has already been noted (*Nature*, 208, 968; 1965), may make more difficult. Sir Frank Francis admitted in his address that without the willing and disinterested co-operation of librarians and the institutions they served the Department of Education and Science would be unable to develop properly the new services of the British Museum, including this new department with its current scientific reference collection of one million volumes on open shelves, with relevant specialist advice and a staff of some 200. He said nothing, however, about the urgent need for action to provide the space which will enable the services to operate nor did he refer to the Ministry of Technology.

Meanwhile, the Library Association is itself concerned about the lack of adequate national planning to organize efficient access to the ever-increasing output of books, periodicals, reports and other documents, and besides its Committee on Scientific Library Services it has a Working Party of its Research Committee on National Reference Services which has issued a preliminary report, *Access to Information*\*. This report, which was discussed recently by Mr. L. Corbett in the *Library Association Record* for May (67, 151; 1965), outlines the proposals for a national bibliographical advisory council and a national bibliographical centre noted here. They are intended to meet the need for a national planning body for such services and for an organization which will provide points of entry into the service available.

At present nothing effective is being done to create a national plan, although the Library Association, the Association of Special Libraries and Information Bureaux (Aslib), the Department of Scientific and Industrial Research (until its dissolution) and the Royal Society have intermittently promoted the development of bibliographical and information services. Research into the development of library, bibliographical and information techniques is at present carried out by several bodies, as is to be seen, for example, in the survey of information needs of physicists and chemists undertaken in 1963-64 by Prof. B. H. Flowers on behalf of the Advisory Council on Scientific Policy and reported in the *Journal of Documentation* (21, 83; 1965). Akin to this is the survey of chemical publications made by Dr. R. S. Cahn for the Chemical Society of which an outline has been published

\* The Library Association. *Access to Information: a National Bibliographical Service*. Pp. 4. (London: The Library Association, 1965.)

in *Chemistry in Britain* for August (1, 358; 1965). Earlier in the year Prof. W. C. Overend opened a parallel discussion of this subject before the Society of Chemical Industry, and at the Library Association's annual conference at Harrogate in May, Mr. K. A. Mallaber, chairman of the Association's Library Research Committee, read what he believed to be the first paper on research read at the Association's annual conferences. Techniques and trends in the effective utilization of engineering information were discussed by Mr. B. E. Holm in a paper published in *Aslib Proceedings* (17, 124; 1965).

The Public Libraries and Museums Act, 1964, gives the Department of Education and Science general oversight of the public library service and it will be responsible for ensuring that workable schemes of inter-library co-operation exist. Besides participating in the co-ordination of effort and determining priorities, its responsibilities include establishment of the National Reference Library for Science and Invention and the National Lending Library for Science and Technology. It is intended that the new Office for Scientific and Technical Information shall bring together all the various growing points, including the needs of university libraries, and see how the various proposals can best be fused into a national policy for developing scientific library and information services. While the Office will promote new services wherever the need is proved, it will not seek to run such services unless no other suitable organization exists.

The new Office was set up after the preliminary report *Access to Information* was completed, but while the Working Party was aware of the proposals on which the new organization was based, it did not consider they offered a complete solution. Like the Heyworth Committee, which recommended a specific enquiry on this point, it saw a similar need for bibliographical organization in the social sciences and the humanities and suggested that duplication of effort could be avoided if the arrangements for scientific documentation served other subject fields as well. A national plan requires effective national machinery.

While the National Central Library is the recognized centre for inter-library lending of books it is still inadequate as a bibliographical information centre. Sir Frank Francis laid some stress on the differences in the needs of the scholar in the humanities as compared with the sciences or technology in this connexion. He thought it unlikely that a central lending library for the humanities would need to duplicate a large part of the holdings of the British Museum and other libraries. The Working Party suggests that to be effective the union catalogues of the National Central Library should include the holdings of major national, university and research libraries, irrespective of whether the material is or is not available for lending. No formal arrangements, however, exist to cover the immense reference potential of the British Museum and such national libraries as the Science Library, the Patent Office Library, apart from many of the smaller special libraries, a few university libraries and the public libraries, and even the *British National Bibliography* covers only a limited selection of Britain's published output.

While some of the services which are required under a national plan are thus in existence, they require extending and co-ordinating. To this end it is proposed that a representative 'National Bibliographical Advisory Council' shall be responsible for determining priorities and promoting and extending such services, as well as for stimulating research and for co-ordinating library and information

service activity. It would also be responsible for improving the co-ordination of bibliographical services, including abstracting and indexing services, for encouraging better provision of reference and bibliographical facilities in libraries, and for acting as a link with international bodies concerned with planning bibliographical and information services. The executive functions would be entrusted to a national bibliographical centre with an adequate professionally qualified staff and charged with establishing a clearing house for information on resources (including compiling and publishing detailed guides to sources of information and bibliographical guides of all kinds as well as sponsoring and organizing regional centres of information); establishing and controlling a full national bibliography, present and retrospective; establishing full union catalogues; carrying out research and serving as a clearing-house of information on research; and acting as the secretariat to the advisory council.

Mr. Corbett discusses the wider background of these proposals in the article already mentioned and in doing so indicates the limitations of the Government's own action in establishing the Office for Scientific and Technical Information. The planning for a National Reference Library gives Britain a unique opportunity to develop this to include first an information division and secondly a research division to stimulate and co-ordinate existing experiments and studies in scientific communication, classification, abstracting, indexing and the use of literature as well as to promote new studies which might arouse fresh interest in these professions and raise their status—a matter with which the present Minister for Education and Science has already indicated his concern. Mr. Corbett dismisses as entirely inadequate the Advisory Council for Scientific Policy's mild reference to the need for the closest liaison between the new organization and the National Reference and Lending Libraries, both of which he would place under the new Office and in close liaison with any branches of the Ministry of Technology serving this field. He does not refer, however, to the probability that there might be some overlap or confusion with the work of the national bibliographical centre proposed by the Library Association: that position could well be clarified by another Scientific Information Conference such as the Royal Society announced in 1948 or by a special investigation by a high level committee. Such a suggestion, however, is advanced to help convince the scientific community and the Government alike of the urgent need for a sound and comprehensive policy.

Mr. Corbett, however, suggests that a reference centre is needed for science and technology such as was proposed and supported by the Advisory Council for Scientific Policy fifteen years or more ago. Although that plan was abandoned ten years ago, Mr. Corbett still sees the need and the possibility of such a centre arranged in three divisions, of which the National Reference Library (to be built on the south bank) would be the basic unit, but dynamically extended to include an information division and a research division. This would be developed from the extensive Patent Office Library and would be the heart of the national centre and energetically administered. Complemented by the other two divisions, its resources would go far to satisfy the needs of British science, technology and industry. If the Library remains under the British Museum Trustees, Mr. Corbett insists that it must work in close harmony and conform in policy with the scientific and technical requirements of the two other divisions and their Council.

Whether such a course is practicable or even advisable seems doubtful. So little progress has been made and the record of frustrations and disappointments is such that there would appear to be sound reasons for separating the reference library entirely from the British Museum and relating it much more closely with the National Lending Library for Science and Technology in the Office for Scientific and Technical Information. We do not hesitate in war to change commanders who fail to win victories, and we should not be more reluctant in peace, in such vital matters as this, to replace those who fail to produce the energy and vision needed for results. The time is long overdue for a full enquiry into the reasons for such prolonged delay: the Estimates Committee has often enquired into matters far less prejudicial to the public interest.

Mr. Corbett makes no such comments himself, but there is a pointed reference to the possible effect of one or two driving personalities on the establishment of a new organization and on attracting to it imaginative staff. He also notes the need for a comprehensive register of scientists and their work—here again the division of responsibility between the Ministry of Technology and the Department of Education and Science does not seem likely to conduce to efficiency. It would probably be far more effective if one Ministry or the other were made responsible for establishing registers of scientists and of scientific research in progress and also studies of scientific and technical manpower.

Organization apart, there can be no question as to the opportunities which confront the new Office for Scientific and Technical Information. Mr. Mallaber's paper displays some of these in the field of research and indicates how random has hitherto been the nature of the subjects investigated. Much of it has been the experimental investigation of theories to establish hypotheses. Librarians have not been particularly enamoured of what might be called pure research—the free collection and association of data from which new and perhaps unexpected information may emerge—but Mr. Mallaber believes that such work needs encouraging, though it is less likely than the former to produce immediately useful results. The third type—development work—has been little pursued in Britain largely on grounds of cost. While he suggests that in the next few years the Library Association should concentrate on securing a full and balanced statement of what research is needed and in creating within the profession an appreciation of the importance and value of research, he looks to the Government as the main source of funds.

Here again there is a question of the relations between the Library Association and the new Office which brings out the vital importance of the professional contribution. That appears also in the compilation by the Colleges of Technology and Further Education Sub-Section of the Association of a revised edition of recommended standards of library provision in such institutions, recently issued under the title *College Libraries*†. These new standards attempt to take into account such developments as the growth of liberal studies and the widening of curricula, and are designed to assist the provision of sound and comprehensive library services in colleges of art, commerce, technology, and further education, etc. They cover resources required for efficiency such as books and

other material, staff, buildings and finance, and in issuing this revised edition the Library Association has rendered a real service to further education. First things come first and while nothing can replace the need for energy and vision and sound judgment at the centre the effectiveness of any national policy depends largely on its continuous support by professional action at all levels.

Still more recently, an article in *The Times* of August 10 stated that temporary accommodation has been found for the National Reference Library for Science and Invention in Central London and that an announcement from the Ministry of Public Building and Works might be expected within the next few weeks. At the same time the article, recapitulating briefly the history of the whole project over at least fifteen years, severely indicted successive Governments for procrastination over a vital matter, and quoted Sir Frank Francis as saying that the whole scheme was in the melting pot at the moment. The article drew a letter on the following day from Sir Frank himself, agreeing as to the vital importance of this Library in connexion with the need for an increased effort in science and technology and maintaining that the plans submitted to the Royal Fine Arts Commission in 1963, worked out in the expectation that the Library would form part of a new Patent Office building, would provide a scientific library service second to none. Nevertheless, quite apart from the uncertainty arising from the Government's severe curb on spending announced in July, Sir Frank admits that uncertainty over the future of the Patent Office has brought matters to an abrupt halt.

Sir Frank gave some facts as to the progress which had been made before this situation arose. A staff of 93 out of the 97 so far sanctioned by the Treasury had been recruited—with the present staff in the Patent Office Library the total is expected to be about 200—and in collaboration with that Library they are already surveying and co-ordinating the collection in the Patent Office Library and the British Museum Library, filling gaps and purchasing up-to-date serials and monographs. The trustees of the British Museum have given strong support to all that has been done and are gravely disturbed at the obstacles to their plans. Sir Frank added that the advisory committee under Prof. H. J. Emeléus, set up by the Trustees to advise on the services of the new library, had not met simply and solely because the delays and uncertainties made nonsense of any such meeting—as they do in fact of any thought of a national policy until the Government wakes up to the vital importance of the matter and realizes the mischief that it and its predecessors have done.

This, as Prof. R. S. Hulton rightly observes in a subsequent letter of August 16, is the real problem. From what E. M. Nicholson is reported to have said, a major culprit at the moment is the Department of Education and Science, but the Ministry of Technology is equally to blame. Certainly it is to be hoped that neither scientists nor industrialists will be deluded by the establishment of a new Office for Scientific and Technological Information while the first essentials for promoting Britain's industrial as well as her scientific and technological effort are neglected and disregarded. It is idle for the present Government to shrug the responsibility off on to their immediate predecessors: if the first post-war Labour Governments had shown sufficient insight and energy the project would have been completed long since; if the present Government had acted on the plans available to it last autumn, to which Sir Frank Francis referred, the project would have been well started.

† *College Libraries: Recommended Standards of Library Provision in Colleges of Technology and Further Education and other Establishments of Further Education*. Pp. 24. Compiled by the Colleges of Technology and Further Education Sub-section of the Library Association (London: The Library Association, 1965.) 6s 8d. (L.A. members 5s.)

What has to be realized, and realized at the top, is that the needs of science and of technology are not to be met on the cheap or by party trickery. They require far-sighted constructive planning, free from party prejudice and dogma, and sustenance by adequate resources which, once committed, are immune from pressure. Only so can serious waste be avoided. There are members enough in the present House of Commons, quite apart from the House of Lords, judging by some recent speeches, who have some understanding of the implications both of a National Reference Library for Science and Invention and of a genuine national library policy. So, is it too much to expect that they, firmly supported by professional associations of scientists and technologists, including the learned societies, should induce the Parliamentary and Scientific Committee to raise the issue and make matters really uncomfortable for any Government to continue to procrastinate? The Government must make the decisions and allocate the resources required to establish the National Reference Library for Science and Invention at the earliest possible moment and make a national policy not only possible but also a reality.

## ADRENAL STEROIDS AND THERAPY— A CRITICAL ASSESSMENT

### Adrenal Steroids and Disease

By Cuthbert L. Cope. Pp. xi+827. (London: Pitman Medical Publishing Co., Ltd., 1965.) 126s. net.

THIS book bears the imprint of the dual qualification of its author as a physician and a biochemist; it is written with an expert knowledge of steroid biochemistry while all the time having the clinician's needs in mind. It has the attraction, nowadays so rare, of a book written by a single author, and yet covers a wide field of medicine and therapeutics, since corticosteroid therapy had, and still has, application far outside the range of diseases of the adrenal gland.

The introductory chapters deal briefly with the chemical structure, the biosynthesis and the occurrence of the natural adrenal steroids. Their concentrations in blood and other body fluids, their metabolic transformation and the estimation of their metabolites in urine are discussed in the next chapters. There is a particularly useful analysis of the origin of the different urinary metabolites. This enables the reader to assess the very variable value of the many biochemical methods employed to test adrenal, pituitary or testicular function by steroid analysis in body fluids. Interesting examples of results of steroid estimations made in blood apparently contradicting findings in urine, or of different methods used in the same body fluid giving divergent answers, will serve as a useful guide through clinical biochemical literature. Tests of adrenal function occupy one chapter; this is followed by a discussion of more general questions, such as the role of the adrenal in stress, the evidence for or against the existence of 'diseases of adaptation', the relation between adrenal steroids and inflammation or immunity. Much space is devoted to the response of the body to prolonged treatment with steroids or with ACTH, to the question whether the response to stress of either the adrenal gland or the pituitary may be permanently inhibited by such treatment, and to the demonstration of the extreme scarcity of reliable data on these problems.

After several chapters dealing with specific adrenal diseases of the type which has been known for many decades, there is a discussion of the discovery of aldosterone, its physiology and pathology, and its synthetic antagonists. The evidence for and against the existence of a steroid which causes loss of sodium occupies one chapter,

and the changing views on the relation between hypertension and the adrenal cortex another. A chapter on the synthetic analogues of cortisol serves as an introduction to the discussion of indications and complications of steroid therapy. It is here that careful consideration has to be given to the multiple actions possessed by adrenal steroids, some of which are desirable in certain conditions and dangerous in others, and to the dangers of long-term effects which may lead to a kind of addiction, to withdrawal symptoms, and to rebound phenomena of the underlying disease. No other medicinal substances are apt, as the steroids are, to obscure the complications of their own administration and to lead to disaster when their administration is stopped. The book concludes with chapters on special problems. One deals with the use of steroids in collagen diseases, in disorders of the blood and in ophthalmology. The fact that the eye can be treated with steroids by local application carries the great advantage of avoidance of systemic signs of overdosage, but this does not mean that treatment need not be cautious and circumspect. One chapter discusses the discovery of substances which inhibit adrenal synthesis by interfering with  $\beta$ -hydroxylation, and another the clinical implications of the observation that certain steroids cause fever. Adrenal function in pregnancy, in the foetus and in the new-born, occupy special chapters. Another is devoted to the recently discovered masculinizing effect on the foetus of treatment of the mother with progestins, and a further chapter to the occurrence of signs of hyperadrenalism in malignant disease not located in the adrenal gland. Each section has its own detailed bibliography.

The author's views are balanced and cautious, and are expressed in a clear, readable style. Diagrams and short summaries help to clarify and recapitulate conclusions drawn from an often bewildering mass of contradictory claims. Fallacies of arguments based on inconclusive evidence are pointed out, and the reader is shown how to distinguish incontrovertible biochemical evidence from data which can be interpreted in more than one way. The clinician interested in the rationale of steroid therapy and in diseases of the adrenal gland will be the first to profit from this book, but the laboratory worker interested in the practical consequences of endocrinological research will find it just as fascinating.

MARTIN VOOGT

## RABBIT THROUGH THE LOOKING-GLASS

### The Rabbit In Eye Research

Compiled and edited by Prof. Jack H. Prince. Pp. xvi+652. (Springfield, Ill.: Charles C. Thomas, 1964.) 87 dollars.

THE publisher's claim that this compendium represents a complete study of the eye of the rabbit is very nearly true. It covers the anatomy of the bony orbit and of the ocular adnexa. There is a detailed account of the morphology and physiology of the globe wherein the chapters on the chemistry and aqueodynamics of the ocular fluids and on the retina and optic nerve are outstanding—the former by its excellence, the latter by its lack of critical appraisal of the material with which it deals. The chapter on the electron microscope is beautifully illustrated and lucid in the extreme. The book concludes with a description of the vascular and neurological systems, and a chapter on the effects of sundry electro-magnetic and ultrasonic radiations.

Why was this well-organized book written at all? Prof. Prince assures us that "while one must always remind oneself of . . . differences during experimental work on the rabbit, the similarities between this animal and man are numerous enough to justify its continued use in the ophthalmic laboratory, with due caution". The extent of

these similarities is a matter of opinion, and the cynic may be forgiven the thought that if cats had been cheap in pre-myomatosis days, a case might now be made for writing a practical book on cats—in spite of their tapeta. Monkeys would be even better. Some examples serve to illustrate this point: the solidity of the walls of the eye-ball of man more nearly compares with that of these tissues in cat than in rabbit. This is a matter one recalls whenever tonometric data are compared for different species, and here we learn (p. 236) that aqueous flow rates in rabbit eyes have to be derived from calibration tables constructed for human eyes. Anyone conversant with the trabecular meshworks in the two species will be pardoned for raising his eyebrows over this extrapolation. Secondly, the function of the rabbit retina differs from that of man more than does the feline. As this difference extends to visual behaviour, and there is no evidence of any typically human photopic apparatus in the rabbit, any hope for a search for cone pigments in the rabbit retina would not appear to be prompted by a marked sense of priority. Again, a considerable part of the section on the electron microscopy of the retina contains examples culled from work on guinea-pigs. Even though there are many similarities between the two species in this respect, this is supposed to be a book on the rabbit eye, not on comparative anatomy.

The price of the book is more than £13. For all the troubles now befalling sterling this is a fearful amount of money. At that price one could expect a more uniformly high quality in graphs, especially when they are "after". But the fact remains that the book is a classic of its kind: those who can afford it will find reimbursement in the time saved in looking for references by using the extensive lists after each chapter and also the excellent index.

R. A. WEALE

## ANTIBIOTICS RESEARCH

Recent Advances in Chemistry and Biochemistry of Antibiotics

By Prof. Hamao Umezawa. Pp. iv+264. (Tokyo: Microbial Chemistry Research Foundation, 1964.) 1800 yen; 5 U.S. dollars.

**R**ECENT *Advances in Chemistry and Biochemistry of Antibiotics*, the first book to be published by the Microbial Research Foundation of Japan, sets out to present a readable account of the more important discoveries in the chemistry, biochemistry and mode of action of antibiotics from 1958 to about mid-1964. So far as possible, a chemical classification has been adopted.

The first chapter deals with sarkomycin, griseofulvin, puberulic and stipitatic acids and a host of other non-nitrogenous antibiotics, many of which, like helvolic acid, cephalosporin *P* and fusidic acid, have structures suggesting biogenesis by way of mevalonate. A brief chapter on the glutarimides precedes polyene antifungal agents and macrolides. The important group of antibiotics derived from amino sugars, including streptomycin, blumycin, neomycin, paromomycin and kanamycin, follows and concludes with a brief account of the mode of action of streptomycin and kanamycin. Brief reference to *N*-glycoside antibiotics is followed by the penicillins and cephalosporin *C* and a lengthy chapter on antibiotics derived from amino-acids and polypeptides. The remaining chapters are devoted to the tetracyclines, aromatic nitrogen-containing and heterocyclic antibiotics, and conclude with a section on miscellaneous substances such as the antibacterial streptolydigin and the antiprotozoal agents teleocidin *A* and *B*. Structural investigation among many of the substances in the final chapter continues, hence classification is biological rather than chemical.

The style of the book is reminiscent of *Annual Review of Biochemistry*, for which much of the material was originally

written before it was decided to publish it in its present form. In one or two places the text tends to be a catalogue of physical and chemical data and, while this is invaluable for reference, it tends to make those sections less readable. Prof. Umezawa has, however, succeeded in producing a text which is brief and comprehensive but not incomprehensible. It is perhaps surprising that there is no mention of cephaloridine, although the closely related 7-(2-thienyl-acetamido)desacetoxycephalosporanic acid and cephalosporin *O*<sub>4</sub> are both included.

A few typographical errors were found; for example, "bond" (line 7, p. 107), "streptomycos" (line 16, p. 179) and "guanidino" (line 23, p. 67) are mis-spelt, and the numeral under the structure for rhodosamine (p. 158) should be *T*-XXIV. According to references 137 and 139, the acetoxy group in formula *I*-LVI (p. 27) is misplaced and it is not made clear if this is a revised structure communicated to the author. The page numbers to references 137, 144 and 145 are incorrect and the symbol for tautomerism and not mesomerism should be used at *HT*-XVIII (p. 183), while acetylenedicarboxamide is described on p. 101 and not 102 as indexed. These very minor criticisms are, however, more than abundantly offset by Prof. Umezawa's masterful handling of this subject. In particular, those chapters dealing with amino sugar, amino-acid, peptide, heterocyclic, glutarimide and nitrogen-free antibiotics reflect his expertise, and the penicillins and tetracyclines are excellently presented.

This book provides a wealth of up-to-date information, which, together with almost fifteen hundred references to reviews and original sources, makes it a valuable addition to the bookshelf of all organic chemists, biochemists, and microbiologists interested in current progress in this field of natural products. Prof. Umezawa has set a high standard for future companion volumes.

A. M. COMBIE

## UNIFORMITARIANISM VERSUS NEO-CATASTROPHISM

Bombarded Earth

An Essay on the Geological and Biological Effects of Huge Meteorite Impacts. By René Gallant. Pp. 256+27 photographs. (London: John Baker Publishers, Ltd., 1964.) 36s. net.

**T**HE great triumph of the British school of geologists represented by Hutton, Playfair and Lyell was to bring order out of chaos simply by postulating the doctrine of uniformitarianism. This assumption removed the need for the continual invocation of catastrophes required by Cuvier's school of thought, with its demand for the 'fixity' of species. The death knell to such ideas was given by Darwin's theory of gradual evolution.

In the past few decades, however, it has become clear that meteoritic impact must have played some part in the shaping of the Earth's features. The basic question at issue is whether the overall effect of meteoritic impact is small or significant. In *Bombarded Earth*, Gallant introduces a hypothesis of neo-catastrophism, the mechanism of which is meteoritic impact, not supplanting uniformitarianism but complementing it.

The first quarter of the book is devoted to a treatment of meteoritic impact, which, by and large, but with certain reservations, represents the consensus among most astronomers and many geologists to-day. From this point of departure, Gallant devotes a chapter to the possibility of shifts of the polar axis and changes in the length of the day by virtue of meteoritic impact on the Earth. Apart from the effect of meteorites in this connexion, such changes of small magnitude are known to occur at present and the causes of some of them are known (Gold, *T. Nature*, 175, 526; 1955). Gallant finds that significantly large shifts can be brought about without fragmentation of the Earth itself only by a relatively long sequence of

impacts by asteroids of reasonable size. He makes his calculations on the basis of conservation of energy in the impact whereas the correct balance to use is conservation of momentum (see the discussion of impulsive forces and Carnot's theorem in J. S. Ames and F. D. Murnaghan, *Theoretical Mechanics*, Dover Publications, New York, 201 and 278, 1958). The former method is not valid but probably gives answers approximately correct.

Gallant discusses next the possibility of slip of the Earth's crust over the underlying mantle by virtue of meteoritic impact, producing continental drift. Since most suggested mechanisms of creating the drift are inadequate (except possibly thermal convection), he postulates sliding of major land masses over the asthenosphere (interpreted as plastic) consequent to meteoritic impact. In later chapters, he invokes this process to interpret the data on palaeontology, palaeoclimatology, and palaeomagnetism that Wegener's hypothesis is frequently called on to explain. In this connexion, he stresses the apparently sudden extinction of various species (such as the dinosaurs at the end of the Cretaceous) as evidence of catastrophism.

However, a rule of thumb exists in the field of the weapon effects of craters from nuclear explosions on the ground, implying that a heavily reinforced concrete structure underground and only narrowly outside the crater formed by the explosion will survive, at least in large part. The important point is the very high rate of attenuation of a shock wave to seismic amplitude in the ground outside of the crater. Thus, it is extremely doubtful that Gallant's mechanism to produce slip of the Earth's crust can be operative.

Gallant refers with examples to the frequently suggested possibility that many of the large and roughly circular features on the Earth are meteoritic in origin. In this connexion, he mentions the theory which I have put forward (*Nature*, 190, 1048; 1961) that the terrestrial ocean basins were formed in this manner, far back in the Pre-Cambrian. However, he does not fall into the absurdity of claiming that all such roughly circular features are meteoritic in origin.

Some errors of fact were noted. On p. 79, meteor velocities for hyperbolic orbits are quoted—now known to be rare or non-existent. The temperature  $10^4$  °C given on p. 180 for impact of a large meteorite is far higher than the actual figure of approximately  $10^3$  °C. The latter value is large enough to produce thermal ionization and thus an intense flash of light in the impact, but not enough to excite nuclear reactions, as stated on p. 181.

An unfortunate aspect of the argument of *Bombarded Earth* is the suggested correlation of impacts of large meteorites with events in the historical records of ancient peoples. Moreover, the discussion in many cases contains a rather larger measure of unverifiable speculation than most specialists would be willing to accept.

J. J. GILVARRY

## INDEX OF CRYSTALS

### The Barker Index of Crystals

Vol. 3. Crystals of the Anorthic System, Part 1: Introduction and Tables. Pp. vi+94. Part 2: Crystal Descriptions A.1 to A.831; Atlas of Configurations. Pp. vii+text. Edited by M. W. Porter and L. W. Codd. (Cambridge: W. Heffer and Sons, Ltd., 1964.) 240s. per two parts.

THE *Barker Index* facilitates the speedy non-destructive identification of crystalline materials. Volumes 1 (*Nature*, 169, 851 (1952)) and 2 (*Nature*, 180, 821 (1957)) covered some 6,500 substances, and this final volume, listing some 800 triclinic (anorthic) crystals, brings a monumental undertaking to a praiseworthy conclusion.

Given any crystal in a standard setting and orientation, a systematic description becomes possible in terms of particular interfacial angles. This principle, conceived by

Federov and rendered workable by Barker (whose *Systematic Crystallography* was published in 1930), faces its most searching test when applied to crystals of the triclinic system. Here, Barker's rule of simplest indices sometimes fails to resolve ambiguities of setting, and much of the credit for overcoming this difficulty, mainly by means of topological and projective relationships, must go to Prof. Terpstra and his colleagues in Groningen. Because of this unavoidable complexity, Volume 3 has nearly double the average number of pages per crystal compared with the previous volumes, while matrix methods of checking angles have involved the use of a computer. Is it, perhaps, a little optimistic of Dr. Perdok to suggest that "the chemist with merely a basic crystallographic knowledge" would be able to use this volume of the Index?

The basic pattern remains similar to that of Volumes 1 and 2. In Part 1, after an explanatory introduction (including worked examples) by Dr. Hey, Mr. Codd gives an account of the use of the bond matrix for calculating crystal angles, and Dr. Perdok surveys the work done in Groningen. Then follows a table of multiple tangents (for the third time!), the main determinative table of classification angles, and more limited auxiliary tables of refractive indices, densities and melting points for confirmatory purposes. Two new features now appear: first a 48-page table, constructed from the output of the Oxford University computer, lists the bond matrix and its inverse for nearly all the crystals; then a 94-page table of configurations is given to facilitate selection of correct crystal setting and orientation. Part 1 concludes with the customary threefold list of substances, although it is to be regretted that some names, for example, A.760, are less informative than they would be had they followed the nomenclature recommendations of the International Union of Pure and Applied Chemistry.

The crystal descriptions, which make up the greater part of Part 2, are not only valuable in their own right, they are a useful supplement to the American Society for Testing Materials (A.S.T.M.) powder index, for there are only 13 substances in common. Part 2 concludes with a colourful 30-page atlas of configurations, provided as an alternative means of crystal setting by reference to standard zonograms.

It is a credit to both editors and printers that accuracy continues high and errors are few—a power missing on p. 42, and a mis-spelt word on p. 49; a formula error in A.269, while A.88 is assigned six A.S.T.M. index lines instead of three. Economy in cross-reference and space might have been achieved by listing the bond matrix immediately after the crystal description, and clarity in the introductory chapter might have been better served by distinguishing figures occurring in the text from those so usefully collected at the end of this section. But the omission of anorthic minerals from the present volume is deliberate. While we applaud the vision of the Barker committee, the workmanship of an international team and the devotion of the joint editors, they promise us a mineral supplement for good measure.

R. HULME

## A SYSTEMATIC REFERENCE TO PALEARCTIC BIRDS

### The Birds of the Palearctic Fauna

A Systematic Reference. Non-passeriformes. Vol. 2. By C. Vaurie. Pp. xx+763. (London: H. F. and G. Witherby, Ltd., 1965.) 147s.

DR. VAURIE'S first volume, covering the Passeriformes, was published in 1959 (*Nature*, 184, 666; 1959); this second volume deals with all the non-passerine species, 559 in number. The complete work provides a new base-line for the taxonomic and geographic study of the species and sub-species of birds native to Greenland, Europe, North Africa, and the northern part of Asia,



It will certainly be a standard work of reference for many years to come.

The work has this clearly defined purpose; and it is not within its scope to give descriptions or illustrations of species, and still less their life-histories. It does, however, mention the distinctive characters of sub-species, in order to indicate the trends of variation. Some information is also given about habitat, which is relevant to distribution, although, as the author points out, its nature varies geographically and seasonally, and can in the longer term be influenced by changing environmental factors.

Thus, Dr. Vaurie has kept to his particular and surely sufficient task of systematic presentation, based on many years of work in the principal museums of Europe and North America; the institution which he serves, the American Museum of Natural History in New York, nowadays has the Rothschild Collection earlier used by Hartert at Tring. The author has worked in close touch with Soviet ornithologists, and this has been of great help in enabling him to deal effectively with the forms found in northern Asia. In the course of his investigations he has published many notes on particular groups, allowing him now to give the end-results without detailed discussion except where it is desired to direct attention to some outstanding problem. All references to other sources have been checked by him with the original publications. The whole massive undertaking bears the stamp of expert authority.

In his taxonomic presentation, Dr. Vaurie directs particular attention to the trends of intraspecific geographical variation. There is a welcome flexibility in dealing with sub-species, which he places in three grades of validity—well differentiated, not well differentiated but acceptable, and unaccepted but worthy of mention (mostly points on a cline).

The other facet of the presentation gives the distribution of each species and sub-species within the region. The information is given in some detail and is a most valuable feature of the book. Extra-limital distribution and the existence of extra-limital sub-species are more briefly indicated.

Although other taxonomists may not accept the author's judgment on every point, the existence of this work will constitute a challenge to state reasons for any different practice. Ornithologists in general may be less willing to follow Dr. Vaurie in certain unexplained departures from the traditional order of species within a genus, or of genera within a family; these sequences have long since ceased to be regarded as more than convenient, and it is not clear that new arrangements have any special merit.

LANDSBOROUGH THOMSON

## TOWARDS A STRATEGY OF MATURITY

### The Scientific Age

*The Impact of Science on Society.* By L. V. Berkner. Pp. xvi + 137. (New Haven and London: Yale University Press, 1964.) 30s.

IN these Turnbull Lectures, delivered at the University of Yale, Mr. L. V. Berkner discusses in succession the economy of plenty, advanced education for a new age, the relations between science and government, science and philosophy, and what he terms "a strategy of maturity". In the first he explains the significance of science and technology for the economy of the United States as a whole. However, while he emphasizes the way in which science and technology contribute to the stability of the economy, his attitude is startlingly different from that of Prof. J. K. Galbraith. He seems inclined to advocate production and change for their own sake: he never challenges the tendency for the process of production to create 'needs'. His more constructive and fundamental thinking is found in later chapters, for

example, when he considers the place of the universities in the changing society of the United States. Despite the close relations to conditions prevailing in the United States, of which he is somewhat critical, his comments on high-school education and university education should be pondered in considering the expansion of higher education in Britain, particularly in relation to the proportion of technicians to postgraduates who are being trained.

In his chapter on the role of the universities he comments freely on Clark Kerr's *The Uses of the University*, and again refers to this in his brief outline of the problems of Government finance for research and the selection of research projects. Here also he is critical of present-day policies and urges that up to 35 per cent of overhead expenses should be allowed against direct costs under research grants as encouraging institutional support for younger and unknown scientists, and the initiation of new, but soundly conceived, projects as well as encouraging better internal administration of public funds and greater freedom for the scientist while reducing Federal control of the institution. Much of his discussion of science and philosophy stems from Lord Snow's *The Two Cultures*, the broad thesis of which he accepts. This he regards as fundamentally a problem of communication. He is concerned that failure to comprehend the character and power of the means available for improving the condition of the bulk of mankind may lead to the degeneration of our civilization. This is a task which calls for the contribution of the humanist as well as of the scientist if we are to achieve the discipline of mind and body that will master the machine.

When he outlines his "strategy of maturity", Mr. Berkner comes nearer to the point of view of Prof. Galbraith. He considers that at present the educational capacity of the United States, particularly with regard to the proportions of learning and skill required, the policies governing the encouragement of science and the attitudes of society generally, are potential sources of instability. Causing instability also in a similar fashion are: the potential elimination of poverty by extending to every level of society the opportunity for higher education; the increasing lack of balance in social opportunity in a world divided between the highly industrialized nations and the underdeveloped areas; and the radical change in the character of war and of justice among nations.

On the educational side, he looks for a solution to the improvements in curricula and in teaching at the elementary and secondary school level which are already leading to progress in individual capacity, as well as to improved articulation between school and university. Improvements which could also help include increasing the opportunities for students to participate in research at an early age with more effective presentation of the idea content in university courses, and the shift of professional emphasis and specialization to the graduate school. However, he suggests that in the next decade in the United States the emergence of the junior college may be the most far-reaching development, and this suggestion also has its implications for Britain. He has some cogent observations on the character of business management and on training for management and more especially on relations between business and industry. He writes with brevity and realism on social change, the problem of poverty and population and the challenge of the underdeveloped peoples. Implicit in it all is the insistence on honest and clear thinking regardless of political prejudice. We must face frankly the real issues of the time, and a strategy of maturity would arise from any rational attitude of mind that evaluates alternatives objectively and tests each step analytically, seeking to utilize the optimum advantages that our scientific culture affords. Outside the American context, Mr. Berkner offers little in the way of constructive proposals, but nevertheless he is as challenging and stimulating for the British readers as for the American audience he originally addressed.

R. BRIGHTMAN



### Measurements for Stresses in Machine Components

By V. F. Yakovlev and I. S. Inyutin; translated from the Russian by J. J. Cornish. Pp. xv+135. (Oxford, London and New York: Pergamon Press, 1964.) 50s.

WHILE this text has all the signs of careful and reliable translation, it remains a somewhat slender and in some respects ill-balanced treatment of experimental stress analysis. The authors support their discussion by 69 reasonably well-chosen illustrations (mainly line diagrams) and 43 bibliographic references—almost entirely selected from Soviet publications. Though a useful book for engineers and research workers deeply concerned with the strength of structures and machine components, it has little general appeal as a teaching text, and the publishers rightly claim no more than that it "... will be of interest in graduate departments of engineering ...".

It is not unduly purist to point out that, despite the title, the book is concerned with the more familiar situation, namely, measurement of strain and subsequent inference of stress. The central theme is devoted to techniques, based predominantly on the electric resistance strain gauge, for assessing the distribution of stress inside solid bodies: the greater part of the text deals essentially with static loading, but the final chapter touches briefly on dynamic and impact conditions. The technique requires that unbonded strain gauges—sufficiently small and close together to justify the assumption of measurement at a point—should be embedded in models (not necessarily full-scale) cast in epoxy resin. Practical aspects are discussed in considerable detail, covering manufacture and positioning of the gauges themselves, appropriate electrical measuring circuits, correct treatment and curing of the resin, together with advice on materials suitable for the moulds. Illustrations of how the procedure may be applied are then given in terms of simple bending of beams, stress concentration at a circular hole in a flat-plate, two mutually perpendicular cylinders which are pressed against each other, and the pressure of variously shaped discs on an elastic half-space. Mathematical analysis is used somewhat sketchily and it is probably at this point that workers having only a limited background in stress analysis will find the book unsatisfactory.

Although the opening chapter ranges over most basic methods of experimental stress analysis, much discussion is superficial. In particular, scant attention is paid to the process of inferring the magnitude and axes of principal stresses from surface strain gauge observations. This particular inadequacy runs through to the main theme of the book; and the inexperienced reader is left much in the dark on how exactly to infer principal stresses at a point where he has no prior knowledge whatever of the axes of the principal strains.

However, despite its weak points, this book will be of interest and practical value to the experienced workers; and this, in fairness, seems to have been the primary aim of the authors.

B. N. COLE

### Theories of Nuclear Fission

By L. Wilts. (Oxford Library of the Physical Sciences.) Pp. x+132. (Oxford: Clarendon Press; London: Oxford University Press, 1964.) 18s.

THE study of nuclear fission processes has been an active field for more than twenty-five years, but many features still remain to be explored experimentally and discussed theoretically. However, it is a good moment to review the progress made over the past decade.

In his small monograph Dr. Wilts discusses present-day models of fission, including the liquid drop, adiabatic and statistical models. He starts with a clear and concise description of the historically important liquid drop model.

The elegance and precision of this model, which has always appealed to physicists, are exemplified by this treatment. Its limitations are explained and then the discussion moves to more recent models. Rather than discuss them in detail the main features are listed, their importance considered, and the region of validity examined. Probably this style of treatment is essential in a monograph. For the same reason only a limited range of the experimental material is covered, but the most pertinent is used in order to test the models. The mathematical presentation is fairly elementary, making *Theories of Nuclear Fission* quite easy to read. The pragmatist may feel that this prevents an exact understanding of some parts of the subject. However, to those unfamiliar with the modern developments in the theory of fission, this monograph will be a useful guide, while to those engaged in the field it is a stimulating review. Altogether it is a well-written and well-presented monograph.

P. A. EGHLESTAFF

**Theory and Methods of Nuclear Reactor Calculations**  
Edited by G. I. Marchuk. Authorized translation from the Russian. Pp. viii+199. (New York: Consultants Bureau, Inc., 1964.) 40 dollars.

*THEORY and Methods of Nuclear Reactor Calculations* is a collection of fifteen papers dealing with neutron transport theory, and in particular methods used in the design of reactors, with a group of three papers dealing with topics such as optical model calculations and inelastic scattering of neutrons by iron. It is a translation by the Consultants Bureau, New York, of the original Russian text published in 1962, and edited by Prof. G. I. Marchuk. The translation has been carried out efficiently.

The papers dealing with reactor theory discuss methods which are useful in practical work—they are mainly numerical in character. The first two papers deal with the application of the spherical harmonics method—a method which has been superseded in many cases by the direct numerical integration *S<sub>N</sub>* methods of Carlson, which are also discussed. Other subjects of technical interest which are mentioned include: the calculation of the burn-up of poisons in reactors, the thermal neutron spectrum in a homogeneous mixture of moderators at different temperatures and neutron resonance absorption. The paper which deals with the optical model of the nucleus is concerned with the calculation of the transport cross-section—in this paper the choice of various parameters in the model does not seem to be always justifiable. However, it is a useful compilation of papers of interest to reactor physicists.

J. H. TAIT

### A Petrography of Australian Igneous Rocks

By Dr. Germaine A. Joplin. Pp. xiii+210. (Sydney and London: Angus and Robertson, Ltd., 1964.) 63s.

THIS short text-book of petrography, presented along genetic lines, is illustrated entirely by descriptions of rock-types occurring in Australia and it has doubtless been written to provide advanced students in Australia with a course of instruction based on local material. The work, however, is well documented and it will be useful elsewhere both as a précis of Australian literature and as a ready source of references to it. The book is in three parts, of which the first is concerned with classification, nomenclature, and textural terms (including brief glossaries of rock names and of textures), the second with the rocks of non-orogenic regions (tholeiites and alkali basalts), and the third with rocks of the orogenic belts (alpine ultramafics, spilites, andesites, granites). Shoehornites and their differentiates occur in both environments. Dr. Joplin has given a lucid exposition of the relationship between magma-type and tectonic environment, and in this connexion her work deserves to be widely read. It contains 39 tables of chemical analyses and is illustrated by about 150 microdrawings.

C. F. DAVIDSON

## INTERACTION OF TECHNOLOGIES\*

By DR. J. W. S. HEARLE

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**THE importance of materials.** The use of materials is central to all technology. The rocket technologist may calculate the thrust needed or define the controls which must be used; the computer technologist may specify the functional operations; the electronic circuit designer may think of the current and voltage relations; the architect may design a building; even the artist or musician may conceive their creations: all these things can be done in the abstract; but for execution of any of the plans real materials must be used. With other technologies, such as chemical engineering, metallurgy, textile technology, and much of mechanical and civil engineering, it is virtually impossible to isolate any aspect of the subject which is not a direct materials problem. Ultimately the solution of any technological endeavour can be reduced to putting the right material in the right place.

Materials science is thus a meeting-ground for many technologies. There are others, such as the provision of power, instrumentation and control, probability and statistics, the similarity of mathematical forms, and the application of the laws of mechanics. These are all areas where the practitioners of different technologies can profit by common discussion. None is more important than the provision of materials with the properties needed to do the job.

**New materials and advancing technology.** We can see in materials science an immediate interaction in technological advance. New materials generate new technical possibilities, and so new industries arise; while, contrariwise, the demands of advancing technologies force the materials technologist to develop new materials. Sometimes the new material triggers the creation of a new utilisation technology, and sometimes the demands of an advancing technology trigger the production of a new material.

This sort of interaction is seen in the relation between electronic engineering and semi-conductors, between fuel technology and rockets, or between aircraft design and advances in metallurgy. It is seen in the development of polymer technology in the past forty years which has led to the vast new industries of plastics, man-made fibres and synthetic rubbers: these in turn have influenced many other technologies—building, adhesives, textiles, tyres, engineering, appliances, furniture, packaging, to name but a few—while, on the other hand, the demands of space technology have recently led to important advances in high-temperature polymer technology. At the frontiers of advancing technology, there is a ferment of ideas associated with advances in materials science.

**Older technologies: the creation of new demand.** In well-established industries using readily available and well-known materials, the problems of materials technology may not be so acute. The materials available may seem to meet all the technical requirements; and the designer can get by for a time without too much thought about materials—merely selecting them as components on the basis of past experience or from a cursory check of tables of standard products. Even here, advances in materials technology must be watched, if for no other reason, in order to ensure that the material being used is the most economical. But, surprisingly, new materials often generate new and unexpected technical demands.

The advent of synthetic fibres provides a simple example. When nylon was introduced in 1938, its obvious technical advantage was toughness, and it was first used where there was an unsatisfied demand for this quality: stronger ladies' stockings, harder-wearing socks, and

better parachute fabrics were among the first uses. But then the development began to follow lines which would not have been predicted by a textile technologist in 1938. Even the requirements in ladies' stockings shifted from long life to extreme fineness—the demands of glamour outweighing the demands of efficiency. Then, with nylon and also the polyester and acrylic fibres which followed, new ease-of-care, drip-dry, wash-and-wear, crease-resistant and pleat-retaining properties were achieved in textile fabrics as a result of the heat-setting characteristics of synthetic fibres. There had been no great public demand for this: prior to 1945, the public had accepted the attributes of a cotton shirt or a wool suit as satisfactory. But once we met the advantages of the new materials, we realized how much we wanted the new properties.

As a consequence, the cotton, wool, and rayon technologists had to set to, and find ways of achieving similar properties in fabrics made from these fibres. Resin finishes which would yield drip-dry cottons, and chemical treatments which would set wool fabrics were discovered. Scientifically there is little reason why these processes could not have been discovered in the great advances in the chemical finishing of textiles in the second half of the last century, or through the work of the strong British textile research associations established around 1920. But it was not until the new material had created the new demand that the problems were tackled and solved. The whole process has been repeated following the enormously successful introduction of stretch yarns made from synthetic fibres: stretch cotton and stretch wool are following. All this is part of a revolution in textile technology, more fundamental than the industrial revolution which brought power to the machines but did not change the materials; and it has come about as a result of interaction between chemical technology, polymer technology, man-made fibre technology, and traditional textile technology—interaction, too, between mechanical and chemical aspects of fibre and fabric technology.

**Materials sciences.** The problems which arise between materials-producing, materials-processing, and materials-using technologies make one group of interactions related to different stages in the life of a particular material. But what about interactions between the technologies of different materials?

Ironically, the various materials technologies have developed with little contact between one another. Although they may have had mutual associates, the metallurgist and the glass technologist, the ceramicist and the textile technologist have not often met.

The lack of contact between materials technologists mattered little so long as each industry was run mainly on a craft basis, utilizing traditional recipes and procedures. The empirical cookery involved in the ageing and ripening of the cellulose solution used in rayon production is of little relevance to the empirical cookery of the iron smelter. It is only with the application of science that a basis for useful contact between materials technologies becomes established. The understanding of the chemistry and of the macroscopic physical behaviour of materials dates from the days of Newton, Boyle, Hooke, Lavoisier, Dalton, Kekulé, Poiseuille, Faraday, and Clerk Maxwell; but it is only in the past fifty years that we have really come to understand the fundamental nature of materials. Even to-day, although we know a lot about gases, crystalline solids, and rubbers, and have mathematical theories of their behaviour, we are still very ignorant about liquids and glasses amorphous solids.

\* See *Nature* of September 11, p. 1121 (1965).

At first, too, as scientists looked at the problems of industrial materials, there was so much to study that each materials science could develop on its own. A basic framework of knowledge had to be established. But now, as a result of the advances in pure science and in the applied sciences of various materials, contact between materials scientists is needed and is likely to yield surprising benefits. Already there are examples which can be quoted.

**Continuity and discontinuity.** In the materials of construction, strength is the most obvious technological requirement. Strength comes from continuity of structure. In metals we have a continuous band of electrons holding the atoms together, and in polymers we have a continuous network or chain of covalent chemical bonds. In strong materials, the structure of which is not so well known, this provides a clue about what we must look for. Thus in glasses there is probably a network structure though this is not so well defined as in polymers. In crystals of small molecules, the extent to which one can get strength depends on how closely the molecules can pack together in order to develop a degree of continuity in intermolecular attractions.

Conversely, discontinuity is a source of weakness. Materials fail at flaws. Here, Griffith's classical treatment of the propagation of fracture from a crack has set off a train of interactions in technology. The Griffith criterion has been applied and modified in glasses, metals, polymers and many other materials. So long as one is dealing with a pure homogeneous flawless material, one's gaze can be narrower: the properties of the particular assembly of atoms are all that matter. The modulus, that is the force needed to produce a given small strain, can be calculated from the deformation characteristics of the particular atomic bonds in the structure; and the result will usually agree well with the values for real materials. But calculations of strength will always yield too high a value, usually orders of magnitude greater than the strength of real materials. It is the unusual points of weakness that must be looked at, and not the behaviour of the bulk of the material. In studies of the rupture of real materials, we can be helped by the relevant work in the technology of all materials. The behaviour of materials at discontinuities is a general problem. A recent book on *Fracture Processes in Polymeric Solids* draws heavily on earlier work in metals and glass. On the other side, Orowan and McClintock at the Massachusetts Institute of Technology have examined the pattern of ridges produced in the fracture of gelatine and the cracks developed during the flexural fatigue of 'Plasticons', and have used the results to explain the similar effects which occur on a much smaller scale in metals.

The importance of flaws as a source of weakness has led to the recent searches for flawless materials: perfect single crystals. Diamond is the classic naturally occurring example; but now we have single crystal whiskers of metals and of materials like silicon carbide. These are fibrous in form, and in their application lessons can be learnt from the old-established fibre technologies of textiles and paper.

Curiously enough, discontinuity can also be a source of strength. It can spread out the forces on a material; it can prevent dangerous stress concentrations; it can interrupt the progress of a crack. Whereas a continuous material may be able to resist very large loads, it is liable to be brittle and to shatter under conditions where a material with the right sort of discontinuity would yield, but would not break. The crystal defects in metals, the amorphous regions in crystalline polymers, and the rubber which can be dispersed in polystyrene in order to toughen it perform this function.

In dealing with a real materials system, the technologist has to keep in mind two separate but interrelated aspects: first, the responses of each part of the system to the local stress; and secondly the influence which this has on the distribution of stress through the system. For example, the absorption of water by cellulose fibres always breaks

cross-links and makes it easier for the molecules to slide over one another: but, in rayon, with short molecules, this lowers the strength; whereas, in cotton, with longer molecules, where breakage by molecular slip is not important, the more uniform distribution of stress results in a higher strength. Lessons like this illuminate our understanding of materials in general.

Another area where work with different materials has interacted is in the statistical problems of failure. Lawrence Balls—one of the first great technologists of the cotton industry—appreciated the 'weak-link effect' in determining the strength of cotton yarn. Then, in 1929, Peirce at the Shirley Institute treated the problem statistically, dealing both with the series problem of a sequence of elements of differing strength and the related parallel problem of a bundle of threads. As one follows the subject, the contributions come from Weibull and Irwin on metals, leading into the whole problem of fatigue in engineering materials; from Spencer-Smith again on textile yarns, dealing with the effect of correlation in strength between neighbouring elements; from Coleman on fibres and polymers, taking account of the time-dependent effects; and from Freudenthal and Gumbel in their work on extreme value statistics, which demonstrates the relevance of the problem in any technological field where one is interested in the rare occurrences at one end of a frequency distribution. Work on cotton yarns could become relevant to work on the interruption of radio control in space: the statistical problems are similar. The intensity of interruption varies from hour to hour, day to day, and year to year as a result of the superposition of several regular and random effects: the variation of thickness of strength of a yarn along its length similarly results from a superposition of different effects.

**Polymers and metals; glasses and liquids.** Ten years ago most scientists concerned with either material would have said that polymers and metals were as different as the proverbial 'chalk and cheese'. But last summer Malcolm Williams of Chemstrand Research Center opened a lecture at the Massachusetts Institute of Technology by presenting pairs of slides, and inviting the audience to decide which were from metals and which were from crystalline polymers. There was close similarity in the morphological forms shown in electron micrographs and in the shape of curves of mechanical properties. The link between the two is the way in which crystallization occurs. The chief determining factors in crystal growth are the critical size of nuclei, the tendency to minimize free energy, and the mass and heat transfer in the region of the growing crystal. These are general effects and they result in similar forms. If crystallization starts in several places we get a domain structure. Imperfections appear as defects and dislocations in the crystal. On applying a force, there is first a small elastic deformation of the crystal itself; but then, as the force builds up, the defects start to move through the system, like a ridge being pushed along in a carpet; crystal planes slide over one another, and we get yielding and permanent deformation. This happens in the drawing of films, metals, and fibres.

The polymer scientist used to think of crystalline polymers as composed of small crystalline regions embedded in a non-crystalline matrix, and consider the deformations of the two parts separately. Now he is realizing that it is also possible to think of materials in the same way as the metallurgist, and consider polymers as a continuous crystalline material loaded with defects. Of course, the behaviour of metals and polymers is not identical: in polymers the atoms are connected in long chains, and this both influences the type of movement which is possible and is likely to lead to larger regions of disorder. Consequently there is still value in the older ideas, as well as the newer ones coming in from metallurgy.

While crystalline polymers have much in common with metals, the examination of the technology of amorphous polymers will benefit from work on glasses and liquids.

The extent to which there is some degree of order in these systems is an important unsolved problem. This in turn reacts on the behaviour of crystalline materials, since the existence of order in the melt will influence the detailed structure of the crystalline solid which is formed. An understanding of the behaviour of a wholly amorphous material may also be a help in understanding more complicated polycrystalline materials: investigations of the cold-drawing of amorphous polymers such as polystyrene are being carried out as a lead to the understanding of drawing of synthetic fibres.

**Fibrous systems.** In living organisms, fibres are of great importance: nerves, muscle fibres and tendons all help to make the body work. Man has long utilized fibres in textiles and paper. Fibres are strong and flexible—two properties which are often opposed, for if you strengthen a structure you usually make it more rigid. In a well-designed fibre system, you can allow the fibres to act independently to give freedom of movement, but cause them to combine to resist rupture.

The flexibility of fibres is to be expected, because they are so fine, sometimes less than ten wave-lengths of light in diameter; but their strength is greater than might be expected. Whereas sheet glass has a strength of about 2,000 lb./in.<sup>2</sup>, commercial glass fibres are nearly a hundred times stronger, and carefully prepared undamaged glass fibres break at 500,000 lb./in.<sup>2</sup>. This is due to the critical dependence of strength on cracks and other flaws—you cannot have a deep crack in a fibre!

Consider a bundle of 1,000 glass fibres. If one fibre is broken, the strength is reduced by 0.1 per cent; but a comparable crack in the surface of an equivalent glass rod would cause a catastrophic drop in strength. Furthermore, if there is some cohesion, due to friction or a binding material between the fibres, then a fibre which is broken in one place will contribute to the strength elsewhere. The degree of cohesion has to be right: if it is too strong, the stress concentration will not be prevented, and the advantages of the fibrous system will be lost, while if it is too weak the whole fibre will slip.

The best-known practical realization of strong fibre-reinforced systems is in reinforced plastics: cotton or paper in phenolic resins, and fibreglass in polyester resin. The plastics technologist is likely to be faced with a choice between woven and knitted fabrics, and with the other complexities of textile and paper technology.

Now there is also an interest in fibre-reinforced metals. Rolls-Royce, for example, are investigating aluminium-coated quartz fibres which can be pressed up into blocks. They are having to solve the problems of melt spinning, just like any other fibre producer. Others are investigating the possibilities of single crystal whisker fibres.

Apart from all the incidental mixing of technologies which comes from the use of fibres in constructional materials, there is the fundamental problem of how fibre-reinforced materials behave. Their technology is not yet well understood. The commercially successful reinforced plastics have been produced by empirical trials, rather than designed on the basis of rational understanding. Theoretical and experimental investigation of a variety of systems is needed.

There is a wider unifying subject here in the structure and mechanics of fibrous systems. Wood and paper, woven and knitted textiles, cords and ropes, the new non-woven fabrics, fabrics coated with flexible thermoplastics, thermo-setting resins or metals reinforced with inextensible fibres, and concrete toughened by the addition of nylon fibres are all different systems; but there are analogous problems in the geometry and statistics of fibre arrangement and in mechanical, electrical, thermal, diffusion and optical behaviour. The acquisition of a facility in dealing with all the systems will come from studying them together.

**Problems of scale.** There are other useful analogues where the scale of a system is changed. Scale down a

fibre assembly a thousand times and you have an assembly of polymer chain molecules; scale it down a hundred times, and you have the fine fibrillar structure which often occurs when polymers crystallize; but scale it up a thousand or more times and you have concrete reinforced with metal wires or rods.

Theoretical or experimental results can be taken over, with care, from one problem to another. The understanding of rubber elasticity helps our understanding of non-woven fabrics. The well-proved analysis of the mechanics of twisted yarn has been applied in an analysis of the mechanics of single plant fibres.

**Rheology and dynamic behaviour.** Mechanical properties are dependent on time. This is always true, though it can be ignored when an elastic deformation is so much more rapid or a viscous deformation so much slower than the time scale of ordinary events. In the many circumstances where time cannot be ignored, the subject of rheology brings together a diverse collection of scientists and technologists. The basic mathematical and experimental methods are the foundations of the subject; but the superstructure spreads out into metallurgy, polymer technology, oil technology, lubrication, adhesion, papermaking, textile technology, geophysics, physiology and medicine, soil mechanics, and other applications.

We find in this subject, too, a link between mechanical and electrical properties of materials. The swinging of dipoles into alignment with an electric field is a mechanical action. So the dynamic response of a material to an alternating electric signal has very close similarity to the response to an alternating stress. Dynamic compliance (the reciprocal of modulus) is analogous to dielectric constant; mechanical creep is analogous to the build-up of electric polarization; and the effect of temperature in loosening up a structure is similar.

The experimental problems of rheology also tax the ingenuity of the electronic engineer, so that we find one of the most important schools of the rheology of liquids in a school of electrical engineering. Other schools are in mathematics, physics, or chemistry departments as well as in many technological departments.

**Dynamics of processing.** It is one thing to investigate materials in the laboratory, or even to examine them in use: it is another thing to examine their behaviour in processing. Speed is then all-important. Polymer crystallization may take hours or weeks in the laboratory, but the man-made fibre being extruded in the factory moves through the crystallization zone at a high speed. A drill pierces metal in a fraction of a second. Large volumes of material flow through pipes at high speed.

In all these situations, the dynamics of processing must be considered. Consider the first example of the production of a man-made fibre. As it emerges from the spinneret, we have first the rheological problem of flow in a viscoelastic medium—within the tube the molecules are oriented, but on emerging they spring back to a disoriented form, and we get a contraction, a bulging, and a reduction in velocity. Then we get problems of hydrodynamics and aerodynamics, as the flow accelerates under gravity but is retarded by the drag of the surrounding gas. All the time, we have a heat-flow problem as the specimen cools; this leads to change in viscosity, and ultimately to solidification and perhaps to crystallization. Finally, there is the mechanical problem of winding up the yarn. If we want to take any measurements during the process, we are immediately led into complex problems of instrument engineering—how do you determine the temperature of a rapidly moving molten threadline, a few microns in diameter? If we want to control the process, we have the problems of control engineering. Can the technology of man-made fibre production possibly be studied in isolation?

The same comment could be made about many chemical engineering and mechanical engineering processes. The

dynamic problems inevitably bring in aspects of many technologies.

*The textile industry.* As final examples, we take two more limited topics. The first is the textile industry—or should it be industries? It used to be. Spinning, weaving, knitting, and chemical finishing were all examined separately. Individual companies had a very narrow range of operation: they often dealt with only a specific aspect of processing of one type of fibre.

This has changed now; the textile technologist must know the whole field. He must be able to select the fibres, and determine the sequence of operations from fibre to finished product. Here we see not so much the interaction of technologies as the merging of several technologies into one, and with the non-woven fabrics coming very close also to paper technology.

*Tyre-cords: a choice of materials.* The other example concerns a choice between materials in a large industrial market with fairly clearly defined requirements. For use as tyre-cords there is a choice between cotton, rayon, nylon, polyester fibre, fibreglass, and steel. All are being used—the natural fibre, the regenerated fibre, the synthetic fibres, the glass, and the metal. This is an example of interaction by competition in use. The user must look at all the technologies; the supplier must watch the technology of his competitors.

Cotton used to be the material used, but in the 1940s it was replaced by rayon, except in bicycle tyres. Then nylon challenged rayon, and there is still a nicely poised balance of technical and economic factors. Incidentally, the strength of rayon tyre-cords has been almost doubled in advances over a period of ten years, due to this competition. So far, the competition was within the textile industry, but with steel wire the competition came from outside textiles. Courtaulds hedged by acquiring a company supplying steel for this purpose! The technological and economic arguments about the different materials go on.

*Advantages of interaction.* In this article, it has been shown how the materials technologies interact with other technologies in use, and in the methods used to process materials. These interactions are fairly obvious. The

user has to handle many materials; and there is competition between materials from very different sources.

Less obvious is the value of interaction between the scientific studies of different materials. Work on other materials can be a useful source of ideas; and sometimes a detailed analysis can be taken over. But perhaps the most valuable general feature is that a comparative investigation of different materials enables the specific to be separated from the general. It is all too easy in dealing with a particular material to attribute phenomena to specific features: to ascribe the characteristic draw-ratio in synthetic fibres to some particular molecular transformation, whereas it is merely a result of adiabatic extension of material with a certain, common form of load-extension curves; or to attribute particular oblique lines of fracture to a particular crystal structure instead of to a stress distribution which would occur in all specimens of the same shape. Luder's bands, first found in metallic fracture, have reappeared in the literature of the fatigue failure of fibres. Or again, changes which were found in the nature of break of reinforced metal specimens as the specimen length was changed were found to be similar to effects which had been previously reported for textile yarns.

By examining the behaviour of a variety of materials, we can build up a coherent body of knowledge in place of an empirical compendium of unrelated facts. We now have the necessary scientific basis, though the complication of applying it to the behaviour of real materials—so different from the abstractions and specially simple examples beloved by the pure physicist—should not be underestimated. On the more practical side, there is much to be gained from collaboration and exchange of ideas in processing methods—cutting, moulding, drawing, forming, and so on—and in instrumentation and control. As the use of materials becomes more and more a rational technology, and less pure industrial empiricism, the benefits of interaction among the materials technologies and with other technologies will grow. For this reason it is encouraging that materials scientists and technologists are increasingly co-operating in various centres and societies, so that the barriers between the technologies are coming down.

## FUTURE OF THE TRISTAN DA CUNHA ISLANDS

By DR. N. M. WACE

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THE plant and animal life of remote islands has long been considered of unusual interest, especially since the classical investigations of Charles Darwin in the Galapagos group<sup>1</sup>. To the earlier explorers, the insular forms of life were matters mainly of curiosity and unco-ordinated speculation; but since the publication of Darwin's *Origin of Species*<sup>2</sup> and Alfred Russel Wallace's *Island Life*<sup>3</sup>, it has been widely accepted that island biota present features of outstanding biological, evolutionary and geographic interest. Much attention has therefore been paid to investigations of the plant and animal life in many remote islands, especially in Hawaii<sup>4</sup>, Juan Fernandez<sup>5</sup>, and the Galapagos<sup>6</sup> and other Pacific archipelagoes<sup>7</sup>, and also in Macaronesia and in the islands of the Indian and Southern Oceans<sup>8</sup>.

Wallace<sup>9</sup> emphasized the devastating effects of the introduced goats on the native vegetation of St. Helena, and a continuing and increasingly important theme of later workers on islands has been the very marked impact of man and his associated plants and animals on the indigenous insular ecosystems<sup>10</sup>. Simpson<sup>11</sup>, in discussing islands as "evolutionary traps and blind alleys", considered that man and the organisms introduced by him have caused more extinction of native species on islands

than on continents. Elton<sup>12</sup> discussed the reasons underlying the sudden changes induced by man, and a recent symposium<sup>13</sup> has been devoted largely to a discussion of man's impact on Pacific islands, and ecological and economic consequences of the rapid changes that he has initiated in them. The instability of island ecosystems is thought to be due chiefly to the small size, lack of diversity and consequent ecological disharmony in their native floras and faunas; the presence of unexploited niches in their ecosystems; and the lack of refugia, and hence of buffering effects on the biota against environmental changes. Introduced herbivorous and predatory mammals, as well as the direct felling and clearing of vegetation, seem to have been the most effective agents used by man in disrupting many island ecosystems<sup>14-16</sup>. Most of the remote temperate and tropical oceanic islands of the Atlantic have long been settled by man, or have been greatly altered by goats and other grazing animals introduced since their discovery by Europeans in the sixteenth and seventeenth centuries<sup>17</sup>.

The temperate and tropical oceanic islands of the Indian Ocean either support large and rapidly increasing human populations which have exterminated much of their indigenous biota<sup>18</sup>, or have been much altered by

the introduction of herbivores<sup>14</sup>. The native biota of all the low-latitude Pacific islands has been to some extent disturbed by pre-European man, except for Juan Fernandez and possibly the Galapagos<sup>15</sup>; but both these groups and almost all the remote 'high' islands of the Pacific have suffered radical alterations to their biotas since their discovery and exploitation by European man<sup>16</sup>. Even the remote islands of higher latitudes in the Southern Ocean which have proved too inhospitable for permanent human settlement have not escaped the introduction of mammals such as rabbits, which are having a profound effect on their native ecosystems<sup>14</sup>.

The four volcanic islands of the Tristan da Cunha group in the mid-South Atlantic (37°–40° S., 10°–12° W.) were uninhabited by man when discovered by the Portuguese in the early sixteenth century<sup>11,12</sup>. Although populations of seals on all four islands were destructively exploited during the late eighteenth century, and Tristan main island supported a human population from 1810 until its evacuation following the volcanic eruption there in 1961, the smaller islands in the group remain substantially unaffected by human activities. Because of their inhospitable climate and terrain, and their remoteness from shipping routes following the advent of steam propulsion at sea and the cutting of the Suez Canal, the islands have largely escaped the later human attention that has characterized almost all other tropical and temperate archipelagoes.

In their present state, the Tristan da Cunha islands therefore represent something unique and of great scientific interest: a group of remote but ecologically diverse oceanic islands in temperate latitudes, with a substantial native flora and fauna in which the pre-human ecological *status quo* is still very largely preserved.

Following the Royal Society Expedition to Tristan da Cunha in 1962, the islands have recently been the subject of a series of geological and biological papers which summarize the present state of knowledge concerning their geological origin<sup>13,14</sup>, and the composition, age, history and relationships of their plant<sup>15</sup> and animal<sup>16</sup> life. Geochemical, palaeomagnetic and geomorphic evidence all suggest that none of the islands is older than mid- to late-Tertiary<sup>13,14</sup>, and evidence of continuing volcanic activity is present in layers of volcanic ash from peat deposits which were laid down during the past few thousand years<sup>17</sup>. The native biota appears to be recently immigrant, and mostly derived from South America; it lacks the deep-seated endemism which is characteristic of the Galapagos, Hawaii, Juan Fernandez and St. Helena<sup>11,16</sup>.

The isolation which has so largely preserved the biota of the Tristan islands from disturbance and alteration at the hands of man is not likely to continue indefinitely. Since the return of the islanders in 1962 and 1963, considerable new construction has occurred and livestock imported to Tristan main island. Strategic, commercial, or other considerations could soon lead to a rapid breakdown of their isolation, with the consequent loss of much that is of unique biological interest<sup>18</sup>.

Because of the preservation of some of the islands from drastic human interference, the Tristan group presents numerous opportunities for research into problems of fundamental ecological and evolutionary interest which are not readily obtainable elsewhere. A close examination of the ecological *status quo* prior to the arrival of introduced plants and animals could contribute much to an understanding of the causes of instability in ecosystems<sup>11</sup> and its possible relationship to the degree of integration within the biotic communities<sup>19</sup>. Owing to the relative simplicity of their impoverished biota, the islands present ecological problems such as those of niche analysis and species interaction, which could more easily be examined there than in a continental setting<sup>20</sup>. Examination of the efficiency of energy fixation, nutrient cycling and gross productivity within the species-poor ecosystem would be of considerable interest for comparison with those of

climatically similar continental regions having a more diverse biota. Biometric and genetic investigations of the Tristan populations could yield much valuable information on the structure and variability of radiating taxa, and on the types of genetic system present, and could contribute to an understanding of the importance of the 'founder principle' in differentiating small immigrant populations<sup>21</sup>.

Gregory<sup>21</sup> has stressed the need for the establishment of aerospora investigations in remote islands, and the Tristan group would make an ideal site for such work. Combined with other investigations on dispersal agents, aerobiological work might also throw some light on the old and still controversial problem of the possibilities of long-range dispersal of plants and animals across wide ocean barriers<sup>22</sup>. The presence of four islands in the Tristan group, of differing ages, sizes and altitudes, adds greatly to their potential for research in these fields. Peat deposits on all of them, containing organic remains of the past five thousand years<sup>17</sup>, together with older remains which have been dated up to 40,000 years B.P. on some islands<sup>23</sup>, make it possible to carry the ecological investigations back into the recent past.

The more pronounced effects of man on some islands than on others makes possible a close examination of the alien species as they extend their range to the less affected smaller islands. An accurate documentation of the human-induced alterations in their ecosystems would be a useful contribution to the understanding of disturbances due to man, which have gone unrecorded elsewhere until the processes involved are very far advanced<sup>13,16</sup>.

The examination of these ecological and evolutionary processes in the Tristan da Cunha islands demands active conservation of their native biota. Owing to their ecological instability, and vulnerability to the effects of man, some control of human activities on the smaller islands will be essential if the scientific interest of their unique ecosystems is not rapidly to disappear. The history of insular biota elsewhere indicates that both herbivorous and predatory mammals (especially goats, sheep, pigs, rabbits; cats, dogs, mongooses and rats) must be rigidly excluded from the smaller islands, and no attempts made to clear the vegetation by firing<sup>14,24</sup>. If the usual more or less haphazard processes of exploitation and development by man are permitted in the Tristan islands, the sorry story of many of the Galapagos group, the Hawaiian archipelago, Juan Fernandez, Kerguelen, Macquarie, St. Helena and many other remote islands, will be repeated there, with the attendant disruption of established ecosystems and reduction or extinction of species, accelerated erosion, and ultimately lowered potential for biological productivity.

Stringent regulations are already in force governing the exploitation of wild life, and the introduction of alien species to the Tristan islands<sup>24–26</sup>. The importance of their observation in relation to the indigenous avifauna has been stressed by the International Committee for Bird Preservation<sup>27</sup>; but conservation experience elsewhere has shown that such regulations are difficult to enforce unless linked to a programme of research in which there is a continuing interest by biologists on the spot<sup>28</sup>. The misunderstandings which led to the importation of goats (since destroyed) to Gough Island in 1958 could easily be repeated with catastrophic results to the existing ecosystems.

If the native biota of the Tristan da Cunha archipelago is to be preserved for investigation, it will be essential to have biologists in the islands who understand the importance of and the need for conservation. This could best be achieved by the establishment on Tristan main island of a small biological research station, from which visits could be made to the other islands for investigation. The setting up of such a research station would enable other scientists with special interests in the islands (for example,



geologists, geophysicists, medical scientists, oceanographers, sociologists) more easily to work there. Instead of the usual rearguard action against commercial, military, or other interests, which so often characterize efforts at conservation elsewhere, conservation together with research could be established together as a first priority in the Tristan da Cunha Islands. The conditions for conservation are relatively simple, since active management is not needed, and it is necessary only to limit human activities and contact with the smaller islands. The conservation of the terrestrial biota and the establishment of a permanent research station on Tristan need conflict with no established commercial interests, and could at the same time become a considerable asset to the recently re-established human community, since the activities of the visiting scientists would lead to demands for both goods and services and the consequent inflow of money and employment of islanders.

The President of the Charles Darwin Foundation for the Galapagos Islands has stressed the importance of the establishment there of facilities for biological research, linked to conservation of their unique and historically interesting biota\*. The Galapagos and the Tristan-Gough group share many of the classical features of oceanic island archipelagos, which have contributed so much to evolutionary thought. They are important to ecological investigations because they provide a range of diverse but naturally simple species-poor ecosystems to be found nowhere else in the temperate and tropical regions of the globe. Although present methods of transport and the increasing demands of man constitute a threat to such vulnerable ecosystems, modern research techniques will undoubtedly allow further valuable scientific information to be obtained from them. Unless a range of undamaged oceanic islands is rigorously protected at once, the next decade may see the irreparable loss of these opportunities for research. It is greatly to be hoped that the International Biological Programme will provide an opportunity for the investigation and conservation of oceanic islands on a global scale, and that the Galapagos Foundation and a research facility in the Tristan da Cunha Islands will be only two in a world-wide network of sanctuaries and study areas.

In the formulation of their ideas on organic evolution, Charles Darwin and Alfred Russel Wallace derived much from their investigations of island life. There are therefore few more appropriate ways in which biologists might commemorate the centenary of the general acceptance of those ideas than by the promotion of research on oceanic islands such as the Galapagos and the Tristan da Cunha group.

**Note.** The Southern Zone Research Committee of the Royal Society established on October 5, 1964, a Tristan da Cunha Study Group. This group is directed to evaluate the scale of interest in research on the four islands of the Tristan-Gough group, and to assess the demand for facilities there. Circulars are being sent to scientists known to be interested in oceanic island problems and to

heads of departments and others on a rather wide scale. The group wishes to hear from anybody concerned with these matters, and correspondence should be addressed to the Convener, Dr. M. W. Holdgate, c/o the Royal Society, 6 Cornwall Terrace, Regent's Park, London, N.W.1.

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## DR. WILLIAM ROXBURGH: THE FATHER OF INDIAN BOTANY

By DR. J. SEN

Indian Botanic Garden, Calcutta

THIS year marks the 150th anniversary of the death of Dr. William Roxburgh and of the publication of *Observations of the late Dr. William Roxburgh, Botanical Superintendent of the Honourable East India Company's Garden at Calcutta, on the Various Specimens of Fibrous Vegetables, the Produce of India, which may prove Valuable Substitutes for Hemp and Flax, on some Future Day, in Europe*, "edited by a friend, to whom he transmitted them

from St. Helena, during his illness; and . . . published at the expense of the East India Company, for the information of the residents, and the benefit that may arise therefrom throughout the Settlements in India" (London: 1815).

Roxburgh died at Park Place, Edinburgh, on February 18, 1815, before the small volume, *Observations*, was published later in that year. It is the first connected



scientific account of the cultivation and utilization of jute and some other fibrous plants growing in India, and is notable for the precise descriptions and presentation of results of experiments conducted by him at the Honourable East India Company's (now Indian) Botanical Garden at Calcutta.

The descriptions of the two species of jute, namely *Corchorus capsularis* Linn. and *C. olitorius* Linn., which yield fibres of commerce, are recorded by him in yet another posthumous publication in three volumes entitled *Flora Indica* (1820-32), which again is the basis of all subsequent Indian botanical works on taxonomy and floristics. This pioneering effort rightly earned for him the title of "the Father of Indian Botany", and until 1872, when the publication of Hooker's *Flora of British India* started appearing in a series, Roxburgh's was the only available single work through which a knowledge of Indian plants could be acquired. In the field of economic botany also, it was the only source of information until the publication of Sir George Watt's *A Dictionary of the Economic Products of India*, the first volume of which appeared in 1889. Roxburgh's other botanical works, particularly *Plants of the Coast of Coromandel* (in 3 volumes) and *Hortus Bengalensis*, and albums containing original paintings of Indian plants, are also well known as accomplishments of a very high order.

Roxburgh first accepted an appointment on the Honourable East India Company's Madras Establishment in 1776, and there he became acquainted with Dr. John Koenig, a pupil of Linnaeus, which no doubt influenced his subsequent work in India. He took charge of the present Indian Botanic Garden in 1793 as its first official superintendent, and with his great foresight he included both purely scientific and commercial aspects of botany in his programme. He established the great herbarium of the Botanic Garden for floristic and systematic studies of Indian plants, and at the same time started the introduction, cultivation and utilization of economic plants in India. (Most of Roxburgh's collections were distributed to the European herbaria by his successor, Dr. Nathaniel Wallich. The present herbarium was in fact made by Sir George King (superintendent, 1871-97) from the nucleus of collections started by Dr. William Griffith (Offg. superintendent, 1842-44) and developed by his successors in office. It is now the 'Central National Herbarium' of India.) Among the many plants he introduced at Samuleotta and at Calcutta, the most important are reported to be cinnamon, coffee, mahogany, mulberry,

nutmeg and pepper. It is also reported that he took an active interest in the improvement of sugar cultivation, in the rearing of silkworms and in the manufacture of silk. He left India in 1813.

Long before Roxburgh started his experiments on fibrous plants at the Indian Botanic Garden, both the species yielding jute used to be variously cultivated by local people. Roxburgh readily realized the great commercial value of jute fibres, and took up experimental cultivation of jute in the Garden, retted his produce, and measured the strength of their fibres in twisted condition before and after tanning or tarring them. Very soon he exported the first hundred tons of raw jute to Britain, and thus paved the way for the development of the jute industry in India. His untiring efforts with jute and a number of other fibres, the samples of which were sent to India House and to the Society (now the Royal Society) of Arts in London, were acknowledged by the award of the Society of Arts' Gold Medal to him on no fewer than three occasions. Many of these fibres are described in the Society's *Transactions* for 1804.

The subsequent history of the modern jute industry is another story, which, however, no doubt owes its origin to the genius of Roxburgh. Its development is a fascinating narrative told in a series of articles by Mr. D. R. Wallace in the *Empire Evening News* of Calcutta and later published as a book—*The Romance of Jute* (Calcutta: 1909), which itself has now become almost a historical document.

Near the 'Great Banyan Tree' of the Indian Botanic Garden, there is a simple monument (on the top of a small mound) to the memory of Roxburgh. The Latin inscription on the monument is by Bishop Heber, and the English rendering of the inscription (as follows) by Reverend Father Santapau written on a separate tablet was fixed last year to the slope of the mound (bearing the monument) for ready appreciation by the people of India whose debt to Roxburgh still remains immeasurable.

Whoever You Be  
If this place soothes the mind with its sweetness  
Or teaches you to think of God with reverence  
You must hold in high honour  
R O X B U R G H  
Formerly the Superintendent of these Gardens  
A man distinguished for his Botanical Science  
And most able planner  
Of rustic pleasure  
His country preserves his remains  
Here lives his genius  
May you enjoy thoroughly  
To his cherished memory his Surviving Friends  
A.D. 1823

## OBITUARIES

### Dr. A. R. Zhebrak

ANTON ROMANOVICH ZHEBRACK, a well-known geneticist of the U.S.S.R., died suddenly on May 20 at the age of sixty-three. He was also a statesman and one of the organizers of the U.N.O. as a member of the U.S.S.R. delegation to the San Francisco International Conference. As a highly educated biologist and a frank man he was a zealous champion against Lysenko. Zhebrak was therefore in disgrace for many years and died debarred from teaching genetics in the Timiriazev Academy of Agriculture in Moscow, from which he had been banished in 1948.

Zhebrak was the son of a poor peasant. As a student he rose fast during the revolution, became a student of the Academy of Agriculture in Moscow, and later a professor of genetics in his *Alma Mater*. The discovery of the inheritance of Zhebrak with crosses of wheat brought him fame and a socialist title. He was president of the Academy of Sciences of the U.S.S.R.

As a stalwart biologist Zhebrak could not reconcile himself to the absurd conceptions of Trofim Lysenko and was his energetic opponent. He was also a patriot and mourned the shame of his native land. In one of his articles in the American press he tried to demonstrate that Lysenko had given some services to the country, but that these services, and not his theories, were the reason for his rise. But unfortunately

professor of botany in the Moscow Pharmaceutical Institute. He died in this post.

Being in disgrace and having no great opportunity for his genetic works, Zhebrak continued them as a private citizen. Some of them have been published since the death of Stalin. The great merit of Zhebrak was that he, as a professor of botany in the Pharmaceutical Institute, continued to prepare new Russian geneticists as his disciples in botany. Dr. Zhebrak was a very kind, compliant, tolerant and honest man, but was irreconcilable to a falsification of science and intolerant of scientific careerists. Many young geneticists, his disciples, met near his coffin to see their beloved teacher on his last journey. Almost all the Russian geneticists were present at the funeral of their friend and companion in arms in the struggle for the scientific truth.

Anton Zhebrak lived until the time when the Russian people and its Government understood the scientific ignorance of Lysenko and the damage of his delirious conceptions for the development of science and agriculture. But the correction of old mistakes continues meanwhile very slowly. In 1948 Lysenko, as a favourite of Stalin, seized the Department of Genetics in the Moscow Academy of Agriculture and for more than sixteen years carried out his absurd genetics for young students. In March 1965 he abandoned this post, mocked and held in contempt by his students, but Zhebrak was still not returned to his old legitimate post—which is now vacant. Nevertheless, we trust that the U.S.S.R. as the country of N. I. Vavilov and A. R. Zhebrak will return to its place of honour in the field of development of scientific genetics and plant breeding.

A. KURZOW

#### Dr. A. C. Graves

DR. GRAVES, Test Division leader at Los Alamos Scientific Laboratory, died suddenly on July 29 while on holiday near Del Norte, Colorado. He was fifty-five years of age.

Dr. Graves, who most recently flew as a scientific crew member during the May 1965 Solar Eclipse Expedition in the South Pacific, had been a member of the Army Scientific Advisory Panel, and scientific adviser at the Nevada Test Site and Pacific Proving Ground since 1955. He was deputy scientific director during 1947-48, and scientific director during 1948-55 of Pacific Proving Ground operations; and test director at the Nevada Test Site during 1951-54.

Dr. Graves was one of the pioneers in the practical applications of nuclear energy. With forty-one other scientists, he helped build the world's first chain-reacting pile—the reactor in the squash court of Stagg Field in Chicago which proved, on December 2, 1942, that a chain reaction could be sustained and controlled. Ever since then Dr. Graves has been intimately associated with atomic energy work.

Dr. Norris E. Bradbury, director of Los Alamos Scientific Laboratory, said: "The tragic death of Alvin Graves

is a great loss to the Laboratory and to the world. I know it does the rest of the Laboratory and the world. He was a very kind, compliant, tolerant and honest man, but was irreconcilable to a falsification of science and intolerant of scientific careerists. Many young geneticists, his disciples, met near his coffin to see their beloved teacher on his last journey. Almost all the Russian geneticists were present at the funeral of their friend and companion in arms in the struggle for the scientific truth.

1942 until 1943 he was with the University of Chicago Metallurgical Laboratory. He joined the staff of Los Alamos Scientific Laboratory in 1943 and became head of the Laboratory's Test Division in 1948.

#### E. W. Golding, O.B.E.

MR. EDWARD WILLIAM GOLDING was born on March 9, 1902, at Northwich, Cheshire, and died at Ewell, Surrey, on June 2, 1965. He was educated at the Sir John Deane's Grammar School and at Manchester College of Technology, where he studied, as student and research scholar, under Prof. Miles Walker. He obtained his degree of B.Sc.Tech. in 1923 and that of M.Sc.Tech. in 1934. On leaving the College, he served for two years in the Research Department of Metropolitan-Vickers Electrical Co., Ltd., at Manchester, and in 1926 was appointed lecturer in electrical engineering at the then University College of Nottingham.

Specializing, at first, in electrical measurements, he later became interested in the applications of electricity to agriculture and horticulture. His investigations of these subjects were undertaken with the meticulous thoroughness which characterized all his work, and his first book, *Electrical Measurements and Measuring Instruments* (1933), now in its sixth edition, was followed by *The Electrification of Agriculture and Rural Districts* published in 1936.

He was, in those days, an enthusiastic and competent golfer, and memories of his burly figure striding purposefully around the Wollaton Park course will always remain with those who knew him there. In 1944 he was transferred from associate member to member of the Institution of Electrical Engineers, and, in 1945, he joined the Electrical Research Association as head of the Rural Electrification Section. To this post was added, in 1948, responsibility for the work of the newly established Wind-power Section. The investigation of the possibilities of large-scale generation of electricity by wind-power soon developed into a major interest which brought him into contact with people from all over the world. He became a member of the Working Group (Windpower) of O.E.E.C., acted as Unesco adviser, on problems of energy, to the Arid Zone Research Advisory Committee, and carried out missions for the United Nations and its specialized agencies in Israel, India, Somaliland, Haiti, Uruguay and Egypt.

Although Golding's writings were part of his work, they were also his main hobby and always gave him great pleasure. In 1955, *The Generation of Electricity by Wind Power* was published. This was followed by two major Food and Agriculture Organization Bulletins: *Windmills for Water Lifting and the Generation of Electricity on the Farm* and *The Potentialities for Rural Electrification in Asia and the Far East*, and a booklet, *Power Supplies*, written for the Overseas Development Institute. Throughout the years he maintained a close association with mining engineering matters and was a past-president (Midland Branch) and Fellow of the Association of Mining Electrical and Mechanical Engineers as well as being examiner for Associate Membership of the Institution of Mining Engineers. He was also a member of the Institution of Mechanical Engineers and a Fellow of both the Royal Society and the Royal Geographical Society. He was awarded the O.B.E. for his services to the mining industry in various countries, and in 1958 the duties of overseas representative were added to his other Electrical Research responsibilities. He continued to travel widely as an adviser on power supplies in remote areas and as a member of many departments in many countries, and in scientific societies, schools and universities. He was, also, a most

active participant at many international conferences and symposia, the most recent being the U.N.C.S.A.T. Conference in Geneva in 1963. In 1960 he was appointed assistant director of the Electrical Research Association, and in this capacity his broad outlook, his range of engin-

ering knowledge and his concern for the well-being of his colleagues were of great value.

He was a generous, likeable man whose passing will leave the poorer all with whom he ever came into contact.

A. H. STODHART

## NEWS and VIEWS

### Physics in the University of Sussex: Dr. D. F. Brewer

DR. D. F. BREWER has been appointed to a chair in physics in the University of Sussex. He was born in 1925, and went to the University of Oxford during the Second World War to take a shortened course in physics. He then spent three years of research in industry before returning to Oxford where he took an honours degree in 1950. For his doctoral thesis with Mendelssohn at the Clarendon Laboratory, Brewer selected work on the properties of liquid helium, a subject to which he has since devoted most of his efforts and to which he has made a great number of important contributions. He became particularly interested in the formation of the helium film from the gas phase and found that the onset of superfluidity is suppressed as the film thickness is reduced. Dr. Brewer further investigated the destruction of superfluidity by vortex formation in channels of varying diameter—research which also led to a new determination of the viscosity of the normal component. All this work was done in Oxford, some in collaboration with D. O. Edwards. He left in 1957 for Columbus, Ohio, where another of Mendelssohn's old students, J. G. Daunt, had been carrying out experiments on the properties of liquid helium-3. There Dr. Brewer was responsible in part for the discovery of the melting curve minimum and in the work on the specific heat below 1° K. He returned for three years to Oxford in 1959 where he determined the specific heat of helium-3 under pressure down to 45 millidegrees. When in 1962 the University of Sussex decided to enter the field of low temperature research, Dr. Brewer was an obvious candidate to lead it. He joined first as senior lecturer, and was appointed reader in 1964. Again the properties of adsorbed helium, now of both isotopes, became his main interest, but various other problems are being investigated in his group, which already numbers ten.

### Microbiology in the University of Sheffield:

Dr. J. R. Quayle

DR. J. R. QUAYLE, senior lecturer in the Department of Biochemistry since 1963, has been appointed to the West Riding chair of microbiology in the University of Sheffield in succession to Prof. S. R. Elsdon, who has been appointed the first director of the Agricultural Research Council's new Food Research Institute at Norwich (*Nature*, 204, 1036; 1964). Dr. Quayle graduated in chemistry at the University of Wales and later obtained a Ph.D. at the same University. On moving to Cambridge—where he obtained a second Ph.D. under the supervision of Lord Todd—his interests began to move in the direction of biology. It was, however, when Dr. Quayle went to California to work with Prof. Melvin Calvin that his transformation to a biologist came about. In Prof. Calvin's laboratory, where he made key contributions to the understanding of the enzymology of photosynthetic carbon dioxide fixation, he made his first contact with micro-organisms and with the technique of autoradiography. In his subsequent career as a microbiologist, carbon dioxide fixation mechanisms have been one of his main interests, and his use of autoradiography to elucidate metabolic pathways of micro-organisms has been a characteristic feature of his research. On returning to the United Kingdom, Dr. Quayle joined Sir Hans Krebs as a

member of the Medical Research Council's Unit for Research in Cell Metabolism in Oxford. Here he had a particularly fruitful association with Prof. H. L. Kornberg and built up an international reputation for himself in working out the metabolic pathways whereby micro-organisms elaborate complex substances commencing with substances as simple as formate or acetate as sole source of carbon. The combination of chemical expertise with a feeling for, and understanding of, living things is rare, but where it exists, as shown by Dr. Quayle's career and numerous publications, it can produce an outstanding microbiologist.

### Solid-State Physics in the Bradford Institute of Technology: Dr. D. Bijl

DR. D. BIJL, reader in natural philosophy at the University of St. Andrews since 1959, has been appointed professor of solid-state physics in the Bradford Institute of Technology. Dr. Bijl was awarded the degrees of 'candidatus' and 'doctorandus' from the University of Leiden. After the Second World War he re-started his experimental work on paramagnetic relaxation. For the latter, together with research on electron spin resonance partly carried out in Oxford during the tenure of a British Council scholarship, he was awarded a Dr.Sc. (Physics) by the University of Leiden. From 1950 until 1955 Dr. Bijl held a Pressed Steel, Ltd., Company research fellowship at Oxford. During this time he pursued two lines of research: thermal expansion of solids at low temperatures and electron spin resonance in crystals at low temperatures. His research group at the University of St. Andrews has developed work in these fields. The future main lines of research which Dr. Bijl expects to develop are in microwave spectroscopy, lattice dynamical properties of solids and semiconductor physics. For the session 1965–66 Dr. Bijl has accepted an invitation to spend a year at Massachusetts Institute of Technology as visiting professor in the Centre for Materials Research and Engineering. When he takes up his appointment at Bradford, Dr. Bijl will be responsible for the development of research and postgraduate studies in solid-state physics and for assisting in the staffing and organization of undergraduate schools.

### Medical Research Council: Advisory Boards

THE Medical Research Council is assisted in the formulation and execution of its research programme by three Advisory Boards, the Clinical Research Board, the Biological Research Board and the Tropical Medicine Research Board. These bodies have the main function of overseeing the progress of work supported by the Council and advising on proposals for such support in their respective fields, and their membership is closely integrated with that of the Council itself. Members of the Clinical Research Board and the Tropical Medicine Research Board are appointed in consultation with the Health Departments and the Ministry of Overseas Development respectively. The following appointments by the Council to the three Boards from October 1 are announced: *Clinical Research Board*, Prof. A. S. Duncan, Department of Obstetrics and Gynaecology, Welsh National School of Medicine, Cardiff, and Prof. T. Cecil Gray, Department of

Anaesthesia, University of Liverpool; *Biological Research Board*, Prof. R. A. Gregory, George Holt professor of physiology, University of Liverpool; *Tropical Medicine Research Board*, Prof. P. C. C. Garnham, Department of Parasitology and Helminthology, London School of Hygiene and Tropical Medicine, and Prof. D. V. Hubble, Department of Paediatrics and Child Health, University of Birmingham.

#### Blood Group Reference Laboratory

DR. K. L. G. GOLDSMITH, formerly deputy director, succeeded Dr. A. E. Mourant as director of the Blood Group Reference Laboratory on September 1. This Laboratory, which is administered by the Medical Research Council on behalf of the Ministry of Health, will continue to deal with all aspects of blood group work with the exception of anthropological and population surveys which will remain the responsibility of the outgoing director. On the same date, Dr. Mourant will transfer to the Council's External Scientific Staff in charge of a serological population genetics laboratory being set up by the Council. This Laboratory, which will incorporate the Anthropological Blood Group Centre (hitherto supported through a grant from the Medical Research Council to the Royal Anthropological Institute), is to be situated at Boundary House, 91 Charterhouse Street, London, E.C.1; its postal address will, however, be Serological Population Genetics Laboratory, c/o St. Bartholomew's Hospital, West Smithfield, London, E.C.1.

#### Ministerial Meeting on Science

UNDER the title "Ministers Talk about Science", a summary and review of the first ministerial meeting on science, October 1963, edited with an introduction and notes by E. G. Meethens, secretary of the ministerial meeting on science, has now been issued by the Organization for Economic Co-operation and Development (Pp. 178. Paris: Organization for Economic Co-operation and Development, 1965. 10 francs; 15s.; 2.50 dollars). Of its five parts, one is a prefatory part, in which Monsieur Lefèvre, Prime Minister of Belgium, comments on the purpose of the meeting generally, and Dr. A. King, director of the Organization for Economic Co-operation and Development scientific affairs, reviews the position of science in the work of the Organization. This is followed, in the second part, by an account of the work of the advisory group on science policy prefatory to the meeting, including the report "Science and the Policies of Government" and some discussion of the theory and practice of science policy generally. In Part 3, J. Salomon reviews the work of international scientific organizations generally, and there is a summary paper on issues of international science policy. Part 4 includes a foreword by J. R. Gass and a paper by C. Freeman, R. Poignant and I. Svernilson on science, economic growth and Government policy, covering first the economic aspects of science and next the problems of Government policy. Part 5 gives a summary of the meeting and the text of the subsequent communiqué.

#### Periodical Publications in the Patent Office Library

THE Patent Office Library is a public, open-access reference library, the use of which is freely available to all. In addition to a world-wide collection of patent specifications and patent journals, it contains 300,000 volumes of periodicals and books covering the whole field of the exact sciences and their associated technologies. The third edition of *Periodical Publications in the Patent Office Library* is a list of periodicals current at the end of July 1964, representing some 9,000 titles (Pp. v+436. London: H.M.S.O., 1965. 28s. net). This has been prepared from a visible-strip index provided in the Library, which is kept up to date by addition of new titles as they are received. In this list, which is primarily intended for use away from the Library, alternative forms

of title, especially alternative language forms, have been added, and in a few cases references have been given from alternative forms of entry for organizations. The publication is divided into five sections. The first deals with U.K. Patent Office publications, the second with the series of overseas patents, and Section 3 the overseas patent and trade-mark indexes, abstracts and periodicals. Section 4 deals with international patent indexes. Section 5, which constitutes the main part of the publication (pp. 13-436), lists alphabetically the scientific and technical periodicals held. Title entries are given if the title is distinctive; if not, the name of the issuing body is entered. The Patent Office Library does not lend its publications, but a quick postal photocopy service is provided, and this is supplemented by an immediate 'while-you-wait' photocopy service for personal callers. The basic charge is 9d. a page.

#### Physical Review

THE more frequent issue, weekly instead of fortnightly, of the *Physical Review* commenced in 1964 and the division into four volumes per annum has not proved sufficient to meet the growing demands for publication. The editors announce that it will be necessary to publish one volume per month in 1966. Because of technical difficulties, however, it will not be possible to offer separate subscriptions in smaller divisions than the present sections A and B. Starting in January 1966, each monthly volume will contain four issues and one comprehensive index issue. Each of the four issues will be devoted to one of the following specific sets of subjects in the following order: (1) atoms, molecules, plasmas; properties of atoms, ions and nuclei in solids; fluids; collective effects in solids; (2) solids (except the topics listed under (1)); (3) nuclei; (4) elementary particles; fields; relativity. The index issue will contain a comprehensive author index and an analytic subject index for all four sections. Members of the American Physical Society are offered the option of receiving (A) issues 1, 2 and index; (B) issues 3, 4 and index, or (AB) all issues. Non-members may subscribe only to all issues (AB). The editors state that it seems inevitable that more than four text issues per month will be needed in 1967.

#### The Geochemical Society of India

WITH the primary objective of fostering national and international co-operation between the geochemists and persons applying geochemical results in any field of investigation, an all-India society, to be known as the Geochemical Society of India, was established on August 20. The Society will have its headquarters at Patna, India. The following are the chief aims of the Society as contained in its statutes: "The principal objective of the Society shall be to foster the study and application of geochemistry in the national and international spheres. In pursuance of the foregoing objective the Society shall: (1) work with any interested group in planning symposia and other types of meetings that are related to geochemistry; (2) sponsor a journal called *The Journal of the Geochemical Society of India*; (3) appoint commissions and committees to study problems that require an all-India or international co-ordination". Among the chief programmes of the Society are the following: (1) All-India co-ordination of work on the Earth sciences; (2) publication of quarterly bulletins containing abstracts and summary data of work under way in India together with notes and news of important researches being carried out in the different institutions of the country as well as elsewhere.

The officers of the Geochemical Society of India are: *President*, Prof. D. N. Wadia; *Vice-Presidents*, Prof. M. S. Krishnan, Dr. A. G. Jhingran, Prof. L. Rama Rao, Dr. Hari Narain; *Treasurer*, Shri A. P. Jain (Patna); *Executive Secretary*, Dr. D. N. Ojha (Patna); *Regional*

**Secretaries**, Dr. A. N. Chowdhary (Calcutta), Dr. D. B. Sikka (Punjab), Dr. B. P. Radhakrishna (Mysore), Dr. S. K. Sen (Kharagpur); **Council Members**, Prof. W. D. West (Sagar), Prof. R. C. Misra (Lucknow), Prof. R. C. Sinha (Patna), Prof. S. N. Wakhloo (J. and K.), Prof. T. C. Bagchi (Kharagpur), Shri H. Nandi (Tatanagar), Prof. S. N. Sarkar (Dhanbad), Prof. S. K. Siddhant (Burdawan), Prof. J. N. Chatterjee (Patna), Prof. I. C. Pande (Chandigarh), Dr. P. Sinha (Patna), Prof. F. Ahmad (Aligarh), Dr. A. K. Saha (Calcutta), Shri S. P. Nautiyal (Bangalore), Dr. Sriramdas (Waltair); **Executive Editor**, Prof. R. C. Sinha (Patna), with an editorial advisory board.

### Building Research

Two important publications have recently been issued as *Building Research Station Digests*, one entitled "Protection against Corrosion of Reinforcing Steel in Concrete" (No. 59), the other "Chimney Design for Domestic Boilers" (No. 60) (London: H.M.S.O. Pp. 8 and 4d. each. 1965). The former is concerned with those factors responsible for unsatisfactory performance of reinforcing steel where corrosion occurs and indicates the requirements for depth and quality of concrete cover which must be met if this is to be avoided. The subject is briefly dealt with under headings: functions of cover, factors favouring corrosion, effects of corrosion, how corrosion occurs, prevention of corrosion, avoidance of defects in practice, materials (aggregates and special reference to chlorides aggressive to steel derived from marine deposits), construction and workmanship, preventing corrosion of steel in concrete floors, and includes examples of failed cover and spoiled appearance of concrete structures due to corrosion and spalling. In the pamphlet dealing with chimney design, emphasis is on specific requirements of modern high-efficiency domestic boilers burning solid fuel, gas or oil, if the chimney is to function satisfactorily without internal damage from effects of condensation or risk to health and safety. It is stressed that the need is "... to provide flue liners or flue blocks for appliances of the controlled combustion type ... recognized in building legislation, but satisfactory performance depends also on the sizing and thermal insulation of the flue". The important headings here are: boiler operation (all fuels), combustion, condensation and chemical attack, relative merits of air dilution and thermal insulation, and design aims.

### Flexible Urethane Foam

In an informative, illustrated article by Charles E. Petty, entitled "Changing the Form of Foam", an account is given of an established industry based on a 'happy accident' some eleven years ago (*Dupont Magazine*, 59, No. 3; May-June 1955. E. I. Du Pont de Nemours and Co., Wilmington, Delaware, U.S.A.). According to William Powers, foam division manager of the Scott Paper Company, this is what happened: "In 1954, our research laboratory was looking for a material to make household sponges. One candidate we tested was urethane foam, which has all these qualities and looks and feels like a sponge. When we tested foam's resistance to a strong caustic solution, however, the thin membrane-like 'windows' that connect its network of tiny, interconnecting strands dissolved away, leaving only a three-dimensional foam skeleton". Although this test did not actually produce the desired sponge, it did reveal the possibilities of a foam product with a highly versatile open-pore structure; such material readily permitted passage of gases and liquids, and also served as a formidable barrier against transported dirt, dust, etc. Foam is essentially 97 per cent air, the remainder being tiny interconnecting strands with enormous dust-holding capacity; it is calculated that a cubic foot of foam with 100 pores per lined inch may have as many as 2,000 ft.<sup>2</sup> of strands to

catch dirt. The basis of this flexible urethane foam is Du Pont's 'Hyleme' organic isocyanates. The article shows (in colour) some of the foam blocks of varied texture, manufactured by the Scott Paper Company. The rise in total consumption in the United States of urethane foam products is certainly impressive; since 1958, usage has risen from 10 million to 200 million pounds per annum. It is on record that while uses for Scott's urethane foam have steadily soared, an acceptable sponge has so far not been developed; "on the contrary, we looked so hard for a sponge, we came up with an open-pore product that won't even hold water".

### Guides to Mineral Exploration

A COLLECTION of articles by officers of the Geological Surveys throughout the British Commonwealth, entitled *Some Guides to Mineral Exploration*, has recently been published by the Geological Survey of Canada (Department of Mines and Technical Surveys. Paper 85-6. Edited by E. R. W. Neale. Pp. 123. Ottawa: Queen's Printer, 1965. 75 cents). The symposium was originally issued in 1964 by the Commonwealth Geological Liaison Office in London, the mimeographed papers of which have only a limited circulation; but in view of the potential value of the work to a wide circle of readers interested in the newer techniques of geological and geophysical search for ore deposits, it has been published in this more readily available form. Five of the eleven articles are by scientists of the Canadian Survey, two by those of the Overseas Geological Surveys organization in London, and one each from the Surveys of Australia, New Zealand, India and Great Britain. The collection was brought together by the Canadian geologist, Dr. E. R. W. Neale, as one of the innumerable activities undertaken during his outstandingly successful two-year term as Commonwealth Geological Liaison Officer in London, which has just come to an end. He is succeeded by Shri G. H. S. V. Prasado Rao, of the Geological Survey of India, to whom a warm welcome is assured.

### Potatoes

THE annual production of potatoes in Great Britain is about 6 million tons, of which 4 million meet the demand for human consumption and 700,000 tons are required for seed; the balance is used for stock, but there is always a considerable wastage. The potato crop is an expensive one to grow and the capital costs increase with the introduction of new machines, so that a comprehensive review from the Ministry of Agriculture, Fisheries and Food of modern techniques is welcome (Bulletin No. 94. *Potatoes*. Pp. iv + 112 + 4 plates. London: H.M.S.O., 1965. 8s. net). Every aspect of the growing and handling of the crop is dealt with by a group of specialists and the material is co-ordinated and edited by C. V. T. Dadd. The sequence is the logical one of starting with notes on the breeding, health, and certification schemes, and finishing with the regulations of marketing and the results of investigations, carried out in various parts of Britain, into the profitability of potato growing. The bulk of the Bulletin, however, is devoted to seed rates for different varieties with particulars of seed treatment, storage and sprouting, cultivations and planting and the fertilizer recommendations for different classes of soil, early potato and seed potato production, the importance of irrigation and potato spraying, methods of haulm destruction and implements for harvesting. There are also discussions on tuber quality, on storage requirements and on the preparation of potatoes for the market. There are nine appendixes concerned with useful details, and an index.

### Soil Ecology and Biology

THERE has been a Biology Commission since the founding of the International Society of Soil Science, but its work has been concerned mainly with microbiology.

However, during the past twenty years there has been a great increase in the science of general ecology based on the biology of reproduction, growth, moulting, digestion and excretion of the various groups that participate in the life of the soil. To provide a suitable means of presenting the work of investigators in this field, a new journal, with an international editorial board, was launched in 1964 (*Revue d'Écologie et de Biologie du Sol*, Tome 1, Fascicule 1, Juin 1964. Published quarterly. Pp. 1-122. Paris: Gauthier-Villars et Cie, 1964. Annual subscription 70 francs). The first volume, consisting of 3 numbers, contains 20 papers totalling 574 pages; there are 10 papers, 140 pages, in Volume 2, No. 1. Two of the papers are in English, the remainder in French.

### Scoring Virulence of Phytopathogenic Bacteria

UNDER appropriate conditions, many phytopathogenic bacteria can express virulence capacities much broader than is indicated by clinical experience. For example, passage through heterologous host plants can result in 'new' virulence characteristics. A likely mechanism would be the selection by the unnatural host plant of postulated naturally occurring virulent individuals in a statistically avirulent population—such population analysis requires a "vegetable white mouse" as a device for scoring virulence. The use of excised bean pods, under carefully specified conditions, together with genetically labelled bacterial cultures, has provided M. P. Starr and D. W. Dye of the Plant Diseases Division, Department of Scientific and Industrial Research, Auckland, with reliable, sensitive and convenient virulence-scoring techniques (*New Zealand Journal of Science*, 8, No. 1; March 1965).

### Phylogeny of the Chimaeroids

C. PATTERSON, writing from the British Museum, considers the Holocephali to be more closely related to arthrodires than to sharks and rays, although not descended from the very similar ptyctodont arthrodires, as Orvig and Stensio have considered probable, but as an independent evolution from pre-arthrodiran stock (*Phil. Trans. Roy. Soc., B*, 249, No. 757. June 10, 1965. *The Phylogeny of the Chimaeroids*. Pp. 101-219+plates 22-28. London: the Royal Society, 1965. 55s. 6d.; 8.30 dollars). The phylogeny of modern chimaeroid fishes is traced back through Mesozoic times to a Palaeozoic sub-order of the Chimaeriformes, the Menaspidoi, which possessed armour, at least to the extent of a dorsal head-shield: the balance of evidence suggests that the Chimaeriformes were derived directly from armoured ancestors with an unmodified hyoid arch, from which stock unarmoured Holocephali of the Carboniferous period, such as *Helodus* and *Chondrichelys*, would also be derived. A fairly clear picture of the head of a Lower Carboniferous menaspoid genus, *Deltopychius*, begins to emerge, partly from material previously ascribed to *Oracanthus*. Fossil remains of Holocephali, other than their tooth-plates, being rare, the microstructure of fin-spines, dermal plates and scales, and teeth provides much of the evidence. Changes in the structure of the dorsal fin-spine and in the evolution of a synchonomorial (placoid) scale from a cycломomial form are found to be parallel to changes which took place in sharks. It is held that the Devonian ancestors of the Chimaeriformes would have possessed a dermal structure like that described as *Eczenatolepis* (Mid-Devonian), which is not accepted here as ptyctodont. The point seems crucial, but this sort of histology is at present very much a specialist's subject. The section on tooth structure is important since it deals with part of the evidence from which the origin of teeth in vertebrates may eventually be understood. The criteria used seem justifiable, for example, presence or absence of pallial dentine, although the outer part of pallial dentine may become metaplastic and form part of the enameloid tissue. A diagram showing the supposed phylogenetic relationships would have helped to a more

critical understanding of this topical problem—have the aphethoid, joint-necked fishes survived in the chimaeras?—likewise a diagram showing the crown and so-called root in different durophagous fishes.

### Brain of the Pearly Nautilus

NOTHING has been known hitherto of the fine structure of the brain of *Nautilus*, and Prof. J. Z. Young's recent paper (*Phil. Trans. Roy. Soc., B*, 249, No. 754: *The Central Nervous System of Nautilus*. Pp. 1-25+plates 1-7. London: the Royal Society, 1965. 21s.; 3.15 dollars) will be of wide interest. The brain proves to be in many ways similar to, but simpler than, that of other living Cephalopoda (Coleoidea), but differs from the coleoid brain in the relatively small size of the optic and large size of the olfactory lobes, and of the rhinophore: the brain is a 'nose' rather than an 'eye' brain, macrosmatic rather than microsmatic. The nerves from the funnel are shown to be connected with the lateral part of the brain, where a small 'magnocellular lobe' is to be found; those from the digital tentacles, however, lead directly into the supracoesophageal cerebral centre, which also receives the nerves from the hood. The tentacles should thus be regarded as cephalic; the name 'Cephalopoda' embodies an ancient error of interpretation. The cerebral connexion of the digital tentacles is of special interest in the light of two other papers by Prof. J. Z. Young (*Phil. Trans. Roy. Soc., B*, 249, No. 755: *The Buccal Nervous System of Octopus. The Centres for Touch Discrimination in Octopus*. Pp. 27-67+plates 8-17. London: the Royal Society, 1965. 18s.; 2.70 dollars), one of which traces elegantly the series of nerve-centres of the feeding processes in *Octopus*—catching the food, poisoning, triturating and swallowing it—while, in the other, the connexion and origin of the inferior frontal system of the brain of *Octopus* are described. This system is already known to be the centre of tactile learning and is now shown to be derived in development from the feeding centres. It is thus suggested that food-discrimination is all-important in developing the learning centres of the brain in the Cephalopoda.

### International Commission on Zoological Nomenclature

NOTICE is hereby given of the possible use by the International Commission on Zoological Nomenclature of its plenary powers in connexion with the following cases, full details of which will be found in the *Bulletin of Zoological Nomenclature* (22, Part 3; August 13, 1965): (1) Validation of the generic name *Oacatus* Vieillot, 1817 (Aves). *Z.N.(S.)* 1647. (2) Designation of a type-species for *Anthonassa* Scudder, 1875 (Insecta, Lepidoptera). *Z.N.(S.)* 1697. (3) Suppression of the specific name *Aphelochus stoueri* Stefański, 1916 (Nematoda). *Z.N.(S.)* 1698. (4) Validation of the generic name *Amblema* Raffinesque, 1820 (Lamellibranchiata). *Z.N.(S.)* 1699. (5) Suppression of the specific names *Volva portus* Linnaeus, 1758, *V. morio* Linnaeus, 1767, *V. ruffina* Linnaeus, 1767, and *Bulla conoidea* Linnaeus, 1767 (Gastropoda). *Z.N.(S.)* 1700. (6) Validation of the specific name *Vespertilio yumanensis* H. Allen, 1864 (Mammalia). *Z.N.(S.)* 1701. (7) Suppression of the generic name *Trichogonia* Rosemeester, 1835 (Lamellibranchiata). *Z.N.(S.)* 1702. Any zoologist who wishes to comment on any of the foregoing cases should do so in writing to the Secretary, International Commission on Zoological Nomenclature, c/o British Museum (Natural History), Cromwell Road, London, S.W.7, before February 13, 1966.

### Races of Man

THE second edition of *Races of Man*, by Sonia Cole, serves as a useful guide to the methods of investigation used in the differentiation of racial types (Pp. 131+13 plates+34 figures. London: Trustees of the British Museum (Natural History), 1965. 11s. 6d.). A brief historical background is followed by descriptive chapters



on physical characteristics, genetical and evolutionary features and blood grouping. Recent advances in the field of human biology have been incorporated. The influence of environment on evolutionary change is one of the more interesting aspects of this investigation; but the rather simplified explanation about the unspecialized nature of the human body is perhaps not the entire story. One hesitates to attribute the lack of specialization entirely to man's achievement of a 'culture', which rendered unnecessary any major evolutionary alteration to his physique. The chapter on racial origins is refreshing in view of the cumbersome terminology still being advanced by some physical anthropologists. The author is surely right in suggesting that only two genera should be distinguished in the early hominids, but it would have been interesting to see the single genus line expostulated. Not everyone would accept the wide geographical range of these early hominids, but the racial and geographical range of *Homo erectus* seems incontestable. The position in the evolutionary sequence of the Great Interglacial European representatives still tends to be avoided. The emergence and appearance of *Homo sapiens* throughout the world make up the remainder, and bulk, of this useful handbook.

#### Ramsay Memorial Fellowships

THE Ramsay Memorial Fellowships Trustees have made the following awards of new fellowships in chemistry for the year 1965-66: General (British) Fellowship to Mr. Moti Lal at the University of Strathclyde; a Glasgow Fellowship to Mr. J. A. Miller at the University of Cambridge; a Canadian Fellowship to Dr. J. F. Skinner at University College, London; a Japanese Fellowship to Dr. H. Suzuki at Bedford College, London; a Spanish Fellowship to Dr. J. A. Burriel Lluna at the Imperial College of Science and Technology, London; a United States Fellowship to Dr. L. L. Ames. The Trustees have renewed the following fellowships for the same year to: Dr. R. D. Levine (General (British) Fellowship) at the University of Oxford; Dr. T. W. Dingle (Canadian Fellowship) at the University of Oxford; Mr. M. J. Baillie (Glasgow Fellowship) at the University of Cambridge; Miss Margaret Kerahaw (New Zealand Fellowship) at the University of Reading.

#### The Harkness Fellowships of the Commonwealth Fund—New York

THIRTY Harkness Fellowships of the Commonwealth Fund—New York are offered each year for study and travel in the United States. Candidates must be British subjects who are citizens of the United Kingdom or present Colonies by birth or by naturalization. Candidates must be between 21 and 32 years of age on September 1, 1966. By that date they should have a degree from a university or the equivalent in qualifications conferred by professional bodies, or an attested level of professional competence in the public service, the professions, the creative arts, journalism, business, industry, or other comparable careers. Candidates who propose an academic career must have experience of post-graduate research. The fellowships are tenable for between 12 and 21 months. Round-trip passages to the United States are provided. Emoluments include living and family allowances, travel in the United States, tuition and research expenses, and other allowances. Selection of Fellows for nomination to the Fund is at the sole discretion of the Committee of Award, which will conduct interviews at Harkness House, London, in February, 1966. Further information and details of the fellowships can be obtained from the Warden, Harkness House, 38 Upper Brook Street, London, W.1. Application, on forms provided by the Fund, must reach Harkness House before November 11.

#### University News:

#### Cambridge

THE following have been elected to fellowships at Churchill College from October 1, 1965 (Title B): Dr.

J. A. Miller, to a senior research fellowship; Dr. J. B. Gibson (Sheffield), to a senior research fellowship.

#### Belfast

THE following lecturers have been appointed: R. N. Hughes (psychology); Dr. J. T. Patterson and Dr. D. J. Stewart (agricultural bacteriology); Dr. B. G. J. Thompson (mechanical engineering).

#### Newcastle upon Tyne

THE following appointments have been made: *Professors*, Dr. R. B. Clark (zoology); Dr. P. Collison (social studies); Dr. J. Brown (psychology); *Readers*, Dr. T. Scratcherd (physiology); Dr. J. G. Buchanan (organic chemistry).

#### London

DR. S. COHEN has been appointed to the chair of chemical pathology tenable at Guy's Hospital Medical School. The following readers have also been appointed: Dr. P. G. Hall (geography, tenable at the London School of Economics and Political Science); Dr. M. M. R. Williams (nuclear engineering, tenable at Queen Mary College); Dr. H. Sawistowski (chemical engineering, tenable at the Imperial College of Science and Technology); Dr. D. W. Turner (organic chemistry, tenable at the Imperial College of Science and Technology). The title of reader in mathematics has been conferred on Dr. L. M. Hocking in respect of his post at University College.

#### Announcements

A SYMPOSIUM on "Man-made Lakes", organized by the Institute of Biology, will be held at the Royal Geographical Society during September 30–October 1. Further information can be obtained from the Institute of Biology, 41 Queen's Gate, London, S.W.7.

THE fourteenth autumn meeting of the Society for Water Treatment and Examination will be held at Leamington Spa during September 29–October 1. Further information can be obtained from Mr. A. W. H. McCanlis, 41 Carshalton Road, Sutton, Surrey.

A SYMPOSIUM on "Management in Research Organizations" will be held at the Manchester College of Science and Technology on September 29. Further information can be obtained from the Registrar, Manchester College of Science and Technology, Manchester 1.

A SYMPOSIUM on "Genetic and Environmental Factors in Human Ability", arranged by the Eugenics Society, will be held in University College, London, during September 30–October 1. Further information can be obtained from the General Secretary, the Eugenics Society, 69 Eccleston Square, London, S.W.1.

THE second international symposium on "Inhaled Particles and Vapours", arranged by the British Occupational Hygiene Society, will be held in Cambridge during September 28–October 1. Further information can be obtained from Dr. J. S. McLintock, Medical Services, National Coal Board, Hobart House, Grosvenor Place, London, S.W.1.

FORATOM (Forum Atomique Européen) Congress 1965 will be held at Frankfurt-on-Main during September 29–October 1, and will consist of a discussion meeting dealing with the subject "Nuclear Energy in Europe—from Raw Material to Electricity Grid". The state of development and future prospects of the use of atomic power for energy supply in Europe will be surveyed and there will be four sections dealing with, respectively: fuels, including uranium sources and fuel elements; present types of nuclear power stations; use of the nuclear power station in the electricity grid; and conclusions concerning the future utilization of nuclear energy in Europe. Further information can be obtained from Foratom-Congress 1965, 5300 Bonn an Rhein, Koblenzer Strasse 240.



## CONSERVATION OF BROADLAND

AN adjournment debate in the House of Commons on July 12 on the Broads as a national park was initiated by Mr. J. Parker almost simultaneously with the publication of the Nature Conservancy's *Report on Broadland*\*.

The *Report* was referred to both by Mr. Parker himself and Mr. B. Hazell, while in replying on the debate Mr. A. Skeffington, the Joint Parliamentary Secretary to the Ministry of Land and Natural Resources, said that the Ministry welcomed the *Report* as stimulating, knowledgeable and imaginative and that he was studying it with great interest. Mr. Parker re-opened the question of clearing the Broads of weeds, which ten years ago he had estimated would cost about £1 million. He pressed for a positive policy with regard to the Broads in the interests of both holiday makers and residents in the area, and hoped that it would be possible to interest the new University of East Anglia in the problems of the area, and to encourage it to assist in research. Mr. Hazell also referred to the need for such a policy, quoting the Strategic Plan for Broadland stressed in the Nature Conservancy's *Report*. Mr. Skeffington agreed that it was essential for the whole future of the countryside and the future of this area to be viewed afresh in the light of a rapidly changing situation, including a considerable increase in population and a great increase in mobility. He said that the Minister was making a thorough and comprehensive review of all the problems associated with the countryside and admitted that this was much overdue, and that the present expenditure of £43,000 by the National Parks Commission under the Act was derisory. The review being made at present would consider not only the needs of the parks already designated but also of possible future parks, which would be areas of recreation calling for greater accessibility than required in the national parks themselves. This review was substantially completed and the Minister hoped to reach a conclusion on this shortly. Finally he admitted that the problem was urgent and that, as the Nature Conservancy's *Report* stated, time was not on the side of Broadland and to do nothing would be to abandon the region to erosion, conflict and decay.

The Nature Conservancy's *Report* follows an earlier draft, and the recommendations now included are based on discussions on this draft. The General Survey contains the factual material, which constitutes the second chapter, and this is followed by one on present trends and another giving the Conservancy's appraisal and conclusions. Recommendations are set forth in the final chapter and these call for a basic policy to conserve and enhance the Broadland environment for holidays and for outdoor recreation as well as research and education. For holidays and such outdoor recreational activities as angling, yachting, dinghy racing, nature study and canoeing, which are essential leisurely pursuits, for research and education and for agriculture, Broadland provides a unique setting. The *Report* directs attention to the need for some understanding of the forces shaping the environment which increase the value of Broadland for basic scientific studies and biological education. Some recreational activities, both now and in the future, could be much better met in other areas, in or near large conurbations, but the total impact of existing demands on Broadland is large, and increasing. There are unmistakable signs that without positive sound policies for the long-term planning, management and development of the region many of its special features will degenerate, and much of its charm and value will be lost. Much of it is ecologically unstable and the restorative processes of

Nature cannot keep pace with the demands on them. Future policies must take full account of all these considerations and the *Report* insists on the need for a strategic plan for Broadland containing both short- and long-term features. Its detailed preparation will call for co-operative effort from many experts, and its implementation will demand full understanding at regional level and encouragement and guidance at national level. Its broad objectives must be the maintenance and enhancement of the Broadland environment; the harmonization of the activities and uses, for example, by space and time zoning; and an expansion programme involving the creation of new Broads and waterways, planned, managed and developed to meet further demands and to offer further scope for zoning. The success of the plan will require acceptance of the precept that long-term planning, management and development of the Broadland's resources of land and water and wild life must be based on research and education. The problems arising from incompatible uses are aggravated by overcrowding, which also prevents the best use being made of a given area. Accordingly, all proposals for expanding the use of Broadland must be based on an appraisal of the social and economic advantages of this use, and must be weighed against any resultant degradation of the environment. This calls for research and study to establish what the real advantages and effects of use are likely to be.

Certain steps can be taken under existing statutory powers and by negotiation, for example, in relation to pollution of the Broadland waterways from whatever source. It is recommended that appropriately constituted expert groups should be established to consider and advise on pollution, the performance of craft intended for use on the waterways, on mooring facilities, on the requirements for caravans, house-boats and 'flat-a-floats' and on a code of behaviour. Discussions between interested parties, phased as part of the plan, should also be initiated to examine the pre-requisites for increased use of those waterways at present closed to navigation or restricted in use. The full use in Broadland of existing powers to cater for the recreational activities on water should be urgently studied by the River Authorities, and discussion should be held to explore the possibilities of extending the application of zoning, both in time and space, as a means of minimising conflict between incompatible uses. Detailed consideration should be given to the practicability of creating new Broadland waterways since these would help to meet existing and future demands for more water space and also to distribute present and forecasted pressures more evenly and to facilitate zoning.

A supplement by certain members of the working party is appended in which the recommendations are based on the fact that at present no single authority can hope to identify all the facts or to provide solutions within its own powers. Accordingly, it is proposed that, as a matter of urgency, the Government should establish machinery to designate "National Recreational Areas", together with an agency to administer them and to provide facilities for outdoor recreation in the countryside generally, and that Broadland should be designated as one of these areas.

Some authority should be invested with financial resources and responsibility for preparing a long-term strategic plan and, pending the completion and adoption of this plan, this authority should seek to ensure the co-ordination and unity essential for the effective planning, management and development of the region. The Conservancy also recommends that as soon as possible a consortium be established of the planning, river, and navigation authorities with an independent chairman of standing, appointed by the Government. Meanwhile the

*The Nature Conservancy. Report on Broadland.* Pp. 98 (27 plates). (London: The Nature Conservancy, 1965.) 17s. 6d.

consortium should be empowered to implement any proposals falling within the powers of the authorities constituting it; to encourage and facilitate appropriate action by other bodies; and to develop support among other bodies and the general public for the policies and measures proposed. It should maintain close working liaison between the interests concerned and create new

facilities for the public enjoyment of Broadland within the strategy of the Conservancy's *Report*, as well as maintain constant review of trends and developments in the area and initiate action to obtain data on them. It should also detect and appraise gaps in the powers required to implement fully the agreed policies, and advise on the economic implications of these proposals.

## RADIOCHEMICALS

FOUR booklets have recently been issued by the Radiochemical Centre, Amersham\*.

The first, *Selected References to Tracer Techniques*, is a revised edition and contains a bibliography of selected publications useful to newcomers to tracer work. The contents consist of titles of general texts and articles dealing with the preparation of labelled compounds, tracer applications, methods of measurement and safety precautions.

*Radioactive Isotope Dilution Analysis* is a second edition of the booklet in which the principle of the analysis is explained and shown to be a sensitive method of determination of many substances in mixtures. It is emphasized that the technique does not require expensive laboratory facilities or materials. The application of the method is illustrated by particular examples.

Problems of radiation self-decomposition of materials are of increasing interest to users of radioactive tracer compounds, and the summary of experience gained from the storage of radioactive compounds at the Radiochemical Centre which is given in the third booklet, *The Stability of Labelled Organic Compounds*, should be very helpful. The information is largely empirical. Data for a number of compounds are listed. The essential factors in the investigation or observation of the decomposition from self-irradiation are the chemical character of the molecule which determines the liability to primary (external) and secondary effects; the purity, the solvents and diluents, the concentration, the specific activity, the temperature and other environmental conditions; and the type and

energy of the radiation from the incorporated nuclide. A detailed list of precautions in handling or storing radioactive organic compounds is included.

The fourth and most recent booklet, *Standards of Activity*, discusses the types of standard available, their certification and use. The activity of a quantity of radioactive material is defined in accordance with the 1962 report of the National Commission on Radiological Units and Measurements (ICRU) (*NBS Handbook 86*) as the number of nuclear disintegrations which occur in the quantity in unit time. The curie,  $5.7 \times 10^{10}$  disintegrations per sec, is the special unit of activity, and activity can be quoted in curies or in its sub-multiples. Activity is not constant with time but decays at a rate determined by the radionuclides present.

Because of uncertainties in purity and half-life, the accuracy to which the activity can be calculated at a given time becomes worse the longer the period from the reference time. Standards of activity have therefore an ephemeral nature and care must be taken in the interpretation of the title 'standard'. The most satisfactory physical form for a standard of activity is a solution in a flame-sealed glass ampoule, but other forms are available. Standards of activity are principally used for the calibration of measuring instruments, and the booklet makes clear the precautions to be taken if full use is to be made of the high accuracy associated with absolutely standardized solutions. In the second half of the booklet the standards available from the Radiochemical Centre are discussed and a complete schedule (operative during 1965-66) of standardized solutions together with details of their guaranteed accuracy and delivery time is given. Each of the four booklets contains a list of references.

S. WEINTROUB

\* The Radiochemical Centre. *Selected References to Tracer Techniques*. Revised edition. Pp. 12. *Radioactive Isotope Dilution Analysis*. Second Edition. Pp. 12. *The Stability of Labelled Organic Compounds*. Pp. 14. *Standards of Activity*. By G. R. Newbery et al. Pp. 27. (Amersham: The Radiochemical Centre, 1965.)

## SCIENCE ABSTRACTS

MEMBERS of the Documentation Research Project section of the American Institute of Physics have carried out a comprehensive survey of published physics literature by a detailed analysis of the contents of the 1961 issues of *Physics Abstracts* (Section A of *Science Abstracts*). A report of the survey has recently been published\*. A total of 20,287 abstracts covering 405 periodicals from 29 countries were scrutinized, and certain details coded and transferred into machine-readable form. The method of coding used is described and illustrated by a code sheet and sample abstract. The specific codes chosen for the country of origin, the periodical, the language and the subject fields, which formed the four major fields of enquiry, are included in an appendix to the report. Each field is dealt with in detail in a separate section of the report. The greater number of papers were devoted to nuclear physics and solid-state physics. About half the articles abstracted during 1961 had been published

during that year. This may be closely related to the fact that *Physics Abstracts* uses the author abstract when available. There were twice as many author abstracts as signed abstracts. Incorporated in the report, in addition to the numerous tables of data and illustrative charts, is a reprint of an article in the *Journal of Chemical Documentation* (4, 157; July 1964), in which a description is given of an investigation of the time-lag of coverage by *Physics Abstracts* with respect to three particular periodicals. The time-lag refers to the time in months between the date of issue of the periodical in which an article appeared and the date of appearance of its abstract in *Physics Abstracts*. The three periodicals were *Physical Review Letters* (an English language journal with no author abstracts), the *Journal of Chemical Physics* (an English language journal with author abstracts for articles but not for Letters to the Editor), and *Zeitschrift für Physik* (a foreign language journal with author abstracts in English or in a foreign language). The investigation showed that the average time-lag was similar for all three periodicals, about three to four months. Many factors contributed

\* *The Journal Literature of Physics: a Comprehensive Study based on Physics Abstracts (Science Abstracts, Section A), 1961 Issues*. By Stella Keenan and Pauline Atherton. Pp. 166. (New York: The American Institute of Physics, 1964.)

to the time-lag, but the analysis indicated that the availability of author or English language abstract could reduce the time-lag by as much as one month.

*Science Abstracts*, which consists of two sections (Section A, *Physics Abstracts*, and Section B, *Electrical Engineering Abstracts*), is produced by the Institution of Electrical Engineers, in co-operation with the Institute of Physics and the Physical Society, and the American Institute of Physics. The director is D. S. Hopper, and advisory editor B. M. Crowther, formerly editor. The editor for Section A is A. Tybulewicz, assisted by seven assistant editors, and for Section B, L. MacQuisten-Wallace, assisted by five assistant editors. During 1964 the original committee of management was replaced by an advisory panel the function of which is to consider the questions of general coverage and production of the *Abstracts* and future automatic retrieval. An important conference of representatives of interested bodies, together with delegations from the United States and from the Science Research Council (then the Department of Scientific and Industrial Research), was held during October 1964. Certain important changes have already taken place in *Science Abstracts*, both in format and in price. The publication in the early weeks of this year of the 1964 author and subject indexes was a welcome surprise. The reduction in time-lag between publication of articles and their abstracts and the increased coverage more than offset the unavoidable greatly increased price. Some years ago, a larger page size and photolithographic reproduction were introduced, but it is only recently that the full advantages of these changes have been utilized. The U.D.C. classification number, which used to be attached to each abstract, was dropped some time ago without any

complaints by users, and to follow the development of physics a gradual but continuous revision of subject headings has taken place. Details of the revision and of the arrangements of entries are all carefully explained and listed in the index numbers. A notable change in the form of the author index took place in March 1965. A new style to be used in the monthly index and in cumulative indexes, which enables an entry card on which the details of an abstract is entered to be arranged easily in index order or to be used several times for different author indexes, has been introduced. A shortened form of the title of an article is used. When the article has several authors, each author's entry appears separately and all the names do not appear together in the entry, but the first author's name is followed by the sign '+' before the abstract number and the co-authors' entries with the '+' sign after their name. Commencing with Volume 68 (1965), *Science Abstracts* will publish the annual cumulative indexes in two parts, each covering a six-month period. The first parts covering the January-June issues have already appeared and the Part 2 (July-December) indexes will be issued after the publication of the December issue. Abstracts are numbered consecutively throughout the twelve monthly issues as previously.

Author and subject indexes covering the period 1960-64 for both Sections A and B are to be published during 1965. The cumulative author index for Section A, *Physics Abstracts*, contains some 240,000 entries covering 2,300 pages of text and is bound in four parts. Its publication date was May 1965. The cumulative subject index with about 370,000 entries is to follow, as also the indexes for Section B, *Electrical Engineering Abstracts*.

## SYNTHESIS OF RIBONUCLEIC ACID BY DIFFERENT REGIONS OF THE EARLY AMPHIBIAN EMBRYO

By PROF. C. H. WADDINGTON, C.B.E., F.R.S., and MRS. E. PERKOWSKA

Institute of Animal Genetics, University of Edinburgh

THE differentiation of the various regions of an embryo into a number of distinct adult cell types must involve the synthesis in each region of a characteristic battery of proteins. According to present ideas, this must necessitate the production in each region of specific messenger RNAs which 'code for' the proteins. It is also necessary that the embryonic cells should provide themselves with the protein-synthesizing machinery, such as ribosomes, with which they are initially not adequately equipped. One should therefore expect that there are well-defined differences in RNA synthesis both in time and space within an embryo. However, even in well-studied forms, such as the vertebrate egg, we are only just beginning to become acquainted with the basic facts.

The technique which was employed first was autoradiography, and it was shown<sup>1</sup> that shortly after gastrulation begins there is a regionally differentiated pattern of RNA (and protein) synthesis, which is most rapid in the organs of the dorsal axis. This synthesis occurs predominantly in the nuclei, and more specifically in the nucleoli, organelles which have long been suspected, and more recently definitely shown<sup>2,3</sup>, to be concerned with the production of ribosomes. However, the nature of the RNA detected in the autoradiographs remained a matter of conjecture. Biochemical methods have also shown that there is more rapid RNA synthesis in the dorsal parts of the amphibian embryo<sup>4</sup>, and that there are differences in the base compositions of the total RNAs of various regions<sup>5</sup>. The method of centrifugation through a density gradient, which can be used to examine the sedimentation constant

of the various RNAs, has been applied by Brown and Litt<sup>6</sup> to material extracted from whole embryos of *Xenopus*. In most of these the RNA had been labelled with phosphorus-32 incorporated into the oocyte, and available by turn-over to syntheses occurring at later stages, but in some experiments <sup>14</sup>CO<sub>2</sub> was supplied directly to embryos. Studies were made on the sedimentation patterns of RNAs extracted from whole embryos at various stages, but no attempt was made to characterize the different regions or tissues.

The main aim of our experiments was to compare the sedimentation patterns of different regions of the embryo at different stages. They therefore had to depend on the dissection of embryos so as to isolate the regions to be studied. This is bound to be a somewhat laborious task, the difficulty increasing with the precision and detail of the dissections. It was therefore first necessary to discover whether sufficient label for the purposes of sedimentation analysis could be got into the RNA of a reasonably small number of embryonic organs, such as would be practicable to dissect. It was also necessary to discover what labels could be taken up by isolated organs; in *Xenopus* substances such as nucleotides are taken up very badly if at all by uninjured embryos<sup>6</sup>.

We have worked mainly with newt (*Triturus alpestris*) embryos, although a few experiments have been made with axolotl material. As label we used uridine-5-<sup>3</sup>H (from Radiochemical Centre, Amersham, England, 24.4 c./mmole). It was found that this penetrates adequately through the cell plasma membranes which are internal to the embryo, but was absorbed very poorly in isolates

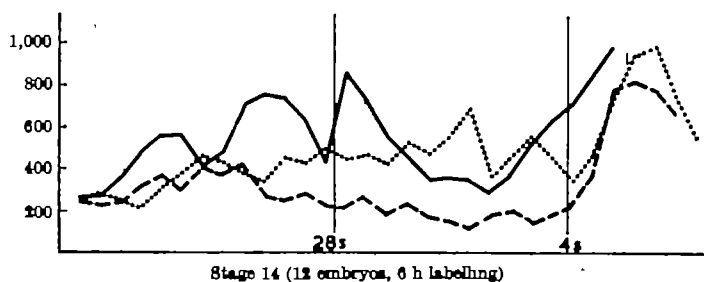


Fig. 1. Sedimentation pattern (heavy material to left) of the three regions of *T. alpestris* embryos at open neural plate stage. —, Axis; ···, ectoderm; ---, endoderm

which are completely covered with the external layer of epithelium, which carries the so-called 'coat'. The intensity of ionization obtainable from a group of explanted organs depends, of course, not only on their mass and the time of exposure to the label, but also on the activity of RNA synthesis. In the preliminary experiment conducted so far, the dissections have been rather crude, merely separating the embryos into three main regions: (a) dorsal axis, including neural system, notochord and somites; (b) endoderm; (c) lateral ectoderm including much of the underlying lateral mesoderm. Using such relatively large fragments, it was found that adequate radioactivity is obtained with no more than 6–8 dorsal pieces (from embryos from early tailbud to hatching stages) exposed to tracer for periods of as little as 2 h. Larger numbers, or longer exposures, are needed for dorsal regions of the open neural plate stage, or for endoderm pieces until near hatching, since in both these regions the rate of RNA synthesis is low. The ectoderm pieces, except from the earlier stages, are very thin, containing little substance, and considerably larger numbers would be required for study of them.

Embryos were freed from the jelly capsules with the usual sterile precautions, and dissected in one of the standard amphibian-embryo salines (Holtfreter's or Barth's) containing antibiotics (penicillin and streptomycin) as well as sulphadiazine. Motile stages were immobilized with *MS 222*, and then a little citrate (to about 0.1 per cent) was added to the dissection medium to facilitate the separation of tissue masses. After exposure to the tracer in solid watch-glasses containing about 1 ml. of medium, the fragments were rinsed in saline, and then processed without being subjected to a chase. After light homogenization, RNA was extracted by the Perry hot phenol method<sup>10</sup>. Since the quantities of RNA in the cultured fragments were not sufficient to give a reading of optical density, an adequate amount of unlabelled *Xenopus* tadpole RNA was added to the preparations, which were then layered on to a 5–25 per cent gradient of sucrose, and centrifuged in the Spinco 25.1 rotor at 2°–8° C for 16 h at 21,000 r.p.m. Fractions were collected in 30–35 tubes, the optical density taken, and the radioactivity measured on a Packard Tri-Carb scintillation counter.

Fig. 1 shows the sedimentation profiles from the three regions of 12 *alpestris* embryos of the open neural plate stage (stages 13–14 of Glaesner) labelled for 6 h. This is the earliest stage from which we have results. It is apparent that RNA synthesis is beginning to become active in the dorsal region, but is scarcely appreciable in the endoderm; in the lateral ectoderm, which was accompanied by most of the lateral mesoderm, there seems to be incorporation into RNA considerably lighter than 28 S, but we do not have results for this region in the immediately succeeding stages of development,

and the significance of this result needs further investigation. The most striking feature of the sedimentation pattern is the importance, in the dorsal region, of incorporation into RNAs which are considerably heavier than 28 S. This pattern is repeated in the dorsal regions from later stages, from tailbud to hatching. Fig. 2 shows the sedimentation patterns from dorsal regions of stages 13–14 (open neural plate, repeated from Fig. 1), from stage 20 (tailbud) and stage 27 (early muscular response). The experiments involved different numbers of embryos (6–10) and different lengths of labelling, and are thus not comparable in the vertical (ionization) scale; the sedimentation patterns have been superposed simply to emphasize the similarity in general pattern, in particular the great importance of heavy RNA.

The sedimentation pattern is somewhat different in the other two regions. In the ectoderm, as already mentioned here, the small mass of the fragments has so far prevented us obtaining clear results, but by stage 27 there is certainly incorporation into 28 S RNA, with

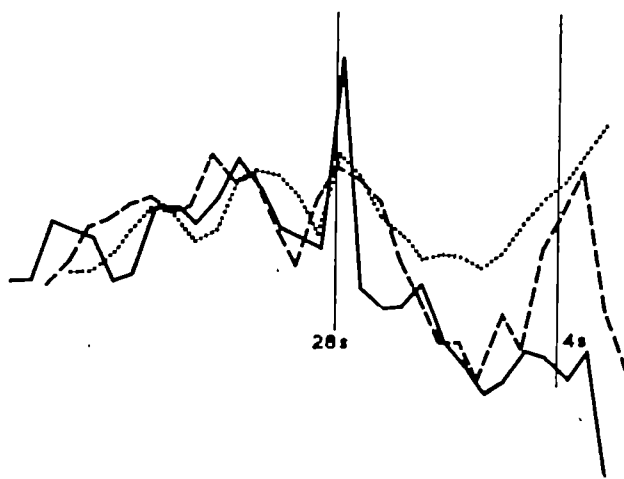


Fig. 2. Comparative sedimentation patterns of dorsal axis at three stages between open neural plate and early muscular response. ···, stage 13–14; ---, stage 20; —, stage 27

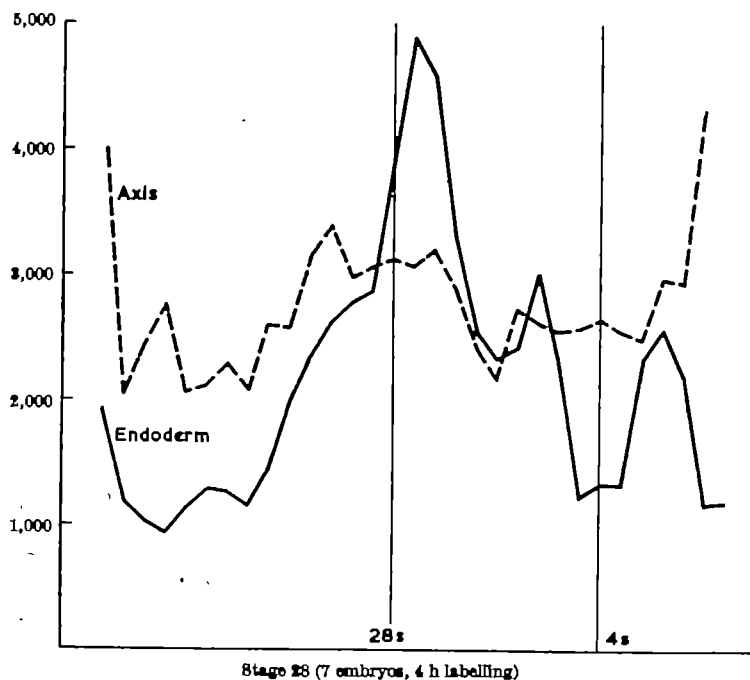


Fig. 3. Comparative sedimentation patterns of dorsal axis and endoderm, shortly before hatching. ---, Axis; —, endoderm

probably some formation of rather heavier material (perhaps about 35 S), but little sign of the much heavier types seen in the dorsal region. In tadpoles shortly before hatching, incorporation into the endoderm has become quite rapid. This is illustrated in Fig. 3, which compares the dorsal region with the endoderm (including the posterior part of the pharyngeal cavity). The mass of the endoderm fragments is considerably greater than that of the dorsal regions, and although the two graphs are plotted on the same co-ordinates, they are not strictly comparable quantitatively. However, qualitatively, they show that the synthesis of heavier types of RNA is much less important in the endoderm than in the dorsal axis. This observation has been made in several other experiments in which these two regions could be compared at stages just before or after hatching.

The significance of the regionally different patterns of RNA synthesis which have been revealed in these preliminary experiments remains to be elucidated. On general grounds one would expect two categories of synthesis to be proceeding: of ribosomal and of messenger RNAs. It would be anticipated that the messengers in different regions would differ in specificities, but there are no grounds on which one might be led to expect them to differ in sedimentation patterns. Concerning the ribosomal RNA, it is difficult to know what one might expect. The ribosomes of different adult tissues do not have RNAs of markedly different sedimentation characteris-

tics, and, although the existence of many DNA cistrons coding for ribosomal RNA<sup>1,2</sup> raises some question whether there may not prove to be tissue-specific differences in these substances, there is again no reason to suppose that these would show up in sedimentation patterns. However, there is evidence that the production of ribosomes may involve, as a first step, the formation of ribosomal-RNA precursors much heavier than 28 S (refs. 2, 9 and 10) and it seems likely that some at least of the heavy material in our sedimentation patterns is of this type. It is not possible to interpret the difference in sedimentation pattern between dorsal axis and endoderm until further analysis has revealed how much of the heavy RNA is ribosomal precursor, and how much messenger or DNA-like. We are planning to use the method of DNA-DNA hybridization to study this problem further.

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- <sup>3</sup> Wallace, H., and Birnstiel, M. L., *Biochim. Biophys. Acta* (in the press).
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## CHANGES IN ELECTRON SPIN RESONANCE SIGNALS OF RAT LIVER DURING CHEMICAL CARCINOGENESIS

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SEVERAL empirical observations and theoretical considerations have suggested a functional relationship between unpaired electrons, such as those associated with free radicals, and carcinogenesis. That aminoazobenzene derivatives most effective as carcinogens may form relatively stable free radicals after being assimilated into tissues was suggested by Kensler, Dexter and Rhoads<sup>1</sup>. Condensed ring compounds which have a relatively strong tendency to acquire an unpaired electron from an alkali metal, forming a relatively stable free radical, are among the more powerful carcinogens<sup>2</sup>. The carcinogenic capability of aromatic compounds may be related to a region of high electron density in the molecule, a condition which might readily occur in a free radical<sup>3</sup>.

Direct observations comparing the concentration of unpaired electrons due to free radicals of metabolic origin in normal and tumour tissues were first reported from this laboratory in 1954<sup>4</sup>. These showed, on the basis of electron spin resonance determination of frozen-dried preparations, that tumour tissue preparations yielded much smaller electron spin resonance signals than comparable normal tissues. Since then we have shown, from measurements on surviving tissues at physiological temperatures, that the electron spin resonance signal at  $g = 2.005$  normally observed in animal tissue such as liver is absent, or nearly so, in surviving tumour tissue<sup>5</sup>.

A direct examination of the relationship between chemical carcinogenesis and the free radicals observed by the electron spin resonance technique in animal tissues has now been carried out. The results reported here show that three chemically different carcinogens, soon after they are administered in the diet, cause the transitory appearance of a distinctive free radical at  $g = 2.035 \pm 0.002$ . This new electron spin resonance signal appears to be the earliest known specific response to the carcinogenic

stimulus. This generalization, if supported by observations on other carcinogenic agents, may provide a useful approach to the problem of cancer detection.

In these experiments electron spin resonance signals were obtained from 50–100 mg samples of surviving liver tissue taken from rats (male and female; Holtzman strain; 150–200 g initial weight) maintained on a semi-synthetic pellet diet. In experiments with *p*-dimethyl-aminoazobenzene (butter yellow) and thioacetamide, the experimental diet contained 0.06 per cent of the carcinogen in a feed deficient in riboflavin (1 mg/100 g diet). In experiments with 2-acetylaminofluorene (AAF) the experimental diet contained 0.06 per cent of the carcinogen in a basic mixture containing 400 mg of riboflavin and 200 mg of DL-tryptophan per 100 g. The control diets were identical with the experimental ones except for the omission of the carcinogens.

At 3–4 day intervals, animals from the experimental and control series were killed, their livers removed and stored on cracked ice for early measurement or stored in the frozen condition for later measurement. For electron spin resonance determination, 50–100 mg of liver slices were prepared, weighed and inserted into a flat electron spin resonance cell in 5 per cent glucose. Measurements were made at 15° C.

The electron spin resonance spectrometer used in these investigations is designed specifically for measurements of aqueous samples at physiological temperatures, and has been described briefly elsewhere<sup>6</sup>. The apparatus was arranged for automatically repeated sweeps of a 200 gauss field, traversed in a 16-sec period, with a modulation amplitude of 16 gauss. The intrinsic time-constant of the instrument is about 500  $\mu$ sec. All measurements were made at a constant value of  $Q$ , thereby fixing the inherent sensitivity of the apparatus. In order to enhance

the signal-to-noise ratio, a series of 50 automatically repeated sweeps were summed in half the channels of a 400-channel multi-channel analyser (RIDL model 34-12B, modified). In the remaining 200 channels a blank was recorded by entering the results of 50 repeated sweeps using the cell containing 5 per cent glucose but no tissue. This blank was automatically subtracted from the experimental signal and the result read out of the analyser by means of an X-Y recorder.

The results obtained in feeding experiments with butter yellow, thioacetamide and AAF are shown in Figs. 1, 2 and 3 respectively, together with the comparable data from control animals. All the liver samples from control animals show the electron spin resonance signal at  $g=2.005$ , half-width 20 gauss, which we have observed previously in liver tissue from a number of laboratory animals<sup>1</sup>. This signal is also present in non-tumorous liver samples from carcinogen-fed animals. However, in all three carcinogen-fed series, the livers exhibit an additional signal not observed hitherto in any normal tissue. In all three cases the new signal occurs at  $g=2.035 \pm 0.002$  and exhibits a half-width of about 20 gauss.

In all three carcinogen series, the  $g=2.035$  signal is transitory. However, Fig. 4 shows that there are striking differences in the times of appearance and disappearance of the observed signal. In rats fed on AAF, the abnormal signal appears within 7 days after feeding starts, rapidly declines in intensity, relative to the normal signal, and disappears after 12 days. When the diet contains butter yellow, the  $g=2.035$  signal makes its first appearance after 14 days of feeding, reaches a maximum intensity at about 20 days and disappears after 40 days. For rats

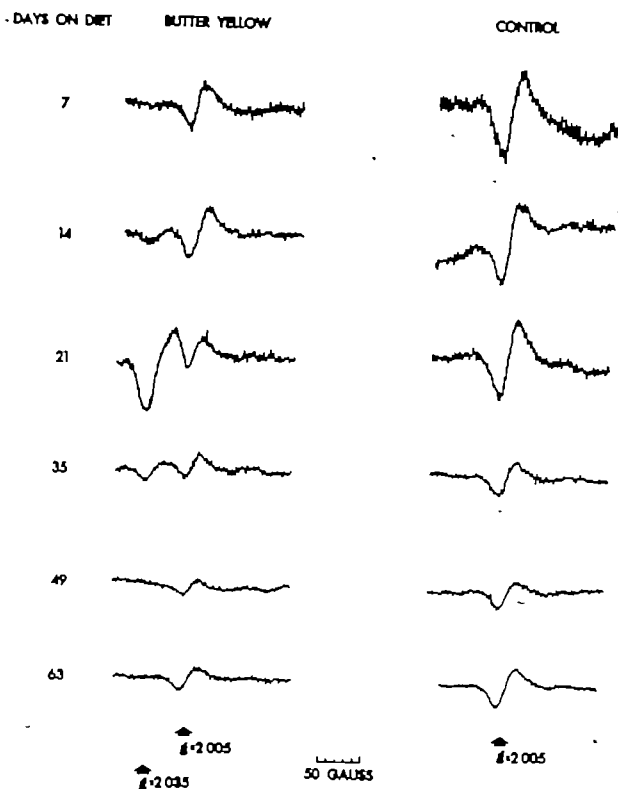


Fig. 1. Electron spin resonance signals from surviving liver samples from rats fed on a riboflavin-deficient diet containing 0.06 per cent of *p*-dimethylamino-azobenzene (butter-yellow), and from rats fed on a control diet lacking the carcinogen. The signals shown represent averages produced by automatic summation, with a multi-channel analyser, of 50 consecutive 16-sec sweeps through a 200 gauss field with a modulation amplitude of 16 gauss; background signals due to the glass cell being automatically subtracted. Measurements were made at an ambient temperature of  $15 \pm 1^\circ \text{C}$ , with the tissue slices suspended in a 5 per cent glucose solution. Instrument gain factors, and the amount of tissue examined (50–100 mg wet weight) varied somewhat from sample to sample so that the absolute heights of the electron spin resonance signals of different samples are not comparable.

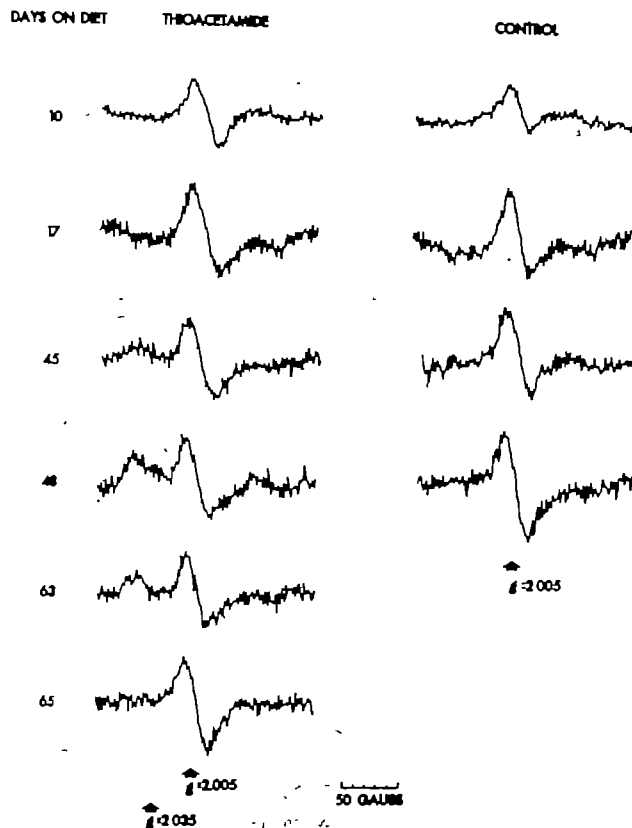


Fig. 2. Electron spin resonance signals of surviving liver samples from rats fed on a riboflavin-deficient diet containing 0.06 per cent of thioacetamide and from rats fed on a control diet lacking the carcinogen. Experimental conditions and instrumental conditions are identical with those described in the legend for Fig. 1.

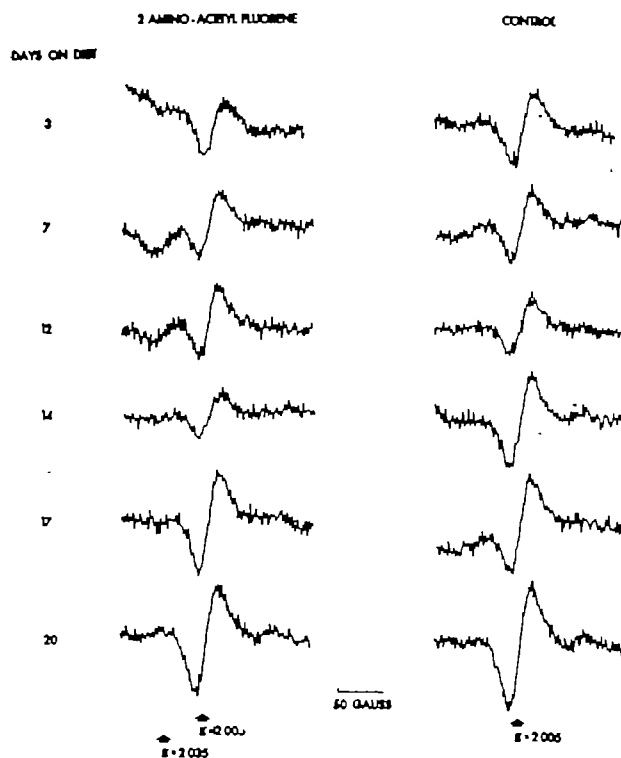


Fig. 3. Electron spin resonance signals of surviving liver samples from rats fed on a diet containing 0.06 per cent 2-acetylaminofluorene (AAF) and from rats fed on a control diet lacking AAF. Experimental and instrumental conditions are the same as those described in the legend for Fig. 1.

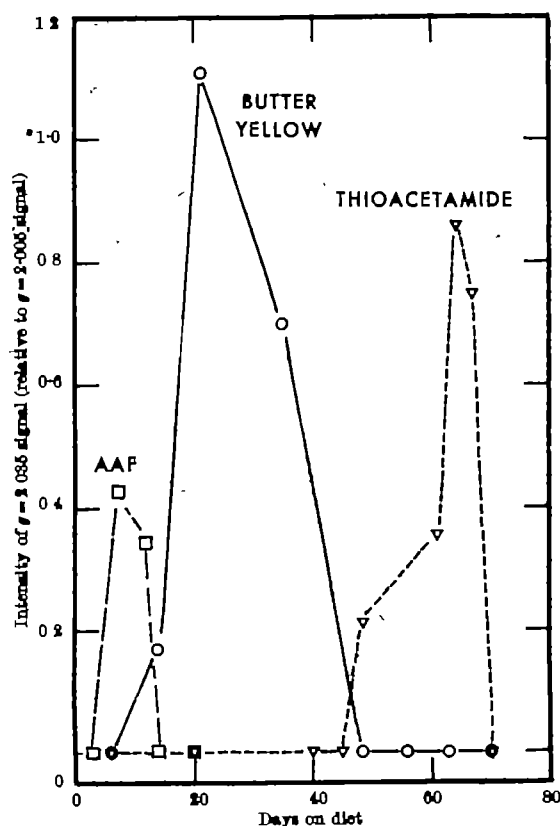


Fig. 4. Variation, with time after the start of the experimental diet, in the intensity of the  $g = 2.035$  signal, relative to the intensity of the  $g = 2.005$  signal in liver samples from rats fed on the three different carcinogens. The ordinate represents the ratio of the amplitude of the  $g = 2.035$  signal to that of the normal  $g = 2.005$  signal of the same sample in the data presented in Figs. 1, 2 and 3.

fed on thioacetamide, the process is even slower, the relevant times being 40, 54 and 61 days.

According to Rees *et al.*<sup>7</sup>, the time required for the appearance of liver tumours in rats fed AAF under conditions similar to those used in our experiments is about 4 months. In the case of rats fed on a butter-yellow diet similar to that used in our experiments, Miller and Miller<sup>8</sup> found that animals developed tumours in about 4–6 months. Rats fed on thioacetamide at the concentration used in our experiments developed tumours only after about 12 months or more<sup>9</sup>. Thus, the abnormal electron spin resonance signal appears, and disappears, long before tumours are in evidence.

Rees *et al.* have reported a series of histological and biochemical observations of livers of rats fed with these three carcinogens. In rats fed with AAF the histological appearance of the liver is 'relatively normal' during the first 5 weeks, although some inflammatory changes and bile duct proliferation are noted<sup>7</sup>. Bile duct proliferation reaches a maximum at 14 weeks. In rats fed on butter-yellow only minimum histological changes are observed in the first 4 weeks<sup>10</sup>. In the next 5 weeks bile duct proliferation increases progressively, and thereafter rather gross histological changes, including nuclear abnormalities, are noted. When rats are fed diets containing thioacetamide, bile duct proliferation is observable only after 6–7 weeks, and histological changes then become gradually more intense<sup>10</sup>. Biochemical investigations of the DNA and RNA contents of livers from rats fed on these carcinogens show no variations which can be correlated with the time required for appearance of tumours<sup>7,10</sup>.

From these results it is apparent that when the abnormal electron spin resonance signal which we have observed in the livers of the carcinogen-fed rats is first detected, that is, 6, 14 and 40 days for AAF, butter-yellow and thioacetamide respectively, histological changes are

minimal and non-specific. In these instances, the electron spin resonance signal at  $g = 2.035$  appears to be the earliest known unequivocal sign that the tissue is later to become tumorous.

Additional evidence that the signal at  $g = 2.035$  is specifically related to the process of carcinogenesis is suggested by the apparent identity of the signals observed in the three experimental series, both with respect to  $g$ -value and half-width. The three carcinogens used in these experiments are quite different in molecular structure; they have in common only their carcinogenic capability, and that they induce the appearance of an apparently identical free radical in the affected liver. Moreover, a series of similar diet studies on rats fed a wide variety of pharmacologically active drugs with no known carcinogenic activity, carried out by Dr. John J. Heise in this laboratory, failed to induce the appearance of the  $g = 2.035$  signal in the liver.

Preliminary efforts have been made to identify the source of the  $g = 2.035$  signal. The abnormal signal is absent from mitochondria isolated from liver of animals fed butter-yellow when the whole tissue exhibits the signal. It has not been possible thus far to observe the abnormal signal in any of the usual fractions of tissue homogenates. Unlike the normal signal at  $g = 2.005$ , which is readily observed in isolated mitochondria, the free radical which gives rise to the signal at  $g = 2.035$  does not appear to withstand the procedures involved in fractionation and isolation.

That three chemically different carcinogens induce the appearance of an apparently identical electron spin resonance signal tends to militate against the possibility that the abnormal signal is due to a free radical form of the carcinogen itself, or of one of its metabolic derivatives. As a first approximation it would appear that the  $g = 2.035$  signal is due to unpaired electrons associated with some cellular component rather than with the carcinogen or a derivative of the latter.

During the course of these experiments we were able to confirm our earlier observation that the electron spin resonance signal at  $g = 2.005$  commonly observed in normal liver tissue is absent from tumour tissue. Fig. 5 shows the electron spin resonance spectra observed concurrently from a butter-yellow induced hepatoma and from a non-tumorous region (as indicated in histological sections) in the same liver. The  $g = 2.005$  signal typical of normal liver tissue is observed in the non-tumorous region, whereas no detectable signal is present in the tumour proper. Fig. 6 shows electron spin resonance

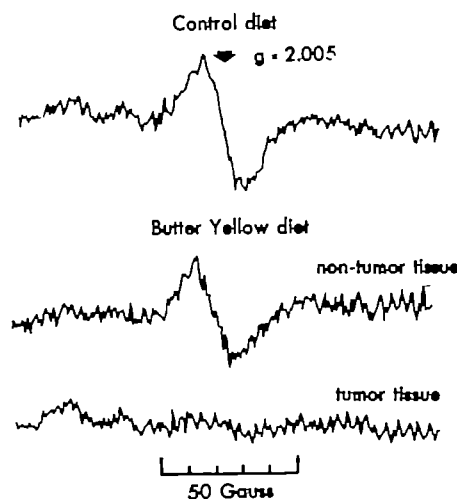


Fig. 5. Electron spin resonance signals from tumorous and non-tumorous regions of the same rat-liver. The liver tumour was induced by feeding a rat on a diet containing 0.06 per cent butter yellow. Electron spin resonance observations were made under the same conditions as described in the legend for Fig. 1.



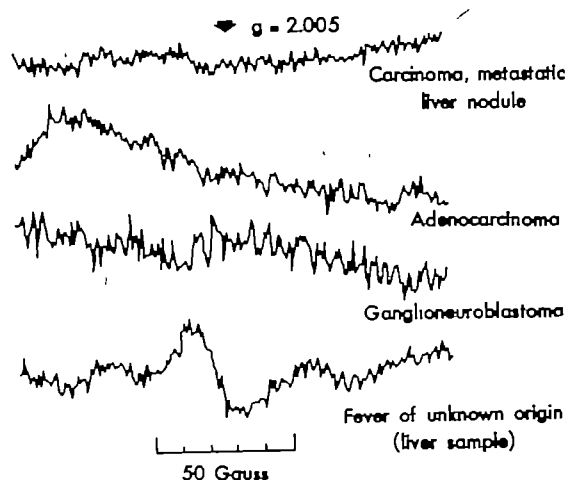


Fig. 6. Electron spin resonance signals from various human liver biopsy samples. All curves except the lowest one are from liver tumours. The latter represents liver sample from a patient with an undiagnosed fever. The electron spin resonance observations were made under the same conditions as described in the legend for Fig. 1.

spectra obtained from several human liver tumours of different origin. In all cases, the  $g = 2.005$  signal, which is typically present in normal liver, is absent in the tumour tissue. The same figure shows that a liver biopsy taken in the case of a non-tumour disease yields the normal signal at  $g = 2.005$ .

The results reported here show that, in the case of the three substances tested, chemical carcinogenesis is associated with certain characteristic changes in the free radicals detectable in rat liver. In all cases a signal at  $g = 2.035$ , which is not present in normal liver, appears shortly after the carcinogen is added to the diet, rises to a maximum intensity and disappears long before carcinoma is evident either grossly or microscopically. At this time the signal at  $g = 2.005$ , which is typical of normal tissue, remains; but this signal disappears when the carcinoma itself develops. Malignant tumours typically exhibit no detectable electron spin resonance signals.

These results are thus far empirical in the sense that we have no evidence as to the biochemical origin of the

abnormal electron spin resonance signal. If, as we suggest, the signal at  $g = 2.035$  is due not to a free radical form of the carcinogen, but to the generation of unpaired electrons in some structure or constituent of the cell itself, then the primary effect of the carcinogen may be on the cell's electron-transport system. Such an effect would be in accord with the concept, long under discussion, that carcinogenesis involves a transformation of cellular oxidation-reduction processes.

The present results suggest a possible new approach to the early detection of the onset of cancer. If the electron spin resonance signal at  $g = 2.035$  is an early and specific sign of later tumour formation, then it should be possible by electron spin resonance analysis of a biopsy to determine whether a tissue with suspicious but not frankly tumorous pathology is likely to later develop into a tumour. So far, this conclusion seems warranted only for the three specific carcinogens which we have studied, but with further investigations of this type it should be possible to determine whether a wider generalization—on which a diagnostic scheme for early detection of cancer might be based—is valid. Such studies are in progress in this laboratory.

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## AN EARLY POTENTIAL EVOKED BY LIGHT FROM THE PIGMENT EPITHELIUM-CHOROID COMPLEX OF THE EYE OF THE TOAD

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A POTENTIAL with no detectable latency was recently found in the monkey retina by Brown and Murakami<sup>1</sup>, and this response proved biphasic in form<sup>2</sup>. It was found by microelectrode methods, and identified by rigorous criteria as generated by the receptors; hence it was named the early receptor potential (RP)<sup>1-3</sup>. Higher stimulus intensities have made it possible to record this response from excised eyes by means of gross electrodes<sup>4</sup>. Investigations by this method have shown that in the pure rod eye of the rat the intervening photopigment is rhodopsin, rather than some accessory pigment<sup>4,5</sup>. Thus the early RP is an extremely rapid consequence of the absorption of light by the photopigment. The physiological significance of this response is of great interest, but is not yet determined. One question which arises is whether this type of response is unique to vertebrate photoreceptors, or whether it occurs in other cells which possess pigments for absorbing light.

A major advantage of studying isolated eyes is that the retina may be separated from the remaining eye cup, and certain variables may be controlled more readily than in eyes *in situ*. The early RP of the monkey is extremely insensitive to anoxia, and even to death of the animal<sup>1,2</sup>. During investigations of the early RP, therefore, one may use the advantages of isolated eyes without sacrificing normality of the response. Thus work was undertaken on the isolated eye of a toad (*Bufo marinus*), which is readily obtainable in Canberra. Observations on the eye cup, after removing the retina, revealed a new rapid biphasic response reported here.

In this work the animal was pitied, and the eye enucleated in normal room lighting, following which three types of preparations were made by dissection under dim red light. In one preparation a cut was made around the eye, so that the cornea, lens and ciliary body could be removed as a unit. This preparation, consisting of the eye cup with the retina, was placed on a cotton

wick electrode soaked with frog Ringer's solution. A second cotton wick electrode made contact with the vitreous humour inside the eye. In a second type of preparation the optic nerve was first shaved off with a razor blade, taking a small portion of sclera with the nerve; this freed the retina from its attachment at the optic disk. The front of the eye was then removed, and a piece of dry cotton wool was shaped to fit the eye cup and inserted into it. After a few moments the cotton adhered to the retina, which was easily removed. The cotton was then used to support the retina, which was mounted with the receptors facing upwards. The cotton was soaked with Ringer's solution, to act as one electrode, and the other wick electrode was placed in contact with the receptor surface near its centre. Microscopic examination of retinas removed in this manner showed the outer segments packed in solid array over the entire receptor surface. There was no indication that any outer segments remained with the eye cup, except perhaps at the cut edges of the retina, and no pigment cells adhered to the receptor surface. Thus the method of removing the retina provided a satisfactory separation between the retina and pigment epithelium. The third preparation was the eye cup without the retina. This preparation could be made by the procedure for isolating the retina, and a number of experiments were performed with this preparation. When the retina is removed from the eye cup, however, the remaining tissue is subject to rapid drying. Ringer's solution could be placed in the eye cup, but it quickly drained out through the hole at the optic disk. This problem was solved by leaving the stump of the optic nerve intact. After removing the front of the eye dry cotton was fitted to the eye cup. The cotton was used to pull the retina free from the eye cup, except for the attachment at the optic disk, which was carefully cut. Ringer's solution was then placed in the eye cup, which was mounted as in the case of the eye cup with the retina.

Each cotton wick electrode passed into a glass tube filled with Ringer's solution. A platinum wire was

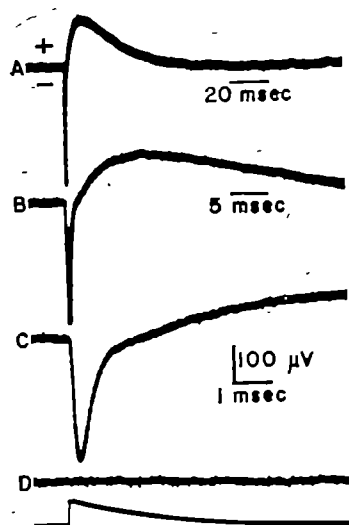


Fig. 1. Responses from the eye cup without the retina. Records A-C are from the same preparation at increasingly faster time bases, to illustrate the time course of the response from the pigment epithelium-choroid complex. Record D is a control response from the sclera alone, after removing the pigment epithelium and choroid from the preparation. The sharp upward deflection in the lowest record shows the time of onset of the stimulus. The voltage calibration holds for records A-D; the 1 msec time calibration holds for records C and D, and for the record showing onset of the stimulus.

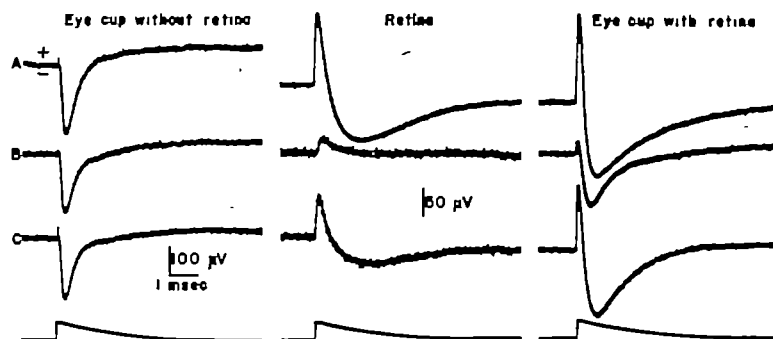


Fig. 2. The effects of light adaptation, followed by dark adaptation, on all three preparations. In each column record A is the first response. The preparation was then stimulated at 1-min intervals for 15 consecutive flashes, and record B is the response to the fifteenth stimulus. This was followed by a 15-min period of dark adaptation, to permit recovery from light adaptation, and record C is the first response after this period of dark adaptation. In all responses an upward deflection indicates positivity of the vitreous side of the preparation. In the bottom record of each column, the sharp break shows time of onset of the stimulus. The time calibration holds for all records. The 100  $\mu$ V voltage calibration holds for records of the left and right columns, and for the top record of the middle column. The amplitude of the response from the isolated retina decreased especially rapidly, however, and the 50  $\mu$ V calibration holds for records B and C from the isolated retina.

inserted into the solution at the other end of the tube, and that end of the tube was sealed by wax. The glass tubes were then wrapped with black tape, to avoid photoelectric artefacts at the fluid-metal junction. Discharge of the condenser in the light source caused a small electric artefact, which was recorded occasionally as a positive pip, as shown in two records of Fig. 2. This proved useful for measuring response latencies. Amplification was by a Tektronix type 2A61 differential amplifier in a type RM 565 oscilloscope. The polarity of all records was such that an upward deflection indicates positivity of the vitreous side of the preparation. The frequency response was flat from 6 to 60,000 c/s, which was sufficient to give undistorted responses.

Light flashes were provided by the condenser-discharge flash of a photographic lamp (Honeywell 'Stroboscan 650'). This lamp was mounted outside the recording room, and the light was led to the preparation by a flexible fibre optics bundle having a numerical aperture of 0.55 (Amar. Opt. type LG5-36). One end of this fibre optics bundle was placed close to the flash tube, the front cover of the lamp having been removed; the other end was placed directly over the preparation. The diameter of the fibre optics bundle was about 6 mm, which fits well to the size of the eyes examined, so an intense brief flash was delivered to the entire preparation. The timing of the flash was monitored by a solid-state photovoltaic detector (Hoffman type HA7E3), and displayed on the second beam of the oscilloscope. This detector has a rise time of about 20  $\mu$ sec, so the onset of the flash was accurately recorded, but the decay in the record appears not to reflect the true time course of the light flash. The effective exposure time of the flash is stated by the manufacturer as 0.7 msec.

Fig. 1 shows responses from the eye cup without the retina. Record A shows that the new response is biphasic in form, consisting of a rapid negative phase followed by a slower positive phase. Following the positive phase, there is no indication that there are any later portions of this response. Record B, at faster time base, resolves both phases of the response, while record C shows more clearly the rapid first phase. Record D was obtained after removing the pigment epithelium-choroid complex from the eye cup, leaving only the sclera, which was partially filled with Ringer's solution. No sign of the new response was obtained under these control conditions, or when the cotton wick electrodes were in direct contact. Thus the responses of records A-C cannot be either electric or photoelectric artefacts. The possibility was also considered that the response might not occur unless Ringer's solution was added to the

preparation. Similar but smaller responses were obtained, however, without adding Ringer's solution to the eye cup. Similar responses were also recorded, although with a higher noise level, when the wick electrodes were filled only with distilled water. Since no Ringer's solution was in contact with the preparation in this case, the new response cannot be a special type of artefact resulting from the addition of Ringer's solution. The new response was not detected from the sclera alone. But it has been detected from the isolated pigment epithelium-choroid complex, after removal of this tissue from the sclera by procedures similar to those for isolating the retina. Thus the new response must be generated by the action of light on the pigment epithelium-choroid complex.

Fig. 2 shows the effects of light adaptation, followed by dark adaptation, on all three preparations. In each column response *A* was elicited by the first flash. The preparation was then light adapted by stimulating at 1-min intervals for a total of 15 flashes, and response *B* was elicited by the 15th flash. The preparation was then left in darkness for 15 min, to observe recovery from light adaptation, and response *C* was elicited by a flash at the end of this recovery period. The response of the eye cup without the retina consistently decreased in amplitude during the period of stimulation, but this may have resulted from slight drying of the preparation. In some cases there was a small recovery of amplitude after the period in darkness, as shown in Fig. 2. But this effect was not always found, and was small in amplitude when it occurred. Thus if there is any effect of this light-adapting procedure on the new response, the effect is very small and has not been clearly demonstrated. Similar results were obtained when light adapting by flashes at 15-sec intervals. Hence the first phase of the new response is remarkably resistant to light adaptation.

In the isolated retina the first and second phases of the early RP were about equal in amplitude prior to light adaptation. By comparison with previously published records, the amplitude of the first phase is exceptionally large. Thus the isolated toad retina is a favourable case for studying both phases of the early RP in the same response. It has previously been shown that the early RP may be abolished<sup>1</sup> or reduced<sup>4</sup> by light stimulation, and in the isolated toad retina the amplitude of the early RP declined rapidly with successive flashes. The second phase declined more rapidly, however, than the first phase. After about the 10th flash the second phase was entirely abolished, but a small first phase remained, and the amplitude of the first phase was rather stable during further flashes. Thus response *B* shows the relatively stable response which could be obtained after about the 10th flash. During the subsequent period of dark adaptation, partial recovery occurred for both phases of the early RP.

The different effects of light adaptation on the early RP, and on the new response, provide a strong distinction between these two responses. It is also of special interest that the second phase of the early RP is more affected by light adaptation than the first phase. This shows that the two phases of the early RP are generated by somewhat different mechanisms. This has likewise been shown by differential blocking of the second phase of the response at very low temperatures<sup>5,6</sup>. Since light adaptation is a normal physiological type of factor, the present findings show a degree of independence of the two phases in their normal functions, as well as in their mechanisms of generation. Light adaptation also provides a convenient method for isolating the first phase of the early RP from the second phase at physiological temperatures. Previous investigations have shown that the quantitative effects of light adaptation become more marked as they are traced through the early stages of the visual system<sup>7,8</sup>. It is noteworthy that this principle holds also for the first and second phases of the early RP.

As the eye cup with the retina was light adapted, the first phase of the response decreased more rapidly than the second phase. The responses became rather stable after about the 10th flash, and in this case response *B* shows a small first phase followed by a larger second phase. Thus the qualitative effects of light adaptation on the whole preparation are superficially opposite to those on the retina alone. This is obviously due to the new response, which is not from the retina, and which strongly resists light adaptation. In this case response *B* must contain a small first phase of the early RP. But what appears to be the second phase of the early RP consists largely of the first phase of the new response. Note that the latency to the second peak of the response decreases with light adaptation, as the early RP declines and as the second peak becomes more dominated by the new response. Thus the new response is crucial for understanding the effects of light adaptation on the whole preparation.

The top half of Fig. 3 shows initial responses from the retina (*R*), and from the eye cup (*E*), which are superimposed for comparison of their time courses. When the stimulus artefact occurred in recordings of the early RP, the response was already above the baseline by the end of the artefact. Since the artefact had a duration of slightly less than 10  $\mu$ sec, the latency of the early RP is even less. This is the shortest latency measured so far, but is still a maximum value, and the rising first phase of the early RP appears to extrapolate to the leading edge of the artefact. Thus the early RP has very little or no true latency on a  $\mu$ sec scale. The new response rises just after the artefact and appears to have a true latency, although a very short one, of the order of 25–30  $\mu$ sec. It also rises somewhat more slowly than the early RP. The time from onset of the stimulus to the initial response peak is about 0.2 msec for the early RP and about 0.3 msec for the new response. The second peak of the early RP occurs about 1.75 msec after onset of the stimulus, while the second peak of the new response occurs after about 11 msec. The first phase of the new response overlaps both phases of the early RP, as shown in Fig. 3, but the second phase of

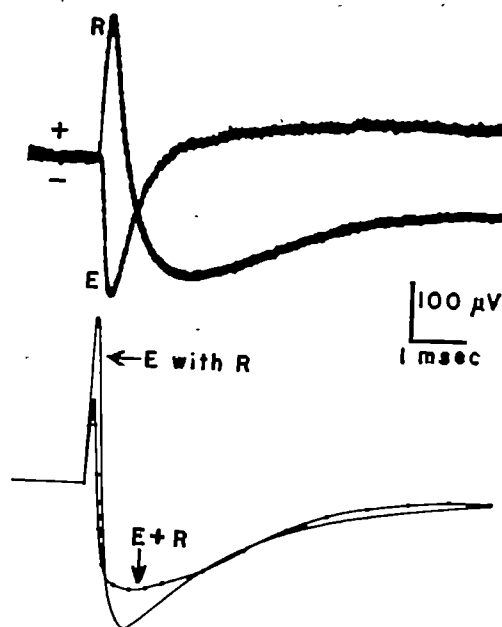


Fig. 3. The top half shows responses of the eye cup without the retina (*E*), and of the isolated retina (*R*), which are superimposed for comparison of their time courses. In the bottom half of the figure the curve labelled '*E + R*' is the sum of the separate responses, and the superimposed curve labelled '*E with R*' is a tracing of the actual response from the eye cup with the retina. The responses used in this figure are the initial responses from the three preparations of Fig. 2.

the new response is much slower than the early RP. The lower half of Fig. 3 shows the sum of responses *E* and *R*, and a superimposed tracing of the initial response from the eye cup with the retina. By comparison with the response of the retina alone, the sum of the two responses has a slightly earlier first peak, a distinctly faster fall from the first peak to the baseline, and an earlier second peak. On these aspects of the time course, the actual response in Fig. 3 fits satisfactorily to the sum of the two curves. Initial responses from the eye cup with the retina consistently showed such differences in time course from initial responses of the isolated retina. Thus the new response also accounts for these differences in time course between initial responses of the isolated retina and of the whole preparation.

In Fig. 3 the peaks of the curve '*E+R*' are not as high as those of the curve '*E* with *R*'. This comparison assumes that the responses of the retina alone, and of the eye cup without the retina, are the same as the responses when they are recorded in the total preparation. This assumption is not fully met, which has intrinsic interest. Light adaptation reduced the initial peak of the early RP more rapidly, and to a greater extent, when the retina was isolated than when the retina was in the eye cup. This is probably because recovery between stimuli was more efficient when the retina was in the eye cup. This is supported by the finding that after the 15 min recovery period the initial peak of the early RP from the isolated retina increased by a factor of only about 3, while that from the retina in the eye cup increased by about 6. This difference in recovery rates may be expected from Dowling's evidence that the pigment epithelium normally acts as a storage site for vitamin A, which is released from the photopigment during bleaching, and that this vitamin A can be returned to the receptors for regeneration of photopigment in the dark<sup>6</sup>. Because of the more efficient recovery processes, the early RP from the retina in the eye cup must be larger than that from the isolated retina. Light adaptation not only reduces the amplitude of the early RP, but in the isolated retina may be seen to slow the rate of rise and delay the initial peak. Thus the early RP of the retina in the eye cup is not only larger; it must also have a faster time course. These factors pertain even for initial responses, because of the light adaptation which occurred during dissection of the eye. These factors seem to account for the lower peaks in the summed response than in responses from the whole preparation. The departure from additivity becomes more marked during light adaptation, as may be expected. Summation of the separate responses in records *B* of Fig. 2 would not predict any appearance of the first phase of the early RP, but such a response always appeared when the retina was in the eye cup.

Previously published records by gross recording methods have all been from whole excised eyes, and some quantitative functions have been determined on excised eyes of the albino rat<sup>4-6</sup>. It is difficult to record from isolated tissues of the rat eye, because of its small size. But I have performed the type of experiment in the right column of Fig. 2 on the whole excised eye of the albino rat, with results comparable to those obtained in the toad. Responses to the first few flashes contained a prominent positive first peak, and the latencies to the first and second peaks of the initial response were about 0.15 and 1.5 msec, respectively. With light adaptation the first peak declined quickly, and in this animal disappeared entirely after the third flash. The negative second peak also declined, although more slowly; its latency decreased to about 0.75 msec, and it proved highly resistant to light adaptation. It seems evident that this negative response of the light-adapted rat eye must consist largely of the type of response found from the pigment epithelium-choroid complex of the toad eye. It has the same polarity, a peak latency which is intermediate between that of the first and second peaks of initial responses, and, most

important, a much higher resistance to light adaptation than the initial phase of the early RP.

Previous investigations have not revealed a distinct positive peak of the early RP in normal responses of the rat eye. This may have been due to more light-adapted preparations, since the first peak disappears quickly with light adaptation. In any case, this absence of the positive first peak suggests that the contribution of the early RP to the responses has been relatively small. Also the latency to the negative peak in published records is in good agreement with the latency I find to the peak of the light-adapted response from the whole rat eye. These findings make it quite likely that investigations of the negative peak of the whole rat eye have pertained largely to the new type of response. Thus it now seems required that separate investigations be conducted on the early RP of the isolated retina, and on the new response of the eye cup without the retina. In microelectrode work the amplitude maxima of both peaks of the early RP of the monkey have been found at about the level of the inner segments of the receptors<sup>8</sup>. Hence the early RP may be separated from the new response to some extent by adjustment of microelectrode depth, but even with this method the early RP is probably contaminated by the new type of response. Isolated preparations seem to offer the best solution to this problem.

Since the albino rat lacks melanin, but appears to have the new response, it is very unlikely that melanin absorbs the light which initiates this response. Thus there is no reason to expect that the new response is generated by choroidal cells. The cells of the pigment epithelium, however, contain numerous myeloid bodies in both frog<sup>10</sup> and albino rat<sup>11</sup>. These are intracellular organelles, the lamellated structure of which is typical of light-sensitive structures<sup>10,11</sup>. When the albino rat is maintained on vitamin A acid these myeloid bodies, like the rod outer segments, degenerate; both structures then regenerate on a normal vitamin A diet<sup>11</sup>. This suggests that the myeloid bodies contain a substance which is synthesized from the same isomer of vitamin A which is required for the synthesis of rhodopsin in the outer segments. Thus the myeloid bodies may contain a photopigment, either rhodopsin or a closely related substance. In good agreement with this possibility is Cone's finding that the negative peak of the rapid response from the whole eye of the albino rat has a spectral response curve similar to the absorption spectrum of rhodopsin<sup>4</sup> and that the cells of the pigment epithelium generate the c-wave of the electroretinogram<sup>12-14</sup>. The c-wave occurs in the toad eye, and is not found from the isolated toad retina<sup>15</sup>. But the c-wave has not been recorded from the eye cup of any animal after removing the retina. Discovery of a rapid response from the pigment epithelium-choroid complex of the toad suggests that the c-wave may be initiated in the pigment cells by an intrinsic photosensitivity. One wonders, in fact, whether the second phase of the response reported here may be a c-wave elicited by the brief light flash. This second phase of the new response seems too slow to compare with the early RP, and probably has a different significance.

It likewise seems especially important to know whether the first phase of the new response is generated in fundamentally the same way as the first phase of the early RP. If so, this is a type of response which is not unique to vertebrate photoreceptors, but which occurs more generally following the absorption of light by a pigment. Opposite polarities of the first phases of the two responses do not seem significant in this regard, since this could result simply from opposite orientation of the generating structures. If the two responses are fundamentally the same, the new response seems especially favourable for investigating the mechanism of generation. This is primarily because of the great stability of the response when elicited repeatedly by intense light flashes. Naming of the new response will be deferred until there is further

information on these problems of its origin and nature, work on which is now being undertaken.

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## ACETYLCHOLINE RELEASE FROM THE CEREBRAL AND CEREBELLAR CORTICES: ITS ROLE IN CORTICAL AROUSAL

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THE relationship between the functional state of the brain and its acetylcholine (ACh) content has been the subject of a number of investigations. Variations in the ACh level have been correlated with physiological changes in nervous activity, such as those occurring in the transition from sleep to wakefulness, or during the action of anaesthetics and convulsants<sup>1-4</sup>. In general, states of depression of the nervous system are associated with an increase in the acetylcholine content<sup>5</sup>. More specifically, the existence of a similar relationship between activity in the cerebral cortex and its ACh content has been demonstrated<sup>6</sup>, and investigations on the release of ACh into pools of eserinized saline on the surface of the exposed cerebral cortex<sup>7,8</sup> support the interpretation that increased cortical activity results in the conversion of 'bound' acetylcholine into a freely diffusible form. The mammalian cerebral cortex contains a substantial amount of choline acetyltransferase, the presence of which is largely dependent on the integrity of subcortical connexions<sup>9</sup>, and it is probable that these are constituted by the cholinesterase-staining fibres, which project from the reticular formation to the cerebral cortex<sup>10</sup>. An association between the reticular 'activating' formation and cholinergic transmission in the cerebral cortex has also been postulated as a result of pharmacological investigations on the electroencephalogram (EEG) of cats with mid-brain hemisection<sup>6</sup>.

An increase in the ACh release during peripheral nerve stimulation which was restricted to the contralateral primary somatosensory cortex has resulted in the suggestion that the cholinergic fibres to the cortex may be associated with the primary afferent pathways<sup>6</sup>, though this has not been supported by pharmacological investigations with topically and iontophoretically applied atropine<sup>11,12</sup>, which failed to block primary evoked responses.

Many cells in the cerebellar cortex are strongly excited by acetylcholine<sup>13</sup>, and the presence of cholinesterase-staining fibres in the cerebellar peduncles<sup>14</sup>, which also contain substantial amounts of choline acetyltransferase<sup>15,16</sup>, suggests that cholinergic transmission occurs in the cerebellar cortex<sup>17</sup>. It was of interest to ascertain whether ACh release also occurred from the cerebellum and to compare this with the results from cerebral cortex.

Cylindrical 'Perspex' cups, with an inside diameter of 1 cm, were held against the surface of the cortex of cats in such a manner that fluid placed within the cup could not leak out<sup>8</sup>. Several cups were used simultaneously in most experiments, distributed bilaterally on the sensorimotor, auditory and visual primary cerebral cortical areas and over the parietal cortex, covering suprasylvian and lateral gyri. Cerebellar cups were placed on the posterior vermal cortex. After placement of the cups, all exposed

cortical surfaces were covered with a 4 per cent agar jelly. The cups were filled for a period of 30 min with a mammalian Ringer-Locke solution containing  $1 \times 10^{-4}$  g/ml. eserine. They were then refilled with 0.4 ml. of fresh eserinized solution which was left in contact with the cortex for periods of 15-30 min. Samples were assayed on the heart of *Tapes virgida*<sup>18,19</sup> and some samples from cerebral cortex were cross-assayed on cat blood pressure. Pentobarbital sodium was used to anaesthetize the cats during these experiments. Four *enéphale isolé* preparations were also utilized to compare the rates of acetylcholine release from unanaesthetized preparations. The use of a highly sensitive bioassay preparation made it unnecessary to atropinize the animals before collecting ACh (see ref. 8). EEG recording from the area within the cups was by monopolar silver leads with an indifferent lead on the neck muscles, and recording screws were inserted into the bone in unexposed areas.

The results from experiments on cerebral cortex are summarized in Tables 1 and 2. Release of ACh from the unstimulated cortex was comparable in all the areas tested; this accords with the finding that the ACh content of different areas of the cat cortex is similar<sup>4</sup>. The amount of acetylcholine released could be increased by direct stimulation of the cortex underlying the cups and by stimulating a variety of peripheral afferent pathways (limb, facial and auditory stimulation). In disagreement with the results described by Mitchell, however, was the finding that peripheral stimulation caused an increase in the ACh output into all cups. Increasing the depth of anaesthesia resulted in a reduction in the release of ACh, and intravenous administration of atropine (1-5 mg/kg) was followed by an elevation in the release. Release from the cortex of *enéphale isolé* preparations into cups on the sensori-motor and parietal cortices was comparable with that from the more lightly anaesthetized cats. Atropine applied topically on three *enéphale isolé* preparations increased the mean rate of unstimulated release from 0.32 to 1.05 ng/min/cm<sup>2</sup>. Fig. 1 illustrates some of the effects on EEG tracings of eserine and atropine applied

Table 1. MEAN RATE OF ACETYLCHOLINE RELEASE FROM UNSTIMULATED CORTICES

A. CEREBRAL <i>Anaesthetized cats</i> Region:	No. of cats	Rates expressed in ng/min/cm <sup>2</sup>		
		Rate in first 30 min	Rate in second 30 min	Rate in first hour
Left sensori-motor cortex	10	0.27	0.39	0.33 ± 0.10 (S.E.)
Right sensori-motor cortex	9	0.14	0.16	0.16 ± 0.07
Auditory cortex	7	0.08	0.27	0.17 ± 0.09
Visual cortex	4	0.09	0.07	0.08
Parietal cortex	2	0.14	0.17	0.16
<i>Enéphale isolé cats</i>				
Right sensori motor cortex	3	0.45	0.45	0.45
Parietal cortex	3	0.165	0.24	0.20
B. CEREBELLAR				
Posterior vermal	6	0.029	0.034	0.0285 ± 0.006

Table 2. MEAN PERCENTAGE INCREASE IN RATE OF RELEASE INDUCED BY VARIOUS MODES OF STIMULATION\*

	Left forepaw	Left forepaw	Facial	Auditory	Direct
<b>A. CEREBRAL CORTEX</b>					
Right sensor-motor cortex	200 (25-650)†	100 (20-210)	100	100 (20-170)	210 (33-400)
Left sensor-motor cortex	250 (0-650)	50 (0-100)	—	110 (0-300)	—
Right auditory cortex	550 (180-660)	—	—	85 (0-190)	—
Right visual cortex	70 (25-110)	—	—	80 (55-100)	—
Parietal cortex	20 (5-60)	—	—	—	—
<b>B. CEREBELLAR CORTEX</b>					
Posterior vermis	—	—	—	—	150 (73-300)‡

\* Rate of release immediately before and after stimulation used to calculate baseline for these figures.

† Ranges.

‡ Mean of 24 observations.

topically on the cortex of *enophthalis* cats. Eserine caused a decrease in the amount of synchronized activity, the EEG more closely resembling the diffusely activated pattern seen during cortical arousal. Application of atropine, in conjunction with eserine, was followed by the development of a sleep-like EEG with synchronization of cortical activity and spindling. This was associated with a failure of previously effective auditory stimuli to induce an arousal response. Intravenously administered atropine caused similar, though even more striking, changes in the EEG comparable with those that have already been described by other investigators, who have also described the dissociation between EEG and behavioural activity that is seen in the atropinized animal<sup>12-13</sup>. In cats, dogs, rabbits and monkeys, atropine induces EEG changes in the conscious animal identical with those of natural sleep (high voltage waves and bursts of spindles) and it has also been observed that atropine makes it difficult to elicit an alerting reaction even though the animal is behaviourally awake. It is evident from the effects that have been described that topically applied atropine will duplicate a substantial proportion of the phenomena which occur after intravenous administration of this drug, which are probably, therefore, the result of an action on the cerebral cortex.

The cerebellar cortex contains only approximately 1/10 of the amounts of acetylcholine and choline acetyltransferase that are present in the cerebral cortex (Phillis, unpublished observations; ref. 16), which is consistent with the finding that the amount of ACh released was proportionately reduced. A release of ACh from the unstimulated cerebellar cortex was consistently demonstrable and this was increased by direct stimulation of the surface of the cerebellar cortex.

Mitchell<sup>6</sup> has discussed the reasons for assuming that the ACh released into cups placed on the cerebral cortex actually originated from within the cortex and is not of

sub-cortical or vascular origin. A comparison of the levels of release from the cerebral cortex in the two series of experiments reveals that the rate was less by a factor of approximately 1/10 in our cats. This is probably the result of Mitchell's use of atropine to increase the level of release and thus facilitate the assay of acetylcholine, since atropine, administered intravenously in doses of 1-5 mg/kg to four cats, caused a mean increase of 0.36 ng/min/cm<sup>2</sup> in the rate of release of ACh. Peripheral stimulation through a variety of afferent pathways caused an increase in the rate of ACh release in all the areas tested. A comparison of the figures in Table 2 shows that the level of increased release was not determined by the modality of stimulation in relation to the specific primary areas involved. Forepaw stimulation caused a comparable increase in the level of release from the ipsilateral and contralateral sensor-motor cortices and from the contralateral auditory cortex. The reason for the disparity between these results and those described by Mitchell, who recorded an increase in the rate of release during forepaw stimulation only in the contralateral sensor-motor area, is not altogether clear. It was noted that high levels of spontaneous release were frequently associated with less dramatic increases during stimulation, and hence it is possible that the high levels of unstimulated release induced by atropine may have prejudiced the likelihood of recording releases during stimulation. It is also possible that atropine may have some action on the reticular formation<sup>14</sup> and that its use was associated with a block of the reticular activating system. In this case, the increase in cups over the contralateral primary somatosensory cortex could have been the result of an activation of local cholinergic circuits in the cortex by primary afferent fibres. The hypothesis that such local circuits may occur is supported by the finding that, after undercutting, 17.3 per cent of the choline acetyltransferase activity remains in the cortex<sup>6</sup> compared with the 2-4 per

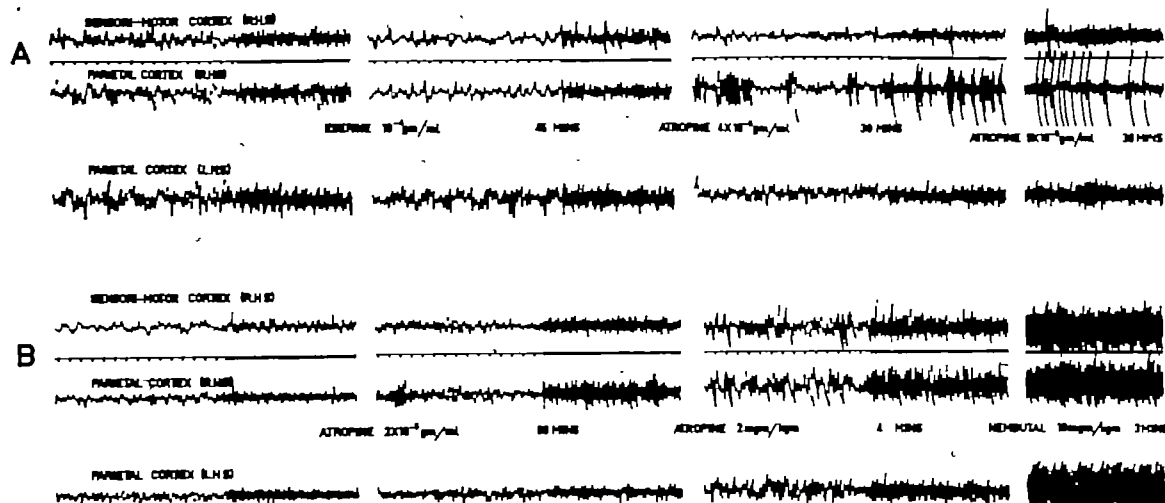


Fig. 1. EEG recordings from two *enophthalis* cats. Drugs applied topically in cups over the right (RHS) cerebral hemisphere. A, Control traces: 45 min after topical eserine,  $10^{-4}$  g/ml; 30 min after topical atropine,  $4 \times 10^{-3}$  g/ml in eserine saline; 30 min after topical atropine,  $6 \times 10^{-3}$  g/ml. B, Control traces: 60 min after topical atropine,  $2 \times 10^{-3}$  g/ml; 4 min after intravenous atropine, 2 mg/kg, 3 min after intravenous sodium pentobarbital (Nembutal), 10 mg/kg. Time calibration, 1 sec.

cent in the distal portion of the sectioned peripheral nerve after a similar time-interval<sup>22</sup>.

The reason for the increased rate of unstimulated release which occurs after the administration of atropine has yet to be established, although it is probably related to the finding that atropine causes a fall in the brain content of ACh (ref. 24). Possible modes of action have been discussed<sup>24</sup>.

The widespread distribution of an increased release of ACh after peripheral stimulation suggests that the pathways subserved by cholinergic fibres must project in a diffuse manner to the cortex. It has already been suggested<sup>6</sup> that the projections of the reticular arousal system to the cerebral cortex may be cholinergic and this would be consistent with the diffuse nature of the cholinergic system described in this report. Further evidence for a cholinergic mediator in the arousal system is forthcoming from experiments on the effects of topically applied eserine and atropine on the cortical EEG. The ability of these compounds to mimic the effects of 'waking' and 'sleeping' states of the EEG, as well as to duplicate the actions that they have when injected intravenously<sup>21,22,23</sup>, suggests that a cholinergic synapse, mediating arousal responses, is likely to occur in the cerebral cortex. The results obtained with such topical applications of drugs do not allow of any interpretation as to whether the sole action of these compounds after intravenous injections is at the cortical level, or whether they also have an action in the reticular formation itself, as suggested by Rinaldi and Himwich<sup>25</sup>. It has been postulated that the effects of atropine on the cortical EEG occur at a suprareticular level<sup>26</sup>.

The results described, on the release of ACh from the cerebellar cortex, are consistent with the suggestion<sup>13,17</sup> that there are cholinergic synapses in this structure. The amounts of acetylcholine released were just above the

threshold level of sensitivity of our assay, and attempts to demonstrate an increase in the rate of release during peduncular stimulation were unsuccessful. However, an investigation of the distribution of acetylcholine-sensitive cells in the cerebellum has indicated that these occur predominantly in the depths of the cortex, and further experiments using push-pull type cannulae<sup>27</sup> are planned to analyse the amounts released at different levels of the cerebellar cortex.

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## RELATIONS BETWEEN STATIONARY AND DYNAMIC PROPERTIES OF RANVIER NODES

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IN an earlier paper<sup>1</sup> we referred to our measurement of stationary current-voltage (CV) characteristics of the Ranvier node, which contains a region of negative resistance. We now try to prove that there is a relation between the stationary CV characteristic and the physiological function, that is, the action potential of the node. In electronics the function of two-electrode circuit elements is referred to their stationary CV characteristics. We have investigated whether the amplifying properties of the Ranvier node depend on its stationary characteristic.

From the electronic point of view the Ranvier node consists of an active two-electrode device which acts between a stable and metastable state. Whenever a stimulus exceeds the constant threshold amplitude, the Ranvier node turns from the stable to the metastable state. A supra-threshold stimulating impulse is amplified to a nerve signal of constant height, that is, to the 'action potential' (AP). Output energy exceeds input energy, but is independent of the amplitude of a supra-threshold stimulus (digital amplification). This function requires that the CV characteristic of the membrane includes a regenerative part with negative resistance. A characteristic like this resembles that of an Esaki diode and can indeed be recorded stationarily, like a material property, independently of the ion environment necessary for normal excitation<sup>1,2</sup>. Hitherto, the stationary negative slope characteristic has only been proved stationarily in depolarised Ranvier nodes which are no longer normally

excitable. Therefore, the following questions arise: (i) Why cannot the stationary negative slope characteristic be recorded in Ranvier nodes with resting potential? and, (ii) can the normal AP, proceeding from resting conditions, also be referred to the stationary negative slope characteristic?

The shape of the non-stationary, that is, the dynamic, CV characteristic of the membrane, measured earlier by Hodgkin and Huxley<sup>3</sup>, Tasaki and Bak<sup>4</sup> and Dodge and Frankenhaeuser<sup>5</sup>, leads to the assumption that the negative resistance is located between +10 and +50 mV relative to the resting potential, but in the region of inward current. Measuring this part of the characteristic with impressed voltage forces the membrane to a current delivery in a direction opposite to the applied voltage (metastable state). Because this active inward current, produced by the membrane, opposed in direction to the applied voltage, lasts no more than 1 msec, the negative resistance cannot be measured stationarily. Yet a slight shift of the operating range, by a depolarization of 20 mV, for example, is sufficient to permit the stationary measurement of a negative resistance, since at this time a small part (between +10 and +20 mV) of the current belonging to the negative resistance is already located in the direction of polarizing voltage relative to the new equilibrium potential. Thus, the inward current corresponds to the direction of the applied voltage and can be drawn from the external circuit. At further depolariza-



tion caused, for example, by a veratridin-containing Ringer's solution or by an increase of the extra-cellular  $K^+$ -concentration, increasing parts of a negative resistance can be recorded. At a depolarization to 60 mV almost the complete range of the negative resistance can be covered. Even though the membrane itself no longer delivers energy—because the resting batteries are discharged—it still possesses the control properties of the normal membrane. Whenever the  $K^+$ -depolarized membrane is repolarized to resting potential, that is, to its normal operating point, by an electrotonus, thus producing an outside energy source, action potentials can be released again, but with amplitudes determined by the corresponding  $K^+$ -depolarization. They are called  $K^+$ -AP.

All circuit elements with a falling OV characteristic are capable of digital amplification ('Esaki' diode, Dynatron). The shape of their output voltage can be made similar to action potentials of nerve fibres. "Depolarization and repolarization threshold" of these artificial action potentials corresponds to the beginning and the end of negative slope part of the OV characteristic, respectively, that is, to the two extreme values of the curve. The peak amplitude is given by the voltage distance between the working point and a defined point on the other branch of positive resistance. In the same way we try to correlate AP and the stationary OV characteristic of the Ranvier node shown in Figs. 1 and 2. Depolarizing stimuli cause a shift of the membrane potential to a level corresponding to a current minimum on the CV diagram, that is, the depolarizing threshold. This leads to a leap of potential into the proximity of  $K^+$  or  $Na^+$ -equilibrium potential. Thus, the metastable state is achieved, and the membrane potential decays until it reaches the value corresponding to a current maximum. A jump in repolarization direction then restores the resting potential.

The depolarization and repolarization threshold as well as the amplitude of  $K^+$ -AP can be directly referred to the stationary characteristic of the excitable membrane measured during  $K^+$ -depolarization. Since a long-lasting polarization to a value equal to  $E_{K^+}$  is not possible in myelinated nerve fibres without damaging the membrane, the characteristic underlying the normal  $Na^+$ -AP can, however, not be recorded stationarily. Thus, only indirect approaches may lead to an answer to the second question. The influence of environmental conditions altering the AP may be used to determine whether they induce at least qualitatively corresponding alterations of

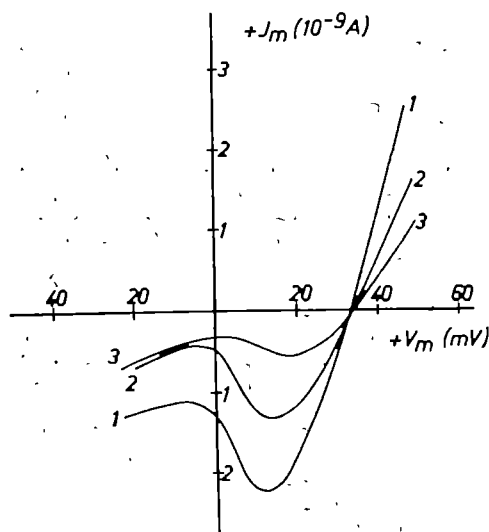


Fig. 1. Changes of the stationary OV characteristic caused by acid and alkaline Ringer's solutions.  $[K^+]$  is kept constant at 40 mM/L. (node 111/68) parameter: pH;  $I_m$ —membrane current, outward current positive;  $V_m=0$  resting potential, restored by an electrotonus, crossing of the characteristics with the abscissa, that is points of zero current, indicates equilibrium potential near  $E_K$ : 33 mV. Curves 1, pH 10; 2, pH 8; 3, pH 6.

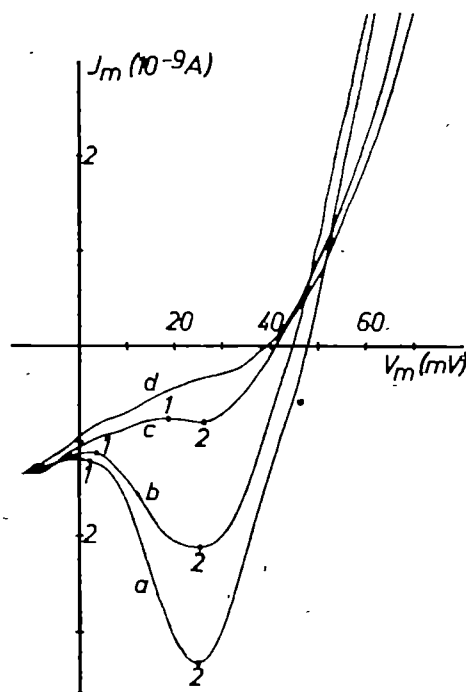


Fig. 2. Changes at the stationary OV characteristic at reduced pH,  $[K^+]$  is kept constant at 80 mM/L. (node 208/68, original curves). Curves a, pH 6-8; depolarization voltage 48 mV; b, c, d, changing to pH 4-6. b, after 1 sec; c, after 5 sec; d, after 9 sec; depolarization voltage 59 mV. (1) Depolarization threshold; (2) repolarization threshold.

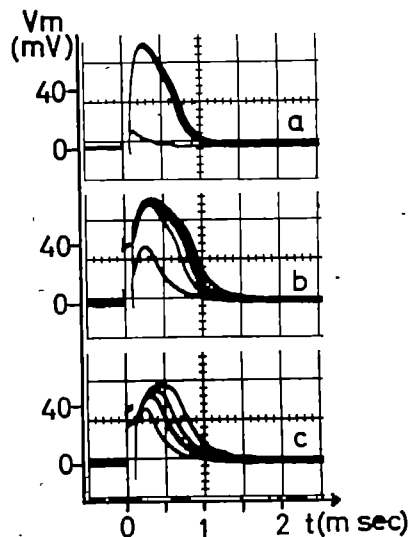


Fig. 3. Changes of action potentials at reduced extracellular pH. Same preparation as in Fig. 2. (a) normal AP (84 mV) sub-threshold response 12 mV; (b) 1-3 sec after changing to pH 4-6. Sub-threshold response 42 mV, increase of repolarization threshold, compared to Fig. 2 (b); (c) after 6 sec amplitude of "AP" is continuously variable between threshold response (42 mV) and maximum amplitude (60 mV) depending on stimulus strength.

the characteristic of the depolarized Ranvier node. In this investigation we have utilized the characteristic recorded at 80 mM  $K^+$  depolarization, which covers almost the complete range of negative resistance.

First, the influence of stabilizing and labilizing conditions was investigated. Stabilizers ( $Ca^{2+}$ -rich, acid, hypertonic and  $SO_4^{2-}$  Ringer solutions) cause an increase in depolarization and repolarization threshold, while a decrease is induced by labilizers ( $Ca^{2+}$ -poor, alkali and hypotonic solutions). If the AP can really be referred to the characteristic, the stabilizers ought to cause a shift of minimum and maximum current potential toward higher depolarization voltage, while these potentials ought

to be shifted toward higher polarization voltage by labilizers. In our experiments, these alterations were found regularly; one example is shown by the alterations of the stationary CV characteristic caused by changes of pH in Fig. 1.

Further experiments were concerned with the investigation of an environmental influence of special interest. Owing to a decrease in the extracellular pH to 4.7, the region of negative resistance, that is, the transitional region between the two branches of positive ohmic resistance, is reduced by shifting the potential minimum current toward the direction of depolarization (Fig. 1c). According to the foregoing considerations the following alterations of the AP might be expected: strong increase of the stimulus threshold, of the amplitude of sub-threshold response and decrease of amplification (AP energy/threshold stimulus energy). Prolonging the action time of the acid solutions, or lowering the pH values, results in a further reduction of the transitional region, and the formerly negative resistance now becomes positive. If

there is a relation between characteristics and AP, in this phase of alteration, 'AP' with a continuously variable amplitude should occur, because the membrane no longer behaves as a regenerative system. At a pH of 4.6, the resistance in the transitional region is reduced to such a degree that it reaches the value of the high-ohmic branch. Thus, the CV characteristic of a simple rectifier appears. By then the Ranvier node should be unexcitable.

In our experiments, all these predicted AP alterations, even the variable amplitudes, could be demonstrated as is shown in the original curves of Fig. 3. They, too, were strictly reversible like the corresponding alterations of the characteristic (original curves, Fig. 2). These results lead to the conclusion that the function of the Ranvier node may be referred to the stationary membrane characteristic.

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## GENETICS OF THE ALKALINE PHOSPHATASE POLYMORPHISM OF THE HUMAN PLACENTA

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**K**INETIC<sup>1</sup>, electrophoretic<sup>2</sup> and immunochemical<sup>3</sup> investigations have shown that the alkaline phosphatase present in human placenta differs in several of its properties from the alkaline phosphatases occurring in other tissues, and suggest that the enzyme is probably peculiar to the placenta. It has also been noted that electrophoretic differences may be found between the alkaline phosphatase extracted from different human placentae. Boyer<sup>4</sup> described three types of electrophoretic pattern and suggested on the basis of their population frequencies that the differences were genetically determined. In this article we show how human placentae may be classified into at least six distinct phenotypes according to the electrophoretic properties of the alkaline phosphatase present. We also give the results of twin studies which indicate that these phenotypic differences depend on the genotype of the foetus, and that at least three relatively common autosomal allelic genes determine this enzyme polymorphism.

Placental extracts were prepared by a modification of Morton's butanol method<sup>5</sup> as used by Boyer<sup>4</sup>. They were subjected to horizontal unidimensional starch-gel electrophoresis at both pH 8.6 and pH 6.0 because it was found that the two sorts of separation were required to give a satisfactory discrimination between the phenotypes. For the separation at pH 8.6 Poulik's *tris*-borate discontinuous buffer system<sup>6</sup> was used, and electrophoresis was carried out at 6 V/cm for 6 h at room temperature. For the pH 6.0 separation the gel was made using a succinic acid/*tris* buffer (0.0025 M in succinic acid, 0.0046 M in *tris*) and the bridge solution consisted of a citric acid/sodium hydroxide buffer (0.41 M in citric acid), and electrophoresis was carried out at 8 V/cm for 6 h in a cold room at +5° C. After electrophoresis the zones of enzyme activity were developed using  $\beta$ -naphthyl phosphate as substrate in a reaction mixture as described by Boyer<sup>4</sup>.

The great majority of placentae can be classified into one or another of six distinct phenotypes according to the pattern of electrophoretic components revealed by these procedures. Fig. 1 illustrates diagrammatically the characteristic electrophoretic patterns seen at pH 8.6 and at pH 6.0. The six phenotypes will be referred to as F, FI, I, SI, FS and S. In phenotypes F, I and S most

of the alkaline phosphatase activity is present in the form of a rapidly moving component. In phenotype F this component is distinctly faster than that in phenotype S both at pH 8.6 and at pH 6.0. In phenotype I the characteristic component has a mobility close to that of the F component at pH 8.6, but at pH 6.0 it is much slower, and has the same mobility as the S component. The phenotypes FS, SI and FI are all characterized by the presence of three rapidly moving components at either one or both pH's used. In each case two of these show mobilities similar to the main components of F, I or S while the third has an intermediate mobility and is also somewhat more intense than the other two. It should be

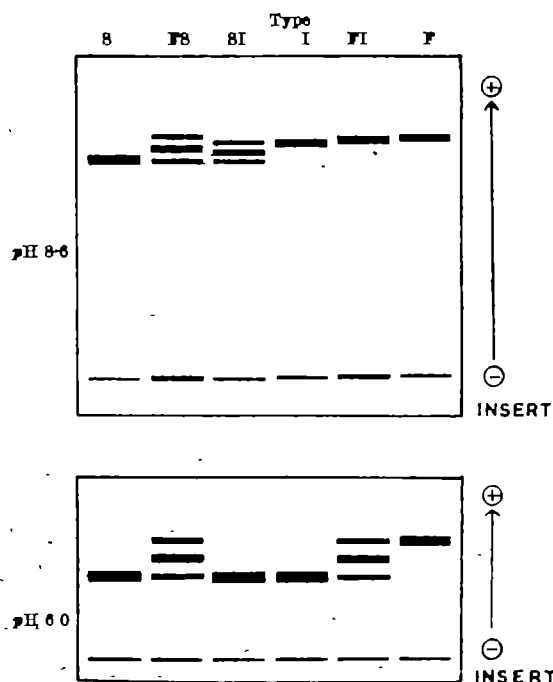


Fig. 1. Placental alkaline phosphatase phenotypes as revealed by starch-gel electrophoresis at pH 8.6 and pH 6.0.

noted that under the conditions described, phenotypes F, FI and I cannot be unequivocally differentiated from one another at pH 8.6, while at pH 6.0 phenotypes S, SI and I are indistinguishable. Thus separation at both pH's is required for complete discrimination of the six phenotypes. It seems likely that Boyer's<sup>1</sup> type A included our types F, FI and I, and that his type AB included our types FS and SI.

In each of the six phenotypes at least one other component which migrates only very slowly and does not move far from the origin at either pH 8.6 or pH 6.0 is also regularly seen. These slow-moving components exhibit slight differences in mobility in the different phenotypes and these have been found to correlate with the more striking differences observed in the major and more rapidly moving components.

The patterns shown in Fig. 1 include only the principal components seen in the various phenotypes. Several minor components may also be demonstrated in each of the phenotypes provided a sufficiently active preparation is used. Types S, F and I, for example, can be shown to have, besides the major rapidly moving component, two slightly faster and one slightly slower minor components. These minor components appear to parallel the mobility differences shown by the major components they are associated with, and do not interfere with the discrimination of the six phenotypes.

For routine typing, extracts were prepared from only a small portion (1-4 g) of any particular placenta. However, a number of experiments have been carried out in which samples taken from different parts of the same placenta were examined separately. In each case the electrophoretic patterns obtained from different samples of the same placenta were found to be identical.

Table 1 shows the incidence of the various phenotypes in a series of 338 placentae obtained from births at University College Hospital, London. They were derived from single pregnancies of women of European origin, but were otherwise unselected. It will be seen that the relative frequencies of the various phenotypes do not appear to be significantly related to the sex of the offspring. In this series there were 6 examples in which the electrophoretic patterns were different from the six standard phenotypes. They included three further phenotypes each being rare, having frequencies of less than 0.01. These rare types will be considered in detail elsewhere.

Table 1. FREQUENCY OF ALKALINE PHOSPHATASE PHENOTYPES IN 338 SINGLE PLACENTAE FROM A HOSPITAL POPULATION

Phenotype	Sex of Infant		Total
	Male	Female	
S	61	80	141
SF	48	63	111
SI	18	14	32
I	4	1	5
FI	7	8	15
F	14	14	28
Others	1	5	6
	153	185	338

In a smaller series of 64 placentae derived from Negro mothers living in England, 54 were of phenotype S, 5 of phenotype SF, 4 of phenotype SI and 1 of phenotype FI. There was thus a marked excess of phenotype S over that found in the European population, a result which is in agreement with the findings of Boyer<sup>1</sup> and which indicates that quite marked population differences exist.

On the basis of the electrophoretic patterns and the relative incidence of the different phenotypes it is possible to construct a simple genetical hypothesis. This is that the six common phenotypes are determined by three autosomal allelic genes ( $P^H$ ,  $P^I$  and  $P^S$ ), phenotypes F, I and S representing the homozygous genotypes ( $P^H P^H$ ,  $P^I P^I$ , and  $P^S P^S$ ), and phenotypes SF, SI and FI the corresponding heterozygotes ( $P^H P^S$ ,  $P^H P^I$  and  $P^I P^S$ ). The characteristic electrophoretic pattern of each of these postulated heterozygous types consists of three components and the mobilities of two of them are identical to those of the main components in the respective homo-

zygous types. The third enzyme component has an intermediate mobility and it seems possible that it may represent in each case a 'hybrid' enzyme containing polypeptides characteristic of the two other components present. The rare phenotypes also show 'triplet' electrophoretic patterns, and they can probably be regarded as heterozygotes for a rare allele and one or other of the commoner alleles  $P^H$ ,  $P^I$  or  $P^S$ . Several different rare alleles probably occur.

From the observed incidence of the six common phenotypes in the population (Table 1) one can obtain estimates of the gene frequencies of the three common alleles  $P^H$ ,  $P^I$  and  $P^S$ . They are 0.27, 0.09 and 0.64. Using these values one finds good agreement between the observed numbers of the different phenotypes and the expected numbers assuming a Hardy-Weinberg equilibrium (Table 2). In this calculation the small number of rare phenotypes have been excluded.

Table 2. OBSERVED AND EXPECTED NUMBERS OF THE SIX COMMON PLACENTAL ALKALINE PHOSPHATASE PHENOTYPES ASSUMING A HARDY-WEINBERG EQUILIBRIUM WITH GENE FREQUENCIES  $r=0.64$ ,  $q=0.09$  AND  $p=0.27$

Phenotype	Postulated genotype	Expected incidence	Population sample		
			Expected numbers	Observed numbers	
S	$P^H P^H$	$r^2$	0.410	135.9	141
SF	$P^H P^I$	$2pr$	0.246	114.7	111
SI	$P^H P^S$	$2qr$	0.115	33.2	32
I	$P^I P^I$	$q^2$	0.008	2.7	5
FI	$P^I P^S$	$2pq$	0.049	15.1	15
F	$P^S P^S$	$p^2$	0.073	24.2	23
		$(p+q+r)^2$	1.001	331.8	332

It is impractical to carry out an extensive family investigation of the ordinary kind in order to study the genetics of a characteristic which is peculiar to the human placenta. However, it has proved possible to test the genetical hypothesis by studying the distribution of the various phenotypes in a series of pairs of placentae derived from dizygotic twins. Such twin pairs can be regarded simply as pairs of sibs. We have been fortunate in obtaining appropriate material from an extensive investigation of twin births which is being carried out in the Birmingham area under the general direction of Dr. John Edwards with support from the Association for the Aid of Crippled Children.

Table 3 summarizes the results obtained in 380 placentae from 190 dizygotic twin pairs. In each case the placentae were dichorionic and twins of the same sex were included when they could be shown to differ in at least one genetically determined characteristic among 6 red cell antigenic systems and 3 polymorphic red cell enzyme systems. All the twin pairs were from parents of European origin. In 112 placental pairs the alkaline phosphatase phenotypes

Table 3. PLACENTAL ALKALINE PHOSPHATASE PHENOTYPES IN 190 DIZYGOTIC TWIN PAIRS

Dizygotic twins	Expected incidence assuming alleles with frequencies $p$ , $q$ and $r$	Expected incidence where $p=0.27$ , $q=0.09$ , $r=0.64$	Expected numbers	Observed numbers
Like pairs				
S S	$0.25r^2(1+r)^2$	0.2754	52.33	53
SF SF	$0.5pr^2(1+p)(1+r)$	0.1049	37.03	37
F F	$0.25p^2(1+p)^2$	0.0324	9.59	6
SI SI	$0.5qr^2(1+q)(1+r)$	0.0531	10.00	11
I I	$0.25q^2(1+q)^2$	0.0024	0.46	1
FI FI	$0.5pq^2(1+p)(1+q)$	0.0172	3.27	4
			108.77	113
Unlike pairs				
S SF	$pr^2(1+r)$	0.1814	34.47	41
S F	$0.5pr^2$	0.0150	3.85	2
S SI	$qr^2(1+r)$	0.0604	11.48	13
S I	$0.5qr^2$	0.0016	0.30	0
S FI	$pqr$	0.0100	1.90	1
SF SF	$p^2r(1+p)$	0.0592	11.23	4
SF SI	$pqr(1+2r)$	0.0354	6.73	7
SF I	$pqr$	0.0014	0.27	1
SF FI	$pqr(1+2p)$	0.0240	4.56	3
F SI	$pqr$	0.0042	0.80	2
F I	$0.5p^2q$	0.0002	0.04	0
F FI	$p^2q(1+p)$	0.0084	1.60	1
SI I	$q^2r(1+q)$	0.0056	1.06	1
SI FI	$pqr(1+2q)$	0.0184	3.50	2
I FI	$pqr(1+q)$	0.0024	0.46	0
			81.27	78
Totals		1.0000	190.04	190

were the same, and in 78 pairs they were different. Rare phenotypes were observed in four additional twin pairs, two being alike and two unlike. These four pairs have not been included in the analysis shown in Table 3.

Since many of the pairs are unlike one can exclude the possibility that the placental alkaline phosphatase phenotype is determined by the maternal genotype, because if this were so none of the pairs should have shown differences. It is possible to calculate the expected incidence of like and unlike placental pairs assuming that the alkaline phosphatase phenotypes are not genetically determined but occur essentially at random. In the 190 pairs one would have expected on this basis to find 63.3 pairs which were alike, and 126.7 pairs which were unlike. There is clearly a great excess of like pairs in the dizygotic twin series, and this, of course, is what would be expected if the placental phenotypes depend on the genotype of the foetus.

With six different phenotypes 21 sorts of twin pair may occur. If the phenotypes depend on the foetal genotype then one may test the genetical hypothesis by calculating the expected incidence of each of the different sorts of sib pair, using the gene frequencies derived from the general population sample (the gene frequencies in the Birmingham twin series were found to be essentially the same as in the University College Hospital single pregnancies

series). The calculation is shown in Table 3 and it will be seen that there is in general very good agreement between the numbers of the different sorts of twin pair observed and the numbers expected according to the hypothesis.

We conclude, therefore, that the placental alkaline phosphatase phenotypes are determined by the foetal genotype and that the polymorphism can be largely accounted for in terms of three relatively common autosomal allelic genes. There appear to be quite wide variations in gene frequency in different human populations, and it is probable that a number of rare alleles also occur which may give rise to unusual enzyme patterns. The biological significance of this polymorphism is still quite obscure, but it may well prove of importance in problems concerned with maternal-foetal interaction.

We thank Dr. J. H. Edwards, Institute of Child Health, University of Birmingham, and his colleagues for assistance in the twin investigation, and Dr. M. Crawford of the Galton Laboratory and Prof. W. C. W. Nixon of the Obstetric Department, University College Hospital, London, for further assistance.

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## CESTICIDAL ACTIVITY OF SUBSTITUTED DIHYDROBENZO-1,3-OXAZINES

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REMARKABLY little has been done to find an ideal cesticide, and the extract of male fern (*Dryopteris filix-mas*), which has been in use from medieval times, is still the drug of choice. Yet this and other drugs like quinaacrine, dichlorophen, hexylresorcinol, arecoline, etc., are rather toxic and their efficacies are not always predictable. During routine screening of compounds for cesticidal activity, two compounds (TQ-OA 119 and TQ-OA 121) were found active in 10<sup>-7</sup> against *Hymenolepis nana* *in vitro*. This article reports the activity of these compounds against *H. nana* *in vivo* and discusses their comparative therapeutic efficacy.

Compounds TQ-OA 119 (3-(3',4'-dichlorophenyl)-2,4-dioxo-6-chloro-dihydrobenzo-1,3-oxazine) (I) and TQ-OA 121 (3-(2'-chloro-4'-nitrophenyl)-2,4-dioxo-6-chloro-dihydrobenzo-1,3-oxazine) (II) were obtained from Firma Dr. Karl Thomae, Biberach/Riss, West Germany. The compounds were insoluble in water, alkali or acid.

Previously in this laboratory the infection of *H. nana* was maintained in mice. But recently we have found that newly weaned rats of the University of Freiburg strain, which we received through the courtesy of Prof. H. Druckrey, are even more susceptible. There is almost 100 per cent 'take'.

Initially, the *in vitro* screening was done following the method of Sen and Hawking<sup>1</sup>, which allows use of alcohol not exceeding 1 per cent of the medium as a solvent for the compounds.

The compounds were then tested *in vivo* in rats, using the technique of Steward<sup>2</sup>. Each rat under experiment was fed with 200 mature eggs of *H. nana*, later starved on the fourteenth day, given the drug orally in 'Tween 80' suspension on the fifteenth, starved on the sixteenth and killed on the seventeenth day. The remaining worms were collected from the intestine and a score was made depending on their size. The results were compared with those from a control group.

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To determine the initial dose for *in vivo* testing the maximum tolerated dose for each compound was first determined. The maximum tolerated dose for TQ-OA 119 and TQ-OA 121 was found to be more than 3 and 3.6 g/kg, respectively. As these were high, a dose about one-sixth the maximum tolerated dose was initially used in rats. The dose was then gradually decreased to find the minimum effective dose which had been taken as the dose which would reduce the average score in the treated animals to one-tenth or less of the average score of the untreated controls.

While comparing the therapeutic efficacy of these compounds with well-known cesticides, it was found that all previous investigations had been carried out in mice. Consequently, for comparison the tests were repeated in

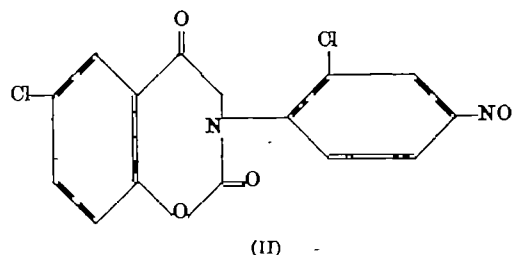
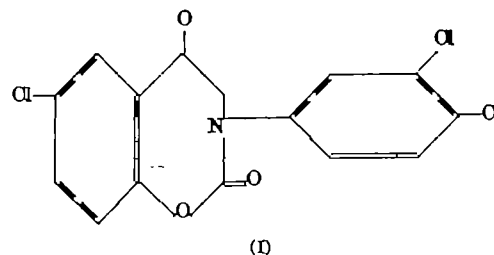


Table 1. SUMMARY OF THE *in vivo* TESTS  
The column headed 'response' gives the number of animals freed from worms and the number of animals treated

Compound	Dose mg/kg	Response	Average score	Remarks
		In rats		
TQ-OA 119	500	3/3	0	Very active
	Control	0/3	11.6	
	500	4/4	0	Very active
	Control	0/4	60	
	200	7/10	21.36	Active
	Control	0/5	302.3	
	200	6/10	10.8	Active
	Control	0/5	183.0	
	100	9/10	1.0	Active
	Control	1/5	45.0	
TQ-OA 121	100	2/10	37.3	Slightly active
	Control	0/5	302.3	
	750	5/5	0	Very active
	Control	0/4	60	
	500	6/6	0	Very active
	Control	0/5	200	
	500	10/10	0	Very active
	Control	0/5	183	
	200	7/7	0	Very active
	Control	0/5	200	
TQ-OA 119	100	3/10	7.1	Active
	Control	0/5	215.00	
	50	0/5	70.5	? Slightly active
	Control	0/5	215.0	
	50	4/9	29.0	Slightly active
	Control	0/5	153.0	
	In mice			
	200	5/5	0	Very active
	Control	0/5	95.08	
	100	4/5	0.58	Very active
TQ-OA 121	Control	0/5	95.08	
	100	4/5	0.5	Very active
	Control	0/5	80.0	
	50	5/5	0	Very active
	Control	0/5	95.08	
	50	4/5	0.5	Very active
	Control	0/5	80.0	
	25	3/5	7.3	Active
	Control	0/5	95.08	
	25	3/5	14.0	Slight action
TQ-OA 121	Control	0/5	80.0	
	200	5/5	0	Very active
	Control	0/5	95.08	
	100	5/5	0	Very active
	Control	0/5	70	
	50	4/5	0.04	Very active
	Control	0/5	95.08	
	50	3/5	3.1	Active
	Control	0/5	80.0	
	25	3/5	21.0	Slight action
TQ-OA 121	Control	0/5	95.08	
	25	2/5	12.7	Slight action
	Control	0/5	80.0	

mice. A summary of the results of *in vivo* tests in rats and mice is presented in Table 1.  
TQ-OA 119 in 500 mg/kg dose cleared all the rats. Though 200 mg/kg dose did not clear all the rats, it removed a substantial amount of worm load, as evident

from the score. 100 mg/kg had less but still considerable action.  
With TQ-OA 121, 750, 500 and 200 mg/kg cleared all the rats. With lower dose of 100 mg/kg only three of the ten rats were cleared, but there was a substantial reduction in the worm load.  
When quinacrine, a well-known oestocidal compound, was tried in 500 mg/kg in rats, four of the ten treated rats died. Only two of the remaining six were cleared, and the treated:untreated ratio of average score was 8.1:183. With lower dose of 200 mg/kg no significant effect was observed.  
In mice, 50 mg/kg of TQ-OA 119 was very effective. Even 25 mg/kg was appreciably effective. With TQ-OA 121, 100 mg/kg removed all the worms from all the treated mice, but 50 mg/kg could not, although the average score was much less than the untreated.  
If maximum tolerated doses for TQ-OA 119 and 121 are taken as 3.0 and 3.6 g/kg, respectively, and minimum effective dose as 50 mg/kg, the therapeutic indexes for these two drugs in mice come to 60 and 72, respectively. The low toxicity of these compounds is proved by the fact that even after administration of maximum tolerated dose in mice the animals did not show any untoward effect during the next seven days and there was no macroscopic changes in the viscera during post-mortem examination. In comparison, the therapeutic index for quinacrine comes to about 8, taking 100 mg/kg as minimum effective dose (Standen, quoting Keeling<sup>3</sup>) and 800 mg/kg arbitrarily as the maximum tolerated dose, which is the LD<sub>50</sub> determined by Bovet *et al.*<sup>4</sup>. Bhat-tacharya and Sen<sup>5</sup> found the therapeutic index to be 5 in the case of BIQ 22. Similarly, safety levels are also low with other known oestocidal drugs.  
The effectiveness of TQ-OA 119 and TQ-OA 121 against *H. nana* in two species of animals (rats and mice) and high therapeutic indexes make them promising as oestocidal drugs. However, TQ-OA 119 in 500 mg/kg and TQ-OA 121 in 150 mg/kg doses when fed orally were ineffective against *Nippostrongylus brasiliensis* infection in rats.  
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A 'FOUR-EYED' FISH FROM THE DEEP-SEA: *Bathylchnops exilis*  
COHEN, 1958

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*BATHYLYCHNOPUS EXILIS* Cohen, 1958, a rare North Pacific mesopelagic fish in the family Opisthoproctidae, has an unusual eye. A spherical protuberance, complete with a well-developed lens, is located on the rostro-ventral portion of the orbit (Figs. 1 and 2). This genus is named *Bathylchnops* (meaning 'deep-lamp-eye') because this structure was originally thought to be a photophore or light-producing organ<sup>1,2</sup>. However, its

size and location prompted us to study the eye of *B. exilis* histologically.  
A specimen, 470 mm in length, was captured in an oblique midwater trawl tow from the surface to 1,000 m depth off Oregon. It was alive and displayed vigorous swimming movements when first captured. This large specimen, which was fixed in 10 per cent formalin, provided the main material for examination. One eye was removed

and embedded in celloidin, and serial sections were cut  $15\mu$  thick. Every tenth section was stained with haematoxylin and eosin and mounted. Representative sections were also stained with silver stains, Mallory's trichrome, and Shorr's connective tissue stain. Two small individuals were also examined. The head of a 107-mm specimen and both eyes of a 110-mm specimen were embedded in paraffin, cut into  $8\mu$  serial sections, and stained with haematoxylin and eosin, Heidenhain's azocarmine-aniline blue stain, alcian blue, alcian blue-periodic acid Schiff, periodic acid Schiff, and luxol fast blue.

The eyes of *B. exilis* are large and directed dorsally (Fig. 1a). The anatomical axis of each eye forms an angle of about  $35^\circ$  with the perpendicular, providing a large dorsal binocular field of vision. The secondary globe



Fig. 1. Head of *Bathylchnops exilis* (470 mm specimen). (a) Dorsal view, (b) lateral view showing secondary globe ( $G_2$ ).

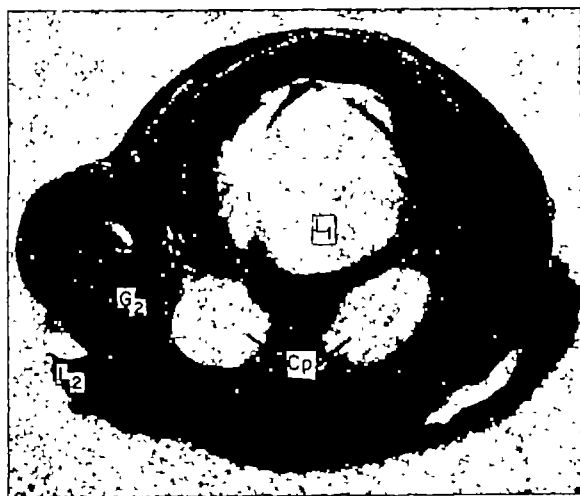


Fig. 2. Left eye (470 mm specimen). CP, Corneal projections,  $G_2$ , secondary globe,  $L_1$ , pupal and lens;  $L_2$ , scleral lens of secondary globe.

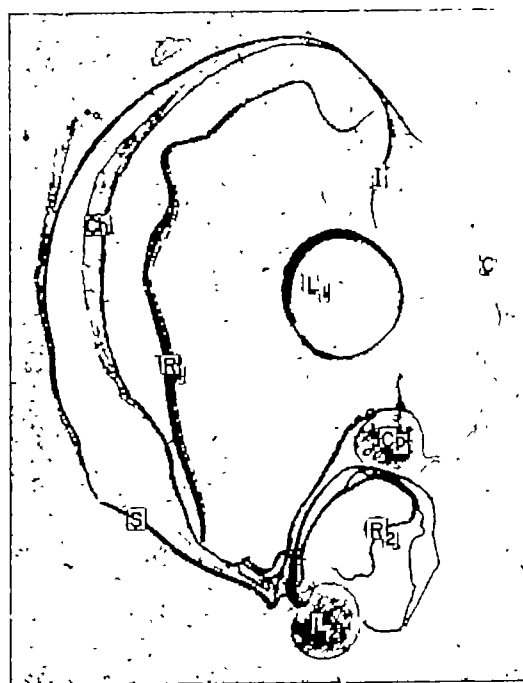


Fig. 3. Section through entire eye (470 mm specimen). C, Cornea; CA, choroid gland; CP, corneal projection; I, iris;  $L_1$ , main lens;  $L_2$ , scleral lens of secondary globe;  $R_1$ , retina of main globe;  $R_2$ , retina of secondary globe; S, scleral cartilage.

is located on the rostro-ventral portion of the main globe, close to the limbus of the cornea. It is directed in a ventral and slightly caudal direction (Figs. 1b and 2). Dimensions of these structures are given in Table 1.

Table 1. DIMENSIONS OF SOME OPTIC STRUCTURES OF A *B. exilis* 470 MM IN LENGTH

	Size (mm)
Main globe	
Anterior-posterior diameter	14.5
Equatorial diameter	18.0
Corneal diameter	18.0
Elevation of cornea above limbus	3.0
Secondary globe	
Anterior-posterior diameter	7.6
Equatorial diameter	8.7
Scleral lens diameter	3.0
Elevation of lens above limbus	2.0
Corneal projections	
Dorsal projection	$5.0 \times 3.0$
Elevation above limbus	2.0
Ventral projection	$3.5 \times 3.0$
Elevation above limbus	2.0

Two peculiar thickenings, appearing as whitish regions in Figs. 2 and 1, were noted near the limbus of the cornea behind and dorsal to the secondary globe. These structures were elevated several millimetres above the limbus (Table 1). They were not found in our small specimens, but were noted by Cohen<sup>8</sup> in *Bathylchnops* more than 112 mm.

A section through the secondary and main globes is seen in Fig. 3. The secondary globe, located on the rostro-ventral part of the main globe, is enclosed by hyaline scleral cartilage. In the ventral part of the secondary globe there is a spherical lens-like structure, consisting of concentrically lamellated connective tissue. This lens is biconvex in sections of the small specimens. The secondary lens is continuous with and differentiated from the adjoining sclera. The possibility of phospholipids in the periphery of the lens, indicated by staining with luxol fast blue, suggests that the refractive index may exceed the usual values for teleostean cornea, which differ only slightly from that of sea-water.

Within the secondary globe is a retinal diverticulum, which is continuous with the retina of the ventral part of the main globe. No secondary optic nerve is present. Where the retina of the diverticulum overlies the inside

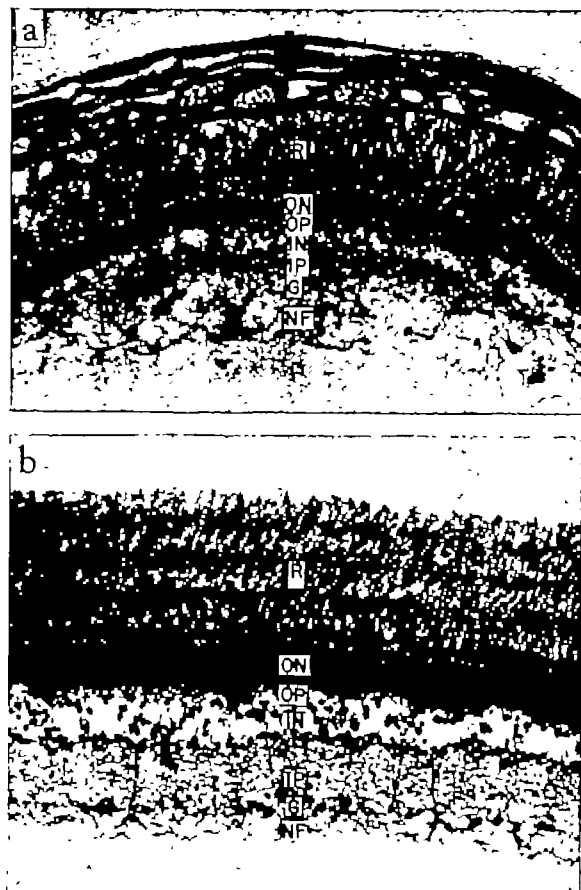


Fig. 4. Retina of (a) secondary globe and (b) main globe. R, Layers of outer rod segments or acromeres; ON, outer nuclear layer; OP, outer plexiform layer; IN, inner nuclear layer; IP, inner plexiform layer; G, ganglion cell layer; NF, optic nerve fibre layer.

surface of the lens of the secondary globe, it is reduced to two layers of endothelium-like cells, continuous with the retina proper and the pigment epithelium respectively. There is no pigment in the layer which is continuous with the pigment epithelium, and the choriocapillaris and choroidal pigment are also absent in this region. Light can thus pass directly from the secondary lens through the unpigmented window in the retinal diverticulum to the normally differentiated secondary retina. A narrow slit-like opening connecting the lumen of the diverticulum with the interior of the main globe was noted by gross dissection. The slit, about 2 mm in length, was covered by an operculum which prevented light from entering the main globe. It opened into the rostro-dorsal portion of the secondary globe. The exposed opening could be clearly discerned when a beam of light was projected on to the secondary lens.

The retina of the secondary globe is very similar to that of the main part of the eye (Figs. 4a and 4b). As with other deep-sea fishes, only rods are present in the photoreceptive layer of the retina<sup>1</sup>. In *B. exilis* the rod layer consists of several layers of outer segments or acromeres. Four layers are found in the thickest portion of the retina in the central part of the fundus (Fig. 4b). The rods are narrow and many occupy the area above a single bipolar cell. The dense outer nuclear layer and the progressive decrease in the number of cells in the inner nuclear and ganglion cell layers indicate a high degree of retinal summation and therefore acute sensitivity.

The two projections on the cornea, located above and behind the secondary globe (Fig. 2), are nearly circular in cross-section (Fig. 3). These structures, which are differentiated from and continuous with the cornea, appear

very similar histologically to the lens of the secondary globe. Muscles, probably derived from tensor chorioideae, insert on the base of the secondary globe, on the corneal thickenings above the secondary globe, and on the sclera. They may be able to move these structures, perhaps to assist in photoreception over a wider field. In other respects the main globe of *B. exilis* shows few deviations from the typical teleostean pattern.

The highly specialized and well-developed eyes of *B. exilis* indicate that photoreception plays an important part in its orientation and ecology. The large eyes are directed dorsally and are separated by only a thin septum. Because of this there is a wide dorsal binocular field of vision. Unlike many other deep-sea fishes, however, *B. exilis* has achieved an enlarged binocular field of vision without resort to the specialized tubular ('telescopic') shape of the eyeball. Binocularity in deep-sea fishes may be useful for space perception in an environment where the usual monocular cues for estimating distance are lacking<sup>2,4</sup>. The well-developed axial musculature and vigorous movement of *B. exilis* while alive indicate that it is a strong swimmer. For such a fast-swimming predator, snapping at small, dimly silhouetted or bioluminescent prey in the dark environment, stereoscopic depth perception would presumably be a distinct advantage. Sensitivity may also be increased by binocular vision which lowers the threshold below that of monocular vision<sup>2,4</sup>. Likewise, the several rows of acromeres in the retina may represent a means of increasing sensitivity without substantially decreasing resolving power<sup>4</sup>.

The specialization of the eyes of *B. exilis* for dorsal binocular vision restricts their visual fields, but the secondary globes compensate for this by providing for light and movement perception in a ventral direction. This is important for detection of prey or predators, or their bioluminescence, in water below. The two peculiar corneal thickenings (see Fig. 2), originally called photogenic tissue, may also serve as lenses and further increase the periscope field of vision. The thickenings are elevated above the orbit so that they can be seen above the lens when viewing the intact eye from behind. Consequently, light caudal to the fish striking these lens-like structures may be refracted on to the lens and hence on to the main retina.

Evolution of the secondary globes appears to be closely related to the development of stereoscopic vision. This is supported by the fact that similar, but structurally far simpler, specializations are present in the tubular eyes of some deep-sea fishes. Analogous structures have been described by Brauer<sup>3</sup> in the related genera *Dolichopteryx*, *Opisthoproctus* and *Winiwaria*. In these fishes a well-developed portion of the accessory retina forms a small diverticulum, in which part of the retinal pigment epithelium and the adjacent choroid are unpigmented, forming a window through which light may be perceived. Unlike *B. exilis*, however, light does not strike a normally differentiated retina but first strikes the rods adjacent to the window. Moreover, none of these other fishes has a secondary lens for focusing light. *B. exilis*, which lacks the wide visual field provided by tubular eyes, has the most highly evolved secondary 'eye'. It also has corneal protrusions. Both structures increase the field of vision while maintaining the advantages of the typical shape of the main globe.

The large *B. exilis* was captured while making oceanic collections under AEC contract AT(45-1)1726. The work was supported in part by a grant from the National Science Foundation (GB 1588) to one of us (W. G. P.).

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<sup>5</sup> Walls, G. L., *Nature*, 175, 996 (1955).

<sup>6</sup> Munk, O., *Vidensk. Medd. fra Dansk naturh. Foren.*, 125, 353 (1963).



# IMMUNOLOGICAL ELECTRO-ADSORPTION METHOD AS APPLIED IN THE CASE OF MICE LEUKAEMIA

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THE immunological electro-adsorption method recently described<sup>1</sup> proved most sensitive in detecting antibodies in cases where only haemagglutinin or complement-fixation tests were available. Both sensitivity and rapidity of the experimental procedure make the method particularly useful. It consists in adsorbing for 1 min or less, on a metalized glass slide, a thin layer of material containing some antigen. The antigen is adsorbed from a solution of a partially purified fraction under the influence of an electric current with the proper polarity, the slide for all cases tested thus far being negatively charged. Then the slide is immersed in a dilute solution of the antiserum to be tested, also in the presence of a weak current, the slide being positively charged this time. It is found that the layer adsorbed after the antiserum treatment is thicker when the serum is homologous rather than heterologous or normal. The thickness of the layers, which runs from a few Å to a few hundred Å, is measured optically with an ellipsometer. As described in ref. 1, it was possible to detect immunological reactions for eight arthropod-borne viruses, and the corresponding antisera could be diluted 1 to 10<sup>4</sup> before specific reaction failed to be observed.

It was of interest to extend this method to other systems, and the results obtained with a virus from leukaemic mice (Friend's virus)<sup>2</sup> are presented in this article. (We thank Dr. Alice Moore for suggesting Friend's virus for a possible testing of the electro-adsorption method and Dr. Charlotte Friend for providing us with an infective extract of spleen.)

The infection of the mice was carried out according to the method described by Friend<sup>2</sup> by injecting a homogenate of spleen of diseased animals intraperitoneally into *N.O.S.* Swiss mice. The antigenic material used for the immunological test was prepared by two methods.

In the first method, described by Friend<sup>2</sup>, an antigenic fraction was obtained from diseased spleens homogenized in saline (the weight of the diseased spleens varied from 1 to 5 g). A clear homogenate containing 20 per cent by weight of the original spleen was obtained after centrifugation at 10,000*g*. This preparation was called antigen stock solution.

In the second and better method, described by de Harven and Friend<sup>3</sup>, the virus was recovered from the plasma of infected mice bled from the axilla. The plasma was submitted to fractional centrifugation and filtered through three 'Millipore' filters of pore sizes 0.65μ, 0.3μ and 0.22μ.

Immunological electro-adsorption tests were performed essentially as described in ref. 1. The adsorptions were carried out on glass slides, 6 mm × 120 mm, coated by evaporation with 'Inconel' (80 per cent nickel and 20 per cent chromium). 1.5 ml. of the antigen solution and 0.8 ml. of the serum diluted 1 to 15 in veronal buffer,

pH 7.5, were used for each test. The slides were negatively charged for the adsorption of the antigen and positively charged for the adsorption of the sera. The duration of the adsorption was 30 sec in every case. The current was maintained at 0.300 m.amp for the adsorption of both antigen and antibodies using a constant current electronic power supply (available from Scitronics, 419 East 73rd Street, New York).

A summary of the results obtained with 17 mice is presented in Table 1. The antigen used for these tests was the stock solution from the spleens diluted 1 to 5 in water. The sera of the infected mice were obtained through orbital bleeding and tested daily until the animals were killed. The number of days elapsed between infection and the time of bleeding is indicated in column 1. Each successive column corresponds to the results obtained with a single mouse. The first group of 5 mice was infected with the stock solution, the second group of 6 mice was infected with the stock solution diluted 1 to 2, and the last group with the stock solution diluted 1 to 4 in saline. The numbers indicate the thickness in Å of the layers adsorbed during the serum treatment. It is apparent that only two days after infection with either the stock solution or the solution diluted 1 to 2, the layers adsorbed from the antisera are significantly thicker than the layers adsorbed from the serum of the same animals prior to infection. This is interpreted as indicating a very rapid antibody formation. Fourteen days after infection the thickness of the layers adsorbed from the sera ranged from 103 Å to 126 Å as compared with 52 Å to 59 Å for normal sera. As an additional control, 6 mice were injected with a 20 per cent homogenate in saline of normal spleen. The thickness of the layers obtained after adsorption of the sera 7 days after injection ranged between 56 Å and 59 Å, the values found for the sera of non-injected mice.

The thickness of the layers obtained after the antigen adsorption was 70–80 Å when the undiluted stock solution was used and 25–35 Å when the solution was diluted 1 to 4 in saline, as was the case for the experiments presented in Table 1.

Similar experiments were carried out with antigen preparations obtained from the blood of infected mice, as already described here. After filtration and centrifugation of the plasma for 30 sec at 30,000*g*, most of the antigenic material remained in the supernatant. The pellet obtained after further centrifugation at 38,000*g* for 1 h was resuspended in saline. It contained some antigenic material which could be adsorbed on the slide since the thickness of the layer adsorbed was 79 Å after treatment with an antiserum and 52 Å after treatment with a normal serum.

In another run, after low-speed centrifugation (15,000*g*) for 90 min and filtration, the plasma was centrifuged at 44,000*g* for 90 min. The supernatant contained some

Table 1

Days	Stock solution					Stock solution diluted 1/2 in saline					Stock solution diluted 1/4 in saline					
0	57	56	57	57	56	54	59	57	56	52	56	53	49	57	56	52
1	66	58	57	64	58	63	56	56	63	55	62	59	56	63	61	58
2	90	78	56	81	77	73	65	67	81	60	71	60	59	56	54	56
3	91	103	66	80	78	85	67	72	100	69	64	..	..	..	..	..
4	..	..	67	102	..	96	..	..	..	66	..	63	..	..	..	..
7	..	..	88	..	115	..	84	73	..	91	63	98	63	..	..	..
8	112	..	..	..	..	..	91	72	..	..	..	68	..	..	..	66
14	121	..	..	118	..	..	..	..	120	..	..	..	..	111	106	120
16	..	..	..	..	..	..	119	..	..	99	..	..	..	..	..	..
18	80	99	..	..	101	93	..	97	..	85	98	106	..	99	..	..

antigenic material, since slides after adsorption of this material could adsorb layers 43 Å, 55 Å, 57 Å, 62 Å and 69 Å thick from sera obtained 0, 4, 7, 18 and 48 days, respectively, after infection. This supernatant was further centrifuged for 3 h at 46,000*g*. The supernatant was inactive this time, but the pellet was found to be antigenic. It took, however, two days for the material to become active after the pellet was resuspended. All centrifugations were carried out at 4°–6° C.

A few experiments were made to test the stability of the antigen as a function of temperature. After serum treatment, the slide adsorbed 50 Å with a normal serum and 46 Å with the antiserum when the antigenic preparation had been heated 15 min at 90° C. Heating the antigen at 50°–55° C for 15 min did not diminish the antigenic properties (93 Å after treatment with the immune serum and 40 Å with the normal serum). After heating the antigen at 65°–70° for 15 min, an increase of 70 Å was observed after immune serum treatment as compared with 40 Å after normal serum treatment. These results indicate that the antigenic activity is more stable than the infectivity of the agent which, according to Friend<sup>4</sup>, is destroyed by heating at 56° C for 30 min.

Some experiments were carried out using extracts of either diseased or normal spleens as sources of antigen to be adsorbed on the slides. Three different types of serum

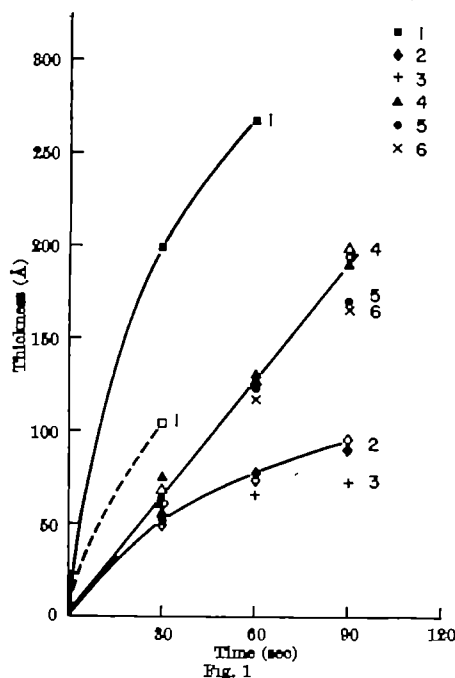


Fig. 1

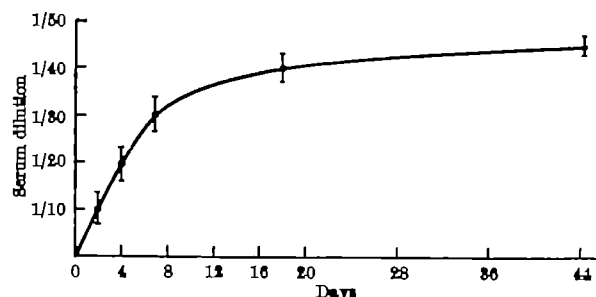


Fig. 2

were adsorbed afterwards on the slides. The results have been summarized in Fig. 1, where the thickness of the layer adsorbed following serum treatment is plotted against duration of adsorption. The antigen stock solution from diseased spleens was used for the experiments summarized by curves 1, 2 and 3, and normal spleen extracts for the experiments summarized by curves 4, 5 and 6. Dashed curve 1 was obtained when the stock solution was diluted 1 to 4 in saline. Homologous serum was used for curves 1 and 4, normal serum for curves 2 and 5, and serum from a mouse injected with normal spleen 7 days before bleeding for curves 3 and 6. It is apparent that a slide coated with an extract of normal spleen (curves 4, 5 and 6) is unable to differentiate homologous from normal serum in contrast to a slide coated with an extract of diseased spleen (curves 1, 2 and 3).

The thickness of the layer adsorbed following antiserum treatment is a qualitative measure of the level of antibodies in the serum. The thickness observed, for a given antigenic preparation, tends to a maximum as the concentration in antibodies increases. A better way of estimating the antibodies titre of a serum by this method is to dilute the serum until the thickness of the adsorbed layer is equal to that observed for a normal serum at the same dilution. This has been done in Fig. 2, where the dilution of a given serum, at which a specific adsorption disappears, is plotted against the time elapsed between the infection of the animal and the moment at which the serum is tested.

In no case could the serum be diluted more than 1 to 50. This is in sharp contrast to the results reported for eight arthropod-borne viruses<sup>1</sup> when sera could be diluted 1 to a million before a specific adsorption disappeared. Nevertheless, it is interesting to note that this new immunological electro-adsorption method seems applicable to a variety of antigens.

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## IMMUNOLOGICAL AND BIOCHEMICAL INVESTIGATIONS OF HUMAN SERUM HAPTOGLOBIN: COMPOSITION OF HAPTOGLOBIN-HAEMOGLOBIN INTERMEDIATE, HAEMOGLOBIN-BINDING SITES AND PRESENCE OF ADDITIONAL ALLELES FOR $\beta$ -CHAIN

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**H**APTOGLOBIN is an  $\alpha_2$ -protein present in the serum of many species which has the specific capacity to bind with haemoglobin stoichiometrically *in vivo* and *in vitro*<sup>1-3</sup>. In 1955, Smithies<sup>4</sup> reported the existence of genetically determined variations in haptoglobin using starch-gel electrophoresis. The three common haptoglobin phenotypes, Hp 1-1, Hp 2-1 and Hp 2-2, can be

now sub-classified into six sub-phenotypes, Hp 1F-1F, Hp 1F-1S, Hp 1S-1S, Hp 2-1F, Hp 2-1S and Hp 2-2, by urea-mercaptoethanol-formate buffer starch-gel electrophoresis after reductive cleavage of the disulphide bonds of the partially purified haptoglobin samples<sup>5,7</sup>. The allele designations are referred to the fast-migrating so-called  $\alpha$ -chains. Additional haptoglobin phenotypes were

recognized in a Brazilian population<sup>8</sup>, and the distribution of the genetic haptoglobin sub-types in various populations have been reported<sup>9,10</sup>. So far, five normal haptoglobin alleles have been found ( $Hp^{1S}$ ,  $Hp^{1F}$ ,  $Hp^{2F}$ ,  $Hp^{2S}$  and  $Hp^{3S}$ ) (refs. 8 and 10). In addition to the usual genetic types, the presence of some electrophoretically abnormal types, for example, Johnson type<sup>11</sup>, and immunologically atypical haptoglobins were also recognized<sup>12-14</sup>.

The molecular structure underlying the polymorphism of each genetic type of haptoglobins has been examined by several investigators using a variety of physicochemical and immunological techniques. Most recently, Shim and Bearn<sup>14</sup> had demonstrated that the antigenic determinants of the haptoglobin molecule reside in both  $\alpha$ - and  $\beta$ -chains, using rabbit antiserum to purified type 1F-1S human serum haptoglobin, and proposed a tentative model for the secondary structure of human type 1-1 haptoglobin.

The work recorded here was performed to elucidate further the antigenic structure of type 2-2 haptoglobin, the composition of the so-called haptoglobin-haemoglobin intermediates, the haemoglobin-binding sites of haptoglobin, and the possible polypeptide compositions of type 2-2 haptoglobin molecules.

The isolation of each genetic type of haptoglobin was performed according to the method as previously described<sup>14</sup>. The purity of the prepared samples was confirmed by starch-gel electrophoresis and immunoelectrophoresis against horse anti-human serum antiserum (Pasteur Institute, lot AH-223-4). Isolation of  $\alpha$ - and  $\beta$ -polypeptide chains of type 2-2 haptoglobin was per-



Fig. 1. Urea-mercaptoethanol-formate buffer (pH 3.1) starch-gel electrophoresis of the isolated  $\alpha$ - and  $\beta$ -polypeptide chains of type 2-2 haptoglobin by gel filtration with 'Sephadex G-75' column. Gel-filtration was carried out at room temperature with 6 M urea-1 M acetic acid, and the other conditions were the same as previously described (ref. 14). Urea-gel electrophoresis was carried out at 15 V/cm for 6 h in the cold (+4°C). Migration upwards. From left, isolated  $\beta$ -chain, reduced 2-2 type haptoglobin, and isolated  $\alpha$ -chain.

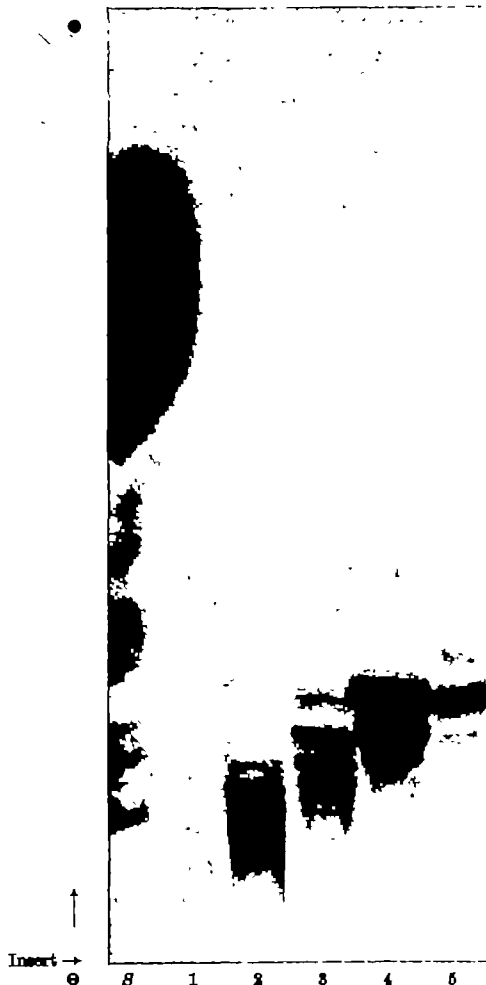


Fig. 2. Starch-gel electrophoresis (borate buffer, pH 8.9) of the gel-filtrated fraction of purified type 2-2 haptoglobin on 'Sephadex G-200' column (3 cm x 70 cm). Gel-filtration was carried out with 0.1 M HCl buffer pH 8.0, containing 1.0 M sodium chloride, at room temperature with a flow rate of 3 ml/h. The protein peak was divided into five fractions. Migration upwards. From left, control whole serum sample, and from part, second, middle, fourth and later part of the protein peak. The pattern clearly demonstrates that slower migrating bands in starch-gel electrophoresis of type 2-2 haptoglobin have larger molecular size than those relatively fast migrating bands.

formed according to the method which was previously applied to type 1-1 haptoglobin using 'Sephadex G-75' column<sup>14</sup>. However, in the case of type 2-2 haptoglobin more prolonged treatment with the reducing agent and incorporation of urea to 6 M into the eluting solution were necessary to obtain clear separation. The isolated polypeptide chain was confirmed by urea-mercaptoethanol-formate buffer starch-gel electrophoresis (Fig. 1).

The relative percentage of the  $\alpha$ -chain in type 2-2 haptoglobin molecules was 33.4 per cent, which indicates the presence of equal numbers of  $\alpha$ - and  $\beta$ -chain in type 2-2 haptoglobin as well as in type 1-1 haptoglobin molecule<sup>14</sup>, if we assume that the molecular weight of  $\alpha$ -chain is about 18,000 (refs. 15 and 16) and that of  $\beta$ -chain is 36,000 (ref. 14) (by indirect calculation). The difference of the molecular size in multiple bands in starch-gel electrophoresis of type 2-2 haptoglobin can easily be shown by gel-filtration with 'Sephadex G-200' column (Fig. 2). Then, if we assume that the multiple bands of type 2-2 haptoglobin indicate the various degree of polymerization of both chains, each band might represent the following composition:  $(\alpha\beta)_1$ ,  $(\alpha\beta)_2$ ,  $(\alpha\beta)_3$ ,  $(\alpha\beta)_4$ , ... in contrast to  $(\alpha\beta)_1$  of type 1-1 haptoglobin. From these considerations, the composition of the multiple bands of 2-1 type might be  $(\alpha\beta)_1$ ,  $(\alpha\beta)_2(\alpha\beta)_1$ ,  $(\alpha\beta)_3(\alpha\beta)_1$ ,  $(\alpha\beta)_4(\alpha\beta)_1$ , ... because artificial mixture of type 1-1 and 2-2 does not represent normal 2-1 type.

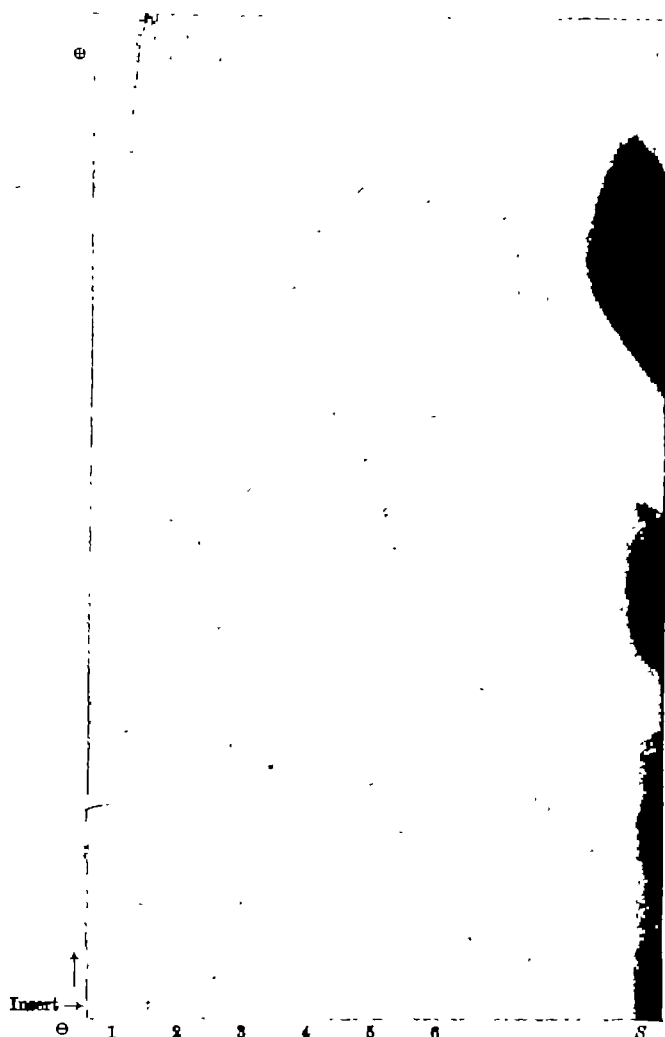


Fig. 3. Starch-gel electrophoretic patterns (borate buffer, pH 8.9, 15 V/cm for 4 h in the cold) of the gel-filtrated fractions of purified type 1-1 haptoglobin sample which was saturated with haemoglobin to 40 per cent of its haemoglobin-binding capacity. Gel-filtration was carried out on 'Sephadex G-200' column (2.3 cm x 55 cm) with 0.1 M pH 7.5 phosphate buffer with a flow rate of 2 ml/h. The protein peak was divided into 6 fractions. Migration upwards. From left, first, second, third, fourth, fifth and sixth fraction. Extreme right, control serum sample. Amido black 10B stained. With benzidine, slow-migrating middle and third bands only were stained, which indicates that the first band is free haptoglobin, and the middle and the third bands are haptoglobin-haemoglobin intermediate and haptoglobin-haemoglobin complex, respectively. The separation was not satisfactory; however, one can easily recognize the differences in the relative amounts of the free, the intermediate and the complex in each fraction. This indicates that the faster mobility of the intermediate than the complex in starch-gel electrophoresis at both pH 8.9 and 6.9 is mainly due to its molecular size.

So far, no direct evidence has been presented on the composition of the so-called haptoglobin-haemoglobin intermediates which appear when haptoglobin is under-saturated with haemoglobin, except a few hypotheses<sup>17,18</sup>. From the determination of molecular weight of the haptoglobin-haemoglobin intermediate Jayle and Moretti<sup>18</sup> believed that the intermediate might consist of two molecules of haptoglobin and one molecule of haemoglobin. However, this finding is inconsistent with the facts that the intermediate migrates faster than haptoglobin-haemoglobin complex in both pH 8.9 borate and pH 6.9 phosphate buffer starch-gel electrophoresis, because in pH 6.9 free haemoglobin migrates slower than 1-1 haptoglobin-haemoglobin complex as shown by Laurell<sup>17</sup>. We added haemoglobin to the purified type 1-1 haptoglobin to 40 per cent saturation of its haemoglobin binding capacity and run gel filtration using 'Sephadex G-200' column with 0.1 M, pH 7.5 phosphate buffer. The separation of type 1-1 haptoglobin-haemoglobin complex, 1-1 haptoglobin-haemoglobin intermediate and free 1-1 haptoglobin was not complete, but partial separation could be achieved

as shown in Fig. 3, which demonstrates that the molecular size of the 1-1 intermediate is smaller than the 1-1 haptoglobin-haemoglobin complex, but larger than free 1-1 haptoglobin. The analysis of the amount of haemoglobin in each fraction showed that the intermediate-rich fraction contained less amount of haemoglobin than the haptoglobin-haemoglobin complex-rich fraction. These data suggest that the 1-1 intermediate consists of one molecule of haptoglobin and half-molecule of haemoglobin. Similar intermediates could easily be recognized in 2-1 and 2-2 type haptoglobins when the sample is under-saturated with haemoglobin. Recently, Nagel and Ranney<sup>19</sup> have shown that haptoglobin binds with haemoglobin *F* ( $\alpha_1^A\gamma_1^A$ ), *A*<sub>1</sub> ( $\alpha_1^A\beta_1^A$ ), *I* ( $\alpha_1^A\beta_1^A$ ) and Lepore ( $\alpha_1^A\beta_1^A$ ), but not with haemoglobin *H* ( $\beta_1^A$ ) and Barts. ( $\gamma_1^A$ ). Therefore, the half-molecule of haemoglobin in the intermediate should be symmetrical dimer ( $\alpha_1^A\beta_1^A$ ,  $\alpha_1^A\beta_1^A$ ,  $\alpha_1^A\beta_1^A$  or  $\alpha_1^A\beta_1^A$  Lepore). The experiment to obtain direct evidence of this hypothesis is now being carried out.

To investigate the antigenic structure and haemoglobin binding sites of haptoglobin molecule, we immunized the rabbits with purified type 2-2 haptoglobin and with the isolated  $\alpha$ - and  $\beta$ -chain of 2-2 type haptoglobin with complete Freund's adjuvant according to the method as previously described<sup>14</sup>. Among four rabbits which were immunized with type 2-2 haptoglobin, one rabbit antiserum showed spur between each normal genetic type as demonstrated by Korngold<sup>15</sup>, which indicates that type 2-2 and 2-1 haptoglobin have more antigenic determinants than type 1-1 haptoglobin. The anti-2-2 serum also showed spur between free haptoglobin and haptoglobin-haemoglobin complex of same genetic type as demonstrated by Beuing *et al.*<sup>21</sup>. The most interesting results were obtained with anti- $\beta$ -chain antiserum which reacted only with free haptoglobin, but not with haptoglobin-haemoglobin complex (Fig. 4). In contrast to anti- $\beta$ -chain antiserum, the anti- $\alpha$ -chain antiserum reacted with both free haptoglobin and haptoglobin-haemoglobin complex. These results indicate that haptoglobin binds with haemoglobin at  $\beta$ -chain, and that most antigenic determinants of  $\beta$ -chain are covered by haemoglobin. Each genetic type of haptoglobin showed identical reaction against anti- $\beta$ -chain antiserum. Therefore, the fact that anti-2-2 serum absorbed with type 1-1 still reacted with both free and haemoglobin-bound 2-1 and 2-2 type, but not with free 1-1 type, which was also demonstrated by Korngold<sup>15</sup>, suggests that the antigenic differences among normal genetic types of haptoglobin reside mainly in the  $\alpha$ -chains.

Recently, Robson and others<sup>22</sup> described some rare haptoglobin types, of which Hp 1-P, Hp 2-P and Hp 2-L

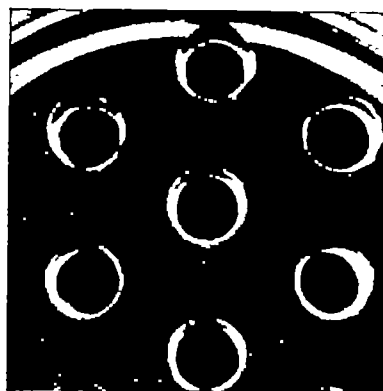


Fig. 4. The reactions of  $\beta$ -chain specific antiserum with free haptoglobin and haptoglobin-haemoglobin complex. Centre well: anti- $\beta$ -serum, lower left well: free 2-2 type serum, lower middle well: 1-1 type; lower right well: 2-1 type serum. Upper left well: haemoglobin-saturated 2-2 type serum; upper middle well: haemoglobin-saturated 1-1 type; upper right well: haemoglobin-saturated 2-1 type serum. The anti- $\beta$ -chain serum reacted only with free haptoglobin and haptoglobin-haemoglobin intermediate (not shown in the illustration), but not with haptoglobin-haemoglobin complex, and showed reaction of identity among each genetic type of free haptoglobins.

seemed to have lost partially their haemoglobin-binding capacity, which suggest the variation in haemoglobin-binding sites of the  $\beta$ -chain. We<sup>22</sup> also observed a haptoglobin variant which completely lost its haemoglobin-binding capacity. These observations suggest that there exist two haemoglobin-binding sites per ( $\alpha\beta$ )<sub>2</sub> sub-unit of the haptoglobin molecules each for half-molecule of haemoglobin, and when only one of them is bound with half-molecule of haemoglobin it appears as the haptoglobin-haemoglobin intermediate. Indirect evidence of the existence of two haemoglobin-binding sites in the haptoglobin molecule has already been presented from this laboratory<sup>24</sup>. So, it is plausible to think that Hp 1-P, 2-P and 2-L (ref. 22) are variants in one of the two haemoglobin-binding sites of  $\beta$ -chain (strictly speaking, per  $\beta$ <sub>2</sub> sub-unit), and the latter type<sup>22</sup> might be a variant in both sites. Therefore, electrophoretically normal but immunologically atypical type haptoglobin<sup>13,14</sup> might be a variant at the parts other than the haemoglobin-binding sites. However, this does not necessarily indicate that the haemoglobin-binding sites are themselves involved in the antigenic determinants, because some abnormal type<sup>22</sup> showed normal immunological properties which suggest that haemoglobin just covers the area of the antigenic determinants of  $\beta$ -chain.

The atypical haptoglobin described by Aly *et al.*<sup>13</sup>, and also by Beuing *et al.*<sup>21</sup>, which was reported to have more antigenic determinants than normal haptoglobin could be explained on this basis, provided the atypical haptoglobin (Marburg) partially lost its haemoglobin-binding capacity, because they demonstrated the spur formation among haemoglobin-saturated samples. It seems that haptoglobin Marburg has no really additional antigenic determinants, or rather, possibly, less than normal haptoglobin, but just a variant in the area of haemoglobin-binding site of the  $\beta$ -chain so that the normal antigenic determinants could still react with the antibodies, while those determinants in the normal haptoglobin are covered by haemoglobin. In fact, haptoglobin Marburg was found to be more antigenically deficient than normal haptoglobin when reacted against our rabbit anti-1-1 type haptoglobin antiserum<sup>25</sup>. This is the reason why the spur disappeared when haemoglobin-saturated haptoglobin Marburg was compared with unsaturated normal 2-1 type haptoglobin<sup>21</sup>.

Family examination of this atypical haptoglobin<sup>13</sup>, 1-P and 2-L types of Robson *et al.*<sup>23</sup>, clearly showed that these types are genetically determined. These observations suggest that the molecular structure of haptoglobin is controlled by two independent genes, one for  $\alpha$ -chain and the other for  $\beta$ -chain. Then, the alleles Hp<sup>P</sup> and Hp<sup>L</sup>, which were designated by Robson and others<sup>23</sup>, should be the variant alleles of  $\beta$ -chain. Therefore, we propose to designate the allele for normal  $\beta$ -chain as Hp<sup>SN</sup>, Hp<sup>P</sup> as Hp<sup>SP</sup> and Hp<sup>L</sup> as Hp<sup>SL</sup>, respectively, to distinguish from those alleles for  $\alpha$ -chains. It is very interesting to note that the biosynthesis of haptoglobin is controlled by a mechanism very similar to that of haemoglobin synthesis and that both  $\alpha$ -chain and  $\beta$ -chain variants exist.

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## THE HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM AND THE MILK-EJECTION REFLEX

THE practice of *Dairy Science Abstracts* of publishing review articles covering a wide range of topics seems to have found favour, since it has become well established. The latest example<sup>1</sup>, under the same title as this article, is published in two parts in successive issues. It is likely to prove tough going for most regular readers since much of it is concerned with various aspects of an area which, following a fairly recent initiative by Unesco, may properly be designated as 'brain research'. However, it is likely to interest many workers in the biological and medical sciences.

It is no novel idea that the discharge of milk from the mammary gland during suckling or milking is an active process involving the unconscious participation of the mother. That the essence of this concept has been intuitively known from ancient times is attested by numerous works of art, some of which illustrate the classical myth which tells of the trick played by Zeus on Hera to immortalize Herakles, incidentally giving rise to the galaxy and also, in some versions, the lilies of the field. The best known representations of this are Tintoretto's canvas 'The Origin of the Milky Way' in the

National Gallery (London), the lower portion of which, depicting the origin of the lilies, is believed to have been excised, and Rubens's later version in the Prado (see ref. 2). The same idea has been empirically exploited to aid in obtaining milk from domestic animals in ancient civilizations and by primitive peoples in to-day's developing countries, as has been recorded in several recent accounts<sup>3,4</sup>, the latest and most detailed of which is that of Amoroso and Jewell<sup>4</sup>.

The concept that milk withdrawal from the mammary gland involves a nervous reflex, in the absence of which only sub-optimal amounts of milk are available, was first propounded more than half a century ago, and its most prominent exponents were Gaines in the United States and Hammond in Britain. However, it is only following the original suggestion of Ely and Peterson nearly twenty-five years ago that increasing knowledge had led to the general acceptance of the view that the reflex is really a neuro-endocrine reflex in which the final stage is the release into the blood from the posterior pituitary of the hypothalamic hormone oxytocin. This octapeptide hormone, now available in synthetic form

because of the classical pioneer work of du Vigneaud, is the agent which causes the contraction of a specific target tissue in the mammary gland, the myoepithelium, so that a large proportion of the milk otherwise held unavailable is squeezed down into the storage cisterns or sinuses from which the suckling or milker can readily remove it. There is now a considerable amount of evidence about various features of the role of the hypothalamo-neurohypophyseal system in the neuroendocrine milk-ejection reflex, and it is this field which is reviewed by Dr. Robert Denamur, who has himself with his colleagues made notable contributions to the subject. His treatment is comprehensive rather than critical, even where, as in consideration of work on blood oxytocin-levels, critical discussion would have been particularly valuable. Despite the fact that in the introduction he disclaims any attempt to compile an exhaustive bibliography, there are no fewer than 885 references.

The article bears signs of a translation from a foreign language, and the reader will sometimes be disconcerted to meet the names of distinguished American workers under an unfamiliar guise, as when van Dyke is shortened to Dyke. This no doubt arises from the over-zealous application of the house rules of an organization the primary purpose of which is, in current idiom, the retrieval of information. There is also, perhaps inevitably, a certain amount of repetition. Nevertheless, Dr. Denamur provides a valuable synthesis of basic scientific knowledge of a subject which is fundamental to a matter of considerable technological importance, namely, how to carry out the milking of domestic animals, especially cows, on a commercial scale with optimum efficiency. As might be expected, this subject has for long been of great interest to Russian animal physiologists and it is interesting to note that their preoccupation has been mainly with segmental nervous reflexes which they believe are elicited by the milking stimulus and, having a shorter latency, precede the neuro-endocrine milk-ejection reflex. The segmental reflex is believed to control the tone of smooth muscle elements in the walls of the cisterns and larger ducts of the bovine udder and, by assisting the flow of milk

and its accommodation in the udder cisterns, to facilitate the action of the neuro-endocrine milk-ejection reflex. Recent reviews of Russian work in this field available in English translation are those of Baryshnikov<sup>4</sup> and Zaks<sup>5</sup>. It should be added that an intriguing element of complexity has been added to the situation by the fact that a few years ago Dr. Denamur himself, as well as some of the Russian workers, obtained results which question the need for the intervention of the central nervous system in the process of milk withdrawal in small ruminants such as sheep and goats; for, in animals with completely deafferented udders, it was possible to obtain normal yields of milk by careful hand-milking. In accordance with this, Folley and Knaggs<sup>6</sup>, in experiments on hand-milked goats, found that more often than not oxytocin could not be detected in the pituitary effluent blood following the milking stimulus even though the milk yields were normal.

It is to be hoped that Dr. Denamur's review will come to the attention of interested research workers whose reading does not normally cover the field of agricultural research. It should be added that reprints of the two parts of the article are available under one cover from the Central Sales Branch, Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, at the modest price of ten shillings for what is in effect a short monograph which will be a useful reference source for some time to come. Credit is due equally to the author for his methodical and logical treatment of what has now become a rather complicated body of information and to the staff of *Dairy Science Abstracts* for the successful completion of an exacting task of editorship. S. J. FOLLEY

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## NECROSIS OF THE PANCREAS, PAROTID AND LACHRYMAL GLANDS ASSOCIATED WITH ENCEPHALOMYOCARDITIS VIRUS INFECTION

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RECENT studies in this laboratory have been concerned with the pathogenicity for animals of selected, antigenically similar strains of encephalomyocarditis (EMC) virus. During the course of this work, necrosis of the adipose tissue of the retroperitoneum and epididymal fat tags was noted in adult mice infected with many of the EMC virus strains under investigation. Since it seemed likely that these changes were the result of retroperitoneal seepage of digestive enzymes released from the pancreas, this organ was examined histologically.

A lesion of the pancreas is found in EMC-infected mice the carcasses of which exhibit necrosis of retroperitoneal adipose tissue. It consists of coagulation necrosis of acinar cells associated with interstitial oedema and a scant infiltrate of inflammatory cells (Fig. 1). The lesion occurs independently of virus dosage in animals inoculated by the subcutaneous, intraperitoneal or intracerebral route. Usually the whole organ is affected, although small clusters of acinar cells are occasionally spared. Necrosis of the islets of Langerhans has been noted, but in most animals the islets and lining cells of the ducts are well preserved.

Histological changes in the pancreas are apparent well before the neurological signs characteristic of EMC infec-

tion develop. The earliest alteration recognizable by light microscopy is swelling of the acinar cells; later there is loss of basophilia and vacuolation of the cytoplasm. Some clumping of chromatin of the nucleus occurs; however, the nucleus often appears intact after the cytoplasm has disintegrated. Ultrastructural changes in the pancreatic acinar cells are evident 48 h after the subcutaneous inoculation of 10<sup>6</sup> lethal doses (LD<sub>50</sub>) of virus. The electron micrographs show prominent alterations in the architecture of the ergastoplasm, dilatation of the cisterns of the endoplasmic reticulum and dissociation of ribosomes from their membranes, swelling of mitochondria and vacuolation of the cytoplasmic matrix. So far, virus particles have not been identified within affected tissue.

The acinar cells of the exorbital and endorbital lachrymal glands and the parotid elaborate polypeptide enzymes and thus are functionally similar to the exocrine cells of the pancreas. They also possess comparable morphological features. Coagulation necrosis of these organs is found in infected animals exhibiting pancreatic lesions. The histopathological changes in the three glands are similar and appear to develop concomitantly. However, as will be discussed here, necrosis of the lachrymal gland is observed

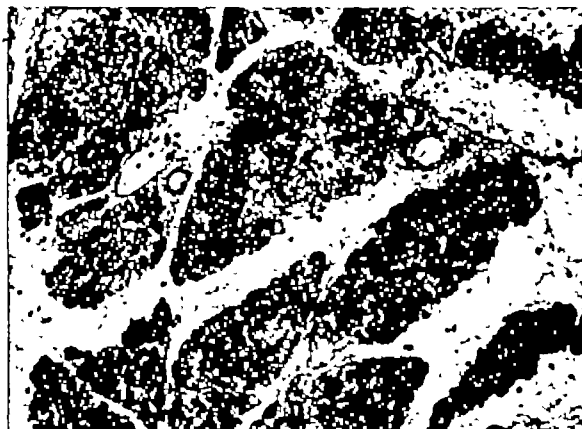


Fig. 1. Representative histological section of pancreas of adult mouse inoculated subcutaneously with  $10^4$  LD<sub>50</sub> of 1138-E strain of EMC virus and killed 72 h later. Note the necrosis of acinar cells, the interstitial oedema and the absence of an inflammatory cell infiltrate. Scattered clusters of intact acinar cells and an islet of Langerhans can be seen.

in mice infected with an EMC strain (1138-M) which causes no changes in the pancreas or parotid.

Titration of the pancreas and exorbital lachrymal glands of subcutaneously inoculated animals have been carried out to determine their virus content. These studies have been complicated by early viraemia and the occurrence of auto-interference at low tissue dilutions. Within 48 h the two organs contain virus in amounts ( $10^4$ – $10^6$  tissue culture LD<sub>50</sub>) which usually exceed the quantities found in other parenchymal organs and blood. The parotid gland has not been tested because a clean dissection of this organ in the mouse is difficult.

Table 1 summarizes data on the occurrence of necrosis of retroperitoneal adipose tissue in adult mice receiving EMC virus having different origins and passage histories. Each of the viruses was tested in groups of mice at decimal dilutions throughout its infectivity range and the animals were killed when moribund. Typical alterations were found in adipose tissue of mice receiving most, but not all, of the virus strains tested. Although histological studies were not carried out routinely in this survey, the association of gross evidence of necrosis of retroperitoneal adipose tissue with the lesion of pancreas is well established.

Studies with two sub-strains recovered from the same 'wild' source<sup>1</sup> suggest that the ability of a virus to cause the glandular lesions is altered by the manner in which it is passaged. A sub-strain (1138-E) transmitted 11 times as a homogenate of brain, intracerebrally, consistently produces coagulation necrosis of the three glands, whereas a second sub-strain (1138-M) passaged 20 times as homogenized heart, intraperitoneally, occasionally causes lesions of the lachrymal gland but not of the pancreas and parotid. The first sub-strain is highly neurotropic in mice; the second causes severe myocarditis but few, if any, neurological signs.

Table 1. OCCURRENCE OF NECROSIS OF RETROPERITONEAL ADIPOSE TISSUE IN MICE INFECTED WITH SELECTED STRAINS OF EMC VIRUS

Virus strain	Passage		Necrosis of adipose tissue
	Mice Route—times	L cell Times	
EMC-M*	10—c. 80	None	V
EMC-M	10—c. 80	5	O
Mengo	Many†	None	N
1138	10—2	None	V
1138-E	10—11	None	O
1138-M	10—20	None	N

\* Decimal dilutions of virus preparations inoculated subcutaneously throughout infectivity range into 13- to 14-week-old male mice.

† Details of passage history not known. 10, brain passaged intracerebrally; IP, heart passaged intraperitoneally; O, consistent finding; V, variable finding; N, never observed.

Several lines of evidence support the conclusion that the lesions are a consequence of EMC virus infection and not the effect of some extraneous substance or unrecognized infectious agent: (1) they are produced by virus strains which have been passaged in several different laboratories using cell cultures or animals or both; (2) they are caused by isolates purified in cell cultures by the plaque technique; (3) they occur with equal severity over a wide range of virus dosage; (4) they do not develop when a virus strain which consistently induces necrosis of the pancreas (1138-E) is used in a mouse neutralization test with antiserum prepared against a strain which does not cause the lesion (Mengo); and (5) they are not found in control mice inoculated with tissues from animals and cell cultures.

All observations so far indicate that the alterations in adipose tissue are due to release of pancreatic digestive enzymes into the retroperitoneal space. The pancreatic lesion and resulting exocrine insufficiency may account for the diarrhoea which I have noted repeatedly in EMC-infected mice during the terminal stages of illness. A non-purulent conjunctivitis also has been observed in some infected animals; histological examination of the eyes and their accessory structures has failed to reveal any cause other than necrosis of the endorbital and exorbital lachrymal glands.

Lesions of the pancreas and parotid glands occur in adult mice infected with Group B Coxsackie viruses<sup>2-4</sup>. Pancreatic lesions have been described in mice receiving certain strains of the virus of foot-and-mouth disease<sup>5,6</sup>. The histopathological alterations in the pancreatic acinar cells produced by members of these two groups of small RNA viruses (picorna viruses) are similar if not identical to those observed in EMC-infected mice.

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## CHANGES IN THE SULPHYDRYL AND DISULPHIDE GROUPS IN BEEF MUSCLE PROTEINS DURING HEATING

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CHANGES of sulphhydryl and disulphide groups in muscle proteins and the formation of H<sub>2</sub>S may be important for the taste and texture of canned meat products.

Methods for the determination of sulphhydryl and disulphide groups in beef muscle tissue (*M. longissimus*

dorsi, 5 days *post mortem*), in myofibrils and in actomyosin gels were developed and the changes of these groups during heating were investigated.

The total content of SH-groups present in muscle proteins can be determined by the reaction with AgNO<sub>3</sub>. Ground tissue, myofibrils or actomyosin, containing



approximately 5 mg protein, are suspended in 35 ml. 0.03 M Tris buffer (pH 7.4), and 1 ml.  $10^{-3}$  M  $\text{AgNO}_3$  (excess) are added. After stirring for 60 min at room temperature,  $1.2 \times 10^{-3}$  moles of glutathione (excess) are added to the filtered solution. The excess of glutathione is titrated by means of an amperometric technique using  $10^{-3}$  M  $\text{AgNO}_3$ . By this method we found for muscle tissue from different animals  $8.8 \pm 0.3$  (S.D.), for myofibrils  $9.6 \pm 1.2$  and for actomyosin gel  $8.8$  moles  $\text{SH}/10^4$  g protein. After denaturation by heating, urea or ethanol, the amount of SH-groups reacting with  $\text{AgNO}_3$  is not significantly increased. Therefore, all SH-groups present in the native muscle proteins are able to react with  $\text{AgNO}_3$ .

When the SH-reagent, *N*-ethyl-maleimide (NEM), is used, only a few of the SH-groups reacting with  $\text{AgNO}_3$  (total SH) can be detected. Ground tissue, myofibrils or actomyosin gel, containing approximately 100 mg protein, is suspended in a mixture of 5 ml. 0.1 M phosphate buffer (pH 6.0) and 5 ml.  $2 \times 10^{-3}$  M NEM (excess). The suspension is shaken for 120 min at 25° C (thermostat) and then centrifuged (14,000g, 5 min) after deproteinization with TCA. The amount of NEM which has not reacted is determined by photometric measurement of the absorption at 300 nm. By means of this technique, we found in myofibrils only  $3.5 \pm 0.1$  moles  $\text{SH}/10^4$  g protein. After denaturation (heating, urea, ethanol, TCA), the amount of SH-groups reacting with NEM increases considerably.

The presence of  $\text{O}_2$  (air) has no influence on the amount of SH-groups reacting with  $\text{AgNO}_3$  or NEM under the conditions used. The amount of disulphide present in tissue, myofibrils or actomyosin can be determined after reduction by  $\text{NaBH}_4$ , which has been introduced into the protein chemistry by Moore *et al.*<sup>1</sup>

0.5 ml. 0.6 M  $\text{NaBH}_4$  (dissolved in 8 M urea) is added to 25 mg of the material. After 60 min reaction at room temperature, the excess of  $\text{NaBH}_4$  is destroyed by adjusting the pH of the mixture to 6.4–6.7 with 0.15 M  $\text{HNO}_3$ . The SH-content of the reduced material is determined by reaction with  $\text{AgNO}_3$  as mentioned here. The SS-content is given by the difference of the SH-groups present after and before reduction. In myofibrils, we found  $6.1 \pm 0.3$  moles  $\text{SS}/2$  per  $10^4$  g protein. The total amount of SH-groups in myofibrils after reduction ranged between 13.3 and 14.8 moles/ $10^4$  g protein.

The cysteine and half-cystine group content obtained chromatographically (ion exchange) after hydrolysis of myofibrils in HCl amounted to only 10 moles per  $10^4$  g protein. Other authors have found similar values (8–9 moles)<sup>2,3</sup>. Apparently, a part of cysteine or cystine cannot be recovered after hydrolysis, even after oxidation to cysteic acid. Probably for this reason, Bárány *et al.*<sup>3</sup> could not find SS-groups in myosins. In our opinion, only the titration of SH-groups in the intact protein after reduction with  $\text{NaBH}_4$  will provide the true value of the cysteine and half-cystine group content.

Heating of myofibrils up to 70° C (30 min) results in a strong increase of SH-groups reacting with NEM (Fig. 1). Apparently, heat denaturation causes an unfolding of the peptide chains and, therefore, a release of reactive SH-groups which before were hidden within the folded structure of the native protein.

The amount of SH-groups of myofibrils reacting with  $\text{AgNO}_3$  is not affected by heating (30 min) up to 70° C (Fig. 2). This is intelligible because all SH-groups in the native protein are able to react with  $\text{AgNO}_3$  under the experimental conditions used. From this result we can conclude that the process of heat coagulation of beef taking place between 50° and 60° C (ref. 4) is not accompanied by formation of new disulphide bonds between the protein molecules. But by heating myofibrils up to the temperatures which are commonly used for canning meat (110°–120°), the amount of SH-groups reacting with  $\text{AgNO}_3$  is considerably decreased (Fig. 2). This effect is greater in the presence of air than under nitrogen. The

decrease of SH-groups at high temperatures is apparently due to an oxidation process which cannot be completely eliminated by treatment under  $\text{N}_2$ , because it is not possible to remove all the air from the tissue or the myofibrils. In actomyosin gels, however, heating under  $\text{N}_2$  does not result in any significant decrease of SH-groups; in this case the air can be almost completely removed.

If myofibrils heated at 120° C for 30 min are treated with  $\text{NaBH}_4$ , almost the same amount of SH-groups reacting with  $\text{AgNO}_3$  is found after reduction as in the reduced unheated myofibrils (Table 1). From this result it is clear that the decrease of SH-groups by heating under these conditions is caused for the most part by oxidation to SS-groups. The increase in the toughness of meat known to be caused by prolonged cooking may be due to such a formation of intermolecular disulphide linkages between the peptide chains of the actomyosin.

Table 1. CHANGES OF SH- AND SS-GROUPS DURING HEATING OF MYOFIBRILS AT 120° C FOR 30 MIN

Unheated samples	SH Moles per $10^4$ g protein	SS Moles/2 per $10^4$ g protein	Heated under	SH Moles per $10^4$ g protein	De-crease %	SS Moles/2 per $10^4$ g protein	In-crease %	S+SS de-crease %
7.2 ( $\pm 0.2$ )	6.1 ( $\pm 0.2$ )		Air	4.8 ( $\pm 0.2$ )	40	8.3 ( $\pm 0.3$ )	35	7
			$\text{N}_2$	5.4 ( $\pm 0.3$ )	25	7.8 ( $\pm 0.3$ )	20	6

However, if the myofibrils are heated at 120° C for a longer time, for example, for 5 h, the amount of SH-groups determined after reduction by  $\text{NaBH}_4$  is considerably less than in the reduced unheated samples (Table 2). Therefore, some SH- and SS-groups are destroyed under these conditions of heating.

Of all the reactions which may be responsible for the destruction of SH-groups, the formation of  $\text{H}_2\text{S}$  comes first into question. For the determination of  $\text{H}_2\text{S}$  released during heating, we modified the method of Marbach and Doty<sup>4</sup>. The sample (2 g) is heated under  $\text{N}_2$  in a closed apparatus; the  $\text{H}_2\text{S}$  formed passes into a trap containing

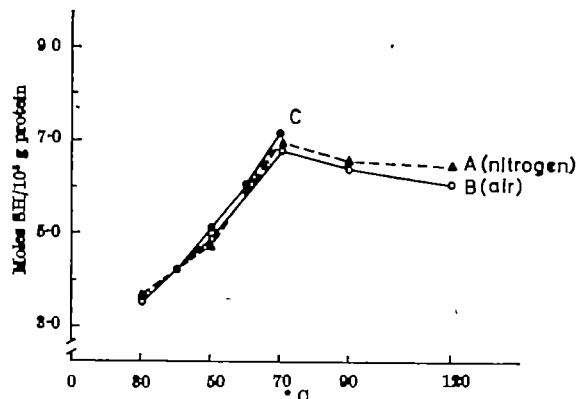


Fig. 1. Effect of heating (30 min) myofibrils on the amount of SH-groups reacting with NEM.

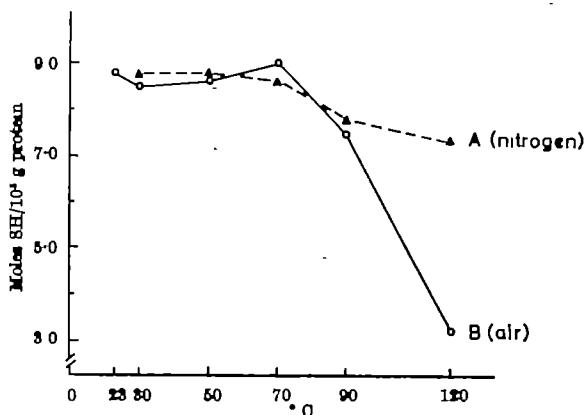


Fig. 2. Effect of heating (30 min) myofibrils on the amount of SH-groups reacting with  $\text{AgNO}_3$ .

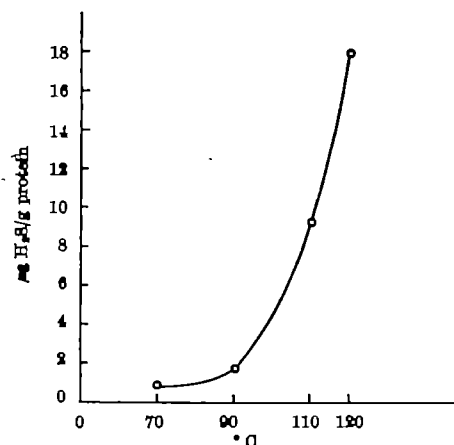
Table 2. CHANGES OF SH- AND SS-GROUPS DURING HEATING OF MYOFIBRILS AT 120° C FOR 5 H

Heated under	SH Moles per 10 <sup>6</sup> g protein	SH Decrease %	SS Moles/2 per 10 <sup>6</sup> g. protein	SS Increase %	S-S decrease %
Air	2.8 (±0.3)	61	7.1 (±0.5)	16	25
N <sub>2</sub>	4.8 (±0.3)	33	6.8 (±0.5)	11	13

Note: the percentage values are related to the values for the unheated samples (compare with Table 1).

NaOH and is determined by photometric measurement (870, 740 nm) of the colour developed after reaction with *p*-amino-dimethyl-aniline and FeCl<sub>3</sub> in HCl. Mercaptans did not interfere with this reaction. As Fig. 3 shows, the formation of H<sub>2</sub>S from myofibrils starts at about 80° C and increases exponentially with rising temperature. The formation of H<sub>2</sub>S increases with increasing time of heating. Heating of the total muscle tissue up to 120° C for 30 min caused the formation of 18.4 and 19.3 µg H<sub>2</sub>S/g protein. This is almost the same as the amount of H<sub>2</sub>S formed by heating the myofibrils (18.7 and 16.8 µg). Therefore, almost the total amount of H<sub>2</sub>S released by heating originates from the structural proteins and not from any water-soluble substances, such as cysteine, present in the tissue. After blocking the SH-groups of the native myofibrils by reaction with AgNO<sub>3</sub>, and also by NEM, heating at 120° C no longer causes the formation of H<sub>2</sub>S. These results show that H<sub>2</sub>S originates from the easily reacting SH-groups of the structural proteins and not from SS-groups or methionine. Fraczak and Pajdowski<sup>6</sup> and recently Mecci *et al.*<sup>7</sup> have suggested that the H<sub>2</sub>S developed during the heating of meat may originate from the SH-groups of the muscle proteins; our experiments demonstrate for the first time that this is indeed the case. All the results reported here could also be obtained with actomyosin.

As our results show, heating up to 70° C causes an unfolding of the actomyosin molecules. At higher tempera-

Fig. 3. Effect of heating (30 min) on the formation of H<sub>2</sub>S from myofibrils

tures, an oxidation of SH- to SS-groups occurs. During longer heating at high temperatures, H<sub>2</sub>S is also formed and this originates from the free or easily reacting SH-groups of actomyosin.

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## AGREEMENT BETWEEN CARCINOMA EXPERIMENTS IN VIVO AND LOW-ENERGY X-RAY IRRADIATION OF THE METALLOPROTEIN CARBONIC ANHYDRASE

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A PHENOMENON characteristic of carcinoma is the sub-normal zinc-level of whole blood of the patients<sup>1</sup>. In earlier papers this sub-normal level has been related either to a decreased content of carbonic anhydrase present in the erythrocytes or to this metallo-protein containing sub-normal zinc<sup>2</sup>. Because of the fact that there have been found significant changes in the zinc-level of blood and urine during and after radiotherapeutic treatment (see hereafter: experiments *in vivo*) it was of interest to examine the influence of X-rays on carbonic anhydrase.

Destruction by X-rays of a metallo-protein in general can be effected, for example, by irradiating into the *K*-absorption band of the metal. Two processes will then occur: that of fluorescence and that of the Auger effect. In both cases important changes regarding the (outermost) valency electrons occur, because the latter have to fill electron holes in the *K*-, *L*-shells, etc. This means that the metal atom can be freed from the organic protein molecule by irradiation of one of the absorption bands of the metal. The metal is then brought into solution in the ionic form.

When a solution of the zinc protein in water is irradiated with a continuum of X-rays, the shortest wave-length being larger than that of the *K*-absorption band of the metal (1.3 Å), there will be scarcely any absorption of the

irradiated energy by zinc. However, by increasing the supply voltage of the tungsten tube, that is, using wave-lengths just below the edge of the *K*-absorption band of zinc, such wave-lengths are absorbed by the metal, and this is freed from the residual organic molecule in the way described here. The higher the operating voltage of the tube, the shorter the minimum wave-length of the continuum, and the greater the chance of freeing zinc.

This effect is illustrated in Fig. 1, which shows how irradiation with the highest energy (case c) releases the zinc in a short time. Using wave-lengths just below the edge of the *K*-absorption band of zinc (curve b), one can also bring about a relatively rapid dissociation. But a very slight and slow-going dissociation occurs if one chooses the tungsten-tube operating voltage 1–2 kV lower (curve a).

It is worth noting that the dissociation processes are of a monomolecular nature. A reaction of the same kind occurs when vitamin B<sub>12</sub>, which contains cobalt, is irradiated.

It should also be noted that only the firmly bound zinc is affected by the releasing process<sup>3</sup>. One should realize that of the zinc which carbonic anhydrase contains, two-thirds is loosely bound, that is, separable by shaking with dithizone<sup>4</sup>, while the remaining one-third is firmly bound, that is, not separable by means of shaking with dithizone.

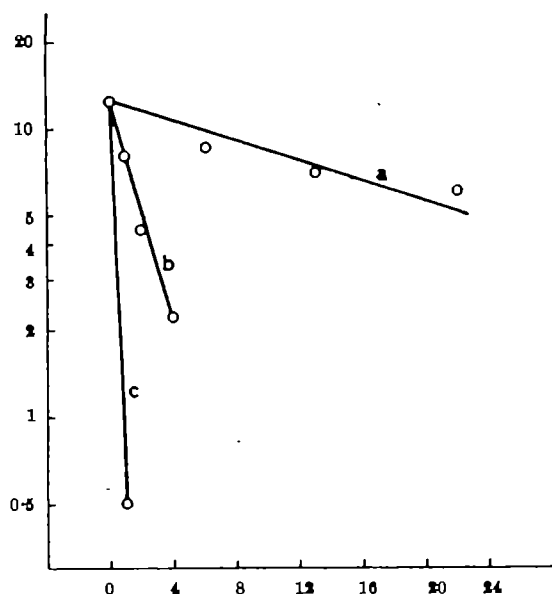


Fig. 1. Abscissa: time of irradiation (h); X-ray tube: a, 9 kV, 20 m.a.m.p., corresponding to 4,700 r./min.; b, 10.5 kV, 20 m.a.m.p., corresponding to 8,000 r./min.; c, 50 kV, 18 m.a.m.p., corresponding to 200,000 r./min. Height of the irradiated specimen: 15 mm; stirring of the solution each 15 min; irradiated surface 4.4 cm<sup>2</sup>; distance X-ray tube to specimen in all cases 8 mm (Philips X-ray spectrograph PW 1520). Ordinate:  $\mu$ g of firmly bound zinc present in a solution of 10 ml. water containing 10 mg of CO<sub>2</sub>-anhydrase after irradiation

It is the latter part which can be released by irradiation with X-rays.

It is of interest to point out here that as a result of the irradiation the activity of the enzyme (that is, the release of carbon dioxide gas from an HCO<sub>3</sub><sup>-</sup> solution) diminishes as the content of firmly bound zinc decreases, as is shown in Table 1.

From these experiments two conclusions can be drawn: (1) X-ray irradiation initiates the effect of bringing firmly bound zinc into the soluble ionogenic form. (2) The activity of the enzyme carbonic anhydrase is correlated with its content of firmly bound zinc (compare also ref. 8).

Wolff<sup>8</sup> has shown that within 24 h after radiotherapy of carcinoma patients the serum zinc of blood increases from 1.1 p.p.m. to 1.6 p.p.m., whereas after 48 h the zinc content falls back to its original value. This result can also be ascribed to releasing the firmly bound zinc.

In experiments carried out in co-operation with Dr. L. J. P. Frank, I have shown that during prolonged radio-

	Table 1 Content of firmly bound zinc present in 10 ml.	Activity (= $\mu$ equiv. H <sup>+</sup> / sec/mg CO <sub>2</sub> -anhydrase) pH range = 6.6-6.4 (ref. 4)
<b>Solution A</b>		
Not irradiated	1.25 $\mu$ g	41.5
After 1 h irradiation (10.5 kV, 20 m.a.m.p.)	0.84 $\mu$ g	8.25
After 4 h (the same)	0.20 $\mu$ g	2.5
<b>Solution B</b>		
After 1 h irradiation (50 kV, 10 m.a.m.p.)	0.00 $\mu$ g	0.2

**Solution A:** a white preparation obtained from Koch Light laboratories; content of firmly bound zinc 0.125 per cent; 1 mg dissolved in 10 ml. water.

**Solution B:** a yellow-greyish preparation from the same firm; content of firmly bound zinc 0.145 per cent; 0.4 mg dissolved in 10 ml. water; this solution showed an activity of 20.

therapeutic treatment (mammary cancer) the zinc content of urine (X-ray fluorescence analysis; bromine as internal standard) can increase from 0.5 p.p.m. to 1.5 p.p.m., which means that again zinc has been brought into the ionogenic form.

In cases where the zinc content of whole blood of carcinoma patients is extremely low, radio-therapeutic treatment does not increase the zinc content of the patients' urine. This is in accordance with experiments carried out together with Dr. H. J. v. d. Berg (unpublished results), showing that carbonic anhydrase prepared from the blood of these patients<sup>9</sup> does not contain any firmly bound zinc.

It is known that one-third of the total zinc amount in whole blood is in the firmly bound state<sup>3</sup>. In earlier work by Addink and Frank<sup>1</sup> it was stated that in severe cases of carcinoma the lowest value of whole blood zinc amounts to 4.4 p.p.m.: a decrease of one-third compared with the 6.5 p.p.m. found for healthy patients. Once again this indicates that firmly bound zinc is absent in such severe cases of carcinoma.

As such patients remain living over a certain period of time, there must be some firmly bound zinc present; but these small quantities are not detectable chemically. One should keep in mind that according to Roughton<sup>7</sup> blood contains at least forty times the required quantity of carbonic anhydrase.

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## A THEORY ON THE MECHANISM OF CARCINOGENESIS BY SMALL DEOXYRIBONUCLEIC ACID TUMOUR VIRUSES

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THE small DNA tumour viruses polyoma, SV40, papilloma and adenoviruses, contain an amount of DNA that can code for only a small number of proteins. Thus the DNA of polyoma, with a molecular weight of about  $3 \times 10^6$  (ref. 1), has about 4,500 nucleotide pairs, and these can code for about 1,500 amino-acids. Since part of this DNA codes for the viral coat protein, and there may be late functions associated with virus development which inhibit cellular DNA synthesis<sup>2</sup> prior to cell lysis, the amount of DNA which remains to initiate carcinogenesis could only be enough to code for a few functions. The purpose of this article is to show that two virus coded functions can be sufficient for carcinogenesis, that the postulated mechanism of carcinogenesis by these two functions is in agreement with all the

present experimental findings, and to indicate the possible application of these findings to carcinogenesis by hydrocarbons.

Experimental results on cell-virus interactions with these viruses can be divided into six main categories.

(A) Early RNA and early protein are synthesized during virus development, before the synthesis of viral DNA<sup>3</sup>.

(B) A nuclear tumour antigen is synthesized early during virus development<sup>4</sup>.

(C) All tumours synthesize a transplantation, presumably cell surface, tumour antigen. For both the nuclear and the transplantation tumour antigen, different tumours induced by the same virus have the same antigen, and different viruses induce the synthesis of different antigens<sup>5</sup>.

(D) Virus infection can lead to an induction of cellular DNA synthesis<sup>6</sup>.

(E) The cell-virus interaction can result either in the development of virions and cell lysis, or it can directly and rapidly induce the transformation of normal cells to tumour cells<sup>7</sup>.

(F) Tumour cells generally do not produce detectable virus<sup>8</sup>.

(A) has been established for adenovirus and polyoma, (B) for SV40 and adenovirus, nuclear localization most clearly with SV40, (C) for polyoma and SV40, (D) for polyoma, and (E) and (F) have been most extensively established for polyoma.)

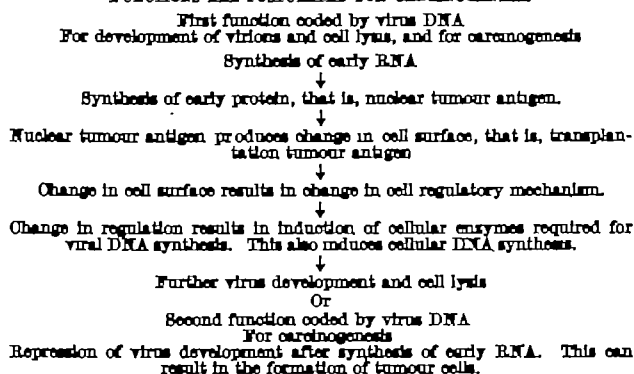
The proposed theory suggests that the two virus coded functions which can explain all these findings are the synthesis of RNA which serves as messenger for the early protein, and a function which represses further virus development after synthesis of this early RNA.

Both the early protein and the nuclear tumour antigen seem to be synthesized at about the same time, and it is suggested in the present hypothesis that the nuclear tumour antigen is the early protein. All virus-induced tumours possess a transplantation tumour antigen, and it is suggested that the nuclear antigen is responsible for the synthesis of the transplantation antigen. This suggestion predicts that the nuclear antigen has enzymatic activity. The synthesis of transplantation antigen can be taken as an indication that there has been a change of the cell surface in comparison to non-infected normal cells. It is assumed that this change in surface changes the cellular regulatory mechanism so as to transform normal cells into tumour cells. The maintenance of the virus-induced cell surface change in the transformed cells, as shown by the continued presence of the transplantation antigen in the tumour cells, would then permit the maintenance of the changed regulatory mechanism. It would follow from the finding that nuclear tumour antigen can be synthesized after virus infection in the presence of inhibitors of DNA synthesis<sup>9</sup>; that the suggested change in cell surface regulation can occur without viral DNA replication. The synthesis of a tumour antigen in the nucleus is presumably due to the fact that virus synthesis takes place in the nucleus.

The suggestion that viral DNA directly codes for the nuclear tumour antigen explains the differences in antigen induced by different viruses. If this assumption of direct coding proves to be correct, the maintenance of nuclear antigen in the tumour cells<sup>10</sup> would be a convincing argument in favour of the assumption<sup>11</sup> that there can be an integration of viral DNA in the tumour cells. The integrated DNA would then be the nucleotide sequence that codes for the nuclear tumour antigen. It would thus be of considerable importance to isolate early RNA, and to characterize it regarding messenger activity for nuclear tumour antigen and for complementarity to virus DNA. It would also be of considerable value for further investigations to have mutants of the same virus that induce the synthesis of different nuclear antigens. The possibility of finding such mutants is suggested by the observation of differences in transplantation tumour antigen induced by different strains of polyoma<sup>12</sup>, and the production of further mutants of this type, together with a marker such as temperature sensitivity<sup>13</sup>, would be highly desirable. Tumour antigens would therefore seem to be the most suitable virus-induced markers<sup>14</sup> for experiments on the possibility of marker rescue in virus-induced tumour cells.

Another finding which has to be explained is the induction of cellular DNA synthesis as a consequence of virus infection, which occurs before the repression of cellular DNA synthesis that precedes cell lysis. It can reasonably be assumed that the amount of viral DNA is not enough to code for all the enzymes required for virus synthesis, so that an induction of cellular enzymes would be required for virus development. The inhibition of polyoma virus synthesis after pre-treatment of cells with mitomycin<sup>15</sup>

Table 1. SEQUENCE OF EVENTS THAT RESULT EITHER IN THE DEVELOPMENT OF VIRIONS AND CELL LYSIS, OR IN CARCINOGENESIS. TWO VIRUS CODED FUNCTIONS ARE POSTULATED FOR CARCINOGENESIS



can also be explained in that the pre-treatment prevents the synthesis of cellular enzymes required for virus development. It is assumed that the change in cell regulatory mechanism induced by the change in cell surface results in the synthesis of cellular enzymes required for virus DNA synthesis. This change in regulation could then also result in the induction of cellular DNA synthesis. This predicts that there can be an induction of cellular DNA synthesis without the replication of viral DNA. This suggested mechanism for the induction of cellular enzymes could also explain the long latent period of these viruses.

It has been shown that the cell-virus interaction can result either in virus development and cell lysis, or in carcinogenesis. Once the messenger RNA for the nuclear tumour antigen has been synthesized, the only other function required to explain this duality in cell-virus interaction is one that represses further virus development. This would then be the second virus coded function for carcinogenesis (Table 1). Expression of this function could result in the growth of tumour cells, whereas a lack of expression would result in further virus development and cell lysis. A low frequency of expression of the virus coded functions required for carcinogenesis and/or the initial transmission of the transformed state to daughter cells, could account for the finding that, even after virus infection of single cell clones, only a minority of the cells undergo the transformation of normal cells to tumour cells<sup>16</sup>.

During growth of the tumour cells after the initial transformation there may be a destruction of the viral genome, or if there is an integration of viral DNA and the nuclear antigen is continuously coded by viral DNA, according to the present hypothesis, the only part necessary to maintain the transformed state would be the nucleotide sequence that codes for the nuclear antigen. Either of these alternatives would explain the finding that the tumour cells generally do not produce detectable virus.

It is of interest to see how far the present theory of carcinogenesis by these viruses can be applied to carcinogenesis by hydrocarbons. It has been shown that carcinogenic hydrocarbons can also induce either cell lysis<sup>17</sup>, or directly and rapidly induce the transformation of normal cells to tumour cells<sup>18</sup>. Tumours induced by these compounds have new transplantation antigens, although in contrast to the situation with virus-induced tumours, a variety of tumour transplantation antigens have been found in tumours induced by any one carcinogen<sup>19</sup>. The maximum number of possible cell surface changes as expressed by differences in tumour transplantation antigens is not known. There are, however, presumably a finite number of possible cell surface changes associated with the change in cell regulation resulting in carcinogenesis. There may thus occasionally be the same change produced by a chemical carcinogen and a tumour virus, and such cross-reacting transplantation antigens have been reported<sup>20</sup>.

With the carcinogenic hydrocarbons it can be postulated that they can directly interact with the cell surface to produce the changes recognized as tumour transplantation antigens, and this may account for the variety of antigens that have been found with the same carcinogen. It would be of considerable interest to know if tumour cells induced by carcinogenic hydrocarbons have a nuclear tumour antigen, as in the case of the virus-induced tumours, and whether a tumour antigen is produced during the process that leads to cell lysis. If the change in the cell surface induced by carcinogenic hydrocarbons can produce changes in regulatory mechanism of the same type as that produced by infection with the tumour viruses, the initial change may also be expressed by an induction of cellular enzyme synthesis. The finding of an induction of some cellular enzymes soon after treatment with carcinogenic hydrocarbons<sup>11</sup> would support the suggestion of a common type of change.

The conclusions drawn here suggest that the further analysis of what have been proposed as the two virus coded functions required for carcinogenesis, and of the changes in synthesis in cells treated with carcinogenic hydrocarbons, could provide crucial evidence on the similarity in the mechanism of carcinogenesis by the small DNA tumour viruses and by carcinogenic hydrocarbons.

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## MEASUREMENT OF THE EXCLUDED VOLUME OF PROTEIN MOLECULES BY DIFFERENTIAL SPECTROSCOPY IN THE NEAR INFRA-RED

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WE have observed that a solution of protein in water measured differentially against water produces a negative near-infra-red difference spectrum. This phenomenon is shown in Fig. 1. Curves A and A' represent the phenomenon as observed. Curves B and B' are the spectra of water, presented here for reference. The relationship between the water spectrum and the phenomenon can be seen by comparing each peak of the spectra. The detailed peak-for-peak correspondence is apparent. Curves A and A' are negative water spectra to a very close approximation.

The apparent anomaly in the difference spectrum of bovine serum albumin (BSA) at  $1.7\mu$  is in the region of the first overtone absorption of the C—H stretching frequency in proteins as shown by Hecht and Wood<sup>1</sup>.

A model system (Fig. 2) is proposed to account for the phenomenon observed. The excluded volume, represented in this scheme as a difference, will, in effect, cause a dilution of the solvent (water) in the sample cell. In regions of the spectrum where the extinction of the solvent far exceeds that of the solute, the absorption will be greater in the reference cell than in the sample cell, and a negative difference spectrum will result. This excluded volume can be represented by the volume occupied by the solute in solution.

If this phenomenon is a consequence of volume excluded to the solvent by the solute, then the following criteria should be met. (a) The total absorption of each peak in

the water spectrum of the sample cell should be decreased and the resultant difference spectrum should represent a negative water spectrum. (b) All solutes, regardless of composition, should give a negative difference spectrum, since all of them occupy volume. (c) Where the volume of a solute is known, the amount of exclusion should be predictable.

Satisfaction of criterion (a) was demonstrated in Fig. 1. Satisfaction of criterion (b) is shown in Fig. 3, where the difference spectra of a number of different solutes in aqueous solution versus water are represented. (The near-infra-red spectra of water and aqueous salt solutions have been studied previously by Aschkinase<sup>2</sup> and Collins<sup>3</sup>, among others.) In each case a negative difference spectrum is obtained. This fact itself is significant, since the differences between large polymers (ionic and non-ionic), a non-ionic small organic molecule, and an inorganic salt are quite extreme for any basis of comparison.

Certain anomalies are observed in comparing the difference spectra of each of these solutions to a normal water spectrum. These anomalies are more pronounced for the more ionic and more freely solvated molecules. Most of the deviations from a true negative water spectrum for protein and sucrose solutions can be accounted for by the positive absorption of the solute. This positive absorption acts to decrease the magnitude of the negative difference spectrum in these regions. Since polyvinylpyrrolidone and sodium chloride have no significant absorption, the

anomalies in their exclusion difference spectra must result from either a different kind or a different amount of interaction between the solute and the solvent. This interaction affects the absorption of the solvent in certain regions and thus produces a difference spectrum related more to the solute-solvent interaction than to the volume of the solute. This is quite obvious in the case of the difference spectrum of a solution of sodium chloride.

We may compare protein, sucrose, and sodium chloride in terms of their mode of solvation in aqueous solution. Both sucrose and sodium chloride have relatively small molecular volumes in comparison to proteins but show

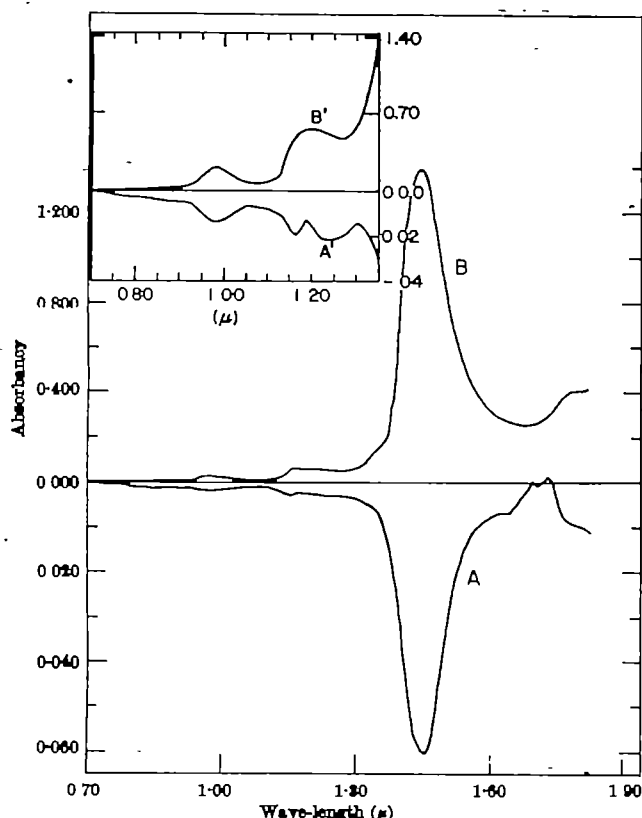


Fig. 1. Difference spectrum of BSA versus water. Curve A, 60 mg/ml. BSA versus water; curve B, water versus empty cell; 1-mm path-length cells thermostatted at 16° C. Curves A' and B', same solutions and conditions as for A and B; 1-cm path-length cells. Crystallized and lyophilized BSA was obtained from Sigma Chemical Co. and used without further treatment.

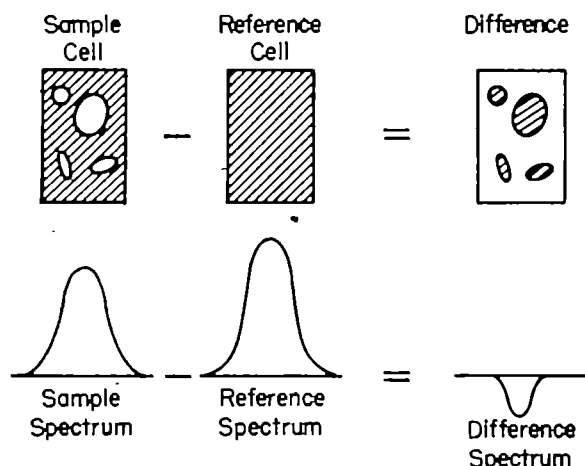


Fig. 2. A schematic explanation of the exclusion phenomenon. The upper line of figures represent the experimental system; the lower line of figures represent the resulting spectral contributions. The hatched areas represent solvent. The irregular open figures represent non-absorbing solute. The algebraic difference between the sample and reference cells is seen to be an amount of solvent equivalent to the volume occupied by solute.

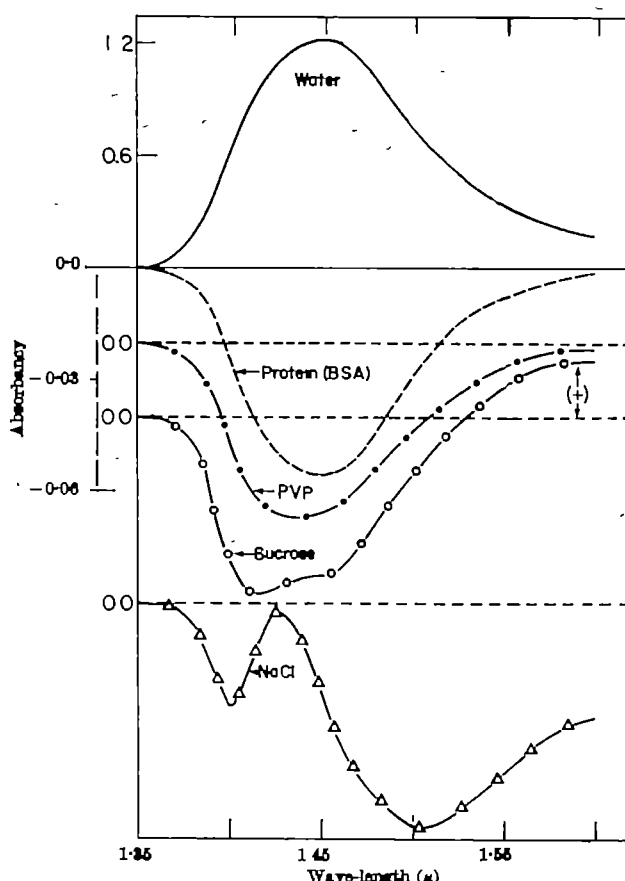


Fig. 3. The difference spectra of several aqueous solutions versus water. Solutions used: ---, 50 mg/ml. BSA; ●, 50 mg/ml. polyvinylpyrrolidone (PVP); ○, 78 mg/ml. sucrose; △, 78 mg/ml. NaCl. The solid line water spectrum is presented for reference. All solutions, except PVP, had the same refractive index at 25° C. The other conditions were the same as those listed for curves A and B in Fig. 1. The PVP used was grade K 80 obtained from Oxford Laboratories, Redwood City, California. It was dialysed to remove salts and lyophilized.

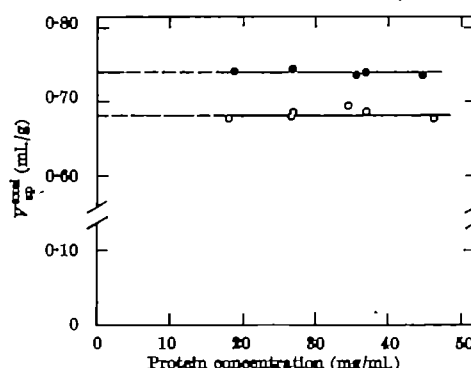


Fig. 4. Excluded specific volume,  $V^{\text{excl}}$ , versus protein concentration. ●, BSA; ○, lysozyme. Experimental conditions are the same as those used for curves A and B in Fig. 1. Crystallized and lyophilized egg white lysozyme was obtained from Sigma Chemical Co. and used without further treatment.

significantly different anomalies in their difference spectra. The fact that a non-ionic sucrose solution gives a difference spectrum closely related to that of a protein solution, while the difference spectrum for an ionic sodium chloride solution is quite anomalous, suggests that solvation of protein molecules differs from that of ionic solutes such as sodium chloride. (We assume here that there is a relationship between the shape of the difference spectrum and the type of solute-solvent interaction.)

To examine the relationship between the volume of a solute and the phenomenon observed (the satisfaction of criterion (c)), solutions of albumin and lysozyme were studied. The results are plotted in Fig. 4 as excluded

specific volume,  $V_{sp}^{rel}$ , versus protein concentration. (We are inferring that this method, though different from Schachman's<sup>4</sup>, measures a similar excluded volume.)  $V_{sp}^{rel}$  was calculated from the following expression:

$$V_{sp}^{rel} = \frac{(A')_{1.44\mu} - (A')_{1.34\mu}}{(A^0)_{1.44\mu} - (A^0)_{1.34\mu}} (1/O)$$

where  $A'$  equals the absorption of the solution at  $\lambda$ ,  $A^0$  equals the absorption of water at  $\lambda$ , and  $O$  equals the protein concentration in g/ml. The protein concentrations were determined spectrophotometrically;  $E_{280}^{1\%}$  (BSA) = 6.60 (ref. 5),  $E_{280}^{1\%}$  (lysozyme) = 26.4 (ref. 6).

The values obtained for  $V_{sp}^{rel}$  appear to be independent of concentration. The average values of  $V_{sp}^{rel}$  for BSA and lysozyme are 0.74 ml./g and 0.68 ml./g respectively. These values are close to the values for the apparent specific volume reported in the literature: BSA = 0.734 ml./g (ref. 7), lysozyme = 0.703 ml./g (ref. 8). This is somewhat more direct evidence of the relationship of this phenomenon to some volume parameter in agreement with criterion (c) and, in fact, suggests that this parameter is

closely associated with the partial specific volume of the protein molecule.

Differential measurement of aqueous solutions in the near infra-red produce negative spectra which are sensitive to both the volume and interaction parameters of the solute. While no definite correlation between the phenomenon observed and these properties can be made at this time, such measurements suggest a useful means of studying these parameters.

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## INDUCED CHANGES IN ORIENTATION OF THE CROSS-BRIDGES OF GLYCERINATED INSECT FLIGHT MUSCLE

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MYOGENIC fibrillar insect flight muscle is known to perform considerable work by means of small-amplitude oscillations both in life<sup>1</sup> and when glycerinated<sup>2</sup>. The structure of the muscle is exceptionally well ordered<sup>3,4</sup>. Electron microscope studies indicate that the ends of the myosin filaments are connected to the Z line via short connecting filaments<sup>5</sup>. The muscle, even when relaxed, has a much higher elastic modulus than relaxed frog or rabbit muscle<sup>1</sup>. Glycerinated insect flight muscle can be relaxed by bathing it in a solution of adenosine-5-triphosphate (ATP), or thrown into a rigor-like state of increased rigidity and decreased rest length by removal of ATP; the transition between these two mechanical states is reversible. We have studied these two states, and the transition between them, by both X-ray diffraction and electron microscopy. Our results show that the transition is accompanied by a re-orientation of cross-bridges between actin and myosin filaments.

**X-ray diffraction of glycerinated muscle in absence and presence of ATP.** Dorsal longitudinal muscles of the giant tropical water bug *Lethocerus maximus* were used. They were immersed *in situ* in 50 per cent glycerol at pH 7 for several weeks at  $-20^\circ\text{C}$  before use. X-ray diffraction measurements were carried out on sets of fibres mounted in a small 'Perspex' cell modified from a design by Dr. W. Brown, in which the X-ray beam passes through 0.3–0.5 mm thickness of muscle, immersed in the appropriate solution and stretched as required. The whole cell could be cooled; most experiments were performed at  $3^\circ$ – $7^\circ\text{C}$ . Copper  $K\alpha$  radiation was used. A specialized X-ray tube has been built in collaboration with Dr. W. Longley, consisting of a fine-focus rotating anode tube which produces an X-ray source of 1.5 mm  $\times$  0.15 mm loaded with 800 W at 40 kV. The X-ray camera was also novel<sup>6</sup>; it consists of a bent gold-plated glass mirror followed by a bent quartz crystal monochromator, the devices being set so as to produce a common point focus on the film. With this apparatus, 20 min exposures at a specimen-film distance of 12 cm showed the main diffraction features described below. Spacings indicated

on X-ray photographs were measured to  $\pm 0.5$  per cent on a Nikon shadowgraph.

In a simple ionic solution which did not contain ATP (50 mM KCl, 5 mM  $\text{MgCl}_2$ , 4 mM EGTA, 10 mM *tris* at pH 7.15) the muscle is known to be in rigor<sup>3</sup>; we have examined this state at  $5^\circ\text{C}$ . In this solution X-ray diffraction showed a highly characteristic crystalline pattern (Figs. 1a and b). There were a set of equatorial reflexions, arising from a hexagonal lattice of side 530–560 Å (refs. 4 and 8), a strongly sampled first-layer line at  $388 \pm 2$  Å spacing, and further layer lines which can all be indexed as orders of  $2 \times 388$  Å (Fig. 2a). Additional pictures obtained with the apparatus adjusted for high resolving power showed that the 388-Å and 194-Å layer lines had no intensity on the meridian. Moreover, all other low-angle meridional reflexions were weak or absent. On stretching the muscle in rigor by up to 5 per cent over its rest length, there was no significant change in the layer line spacing.

When the solution was changed to one containing 5 mM ATP plus the same ingredients as before, the muscle relaxed, both at room temperature<sup>3</sup> and when cooled, the X-ray diffraction pattern changed dramatically (Fig. 1c). The 388 Å layer line was greatly attenuated and strong meridional spots appeared at 146 and 73 Å (orders of  $146.1 \pm 0.3$  Å; Fig. 2b). The spacings of these spots were likewise unaffected by applying 5 per cent stretch to the muscle. On removal of the ATP by rinsing in ATP-free solution the muscle reverted to the rigor pattern. This cycle could be repeated several times, and could be performed either at room temperature or when the cell was cooled.

**Electron microscopy: experimental.** For electron microscopy, specimens were prepared by adding 1/10 volume of collidine-buffered<sup>7</sup> 25 per cent glutaraldehyde to the solution (at  $0^\circ\text{C}$ ), bathing either relaxed or rigor muscle in the same cell as that used for the X-ray diffraction. Thus fixed specimens could be observed directly in the X-ray beam. The action of the glutaraldehyde in the cold did not affect the X-ray diffraction pattern; the charac-



teristic patterns of relaxed or rigor muscle were still visible after fixation. The specimens were post-fixed in osmium tetroxide, dehydrated in acetone and embedded in 'Araldite' or 'Epon' (Luft's 'Mixture A'<sup>10</sup>); some were stained with phosphotungstic acid in methanol before embedding. Phosphotungstic acid-stained, 'Araldite'-embedded specimens were also observed in the X-ray beam—a method of checking the state of the fixed specimen which was suggested to us by the work of Elliott<sup>11</sup>. They showed the characteristic relaxed or rigor diffraction patterns, at 2 per cent lower meridional spacing, and with much higher intensity in the high-angle meridional reflexions. The spacings, 143.2 and 380 Å, agree with those found by X-ray diffraction in embedded specimens prepared from fresh blowfly (*Calliphora*) flight muscle<sup>11</sup>.

Longitudinal sections of embedded specimens were cut on a Porter-Blum microtome, using a diamond knife, and were stained for 5–20 min with either uranyl acetate<sup>12</sup> or potassium permanganate<sup>13</sup>, followed by 1–5 min additional staining with lead citrate<sup>14</sup>. They were examined in a

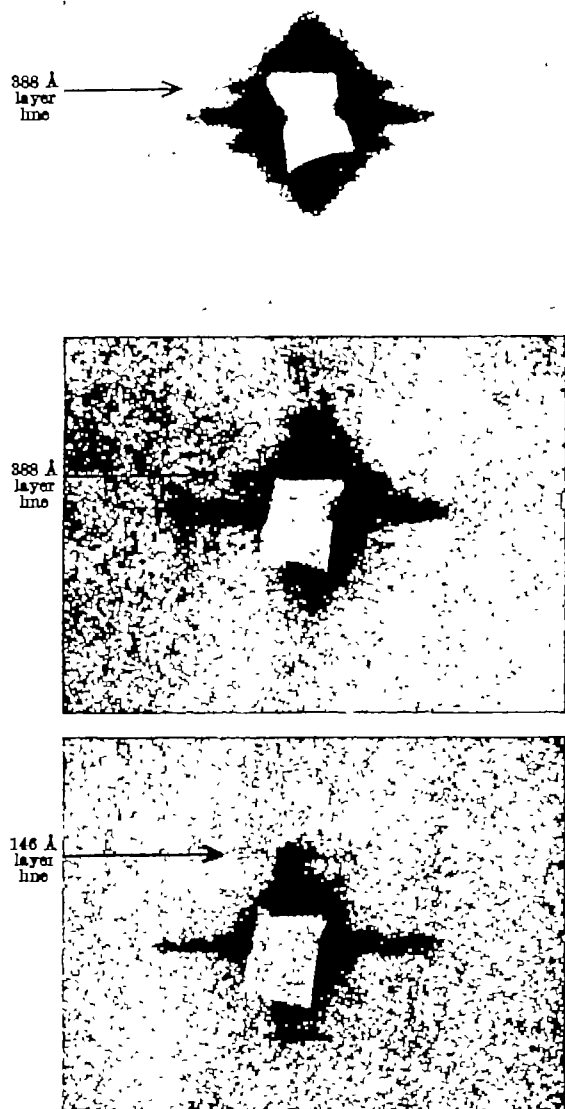


Fig. 1. *a*, X-ray diffraction pattern from glycerinated flight muscle of *L. maceratus* in rigor. X-ray beam at right angles to fibre axis. Exposure time 1 h. Note the 388 Å layer line and the effects of the lattice on the zero and first layer lines. *b*, Same as *a*. Exposure time 20 min. Note the weakness of any meridional reflexions. *c*, Same as *a*, but in presence of 5 mM ATP. Exposure time 20 min. Note the strong 146 Å meridional reflexion.

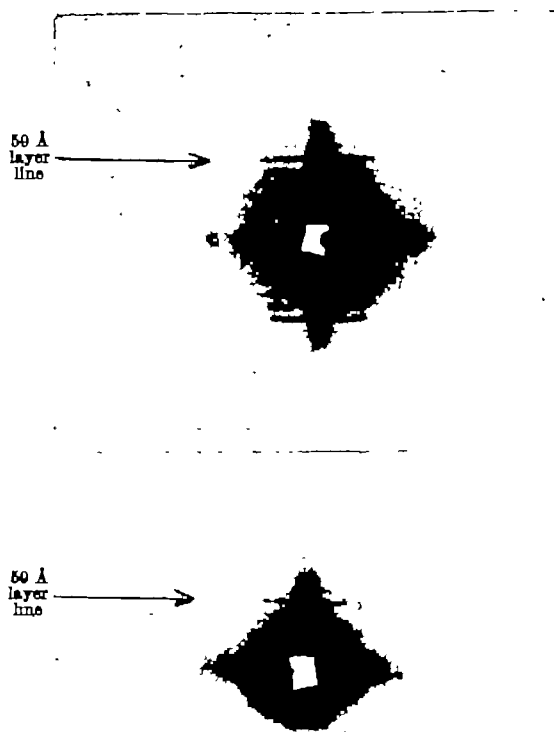


Fig. 2. *a*, Same as Fig. 1*a*. Exposure time 15 h. *b*, Same as Fig. 1*a*. Exposure time 15 h. ATP solution replenished periodically. Orders of 146 Å can be seen together with actin reflexions.

Siemens Elmiskop; magnification was calibrated to  $\pm 1$  per cent, using a method derived from refs. 15 and 16. In insect flight muscle, each actin filament lies midway between two myosin filaments<sup>4,5,6</sup> (Fig. 3). Sufficiently thin sections should therefore sample single layers of filaments, composed of thin filaments only in some regions and alternately thick and thin filaments in other regions. Our 200–400-Å-thick sections contained regions of each type (Fig. 3). Cross-bridges between actin and myosin filaments were clearly visible in single filament layers containing both filament types. The ends of the cross-bridges attached to actin were visible in single filament layers containing actin filaments only. The cross-bridges were in good transverse register across each myofibril, which facilitated measurements of the axial periodicities associated with the bridges. Strong diagonal periodicities resulted from the combined transverse and axial regularity, especially in thick sections (600–1500 Å), and also proved useful in measuring axial periods. The axial periods were obtained as average values over intervals of 10–60 successive repeats, from sections cut with the fibre axis parallel to the knife edge.

**Specimens fixed in rigor.** In specimens fixed in rigor a 380-Å period was easily seen in myofibrils in thick sections; no other period was evident. Thin sections demonstrated a regular polarized arrangement of cross-bridges in single-filament layers containing alternately thick and thin filaments. The bridges were slanted at a mean angle of about  $45^\circ$  to the filament axis; there was appreciable variation about this mean position. The bridges joined the actin in symmetrical pairs, forming actin-centred chevrons which pointed away from the Z band and towards the M line (Figs. 4*a* and *b* and 6*a*). This resembles the polarized 'arrowhead' pattern observed by H. E. Huxley in either *F*-actin or native *I* filaments which had been complexed with heavy meromyosin. The polarization is the same as

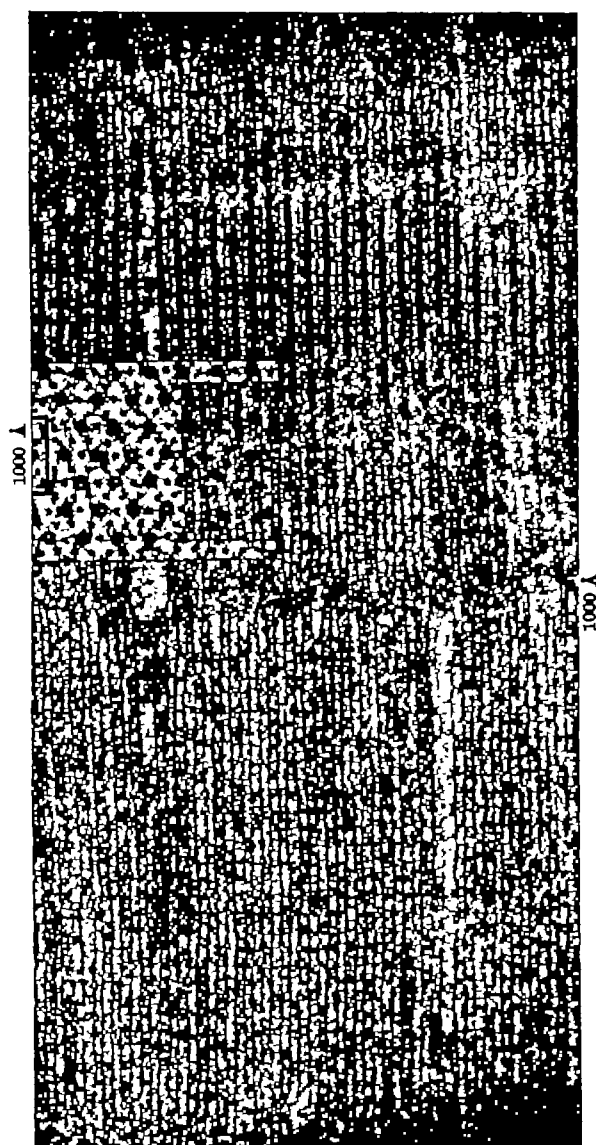


Fig. 3. Electron micrograph of one sarcomere of glycerol-extracted *L. marinus* indirect flight muscle, fixed in rigor with glutaraldehyde, post-fixed with  $\text{OsO}_4$ , sectioned with fibre axis parallel to knife edge, double stained with uranyl and lead salts. Inset shows filament lattice. Longitudinal section about 300 Å thick shows single filament layers. 380 Å period associated with cross-bridges in rigor, seen as a series of faint transverse bands, is visible in both varieties (actin-myosin and actin only) of single filament layer. Magnification  $\times 60,000$  (inset  $\times 100,000$ ).

that seen in heavy meromyosin-treated *I* filaments which remained attached to the *Z* band<sup>17</sup>.

In our sections, the chevrons repeated axially at 380 Å (range 370–390 Å), each repeat marked either by a single chevron or by a closely spaced pair of chevrons (Fig. 4a). The axial separation between the chevrons of a pair was 120–180 Å. In regions where the single filament layer included only actin filaments such as those illustrated in Fig. 3, a 380-Å repeat of projections along these filaments was also visible. Although the chevron appearance was less clear, it was sufficiently well indicated to justify the interpretation that these projections represented cross-bridge terminations attached to actin, originating from myosin filaments which had been excluded from the section. Since the 380-Å period associated with cross-bridges was present in sections containing only actin filaments, we supposed it to be the period of the actin attachments. Comparable information about the period of the myosin attachments has not been obtained since single layers of myosin filaments also contain actin. Moreover, variation in the number, angle and apparent

length of bridges has obscured interpretation of the myosin period in rigor.

*Specimens fixed in relaxation.* Sections of specimens fixed in relaxation demonstrated significant structural differences between this state and the rigor state. Thick sections showed two periods within the *A* band. The longer period, again 380 Å, could be identified with a regular dense beaded appearance along the actin filament images in suitably oriented fibrils. A finer period, a regular but faint transverse banding, seemed to be a thirding of the long period on casual inspection; careful measurements of forty to sixty repeats indicated 143 Å as the best value.

Thin sections showed that the polarized configuration of cross-bridges was absent in relaxed muscle. Instead, the

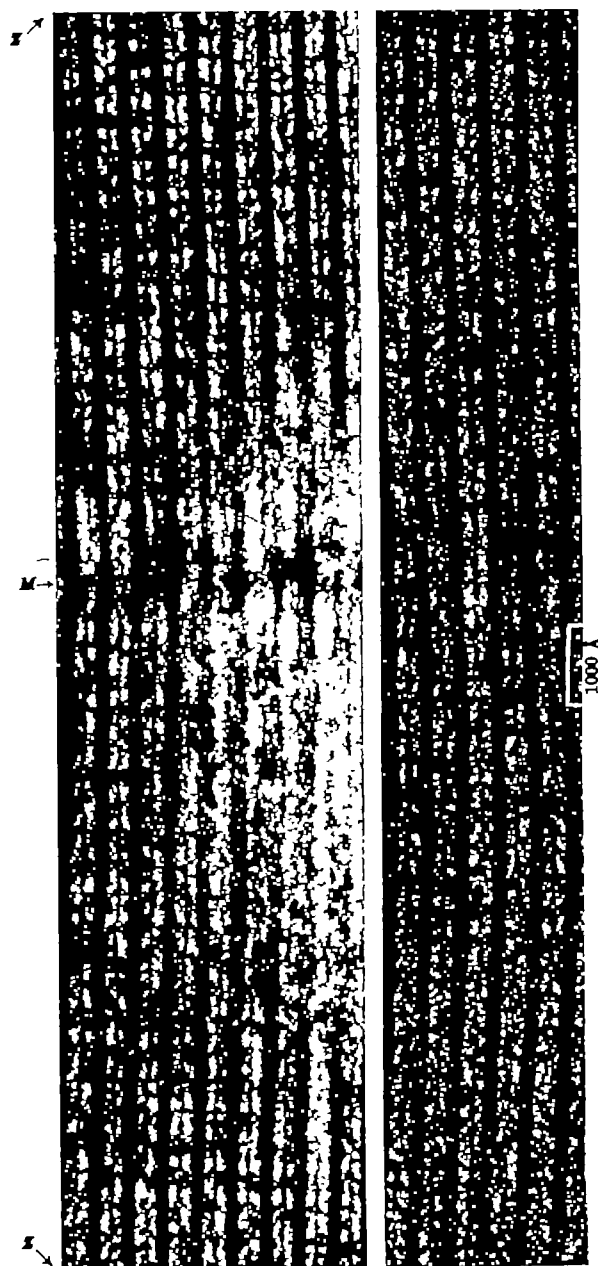


Fig. 4. Electron micrographs of 'Bpon' sections of same material shown in Fig. 3, fixed in rigor, double stained with uranyl-lead (a) and permanganate-lead (b). Field includes centre of *A* band. *Z* bands lie beyond top and bottom of field and provide attachment for two sets of actin filaments which, in this example, just meet at *M* line. The slanted cross-filaments which form actin-centred chevrons pointing toward *M* line (location of thickenings on myosin filaments), which traverses the filament array just above centre of figure. Contrast of Fig. 4a has been enhanced by a copy negative stage. Magnification  $\times 108,000$ .

cross-bridges were essentially perpendicular to the filaments. A regular myosin period of about 140 Å was marked by a varying sequence of fine serrations and bands which characterized the images of the myosin filaments. Many of these features clearly represented cross-bridges parallel to the section plane and extending to meet adjacent actins in the same filament layer; the knobs and bands comprising the remaining features were interpreted as cross-bridges directed out of the plane of the section (Figs. 5 and 6).

In thin sections of relaxed muscle, the 380-Å period was less prominent than in comparable sections of rigor muscle. It was best seen in single-filament layers of actin only, where it was marked by recurrent thickenings or

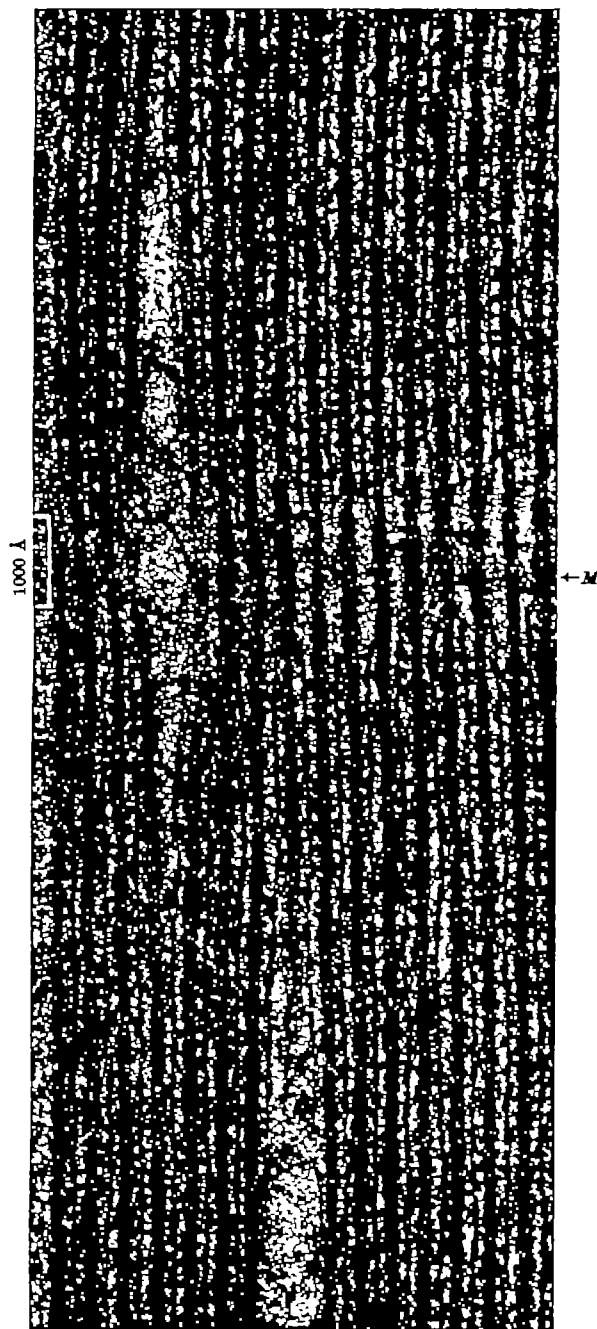


Fig. 5. Electron micrograph of glycerinated *L. marinus* flight muscle relaxed with ATP-EGTA treatment and fixed in this state by adding glutaraldehyde to bath. Further treatment was as for Fig. 3, except embedding medium, 'Araldite' here, and stain, permanganate-lead here. Field shown is comparable to Fig. 4. In this example, actin filaments from two half-sarcomeres are not touching. Cross-bridges are perpendicular to filaments, and have a 143 Å axial period. Magnification  $\times 120,000$ .

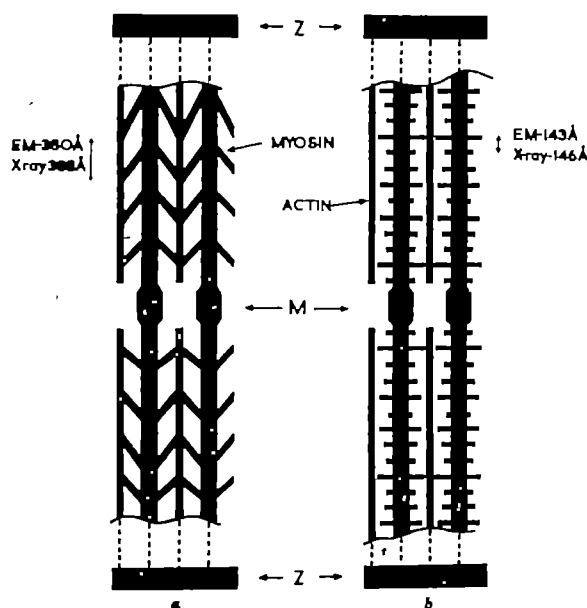


Fig. 6. Diagrams of single layers of actin and myosin filaments showing cross-bridge positions found to be characteristic of (a) rigor and (b) relaxed states of glycerinated insect flight muscle. Thick filaments represent myosin, from which cross-bridges extend towards actin (thin) filaments. In (b), the variation in length of projections represents varying azimuthal positions of bridge pairs around myosin filaments. The exact azimuthal interval is not known but is apparently near  $60^\circ$ .

projections along the filaments. In single-filament layers including both actin and myosin, it was evident only in large samples of a single layer, often only when viewing the micrograph obliquely across the filaments. This period evidently arose because a significant proportion of the cross-bridges directed parallel to the section plane were localized within narrow transverse zones which recurred along an average 380 Å axial repeat. This meshing between the 380 and 143 Å periods could not be readily recognized or explored in the image details of individual actin or myosin filaments.

**Pattern of cross-bridges in relaxation and rigor.** In order to correlate these findings with the X-ray diffraction observations we suppose that the low-angle diffraction pattern with 146 Å period arises from the cross-bridges<sup>4</sup>, whereupon it appears that the cross-bridges of glycerinated insect flight muscle have the following properties:

(a) In relaxation they are attached to the myosin at regular 146 Å intervals, thereby giving rise to the X-ray meridional reflexions.

(b) In relaxation, most of the bridges are at right angles to the filament axis, as seen in the electron microscope and shown by the strength of the 146- and 73-Å meridional reflexions of the X-ray diffraction pattern.

(c) In ATP-free rigor, most of the bridges take up an angled orientation to the filaments, as is demonstrated in the electron microscope and deduced from the weakness of meridional reflexions in the X-ray pattern; meridional intensity is proportional to the strength of electron density variation of the whole structure projected on to the fibre axis, and, if the slope of the cross-bridges is sufficient to cause them to overlap in this projection, the density variation will be greatly reduced or abolished.

(d) In ATP-free rigor, the bridges attach to the actin in symmetrical pairs. These pairs repeat every 388 Å along the filament axis, producing the off-meridional X-ray layer lines and the chevrons seen in the electron micrographs. This repeat distance is very close to the length of one half-turn of the actin helix in glycerinated insect flight muscle, as deduced from the high-angle actin reflexions seen in these diffraction patterns (Fig. 2a, the detailed argument will be presented elsewhere<sup>11</sup>), so that the attachment appears to be determined by the conformation of the actin itself. This is incommensurate with the 146 Å period of myosin in relaxed muscle.

The rigor state is not geometrically perfect, since some cross-bridges are able to link to the same actin at much less than 388-Å spacing, causing the double chevrons, and the angle between bridge and filament is somewhat variable. Nevertheless, the state seems, on the average, fairly crystalline, for the effects of a lattice (which breaks up the X-ray diffraction intensity into a series of spots) are well marked on the zero, first and second layer lines. We have no evidence that the rigor attachment of the cross-bridges changes the length of the myosin filaments themselves; the invariance of the 148 Å meridional spot and 388 Å layer line on stretch indicates that neither the myosin nor the actin filaments are readily extended. X-ray diffraction photographs which include weak 148 Å meridional reflexions have been obtained from rigor specimens after prolonged exposure. This suggests that a regular periodic structure, probably associated with cross-bridge origins along the myosin filaments, is retained in rigor, although other interpretations of this result are possible.

It is attractive to suppose that changes in the position of cross-bridges similar to those shown in Fig. 6 cause the active oscillation of the flight muscle. Although such a mechanism for insect flight muscle activity is hypothetical it gains plausibility from recent X-ray studies of vertebrate muscle which indicate that the cross-bridges move in frog sartorius muscle during isometric tetanus<sup>13</sup>.

This work forms part of a collaborative effort between the Department of Zoology, Oxford, and the Laboratory of Molecular Biology, Cambridge. We thank Prof. J. W. S. Pringle for suggesting the suitability of this muscle for structural studies, and Dr. H. E. Huxley for many valuable discussions. One of us (M. K. R.) is supported by a U.S. Public Health Service post-doctoral fellowship (2-F2-NB-21075-02), and another (R. T. T.) thanks the Agricultural Research Council for support.

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## ACRIFLAVIN-INDUCED LOSS OF KINETOPLAST DEOXYRIBONUCLEIC ACID IN *Crithidia fasciculata* (Culex pipiens STRAIN)

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THE kinetoplast is a DNA-containing, membrane-limited organelle peculiar to protozoa belonging to the Trypanosomatidae<sup>1</sup>. Bodonids contain a similar organelle<sup>2</sup>. This organelle was first demonstrated by Rabinowitch and Kempner<sup>3</sup>, who found, to their surprise, that it was stained by dyes which until then had been thought to stain only nuclei. Almost a generation later Janicki<sup>4</sup> found that kinetoplasts stain as do mitochondria, and, more particularly, as shown in this article, are Feulgen positive<sup>5</sup>. In 1910, Werbitaki<sup>6</sup> and, later, numerous others noted that trypanosomes appeared to lose their kinetoplasts after treatment with certain acridine dyes such as acriflavin. This loss of ability of kinetoplasts to stain with nuclear dyes was thought to indicate loss of the organelle induced by acriflavin and hence the resultant organisms were termed 'akinetoplastic' (AK).

The advent of electron microscopy caused a reassessment of the definition of akinetoplasty when it was shown that acriflavin-treated Trypanosomatidae still retained the kinetoplast envelope<sup>7</sup>; only the electron-dense highly polymerized DNA was no longer evident. In this article, we retain the term akinetoplasty but think of it as a corruption of altered or atypical kinetoplasty, that is, 'AK', where AK corresponds to loss of DNA as monitored by loss of Giemsa or Feulgen stainability or loss of the fine fibrillar electron-dense material in the kinetoplast<sup>8</sup>.

Our experiments were carried out exclusively with Trypanosomatidae, most of which belonged to the genus *Crithidia* (seven species). Although there are quantitative generic differences, the trends are the same. Comprehensive comparison of species and genera and of the kinetics of acquisition of the akinetoplastic state will be reserved for a later report. In this article, we restrict our discussion to the effects of short exposure to acriflavin on the kinetoplasts of *Crithidia fasciculata* (Culex pipiens strain) ATCC 12857.

The organisms were maintained in an undefined medium<sup>9</sup>, grown at 24°–26° C and transferred at intervals

of two weeks. Cells to be used for inoculum were also grown in the undefined medium until they reached near-peak log growth (as assessed by following the growth curve densitometrically). Then 1.0-ml. aliquots of cells plus medium were added to 4.0-ml. aliquots of the same medium which contained neutral acriflavin (0–40 µg/ml.). Samples of cells were collected immediately after inoculation (day zero) and at daily intervals thereafter for a week. The samples were smeared on slides, stained with Giemsa stain and the number of AK organisms counted: 300–600 organisms were counted for each determination, which was then expressed as a percentage of AK.

Fig. 1 illustrates the percentage of AK produced at different acriflavin concentrations. Loss of highly polymerized DNA in kinetoplasts occurs rapidly: by 24 h there is already significant AK, for example, 20.3 ± 1.9 per cent at 8 µg/ml. to a peak of 59.5 ± 2.1 per cent at 24 µg/ml. acriflavin (see also Fig. 1 in ref. 10). It can be seen that after peak AK production in the presence of 24 µg/ml. acriflavin, exposure to yet higher amounts of acriflavin resulted in a smaller percentage of AK. This reduction rather than maintenance of the total percentage of AK when concentrations of acriflavin were used which were greater than that which gives peak AK production is not restricted to one organism: the effect is also shown with *C. fasciculata* (Anopheles strain); *C. fasciculata* (Nöller strain); *C. oncopeltii*; *C. luciliae*; and *C. species from Buryophthalmus*. For all these organisms, peak AK production at 24 h occurred in the presence of 24 µg/ml. acriflavin but absolute percentage AK produced in each case varied with the organism used.

Interpretation of our results depends on considering a direct action of acriflavin with kinetoplast DNA in much the same way as has been described for various effects of acriflavin on isolated, purified, microbial DNA. In such experiments, Lerman measured the effects on DNA X-ray diffraction patterns and viscosity, by the addition of acriflavin<sup>11</sup>. In order to explain his results, he suggested

Table 1. DIFFERENCE IN PERCENTAGE AT (MOLES PER CENT) BETWEEN NUCLEAR AND KINETOPLAST DNA AS A GAUGE OF SENSITIVITY TOWARDS ACRIFLAVIN-INDUCED AKINETOPLASTY

Organism	% AT in nuclear DNA	Kinetoplast DNA	Peak % AK after 24 h†
<i>Crithidia fasciculata</i> ( <i>Oxalis pipiens</i> strain), ATCC 12857	42?*	66?*	59±2.1
<i>O. fasciculata</i> ( <i>Oxalis pipiens</i> strain, Nöller isolate), ATCC 12858	42?*	66?*	49±2.0
<i>O. fasciculata</i> ( <i>Anopheles quadrimaculatus</i> strain), ATCC 12745	46	61	33±5
<i>O. oncopelti</i> , ATCC 12962	47†	66†	41±1

\* Results from ref. 13. The authors did not specify which of the two organisms were used. From our results on the percentage of AK we would expect differences in base ratios of the two organisms.  
† Results from ref. 10, Fig. 1. Other authors (for example, ref. 13) have obtained somewhat different results.  
‡ Our results, which are given here, are compared with differences between nuclear and kinetoplast base ratios in Fig. 2.

that the planar acriflavin molecule is bound to DNA by becoming intercalated into the spaces between the base pairs of the DNA helix so that the plane of the acriflavin molecule lies perpendicular to the helix axis like the filling of a sandwich composed of upper and lower nucleotide pairs with acriflavin in the middle<sup>11</sup>. The results of Tubbs *et al.*<sup>12</sup> suggest some reasons for the differences in acriflavin sensitivity of different organisms and in our case for the preferential attack of acriflavin on kinetoplast rather than nuclear DNA (even though these investigators also worked exclusively with isolated, purified DNA). They showed that binding of acriflavin to DNA was dependent on certain characteristics of DNA including: (a) the base composition. They further speculated that affinity to sites is in this order—AT:AT > AT:GC > GC:GC; (b) the order of the bases; (c) the macromolecular configuration of the DNA.

Of particular pertinence to our results is the finding that acriflavin had greater affinity for high AT-containing DNA<sup>13</sup>. In all cases where it has been charted, kinetoplast DNA (or—as it was termed in some investigations—satellite band DNA) has been shown to have a higher percentage of AT than does nuclear DNA (Table 1). It is then reasonable to consider that kinetoplast DNA is preferentially attacked because of its comparatively higher content of AT. That such a relationship exists is shown by plotting the difference between nuclear and kinetoplast DNA (given in Table 1) against the peak percentage of AK (after 24 h exposure to acriflavin) for three crithidias (Fig. 2).

The questions still remaining unanswered are: (a) What difference between kinetoplast (satellite) and nuclear DNA must exist before there is a preferential attack on the higher AT-containing species? For the genus *Crithidia*, a difference of 10–15 would seem to provide a safe margin for predicting 10–20 per cent AK in 24 h. (b) How can we explain the reduction in percentage AK at very high

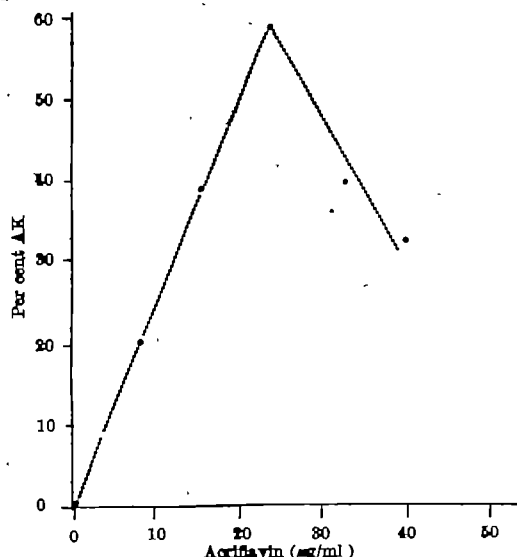


Fig. 1. Effect of exposure of *C. fasciculata* (*Oxalis pipiens* strain) to acriflavin on the production of akinetoplasty (AK)

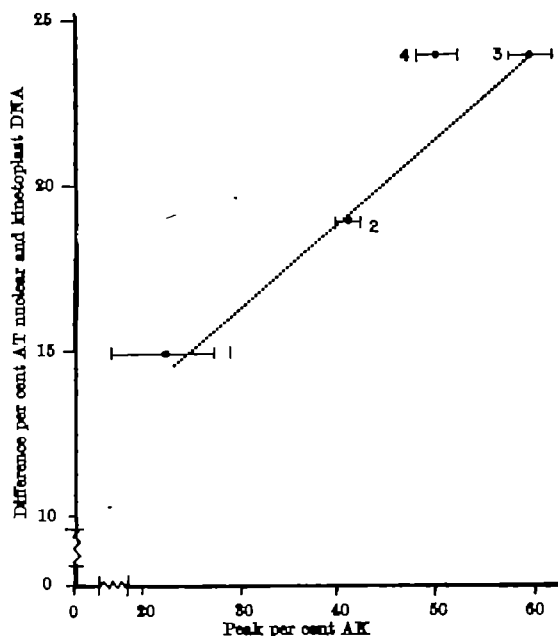


Fig. 2. Relationship between peak percentage AK and magnitude of difference between nuclear and kinetoplast DNA percentage AT (data derived from Table 1). The organisms used were (1) *C. fasciculata* (*Anopheles* strain); (2) *O. oncopelti*; (3) *C. fasciculata* (*Oxalis pipiens* strain); (4) *C. fasciculata* (*Oxalis pipiens* strain, Nöller isolate). Although there is no proof, based on our own experience in distributing trypanosomids to other workers and the close correlation with the results of our experiments with akinetoplasty, we believe that Schildkraut *et al.* (ref. 13) used organism 3.

acriflavin concentrations? Two possibilities seem attractive: (1) At low concentrations of acriflavin, most of the dye enters the cell and eventually becomes intercalated between AT:AT and perhaps AT:GC sites of the kinetoplast DNA. When the concentration of acriflavin is raised, the probability of dye attachment to the surface of DNA<sup>14</sup> is also raised. Since we are dealing with whole cells rather than isolated DNA, it is also possible that some of the dye never reaches its primary sites inside kinetoplast DNA but instead becomes coated on either the cell or kinetoplast membrane, blocking further passage of acriflavin. Blockage would become more profound as acriflavin concentration increases. (2) As concentration of acriflavin is increased, the preference towards attack of DNA containing a higher percentage AT is lost, since enough dye is available to saturate sites requiring binding by intercalation in both nuclear and kinetoplast DNA. When both nucleus and kinetoplast are thus attacked, the cell is moribund and soon lyses, frustrating attempts to count it. The fact that there is a reduction in both optical density (a measure of cytoplasmic mass) and total cell count of cultures containing high levels of acriflavin makes this possibility the more attractive at this time.

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## LETTERS TO THE EDITOR

## ASTROPHYSICS

## Rotation of the Galaxy NGC 1316 associated with the Radio Source Fornax A

THE peculiar *So* galaxy NGC 1316 lies between the two intense regions of radio emission that constitute the double radio source Fornax A (refs. 1 and 2). The radio centroid lies within the optical boundary of the galaxy. The physical association of the galaxy and the radio source has been put beyond doubt by Arp's discovery<sup>3</sup> of extremely faint optical radiation emanating from curved pathways connecting each radio emitting region with the galaxy. In the immediate vicinity of NGC 1316 these pathways appear to form extensions to the major axis of the main elliptical body of the galaxy. This major axis makes an angle of about  $45^\circ$  with the line joining the radio maxima. Further out the pathways curve sharply and enter the radio emitting region.

A striking feature of NGC 1316 is the presence of a patchy lane of dark absorbing material<sup>4,5</sup> which, as projected on the plane of the sky, appears, at first sight, to lie along its minor axis. Closer inspection shows that this dust lane delineates a spiral pattern. The relationship of these features is indicated schematically in Fig. 1. In this figure the points A and B represent the maxima of radio emission. They are enclosed by the  $4^\circ\text{K}$  brightness temperature contours at 2,650 Mc/s determined with the Parkes 210-ft. radiotelescope by R. M. Price and F. F. Gardner<sup>6</sup>. Between A and B the elliptical outer isophotes of the main body of NGC 1316 are indicated, and within this—in a very schematic way—the spiral pattern delineated by the patchy dust distribution is shown. The dashed lines represent the run of the optical extensions found by Arp.

The Fornax A-NGC 1316 system shows some superficial similarities to that of Centaurus A and the associated galaxy NGC 5128 (ref. 7). Here, too, faint optical extensions to the major axis connect the galaxy with the outer radio regions and a lane of dark material lies along the minor axis of the elliptical<sup>8</sup>. In this case it has been found that the rotation axis of the galaxy, when projected on the sky, lies along the major axis<sup>9,10</sup>. It has been suggested<sup>8,11</sup> that the origin of double radio sources of the kind considered here is connected with violent events of the kind known to occur in the nuclei of some galaxies. The faint optical extensions show that there is some physical connexion between the radio-emitting regions and the

associated galaxy. If this physically connecting pathway—whatever its nature may be—runs between the galactic nucleus and the radio-emitting regions, then, in the case of the Centaurus A system, it emerges from the nucleus of NGC 5128 along its rotational axis. A similar situation exists in the double radio source 3C83 (ref. 12). Here again faint optical extensions coincide with the projected rotational axis.

It is the purpose of this note to point out that the structure of the Fornax A-NGC 1316 system is essentially different.

Spectra of NGC 1316 have been obtained with the nebular spectrograph attached to the 74-in. reflector at Mount Stromlo Observatory. These spectra, on Kodak IIa-O emulsion, have a reciprocal dispersion of 86 Å/mm. They were obtained with the galactic nucleus held fixed on the slit in conditions of good seeing and the slit orientation was different for different exposures. The tilt of the H and K absorption-lines of Ca II (with respect to lines in the spectrum of the comparison arc) has been measured on these spectra. The tilt is maximum when the slit is orientated north-east-south-west, that is, when the slit lies along the major axis of the galaxy. The tilt is zero when the slit lies along the minor axis. The projected rotational axis of NGC 1316 therefore lies along the minor axis, in contrast to the situation found in NGC 5128.

From the broad absorption lines it is not possible to measure a detailed rotation curve, but from the value of  $6^\circ$  which was obtained for the mean tilt when the slit lay along the major axis we find a mean projected rotational velocity gradient of 80 km/sec/kpc for a region extending 1.5 kpc on either side of the nucleus. In deriving these figures a distance of 15 Mpc to NGC 1316 was adopted. Relative to the galactic nucleus the north-east side of the galaxy is approaching and the south-west side is receding.

These observations show that in the immediate vicinity of the galaxy the optical extensions lie in the symmetry plane normal to the rotational axis. It is consistent with observations to suppose that the dark material also lies in this plane which, on this picture, is observed under appreciable inclination. The outermost parts of these 'dark spiral arms' are much more clearly seen on the north than on the south side of the galaxy, and this suggests that the north-west side is nearest to us. It is probable therefore that the spiral pattern of the dark material is rotating with trailing arms.

If there is a pathway connecting the nucleus of NGC 1316 with the radio-emitting regions it is tempting to believe that it lies in the galactic plane, that it is of trailing spiral form and that it is delineated within the main body of the galaxy by dark material and outside it by the optical extensions discovered by Arp.

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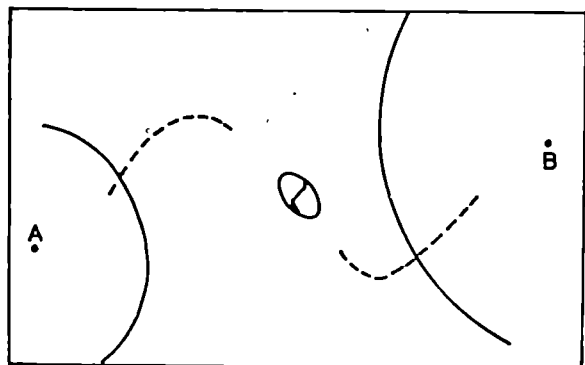


Fig. 1. Schematic diagram of the NGC 1316-Fornax A system. North is at the top and east to the left. The distance between the two radio maxima A and B is 30 min of arc.

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### Maxima of the Eleven-year Cycle of Solar Phenomena

It is generally accepted that during the last four cycles of solar activity the intensity of the maxima has been steadily increasing.

The term 'solar activity' is generally intended to include all the solar phenomena which vary systematically with the solar cycle of about 11 years<sup>1</sup>. But, as it has been already pointed out<sup>2</sup>, in some cases it would be more correct to use the terms spots activity, faculae activity, plages activity, filaments activity, prominences activity and so on instead of the ambiguous term solar activity.

In this report we point out that it is not accurate to refer to an increase of intensity in the maxima of solar activity in the last four cycles, but only of an increase in the maxima of the sunspot cycle.

In Figs. 1 and 2 we have plotted the yearly means of indices of some phenomena of solar activity for the cycles 16-19.

For a better interpretation of the data shown in Figs. 1 and 2, the mean value  $\bar{I}$  according to the formula:

$$\bar{I} = \frac{1}{3} (I_{M-1} + I_M + I_{M+1})$$

where  $I_{M-1}$ ,  $I_M$ ,  $I_{M+1}$  are the values of the index  $I$ , one year before the sunspot maximum, the year of the sunspot maximum and the year after the sunspot maximum, respectively, has been computed for the cycles 16, 17, 18, 19 separately for the indices considered. In Figs. 3 and 4 the deviations of the  $\bar{I}$  values from their mean are plotted. They are expressed as a percentage of the mean.

A careful examination of the figures shows that, although the intensity of the maxima of the sunspot cycle has been increasing during the past four cycles, the intensity of the maxima of the other phenomena has been almost constant during this period. Furthermore, it seems that the intensity of the maxima of the faculae

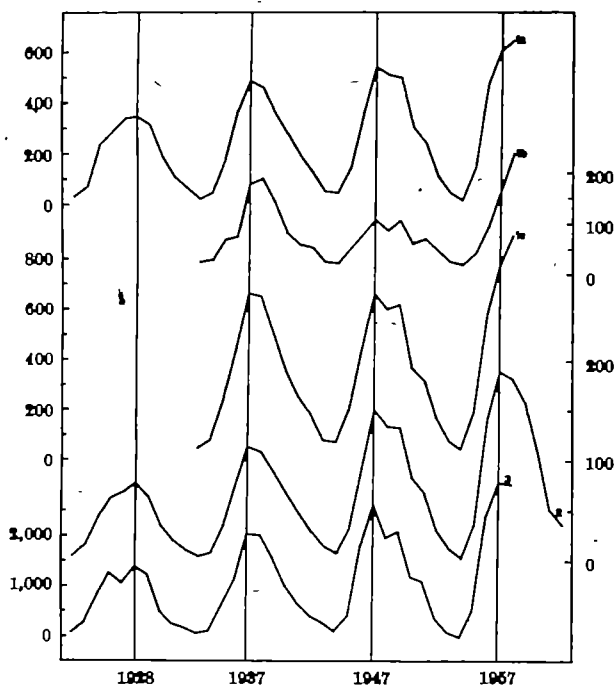


Fig. 1. Yearly values of indices of sunspots: 1a, yearly number of sunspots groups lasting for two or more days (Greenwich); 1b, yearly number of sunspots groups seen on one day only (Greenwich); 2, yearly number of sunspots groups (Greenwich); 3, yearly means of the corrected daily area for whole spot expressed in millionths of the Sun's visible hemisphere (Greenwich)

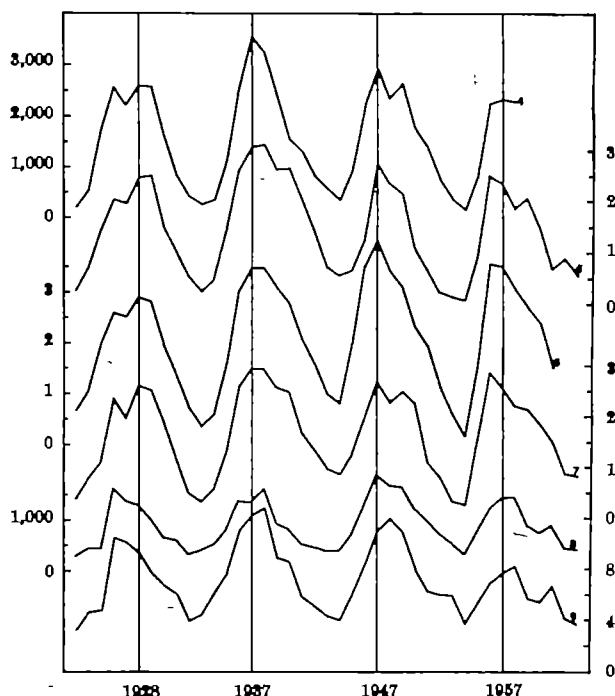


Fig. 2. Yearly means of indices of solar phenomena: 4, yearly means of the daily corrected area of the faculae expressed in millionths of the Sun's visible hemisphere (Greenwich); 5, yearly means of the daily character figure of the H $\alpha$  plages (Zurich, Aroseti); 6, yearly means of the daily character figure of the H $\alpha$  filaments (Zurich, Aroseti); 7, yearly means of the daily character figure of the H $\alpha$  prominences (Zurich, Aroseti); 8, yearly means of the daily projected area of the H $\alpha$  prominences expressed in unit of prominence (Aroseti); 9, yearly means of the daily number of H $\alpha$  prominences (Aroseti)

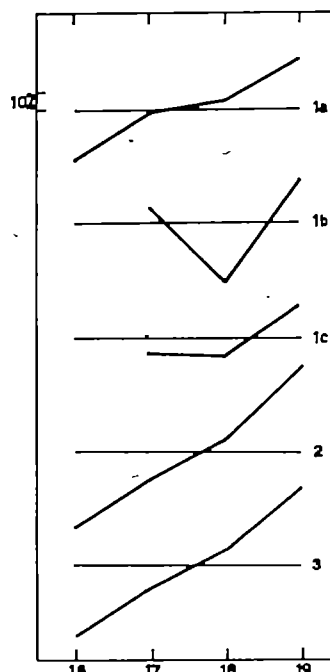


Fig. 3. Deviations of  $\bar{I}$  values from their mean. The deviations are expressed as percentage of the mean. The indices are numbered according to Fig. 1

cycle, H $\alpha$  plages cycle, and H $\alpha$  prominences cycle has been decreasing during the past three cycles. Even if we confine ourselves to the sunspot cycle, we see that the indices 1 vary much less than the indices 2 and 3.

According to these considerations, the results of some recent investigations concerning the existence of long-



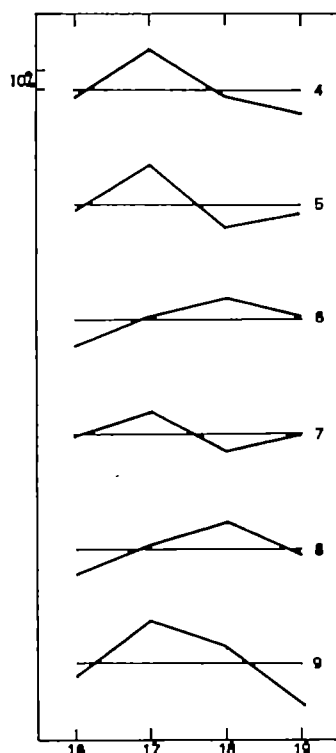


Fig. 4. Deviations of  $I$  values from their mean. The deviations are expressed as percentage of their mean. The indices are numbered according to Fig. 2

term variations in the relationships between ionospheric and solar activity indices<sup>3</sup> may perhaps be reconsidered.

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## PHYSICS

### A Suggested Method for Increasing the Resolving Power of a Spectrograph

EARLIER I reported some experiments in which interference phenomena produced by different parts of the diffraction image of a telescope were examined<sup>1-3</sup>. It has been shown<sup>3</sup> that by observing the Airy disk in this way, by means of interference it is possible to resolve finer details in an image than can be recorded from the intensity distribution only. I believe that the same principle may prove of value for increasing the resolving power of a spectrograph.

The principle of such measurements is given in Fig. 1, showing a grating spectrograph with the entrance slit in  $S_1$  and an exit slit in  $S_2$ , after which the light is recorded by a phototube. In front of the slit  $S_2$ , a double image unit is placed composed of a Wollaston prism  $W$  of small deviation, a quartz retardation plate  $Q$  and two polarizers  $P_1$  and  $P_2$  in  $45^\circ$  position. If the two rays produced by the double image prism pass the slit  $S_2$  simultaneously, interference phenomena can be observed, say, by means of a phototube  $Ph$ . The Wollaston prism acts as a Babinet wedge as well, and when it is displaced sideways intensity variations are recorded by the phototube  $Ph$ .

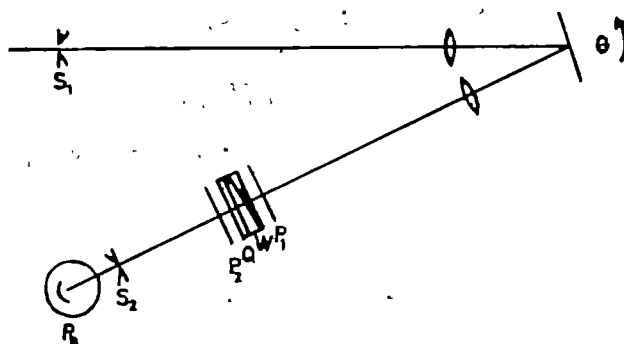


Fig. 1

Each wave-length in the spectrum will appear as two adjacent images, and because of the diffraction image of the spectrograph (and limited resolving power of the grating and finite size of the slits) these will be broad enough to make it possible in both cases for some adjacent wave-lengths to contribute to the light passing the slit  $S_2$ . Let the intensity in the slit of one of these images be  $i_1$  and the intensity of the other image be  $i_2$ , then their contribution to, say, a dark fringe phenomenon is given by:

$$I_{\min} = i_1 + i_2 - 2\sqrt{i_1 i_2} \quad (1)$$

Other neighbouring wave-lengths will give similar contributions and the observed intensity  $I_{\min}(\lambda_0)$  will be obtained as:

$$I_{\min}(\lambda_0) = \int_{-\infty}^{+\infty} J(\lambda) I_{\min}(\lambda - \lambda_0) d\lambda \quad (2)$$

Here  $\lambda_0$  is the wave-length corresponding to the position of the slit  $S_2$  and  $J(\lambda)$  the true intensity distribution in the spectrum.

Expression (2) is of the same type as the well-known integral equation describing the effect of limited resolving power of a spectrograph<sup>4</sup>. The known function  $I_{\min}(\lambda - \lambda_0)$  has, however, a very different shape from that in the conventional theory of resolving power; it can in fact be given different properties by changing the separation between the double images.

In order to exemplify this, a calculated function  $I_{\min}(\lambda_0)$  is given in Fig. 2, where the function  $J(\lambda)$  is given by two adjacent narrow emission lines with a separation  $\Delta$ , which in this case is 7/8 times the separation  $d$  between the double images and 7/16 the size of the diffraction image of the spectrograph assuming a rectangular aperture of the lenses. Fig. 2 gives  $I_{\min}(\lambda_0)$  as well as  $I_{\max}(\lambda_0)$  assuming narrow slits  $S_1$  and  $S_2$ . Without the double image device, scanning the intensity (by turning the

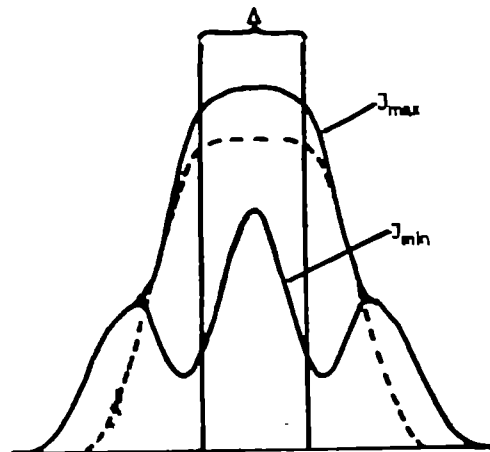


Fig. 2

grating) would give instead the dotted curve of Fig. 2. Moreover, for a singlet the central maximum of the  $I_{\text{min}}$  curve is replaced by a deep minimum. It is evident, therefore, that the method of using interference between different parts of the diffraction image increases the resolution. When making this calculation the intensity distribution within the diffraction image has been assumed to follow a  $(\sin x/x)^2$  curve, where  $x$  is the distance from the centre of the image.

Experiments have been made by using artificial spectral lines made up by two slits polarized in perpendicular directions. The phenomena with the two minima in Fig. 2 for the  $I_{\text{min}}$  curve are clearly revealed in these experiments. So far the instrumental arrangement has made necessary the use of optical systems of the focal ratio  $f/800$  or similar. In our instrument the separation of the beams is one minute of arc only.

Instead of examining the function  $I_{\text{min}}(\lambda_0)$  it may be preferable to measure the degree of visibility\* or only  $I_{\text{max}}(\lambda_0) - I_{\text{min}}(\lambda_0)$ . In the latter case when assuming diffraction according to a  $(\sin x/x)^2$  function only, we find:

$$I_{\text{max}}(\lambda_0) - I_{\text{min}}(\lambda_0) = \frac{4}{\pi} \int_{-\infty}^{+\infty} J(\lambda) \frac{\sin\left(\lambda - \lambda_0 + \frac{d}{2}\right)}{\lambda - \lambda_0 + \frac{d}{2}} \cdot \frac{\sin\left(\lambda - \lambda_0 - \frac{d}{2}\right)}{\lambda - \lambda_0 - \frac{d}{2}} d\lambda \quad (3)$$

In this formula the size of the diffraction image produced by the (rectangular) aperture of the spectrograph equals  $\pi$ .

In Anacapri, we have recently performed some successful experiments where the slit  $S_1$  and photo tube  $Ph$  of Fig. 1 have been replaced by a photographic film. By photographing the solar spectrum in this way, about 6000 Å (with our Babcock grating in fourth order) interference phenomena have been clearly traced in the Fraunhofer lines. In our first experiment the displacement of the two superimposed spectra has been 0.10 mm. The diffraction image of the spectrograph (rectangular aperture) has been 0.12 mm and the image of  $S_1$  on the photographic film 0.03 mm. As can be inferred from Fig. 3, fairly strong interference fringes are present in the continuous spectrum with this arrangement. Probably the limited resolving power of the grating has contributed to the interference phenomenon.

The two spectra in Fig. 3 showing no fringes have been obtained without the Wollaston prism unit, whereas the other two spectra have been taken with different settings of the prism, so that the maxima of the spectrum above have been replaced by minima in the spectrum below. An inspection of the Fraunhofer lines in these two spectra is of great interest. In fact, in the (broad) intensity maxima a single spectrum line appears as a doublet, whereas in the (broad) minima the same line appears as a broad and remarkably dark singlet. Already from this first experiment it seems evident that the effects predicted by me are present, and that the new method of studying



Fig. 3

interference may find interesting spectrographic applications.

However, only extensive experiments will prove if a real gain is obtained with the suggested method. We have to consider that there will be a certain reduction of light, even if the resolving power is increased. The use of 'helium lenses' or similar may prove better in this respect.

I thank Mr. U. Ericson and Mr. Y. Sundblad for valuable help with the preliminary work made in 1964\* and Dr. A. Wyller and Mr. U. Kusoffsky for assistance with the final spectrographic tests.

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## The Temperature Scale

Most of the points raised by Prof. J. C. Georgian<sup>1</sup> on the subject of the temperature scale<sup>1</sup> are discussed in Chapter 23 of *A Textbook of Heat* by Allen and Maxwell (1948). The authors refer to a paper by Lewis and Adams<sup>2</sup> making the very point about the dimensions of temperature, specific heat and enthalpy made by Prof. Georgian and proposing a temperature scale that would make  $PV = n\theta$ , where  $n = \text{Avogadro's number}$ . They also discuss a proposal by Planck which seems to have been essentially that now made by Prof. Georgian.

There are, however, certain objections to these proposals. Unlike, for example, the Reynolds number, specific heat and enthalpy are dimensionless only so long as we bear in mind their relationship to water, for they rely for the invariance of their nominal numerical values on the retention of liquid water as the nominal datum. A change of a temperature scale from Kelvin to Georgian would therefore result merely in a change of the value of  $J$  that had to be used in equations relating temperature and heat, unless we were prepared to re-calculate all our specific heat in terms of the relationship of the material to a perfect gas rather than to water. Furthermore, the unit of energy is nominally based on the properties of water (via the definition of the unit of mass), and to proceed to define temperature by invoking another substance altogether (perfect gas) is, I submit, unnecessary and undesirable.

I have already proposed<sup>3</sup> that the calorie and the factor  $J$  could be eliminated simply from temperature equations by the replacement of the Kelvin scale by one having  $100 \times J$  degrees between the ice point and the steam point. I suggested<sup>3</sup> that such a degree should be called the degree Joule. I think that the adoption of such a scale would be easier and more satisfactory than would the adoption of Prof. Georgian's scale, as it would not require any properties to be re-calculated, and I think it would commend itself more readily to practical users.

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I WISH to thank Mr. Laws for refs. 2 and 3.

I was aware of ref. 2 and, in addition, Tolman<sup>4</sup> has an interesting discussion of the dimensions of temperature. Only Tolman wishes to give temperature the units of  $ML^2/T^2$ , that is, allow the molecular mass number (molecular weight) to take on the dimension of mass. However, Porter<sup>5</sup> in his book *Method of Dimensions* explains clearly that the dimension of temperature is indeed  $L^2/T^2$ .

I cannot accept the objection by Mr. Laws to my temperature scale, as in using the MKS system any relationship to water is now irrelevant, because the kilogram mass is defined as a platinum-iridium cylinder deposited at the International Bureau of Weights and Measurements at Sèvres, France. The unit of energy is not based on the properties of water but is defined as one Newton-meter equals one Joule. Hence, all forms of energy will have the same unit, be it heat, mechanical, electrical, chemical, or atomic energy.

The purpose of my temperature scale is to base the scale on the MKS system and, thereby, eliminate the conversion factor  $R$ , in the same manner as the conversion factors  $J$  and  $g$ , have been eliminated in the MKS system.

The proposal of Mr. Laws of adopting a temperature scale in order to preserve the tabulated numbers of  $c_p$  is unnecessary as these values can be readily converted to the MKS system from presently tabulated values in absolute molal units. For example, the National Bureau of Standards has tabulated thermal properties of gases in its *Circular 564* (ref. 6) in dimensionless molal values. Using the MKS temperature scale, it can easily be seen that the ideal gas relation between specific heat at constant pressure and volume becomes:

$$c_p - c_v = \frac{1}{M} \quad (1)$$

where the usual gas constant  $R$  is replaced by  $1/M$ . The specific heat at constant pressure then becomes:

$$c_p = \left( \frac{\gamma}{\gamma - 1} \right) \frac{1}{M} \quad (2)$$

and at constant volume:

$$c_v = \left( \frac{1}{\gamma - 1} \right) \frac{1}{M} \quad (3)$$

The specific heats of gases can be readily determined by dividing the molal values by the molecular mass number  $M$  (molecular weight). I submit the following short table of specific heats at constant pressure for use with the MKS system of units and temperature scale.

Gas	Specific heat $c_p$ at 1 atmosphere and $241.11 \times 10^4$ J/kmole temperature (290° K)
Air	0.1310
Argon	0.0627
Hydrogen	1.717
Nitrogen	0.1252
Oxygen	0.1105
Steam	0.2406 ( $\gamma = 1.3$ )
Carbon dioxide	0.1014
Carbon monoxide	0.1253

If other properties of fluids are required it is possible to re-calculate the properties of fluids very rapidly with the digital computer by substituting the proper coefficients in the appropriate equations.

I have, since writing the original proposal<sup>1</sup>, had a series of thermometers manufactured using Joules per kilomole as a scale; and I am now using them in the laboratory and requiring students to become familiar with them. There was only a nominal surcharge for making these thermometers, and with wide adoption of the scale the price of the thermometers would be the same as present centigrade or Fahrenheit thermometers.

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## Gauge Co-ordinate Group of Physical Theory

RECENTLY, a characterization of the larger exceptional Lie groups has been obtained which throws considerable light on what their role may be in physical theory<sup>1</sup>. They are, namely, automorphisms of Clifford algebras of the special form  $\Gamma^{(n,n)} = Q_n \times Q_n$ ; that is, tensor products of normed division algebras  $Q_n$  of  $2^n < 8$  elements. If  $n = 3$ , the groups are  $F_4, E_6, E_7, E_8$  for  $n = 0, 1, 2, 3$ .

The Dirac algebra is  $\Gamma^{(3,3)}$  restricted by reality conditions so that its group is  $D_5$ , the rotation group of six dimensions, isomorphic to the conformal group of spacetime which leaves invariant Maxwell's equations. The way this algebra appears in Dirac's equation (that is,  $\Gamma^* = \{\rho_1 \sigma_i, 1, \rho_3\}$ , with  $\sigma_i, \rho_i$  imaginary quaternions) causes the splitting:

$$D_5 \sim D_4 + A_1 \quad (1)$$

into the Lorentz group and the gauge group of isotopic spin.

The theory of strong interactions<sup>2</sup> has required the enlargement of the gauge group to  $A_5$ , and more recently<sup>3</sup> to  $A_6$ . In the latter case, then, we might expect a role for  $E_6$  with the approximate splitting:

$$E_6 \sim E_5 + A_1 \quad (2)$$

$E_5$  is the group of the sphere  $S_5$ , which is unique in possessing a triality principle. Elsewhere<sup>4</sup> I have given some reasons for believing that it is the group of a classical phase space. It is essential for this interpretation that it involves a cosmological model, that is, at least one of the invariants of the group has a cosmological significance. Without this feature a splitting such as (2) could not have much depth, since it is necessary for the consistency of a theory of measurement. The latter requires three kinds of macroscopic field: (i) metrical (gravitation); (ii) interaction (electromagnetic); (iii) creation ( $C$ -field). It has been shown<sup>5</sup> that the  $C$ -field is necessary for unambiguous interpretation of the 'arrow of time'.

The splitting (2) depends on algebraic specialization of the field of definition of the group, and a crucial question is: What is this specialization? This must provide a source of purely numerical invariants, such as fine structure, mass ratios, etc.

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## GEOLOGY

### Significance of Burrowing Structures in the Origin of Convolute Laminæ

THE origin of convolute laminæ in sedimentary sequences has long been disputed<sup>1-4</sup>. The two major hypotheses for the origin of convolute laminæ are: (1) deformation by current drag<sup>1,2</sup>, and (2) deformation by subsequent loading<sup>3</sup>. Exceptionally well-exposed strata of Miocene age, containing convolute laminæ and burrowing structures formed by mud-feeding organisms, occur at Kaiti Beach, near Gisborne, North Island, New

Zealand. The relationship between the burrows and the convolutions presents a new line of evidence regarding the origin of convoluted laminae.

The section of Kaiti consists of rhythmically bedded graded sandstone and mudstone, interpreted to have been deposited largely by turbidity currents. Convoluted laminae are present in most of the sandstone beds. Individual beds containing convolutions are of uniform thickness throughout their exposed length and the convolutions have no apparent directional qualities nor current directional significance.

Burrowing structures formed by marine worms and holothurians (?) are present in most of the mudstone beds. Holothurian (?) burrows are sinuous, cylindrical structures, up to one and one-half inches in diameter, and are approximately parallel to bedding planes. They are concentrated at the base of the mudstone and occur at uniform depths, usually not more than 3-4 in., below the basal sand of the overlying rhythm.

The uniformity of distribution in depth and area of the burrowing structures in almost every rhythm suggests that between the passage of each turbidity current a stable bottom population was developed.

Burrowing structures cut across the convoluted laminae in a dozen or more beds and clearly post-date the convolutions (Fig. 1).

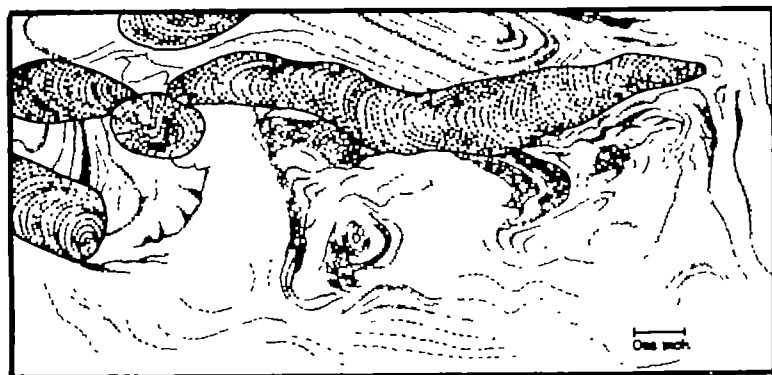


Fig. 1. Tracing made from photograph showing convoluted laminae cut by holothurian (?) burrowing structures

Burrows made by worms are generally straight and show no signs of deformation. In addition, none of the laminae appears to be folded against the massive burrows.

The age relations of burrows and convolutions, the lack of deformation of the worm burrows and the relatively shallow burrowing depth of the holothurians (?) all strongly suggest that current drag rather than subsequent loading formed the convolutions. Local erosion and truncation of antilinal crests of convolutions within a single bed by the overlying turbidite also supports this interpretation.

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## Potassium-Argon Ages of some Aberdeenshire Granites and Gabbros

POTASSIUM-ARGON age determinations have been made on specimens of granites and gabbros representative of the 'younger' Caledonian plutonic intrusions of north-east Scotland. Unlike the 'older' igneous rocks these 'younger' intrusions post-date the earlier fold movements and acme of regional metamorphism in the Dalradian schists<sup>1</sup>. The exact relation of the gabbro intrusions to the established sequence of movements and metamorphisms in the Dalradian rocks has received considerable attention<sup>2-4</sup>. It is evident that their emplacement post-dates the large recumbent ( $F_1$ ) fold structures of north-east Scotland (Benff Nappe), the second phase ( $F_2$ ) structures and the static (post- $F_2$ ) climax of regional metamorphism; also the evidence strongly favours deformation of the gabbros by the Boyndie-Buchan group of folds ( $F_3$ ) and is suggestive of their intrusion as a single large sheet<sup>5</sup>.

The potassium-argon ages for three of the basic masses show a spread from 460 to 498 million years. If the gabbros were originally intruded as a single sheet, then the spread of ages can be regarded as due to varying degrees of overprinting caused by metamorphic effects associated with the post-gabbro fold movements ( $F_3$ ). Thus the minimum age of intrusion is that represented

by the results of 486 and 498 million years obtained for the Haddo House mass and the true age could well be somewhat greater. This result is important with respect to the age of the earlier fold phases affecting the Dalradian rocks. Johnson and Harris<sup>6</sup> found that the supposed Arenig rocks of the Highland Border Series are affected by the earliest folds found in the Dalradian schists; this, on the time-scale at present accepted<sup>7</sup>, would mean that the Dalradian recumbent nappe structures were formed more recently than 500 million years ago. On the other hand, the new radiometric evidence strongly suggests that the first and second fold phases in the Scottish Dalradian rocks took place in pre-Arenig times, and emphasizes the doubt as to the precise age of the 'Arenig' rocks of the Highland Border Series. Also geological evidence from Co.

Mayo<sup>8</sup> and Tyrone<sup>9</sup> indicates that the Irish Dalradian underwent pre-Arenig movement and metamorphism.

Bisset<sup>11</sup> was able to divide the 'younger' granites in the area of Aberdeen into an earlier group, which he referred to as the Skene complex, and a later group including the red granites of Bennachie and Hill of Fare. The red granites are clearly members of the late or post-Silurian Newer Granite suite and the dated specimen of Bennachie granite yielded an age of 404 million years. On geological grounds the earlier Skene complex was also stated by Bisset to be a member of the Newer Granite suite and like the red granites is therefore younger than the gabbros. The Skene complex includes dioritic rocks and a variety of granitic types, all of which show complex injection relations with the country rocks, while the later red granites are relatively homogeneous masses with clearly defined sharp contacts. Felsite and lamprophyre dykes cut the Skene complex but not the red granites of Bennachie or Hill of Fare. The dated specimen from the Craigenlow quarry is a type assigned by Bisset to the porphyritic granites of the Skene complex. As a result of recent extensive quarrying, the rock can be seen to represent an advanced stage migmatite which remains as ghosts of an earlier metamorphic foliation. Superposed on the latter, however, is a complex fluxion foliation—the rock having sheared and re-healed repeatedly during movement. These shearing movements have sometimes been sufficiently strong to break up earlier lamprophyre intrusions, so that the latter occasionally occur as xenoliths in the

Table 1

Locality	Rock type	Mineral	Potassium oxide (per cent)	<sup>40</sup> Ar (mm <sup>3</sup> wt (g)	Atmospheric contamination (per cent)	Age (m.y.)
1. Maud mass, Alkey quarry	Biotite, hypersthene tonalite (norite)	Biotite	8.70	1.497 × 10 <sup>-1</sup>	46.8	460 ± 3
2. Maud mass, Alkey quarry	Granite (related to Maud mass)	Biotite	7.96	1.370 × 10 <sup>-1</sup>	17.4	460 ± 5
3. Haddo House mass, Auchedly granite quarry	Norite (contaminated)	Biotite	7.96	1.376 × 10 <sup>-1</sup>	18.0	462 ± 4
4. Huntly mass, Huntly Castle	Norite (contaminated)	Biotite	7.43	1.400 × 10 <sup>-1</sup>	19.5	498 ± 5
5. Skene complex, Craigenlow quarry	Grey granite	Biotite	7.43	1.368 × 10 <sup>-1</sup>	50.3	486 ± 6
6. Bennachie mass, Piffche quarry	Red granite	Biotite	8.37	1.477 × 10 <sup>-1</sup>	19.4	470 ± 5
			8.23	1.357 × 10 <sup>-1</sup>	16.7	416 ± 2
			8.28	1.350 × 10 <sup>-1</sup>	20.1	414 ± 2
			6.91	1.029 × 10 <sup>-1</sup>	20.5	404 ± 5
			6.91	1.029 × 10 <sup>-1</sup>	19.6	404 ± 5

$\lambda_e = 0.584 \times 10^{-18} \text{ yr}^{-1}$ ,  $\lambda\beta = 4.72 \times 10^{-18} \text{ yr}^{-1}$  Volume of argon-40 (radiogenic) in mm<sup>3</sup> H.T.P.

rock they formerly intruded. The ages of 416 and 414 million years obtained for the Craigenlow specimen are consistent with those geological features which suggest that the Skene complex is an earlier and probably deeper seated member of the 'younger' or Newer Granites than the nearby red granite of Bennachie. It is here suggested that the Skene complex may in some respects be analogous to the Rogart granite in Sutherland which is also an early, deep-seated, member of the Newer Granite suite and has yielded ages of 424 and 416 million years<sup>13</sup>.

The contact relations of the red granites suggest that at the time of their intrusion the temperature of the country rocks was distinctly lower than that of the granites. Elsewhere, for example, at Lochnagar, the mode of intrusion suggests that the country rocks were locally brittle when the red granites of north-east Scotland were intruded. The difference in ages of the Bennachie granite (404) and the Skene complex (416 million years) presumably therefore reflects the cooling history of the country rocks into which the granites were intruded.

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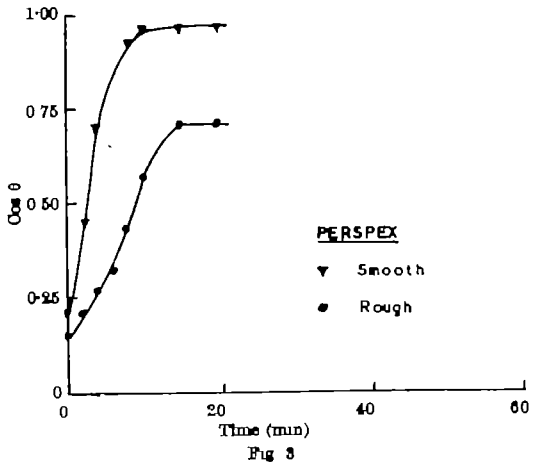
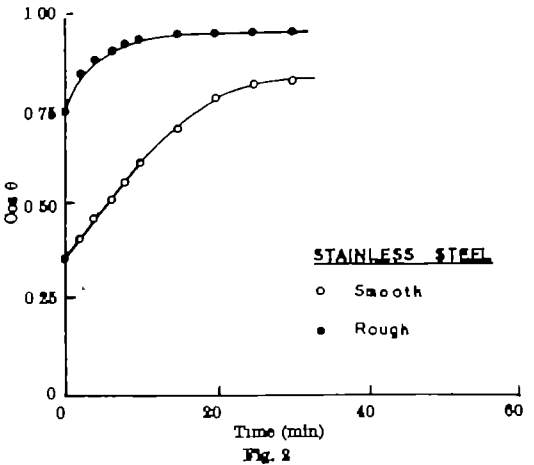
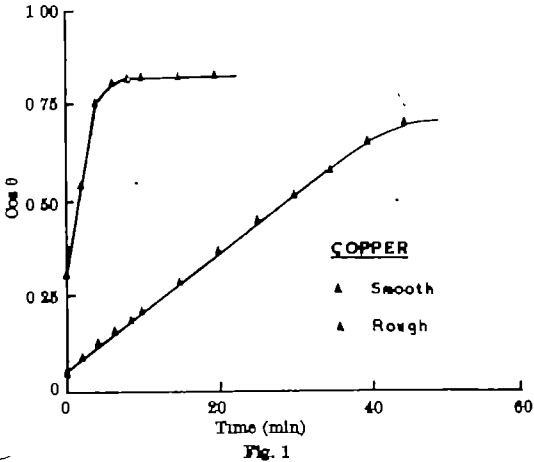
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ENGINEERING

Measurement of Contact Angles in the Presence of Mass Transfer

Low distillation and absorption efficiencies in gas/liquid contacting equipment have been attributed to liquid film breakdown and underwetting. A study of a model simulating these conditions has resulted in observations of contact angles when mass transfer is occurring.

Results are presented here for the contact angles of water on stainless steel, copper, graphite and 'Perspex' surfaces in the presence of a saturated ethanol/air vapour.



The solid surfaces were prepared in pairs of different roughness and before each experiment were carefully pretreated and dried.

The effect of the ethanol vapour in each case was to cause the sessile drop of water to spread over the surface and the contact angle to be reduced until an equilibrium was reached. This is attributed to the absorption of ethanol by the water droplet causing a reduction in surface energy and to adsorption of ethanol on the solid surface.

Wenzel<sup>1</sup> has shown that, for contact angles measured without mass transfer under isothermal conditions on differing roughened surfaces,  $\cos \theta^1 = r \cos \theta$ , where  $\theta^1$  is the contact angle on a roughened surface and  $\theta$  is the true contact angle on a perfectly smooth surface.

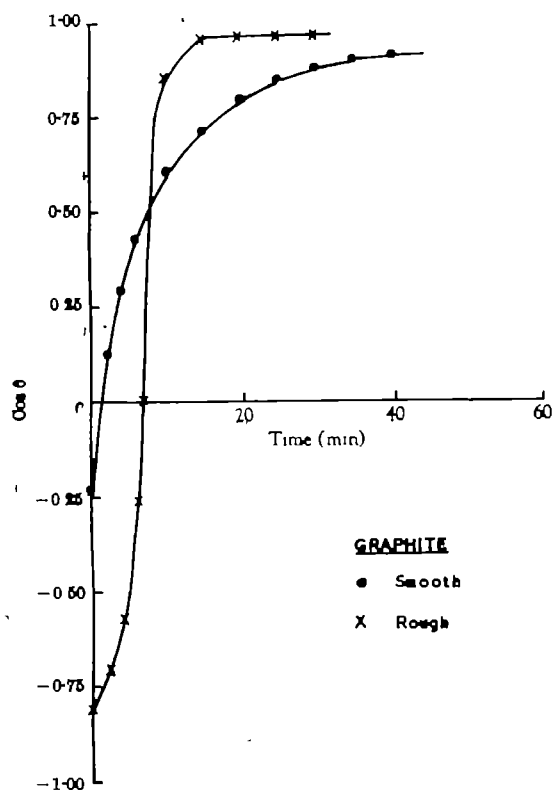


Fig. 4

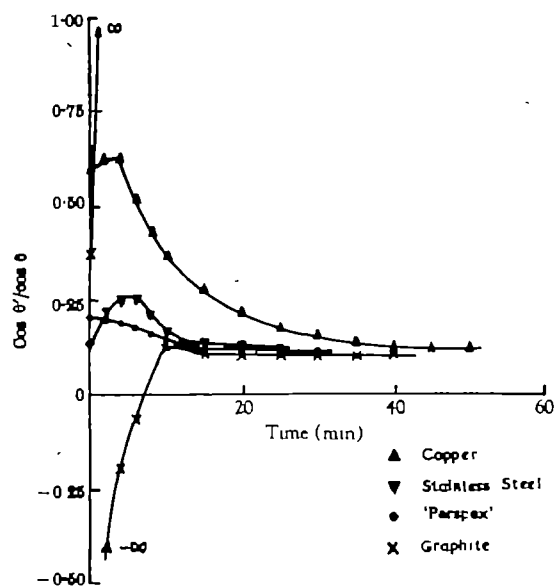


Fig. 5

Table 1

	Roughness m.	Contact angle Initial	Equilib.	Time to reach equilib.	Spreading coeff. Initial	Equilib.
					dynes/cm	
Copper	5-10 × 10 <sup>-4</sup>	87°	45°	45 min	-68.2	-14.8
	50-60 × 10 <sup>-4</sup>	72°	33°	8 "	-49.6	-7.9
Stainless steel	5-10 × 10 <sup>-4</sup>	60°	35°	25 "	-46.5	-8.7
	50-60 × 10 <sup>-4</sup>	45°	20°	15 "	-18.5	-2.0
'Parapex'	5-10 × 10 <sup>-4</sup>	81°	45°	12 "	-60.6	-14.3
	50-60 × 10 <sup>-4</sup>	78°	15°	10 "	-57.0	-1.65
Graphite	5-10 × 10 <sup>-4</sup>	102°	20°	40 "	-87.0	-2.9
	50-60 × 10 <sup>-4</sup>	145°	18°	15 "	-129.0	-2.4

Fig. 5 indicates that for any particular time before equilibrium is reached, since the absorption rate by the liquid and the roughness ratio for each material are equal, the contact angle is most markedly influenced by the adsorption of ethanol on the solid surface.

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## CHEMISTRY

### A New Approach to Desorption of Surface-active Substances at Liquid-phase Boundaries

In order to investigate the kinetics of the formation of adsorption layers at liquid/gas and liquid/liquid boundaries we have made use of the methods of surface tension measurement, including the sessile bubble or drop technique<sup>1</sup>. Furthermore, an attempt has been made to investigate the possibility of desorption from the adsorption layer by replacement of the surfactant solution around the bubble (drop) by a pure solvent in static conditions (without disruption of the bubble or drop and the adsorption layer).

The experiment was carried out in the following way. A special thermostatic optical cell with a device for maintaining liquid flow at a constant hydrostatic pressure is filled with a surfactant solution, in which the sessile bubble is formed. The change in surface tension is then recorded in the usual way (photography with subsequent measurement of the bubble image and calculation of surface tension from corresponding tables). Then, after a given time of surface-layer formation, sufficient pure solvent is slowly pumped through the cell (approximately 3 l. per 20 min.) to remove completely the dissolved substance. The time of such 'washing out' of the cell depends on the rate of solvent flow, solution concentration and cell construction.

Surface tension is constantly recorded during this process of 'washing out' and afterwards. Once this stage of the experiment is over, a new bubble is formed in the washing substrate and the surface tension of the substrate is again recorded.

Using this method we were able to show the desorption of surface-active molecules from adsorption layers. Typical results for sodium oleate are given as an example: in addition to the surface tension curve characterizing the process of desorption (the increase of surface tension from 33.3 ergs/cm<sup>2</sup> for 0.01 per cent solution at 20° C and pH 11.0 to 72.5 ergs/cm<sup>2</sup>), the surface tension value (72.5 ergs/cm<sup>2</sup>) after replacement of the solution by water (at the same pH value) and the surface tension of the washing substrate (72.5 ergs/cm<sup>2</sup>) are very close to the value for pure water under similar conditions.

On the other hand, we were able to show experimentally the irreversibility of adsorption of the macromolecular surface-active substances at the liquid/gas surface using, for example, polyvinyl alcohol. The adsorption layer formed (surface tension of 68.0 ergs/cm<sup>2</sup> for 0.3 per cent solution) does not show any signs of desorption after the replacement of the solution by pure water for a long time (surface tension has not changed after 20 h), while the

surface tension of the washing substrate is greatly increased (72.5 ergs/cm<sup>2</sup>) and corresponds to the value of pure water. It clearly shows that polyvinyl alcohol molecules, in any event for a long time, cannot leave the adsorption layer.

It should be pointed out that the question of reversibility or irreversibility of macromolecular adsorption has been considered theoretically<sup>3</sup>.

Moreover, the suggested method makes it possible to consider the question of adsorption equilibrium at liquid interfaces as a true thermodynamic equilibrium since it enables one to obtain the desired concentration of solution under investigation from two approaches (from lower and higher concentrations).

Besides desorption studies and the problems connected with it, the suggested method opens up great possibilities for research under static conditions of various types of interactions in adsorption layers and makes it possible to carry out successive measurements at the same undisturbed surface (adsorption) layer.

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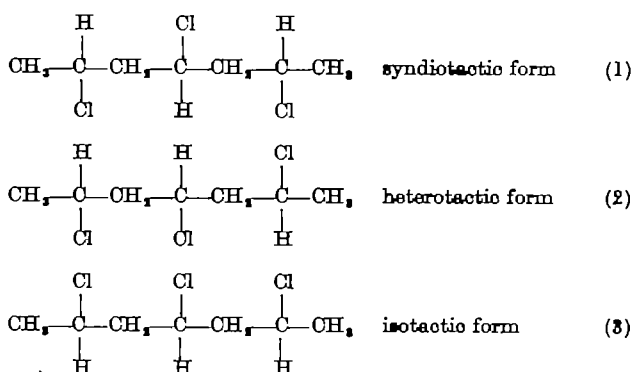
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### Gas Chromatographic Separation of Stereoisomeric Trichloroheptanes using Organo-clay Complexes

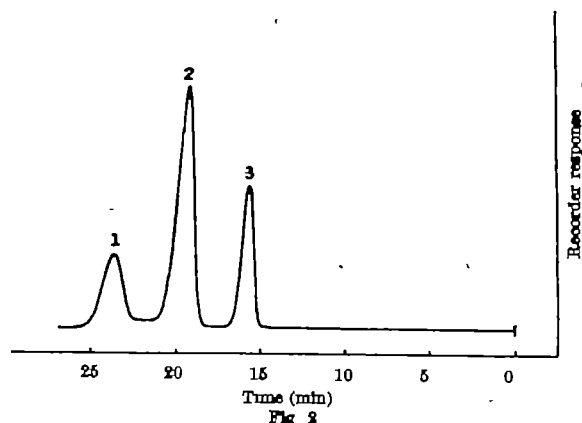
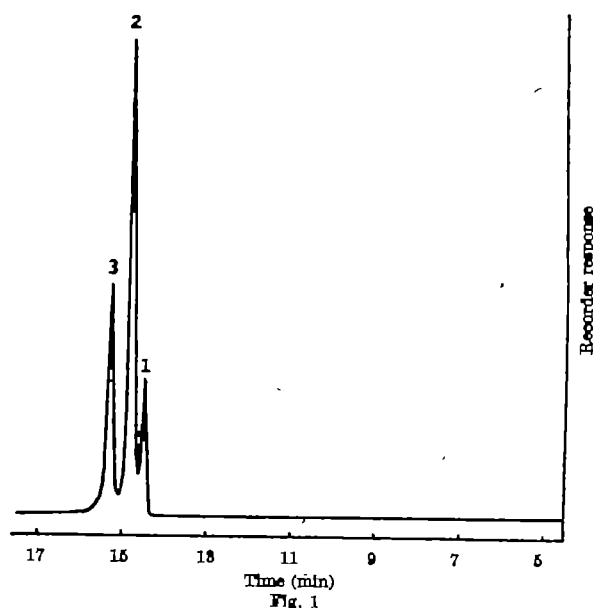
SINCE White<sup>1</sup> has shown that dimethyldioctadecyl ammonium bentonite is particularly suitable as a stationary phase for the chromatographic separation of aromatic and aliphatic compounds, several authors have shown the ability of this material to separate aromatic isomers, for example, xylenes, toluidines, cresols<sup>2</sup>, dichlorobenzenes<sup>3</sup>. The performance of organo-clays was improved by modification with conventional liquid stationary phases<sup>4</sup>.

Here we report the separation of aliphatic stereoisomers of the 2,4,6-trichloroheptane which exists in three forms:



The presence of these isomers in 2,4,6-trichloroheptane has been proved by gas chromatography on 'Apiezon L'-coated capillary column (Fig. 1), the sequence of peaks being: (1) syndiotactic; (2) heterotactic; (3) isotactic form as confirmed by spectroscopic analysis<sup>5</sup>.

In chromatographic columns filled with conventional stationary phases<sup>6</sup> we were not able to obtain any separation of isomers even when a 10 m column with 'Carbowax' as the stationary phase was used, which showed very good



selectivity in separation when two isomeric 2,4-dichloropentanes<sup>7</sup> were used.

On the other hand, an excellent separation was achieved with a 2-m column filled with dimethyldioctadecyl ammonium bentonite modified with 'Apiezon L' (Fig. 2). With this column, in contradistinction to the capillary one, a reversed elution order was observed, the isotactic form appearing first, the syndiotactic form being most retained. The selectivity of organo-clay complexes for this kind of separation may be explained by specific absorption forces of an electronic nature between the sorbate and chlorine atoms of single stereoconfigurations of the solute.

The chromatographic runs were performed on Fractometer 116E (Perkin-Elmer), the separating conditions being as follows: 50 m stainless steel capillary column of internal diameter 0.25 mm coated with 'Apiezon L', column temperature 150° C, nitrogen carrier gas velocity 1.0 ml./min, sample size 0.1 µl., split ratio 1:200, flame ionization detector (Fig. 1).

A typical chromatogram on Fig. 2 was obtained with a column 200 cm long x 0.38 cm bore filled with 15 ww per cent of equal parts by weight of 'Bentone 34' (F. W. Berk and Co., Ltd.) and 'Apiezon L' on 'Celite' (40-60 mesh) prepared according to Mortimer<sup>8</sup> at a column tem-

2,4,6-Trichloroheptane isomer	Relative retention volume at 150°	
	'Apiezon L'	'Bentone 34'
Syndiotactic	0.97	1.23
Heterotactic	1.0	1.0
Isotactic	1.05	0.80



perature of 150° C, a hydrogen carrier gas velocity of 50 ml./min, a sample size of 0.1  $\mu$ l., and a hot-wire conductivity detector.

The relative retention volumes of isomers are given in Table 1. It should be noted that with increasing sample size the elution peaks become asymmetric and the separation factors decrease. In spite of these circumstances it was possible to perform, under proper conditions and column size, preparative separation of 20- $\mu$ l. samples of 2,4,6-trichloroheptane and isolation of pure stereoisomers.

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### Relationship between Volume Change on Fusion and the Volume of Defect Formation in Ionic Crystals

THE molar volume change on fusion of an ionic compound,  $\Delta V_{\text{fusion}}$ , is relatively easy to determine. It may be found directly, by observing the change in length of a column of fused salt in a capillary when this is cooled through the freezing temperature, or indirectly by measuring the freezing point of the salt as a function of pressure and applying the Clapeyron–Clausius equation. The literature now contains reliable values of  $\Delta V_{\text{fusion}}$  for the common inorganic salts<sup>1,2</sup>.

It is well known that ionic solids may contain defects of the Schottky (vacant site) or Frenkel (interstitial ion) type, and that migration of these defects gives rise to diffusion and electrical conductance in the solids. Since the individual defects in an ionic crystal bear an excess charge relative to the undistorted lattice, the condition of electrical neutrality requires that a given crystal must contain at least two types of defect bearing opposite excess charge. For each cation vacancy in a stoichiometric crystal containing ions of non-variable valency, there must be either an anion vacancy or an interstitial cation elsewhere in the crystal. In a particular compound, one or other of these possibilities usually predominates; for example, the alkali halides are known to contain cation and anion vacancies<sup>3</sup>, and the silver halides mainly cation vacancies and interstitial cations<sup>4</sup>. In  $\text{CaF}_2$ , the complementary defects are anion vacancies and interstitial anions<sup>5</sup>.

The molar volume of formation of defects,  $\Delta V_{\text{form}}$ , is defined as the volume increase associated with the formation of one mole of each of the complementary defects in an initially perfect crystal.  $\Delta V_{\text{form}}$  is relatively difficult to determine experimentally; values are at present available only for KCl, NaCl, AgBr and  $\text{KNO}_3$  (refs. 6–8). It is found from the isothermal pressure coefficient of the electrical conductivity of the crystal; measurements must be carried out on the pure substance, and also on a sample into which additional defects have been introduced by adding traces of divalent cations<sup>6</sup>. It has been found that, for crystals containing vacant cation and anion sites in equal numbers,  $\Delta V_{\text{form}}$  is about 1.5 times the molar volume of the salt, whereas for silver bromide  $\Delta V_{\text{form}}$  is only about 0.5 times the molar volume. The magnitude of  $\Delta V_{\text{form}}$  relative to the molar volume of the solid,  $V_s$ , therefore gives an indication of the types of defect predominant. (If there were no distortion of the

crystal lattice around each defect,  $\Delta V_{\text{form}}$  would equal  $V_s$  for crystals containing anion and cation vacancies, and would be zero for crystals containing cation vacancies and interstitial cations.)

The purpose of this communication is to point out that there appears to be a proportionality between  $\Delta V_{\text{form}}$  and  $\Delta V_{\text{fusion}}$  for all crystals, irrespective of the types of defect present in the solid (Table 1).

Table 1

Salt	$V_s$ , just below m.p.	$\Delta V_{\text{fusion}}$	$\Delta V_{\text{form}}$	$\Delta V_{\text{fusion}}/\Delta V_{\text{form}}$
KCl	41.00	7.2	67	0.11
NaCl	30.2	7.5	43	0.18
AgBr	31.1	2.5	16	0.16
$\text{KNO}_3$	52.5	1.73	11	0.16

(all volumes in ml.mole<sup>-1</sup>)

The ratio  $\Delta V_{\text{fusion}}/\Delta V_{\text{form}}$  is formally equal to the degree of defect formation (that is, the fraction of lattice sites vacant, or fraction of interstitial sites occupied) which would have to occur in the solid in order to produce a volume change equal to  $\Delta V_{\text{fusion}}$ . It is seen that  $\Delta V_{\text{fusion}}/\Delta V_{\text{form}}$  lies in the range  $0.15 \pm 0.04$  for the four salts for which data exist. The X-ray diffraction work carried out by Levy *et al.*<sup>9</sup> on fused alkali halides showed that the average co-ordination number in salts having the rock-salt structure falls from six to about five on fusion. If this change were brought about by introduction of defects into the crystalline solid, the degree of defect formation required would be about 0.17. This is suggestively close to the figures in the last column of Table 1, and leads me to propose the following rules:

(1) The fusion of an ionic crystal is equivalent to the introduction of disorder into the crystalline lattice to the extent of  $15 \pm 4$  per cent.

(2) The defects introduced on fusion are of the same type as those already present in the crystal below its melting point.

Thus, fused silver halides may be thought of as containing an approximately intact bromide lattice, with 85 per cent of the silver ions occupying normal sites and the remaining 15 per cent on interstitial sites, while fused alkali halides have approximately the rock-salt lattice each with 15 per cent of the cation and anion sites vacant. These pictures are approximate in so far as the vestiges of lattice structure which remain in the melt are insufficient to impart the property of rigidity.

The converse of the above rules may be written:

(3) Those ionic compounds for which  $\Delta V_{\text{fusion}}/0.15$  is less than about one-half of the molar volume have cation vacancies and interstitial cations, or anion vacancies and interstitial anions, as predominant defects in the solid. Those compounds for which  $\Delta V_{\text{fusion}}/0.15$  is about 1.5 times the molar volume contain predominantly cation vacancies and anion vacancies.

This third rule may be the most useful of the three, because it enables us to predict the type of defect present in a solid from a knowledge of its volume change on fusion. This quantity is relatively easy to determine, whereas the determination of  $\Delta V_{\text{form}}$  (on which conclusions as to type of defect have usually been based) requires the difficult and painstaking measurement of the pressure coefficient of the electrical conductivity.

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**Relationship between the 3,3',5-Triiodo-L-thyronine Content of Thyroid as determined by a Thin-layer Chromatographic Method and the Biological Potency assayed by a Rat Anti-thiouracil Goitre Method**

In a recent paper<sup>1</sup>, we described a method which could be used for identifying thyroxine in a feeding-stuffs additive; its separation from the other components present in the hydrolysed iodinated protein was effected on paper and on starch-bound cellulose powder thin-layer plates.

If the procedures referred to therein are applied to hydrolysed thyroid, both paper and thin-layer chromatograms are capable of effectively resolving a substance having an  $R_f$  value corresponding to thyroxine as a well-defined spot. The spot with  $R_f$  value corresponding to triiodothyronine is, however, poorly defined on paper chromatograms, but the thin-layer chromatogram shows a distinct spot which can be assessed visually against triiodothyronine standards.

Since it is known that triiodothyronine ( $T_3$ ) makes a far greater contribution than thyroxine ( $T_4$ ) to the activity of thyroid when assayed by the rat anti-thiouracil goitre method, it was thought that the triiodothyronine content of hydrolysed thyroid, as determined by visual assessment of thin-layer chromatograms, should provide some indication of the potency. When two samples of thyroid were examined by this means, the quantity of material in the triiodothyronine position present in one sample was distinctively different from the quantity in the other. The first sample was reported to have one-third of the biological activity of the second when subjected to the rat anti-thiouracil goitre test and we estimated that the spots on the thin-layer chromatograms corresponded to 0.025 and 0.06 per cent of triiodothyronine respectively. A third sample, said to be 'biologically unsatisfactory' (the source and type of bio-assay are unknown), showed only a very faint spot, equivalent to about 0.005 per cent of triiodothyronine.

The conditions necessary to obtain spots well separated from other materials and in good alignment with triiodothyronine standards have been worked out in detail. The hydrolysis and extraction of active materials into *n*-butanol are based on the procedure described by Devlin and Stevenson<sup>2</sup>. Thin-layer plates prepared by the method previously described<sup>1</sup> should be used, and should run at a

temperature greater than 20°C and preferably near to 26°C in the *t*-amyl alcohol and ammonia solvent system described by Barker<sup>3</sup>, special precautions being taken to ensure that the atmosphere is saturated with ammonia. The FFCA reagent of Gmelin and Virtanen<sup>4</sup> is the preferred spray reagent for spot location. The chromatogram is undisturbed by the water-washing necessary to remove excess reagent after spraying. The procedure is improved when a 'blank' enzyme hydrolysate is superimposed on the triiodothyronine standards, so that the hydrolysed sample and standards run in the same environment, as shown in Fig. 1.

This work was undertaken as a result of our membership of Panel 7 of the Joint Committee of the Pharmaceutical Society and the Society for Analytical Chemistry on Methods of Assay of Crude Drugs. The samples of thyroid were selected from those used for work by Panel 7 and bio-assays were supplied by Mr. K. L. Smith of Boots Pure Drug Co., Ltd., Nottingham. Collaborative tests on the method based on these principles is at present being carried out by members of Panel 7 to confirm that the apparent correlation with biological activity can be substantiated on a wider range of samples.

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## BIOCHEMISTRY

### Effect of Magnesium Deficiency on Plasma Alkaline Phosphatase Activity

MAGNESIUM activates many enzymes *in vitro*, but very few disturbances in enzymatic activity have been demonstrated during magnesium deficiency *in vivo*<sup>1</sup>. The alkaline phosphatases from various mammalian tissues are among the enzymes known to be activated by magnesium in isolated systems. This communication reports investigations of the effect of magnesium deficiency on plasma alkaline phosphatase activity in the rat.

Two groups of female Wistar albino rats of initial weight 100 g were fed with magnesium-deficient and control diets respectively for 15 days. All animals received an amount of food equal to that consumed by the deficient rats; this gave an initial food intake of 10 g reducing to 8 g/rat/day. Distilled water was provided *ad lib.* throughout the experiment. The magnesium-deficient and control diets were prepared as described previously<sup>2</sup>; their magnesium contents were 0.2 mg/100 g (deficient) and 78 mg/100 g (control). The rats were anaesthetized with ether and killed by bleeding from the heart. Plasma was separated from heparinized blood within 0.5–1 h.

The plasma from each magnesium-deficient rat was divided into two and the magnesium concentration in one portion was raised to normal by adding to it 0.83 per cent of its volume of a magnesium chloride solution containing 165 mg/100 ml. of magnesium. The alkaline phosphatase activity was measured in all samples of plasma, as described by King and Wootton<sup>3</sup>, after standing overnight at 4°C. The measurements were repeated after storage for a further six days to allow ample time for the added magnesium

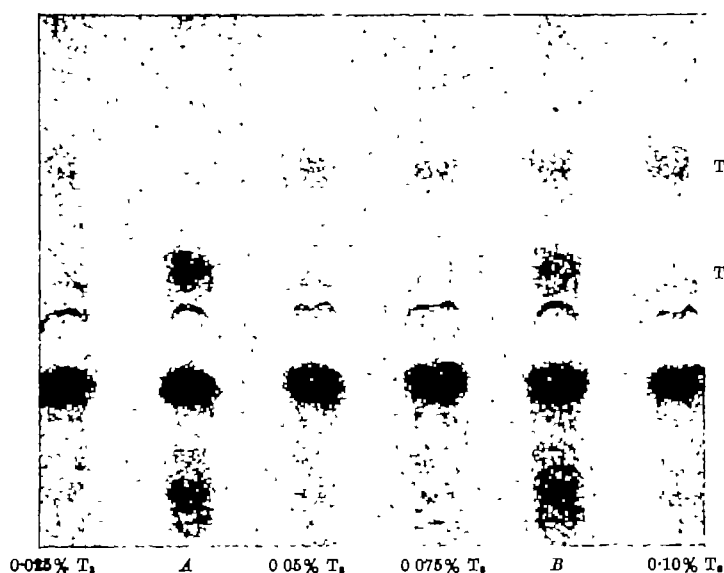


Fig. 1. Thin-layer chromatogram showing 6 µl. of each of two samples of enzyme-hydrolysed thyroid, A and B, alongside increasing quantities of triiodothyronine ( $T_3$ ). Six µl. of a 'blank' enzyme hydrolysate have been spotted over each spot of triiodothyronine.

to combine with the enzyme. Magnesium concentrations were determined by atomic absorption spectrophotometry<sup>4</sup>.

The alkaline phosphatase activity in the plasma from magnesium-deficient rats was only half that found in the control animals ( $P < 0.001$ ) (Table 1). Addition of sufficient magnesium chloride to restore the magnesium concentration to normal in the plasma from deficient rats raised the alkaline phosphatase activity significantly ( $P < 0.001$  and  $P < 0.01$  after storage for 1 and 7 days respectively), but did not restore it to the level found in the control animals ( $P < 0.001$ ). No consistent difference in alkaline phosphatase activity was observed between the measurements made after storage for 1 and 7 days, indicating that reactivation by the added magnesium was complete after storage overnight.

Table 1. RELATION BETWEEN ALKALINE PHOSPHATASE ACTIVITY AND MAGNESIUM CONCENTRATION IN THE PLASMA FROM CONTROL AND MAGNESIUM-DEFICIENT RATS

Group	Magnesium concentration (mg/100 ml.)	Alkaline phosphatase activity (King-Armstrong units) after storage for	
		1 day	7 days
Control	1.77	40.3	43.9
	$\pm 0.04$	$\pm 3.9$	$\pm 4.4$
Mg-deficient	0.43	20.5	19.5
	$\pm 0.02$	$\pm 3.0$	$\pm 1.9$
Mg-deficient + MgCl <sub>2</sub>	1.87	25.0	23.3
	$\pm 0.04$	$\pm 2.6$	$\pm 2.4$

The values indicate mean  $\pm$  S.E.M.  $n=12$  for control group;  $n=15$  for the other groups.

A reduction in enzymatic activity may be due to either a decrease in the amount of enzyme present or a fall in the rate of operation by a normal amount of enzyme. The increase in alkaline phosphatase activity observed after adding magnesium to plasma from magnesium-deficient rats indicates that the efficiency of the circulating enzyme is reduced by a lowered concentration of magnesium in the plasma. As the enzymatic activity in the plasma of deficient rats was still lower after the addition of magnesium than in the plasma from control rats, it appears that the concentration of the alkaline phosphatase enzymes in the plasma may also be reduced by magnesium deficiency.

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### High-sulphur Proteins as a Major Cause of Variation in Sulphur Content between $\alpha$ -Keratins

A common feature of the wide variety of mammalian tissues classified as  $\alpha$ -keratins is that they may be split into two separate protein fractions<sup>1-3</sup> bearing a general resemblance to the 'high-sulphur' and 'low-sulphur' protein-fractions first recognized in wool<sup>4,5</sup>. However, in these  $\alpha$ -keratins which include hair, fur, wool, horn, nail, hoof, quill and baleen, the percentage of sulphur present varies greatly and covers the approximate range 2-6 per cent<sup>6-8</sup> (Table 1). In general the horny keratins contain smaller amounts of sulphur than do the hairs and so the sulphur content of sheep horn, for example, is often lower than the sulphur content of wool from the same animal. It has been suggested<sup>7</sup> that these differences in sulphur content can be used as a means of distinguishing keratins from one another, but in the case of wool it is now well established that there is a characteristic range

of values of sulphur content (about 2.0-4.2 per cent) rather than a particular value for this keratin. A review of the relevant literature is given by Reis and Schinckel<sup>10,11</sup>. A sheep will produce wool with a high sulphur content during high levels of nutrition or during the abomasal infusion of sulphur-containing amino-acids; on the other hand, wool with a much lower sulphur content will be produced when the animal is on a copper-deficient or low-nutritional diet<sup>12-13</sup>. Moreover, these variations in the sulphur content of wool have been shown to originate primarily in the 'high-sulphur' proteins which change both in amount and in sulphur content<sup>1,14-16</sup>.

In recent work, Gillespie and Inglis<sup>8</sup> have shown that, when  $\alpha$ -keratins are compared, they differ greatly both in the amount and in the type of 'high-sulphur' proteins which they contain. These results, when combined with analytical data<sup>9</sup> on the 'low-sulphur' proteins (Table 1), strongly suggest that the main difference between  $\alpha$ -keratin types lies in variations in the 'high-sulphur' group of proteins. The values presented for the proportion of high-sulphur protein in each keratin are underestimates because of the difficulty of measuring the amount of this protein bound to the 'low-sulphur' fraction and removed from it during purification. It is not possible to make a sulphur balance because of the uncertainty about the amount of 'high-sulphur protein' present and because of the uncertainty of the composition of the unextracted residue (10-15 per cent of each keratin).

Table 1. SULPHUR ANALYSES OF S-CARBOXYMETHYL PROTEINS FROM VARIOUS  $\alpha$ -KERATINS

Keratin Type *	Low-sulphur protein			High-sulphur protein		
	% S †	% S ‡	Sulphur contributed to keratin g/100 g	% S †	% S ‡	Sulphur contributed to keratin g/100 g
Raccoon hair	5.6	2.3	1.0	45	7.1	3.2
Monkey hair	5.2	2.3	1.2	39	7.1	2.8
Guinea-pig hair	4.8	2.1	1.2	34	7.2	2.4
Rabbit hair	4.2	2.3	1.4	29	7.2	2.1
Bovine hair	4.0	1.7	1.0	29	5.9	1.7
Wool (sulphur-enriched)	3.8	1.9	1.1	81	5.8	1.8
Wool (control)	3.1	2.1	1.4	23	5.0	1.1
Whale baleen	3.0	2.3	1.8	13	5.8	0.70
Porcupine quill	2.6	1.7	1.2	17	5.9	1.0
Sheep horn	2.2	1.6	1.2	13	5.2	0.62
Rhinoceros horn	1.9	1.4	1.2	7	4.3	0.80

\* Origin and preparation of keratins<sup>8</sup>.

† Sulphur estimation by oxygen flask combustion<sup>14</sup>.

‡ From amino-acid composition, using Spence amino-acid analyser.

§ Preparation of proteins<sup>14,17</sup>.

\* In this computation the relation was used: percentage low-sulphur protein = 100 - (percentage high-sulphur protein + 10); it being assumed that each keratin contained 10 per cent of membranes, scales and nuclear remnants.

Nevertheless it is clear that 'low-sulphur' proteins, although not identical in sulphur content, contribute a fairly constant amount of sulphur to each keratin, whereas there is at least a ten-fold variation in the amount of sulphur contributed by the 'high-sulphur' protein. It is therefore proposed as a general hypothesis that variations in sulphur content between  $\alpha$ -keratins are primarily due to the combined effects of variations in quantity and sulphur content of constituent 'high-sulphur' proteins. This would appear to be applicable to both inter- and intra-species differences.

The implications of this are important, for if all  $\alpha$ -keratins have a common mode of synthesis in which there is a primary synthesis of 'low-sulphur' proteins (as  $\alpha$ -filaments), and at a later stage in development the 'high-sulphur' proteins are produced in a secondary synthesis and are inserted between the filaments as an amorphous matrix<sup>18,20</sup>, then there must be a common regulatory mechanism which decides both the amount and type of 'high-sulphur' protein which is produced by a follicle. Although the nature of this mechanism is unknown, certain factors which influence it are known. Species is important, for it is probable that the hair of some animals (for example, the raccoon) is consistently higher in 'high-sulphur' protein than that of others (for example, the sheep). The end-use of the keratin is

possibly important and the horny keratins may owe their particular physical properties to their richness in filaments and comparative paucity in matrix proteins<sup>8</sup>. Diet is involved, at least in so far as the supply of *S*-amino-acids is concerned, although judging from experiments with sheep its effects are restricted to within certain limits<sup>18</sup>. A study of this control mechanism may provide a valuable insight into the processes by which keratins are formed.

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### Interaction of 4-Dimethylaminoazobenzene and Dietary Fat in the Regulation of Hepatic Microsomal Ascorbic Acid Synthesis

An increase in urinary ascorbic acid excretion in rats has been demonstrated to occur after an injection of 4-dimethylaminoazobenzene (DAB) and also after the injection of several methylated derivatives of this carcinogen<sup>1</sup>. Other types of compounds, such as chloretone, aminopyrine, meprobamate and phenylbutazone, also increase the urinary excretion of ascorbic acid in rats<sup>2,3</sup>. It has been suggested that these compounds stimulate the biosynthesis of glucuronic acid as an adaptive response for detoxication processes, and that some of the glucuronic acid is then utilized for the synthesis of ascorbic acid.

Some hepatic microsomal enzymes which metabolize foreign substances have been noted to increase in activity after the administration of several carcinogenic hydrocarbons<sup>4</sup>. In addition, alterations in the swelling properties of hepatic microsomes have been noted after the administration of carcinogenic azo dyes<sup>5</sup>. The induction of hepatomas by azo dyes appears to be influenced by the quantity and type of fat in the diet. Corn oil accentuates the formation of DAB-induced hepatomas, whereas a fat-free diet, or hydrogenated coconut oil, appears to inhibit tumorigenesis<sup>6</sup>. The effects of dietary fatty acids may be mediated via their incorporation into phospholipids. Since phospholipids probably form the structural matrix for RNA and enzyme systems in the microsomes<sup>7</sup>, changes in the fatty acids of these phospholipids might affect the activity of the enzymes, or influence the effects of carcinogenic compounds on the enzymes.

To investigate the combined influence of dietary fat and DAB on hepatic microsomal ascorbic acid synthesis, six groups of three-month-old Sprague-Dawley rats (8 rats/group) were fed synthetic diets consisting of sucrose, casein, cellulose, choline, a salt mixture and a vitamin mixture (containing 2 mg/kg riboflavin but

adequate in all other vitamins), with additions as follows: Group I, no additions (fat free); Group II, fat-free + 0.08 per cent 4-dimethylaminoazobenzene (DAB); Group III, 20 per cent hydrogenated coconut oil (HCNO); Group IV, 20 per cent HCNO + 0.08 per cent DAB; Group V, 20 per cent safflower oil (SFO); Group VI, 20 per cent SFO + 0.08 per cent DAB. After 4 months on the diets the rats were killed by intraperitoneal injection of sodium pentobarbital ('Nembutal'). Livers were excised and homogenized, and the microsomes isolated by ultracentrifugation by the method of Hogeboom<sup>8</sup>. Microsomal L-ascorbic acid synthesis from D-glucuronolactone was investigated *in vitro* using the method of Chatterjee *et al.*<sup>9</sup>. The test system contained 0.25 ml. of the microsomal suspension (equivalent to 250 mg of wet liver tissue), and an additional 2.25 ml. of the following composition and concentration: 0.02 M sodium phosphate buffer (pH 7.4), 0.01 M D-glucuronolactone, and 0.05 M KCN. The mixture was incubated at 37° C for 2 h in air. Ascorbic acid was determined by adding 0.5 ml. 30 per cent metaphosphoric acid to the incubation mixture and titrating against a standard 2,6-dichloroindophenol solution. The microsomal protein content was determined by the method of Gornall *et al.*<sup>11</sup>. The probability (*P*) that apparent differences in the data were due to chance was calculated by the *t* test.

Table 1. MICROGRAMS OF L-ASCORBIC ACID FORMED/MICROGRAM OF PROTEIN

Diet	Without DAB	With DAB
Basic (fat-free)	25 ± 1.3	30 ± 2.1
Basic + HCNO	24 ± 1.3	28 ± 1.9
Basic + SFO	25 ± 0.9	26 ± 2.1

The degree of synthesis of ascorbic acid by hepatic microsome suspensions from rats on the various diets with and without DAB is indicated in Table 1.

Previous investigations have demonstrated that dietary components can affect enzymatic activity<sup>12</sup>. In particular, essential fatty acid deficiency in the rat has been shown to result in alterations in several hepatic enzymes<sup>13,14</sup>. Succinic, glutamic and butyric dehydrogenases were found to be reduced in activity, and cytochrome oxidase and choline oxidase were increased, but there was no change in succinic oxidase activity. In the present work, the amount or type of dietary fat alone appeared to have no effect on the microsomal enzyme system for the synthesis of ascorbic acid, indicating that the maintenance of these enzymes is probably independent of exogenous essential fatty acids.

Some investigations have shown that, in general, in hepatomas there is a deficiency of drug-metabolizing enzymes, such as the azo dye reductase and *N*-demethylase systems<sup>15</sup>. The O3H mouse hepatoma, however, retains the microsomal enzyme that converts L-gulonolactone to L-ascorbic acid<sup>16</sup>. Similarly, in the present investigation, representing a pre-neoplastic stage in DAB carcinogenesis (none of the rats had developed overt tumours), the enzyme system converting D-glucuronolactone → L-gulonolactone → L-ascorbic acid was shown to be intact in microsomes of rats fed DAB with safflower oil. Furthermore, ascorbic acid synthesis was increased above normal in the liver microsomes of rats fed DAB with a fat-free diet, and also increased, although to a lesser extent, in those fed DAB with hydrogenated coconut oil. In a previous investigation by Morin<sup>17</sup>, it was shown that DAB administration resulted in an increased percentage of oleic acid and decreased stearic acid in the choline containing phospholipids (particularly the lecithins) in the hepatic microsomes of rats on a fat-free diet. Similar alterations, although less marked, were produced by feeding DAB with hydrogenated coconut oil, but not with safflower oil. Evidence from several investigations suggests that the mechanism of action of most drugs in increasing enzymatic activity is to increase the amounts of the enzymes via an increased microsomal protein synthesis<sup>18</sup>. Since the increases in ascorbic acid-synthesizing activity

produced by DAB in each dietary group parallel the microsomal phospholipid changes, it may be postulated that a specific substitution of oleic for stearic acid in the lecithins of the microsomal membrane alters the arrangement of the RNA it supports such as to produce increases in the synthesis of specific proteins.

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## Acetylcholine in the Electric Organ of *Torpedo*

Feldberg and Fessard<sup>1</sup> have shown that the electric organ of *Torpedo marmorata* is activated by acetylcholine (ACh) and that it contains a substance like ACh, as determined by bioassay, equivalent to between 40 and 100 µg ACh/g tissue. It has been suggested recently<sup>2,3</sup> that not all the material extracted from nervous tissue and bioassayed as ACh is in fact ACh. Since, so far as we are aware, the ACh-like activity extracted from *Torpedo* is the highest reported for any vertebrate tissue, confirmation of the nature of the active substance and a study of its metabolism in the electric organ are of interest. As a necessary preliminary we have reinvestigated the content of ACh by three different extraction procedures.

In our first experiments the tissue was extracted with acid-ethanol according to Crossland<sup>4</sup>. In subsequent experiments this method was compared with the usual trichloroacetic acid (10 per cent, w/v) procedure and with a perchloric acid extraction technique developed in this laboratory<sup>5</sup>. Samples of tissue (1 g or less) were minced with scissors in two volumes of 0.2 N perchloric acid and then rapidly and thoroughly homogenized in "Teflon"-glass hand-operated homogenizers. The suspensions were centrifuged, the supernatants stored at 4° C and the precipitates re-homogenized three times with two volumes of water and recentrifuged. The supernatants were combined and brought up to pH 4 with 0.2 N sodium hydroxide. The extracts, in a final dilution of 1 in 100, were assayed against acetylcholine chloride on the frog rectus abdominis muscle, guinea-pig ileum and the dorsal muscle of the leech; the activity was the same on all three test objects. Alkali-boiled controls were used in all assays. The average values from two series of experiments in which perchloric acid and acid-ethanol were used, and where perchloric acid and trichloroacetic acid were compared, are shown in Table 1. These results were from extractions performed at 0°-

10° C. The yields were lower when the tissue was initially frozen with liquid nitrogen, an observation in agreement with Hobbiger and Werner<sup>6</sup>. The very low activities obtained with acid-ethanol can be attributed to the failure of the extractant to inactivate the tissue cholinesterase completely. If the minced tissue was left in contact with eserine (10<sup>-4</sup> M) or diisopropylphosphorofluoridate (10<sup>-4</sup> M) at 0° C for 30 min or longer before extraction with acid-ethanol the ACh-levels were comparable with those obtained using perchloric acid. The range of activities obtained with perchloric acid was between 65 and 130 µg ACh/g tissue, suggesting that the values reported by Feldberg and Fessard<sup>1</sup> may be on average too low rather than too high. Since one of the objects of the investigation was to characterize chemically the active substance which by bioassay appears to be ACh, perchloric acid extraction seems to be the method of choice. Trichloroacetic acid has the disadvantage that the procedure is more time consuming. Moreover, it does not precipitate phospholipids so effectively<sup>7</sup> and may interfere with the chromatography of ACh<sup>8</sup>. Results of the chemical investigation will be published separately.

Table 1. COMPARISON OF METHODS OF EXTRACTION OF ACETYLCHOLINE FROM THE ELECTRIC ORGAN OF *Torpedo*

	ACh content (µg/g tissue)		
	perchloric acid	acid-ethanol	trichloroacetic acid
Series 1	86	25	—
Series 2	70	—	68

We have some evidence that endogenous synthesis of ACh may occur both in the excised whole organ and in minced tissue. Samples of tissue allowed to remain at room temperature for periods of up to 2 h contained more ACh activity than those extracted immediately the fish was killed. The largest increase was equivalent to 60 µg ACh/g tissue in a sample left for 1 h at room temperature. No increase in ACh activity was found in samples stored at 0° C. *In vitro* estimation of choline acetyltransferase activity in cysteine-sucrose tissue homogenates showed that the enzyme was capable of a rate of ACh synthesis of 5-8 mg ACh/g tissue/h.

These experiments largely confirm the original observations of Feldberg and Fessard<sup>1</sup>. In addition a new and convenient method of extraction has been developed which forms a basis for further study of the metabolism of ACh in the electric organ of *Torpedo*.

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## Red Pigments of *Dactynotus rudbeckiae* and *D. ambrosiae* [Homoptera, Aphididae]

THE extensive investigations of Lord Todd and his colleagues<sup>1</sup> have shown that several related perylene-quinone pigments, the erythroaphins (Fig. 1), can be obtained from many aphid species; the two most completely investigated representatives, the erythroaphins *fb* and *af*, are stereoisomers of the formula C<sub>22</sub>H<sub>12</sub>O<sub>6</sub>. These pigments, however, are not present in the live insect, but are formed through post-mortem enzymatic transformations of yellow precursors, the protoaphins, which do not contain the perylene nucleus<sup>2a</sup>. In the course of research on naturally

occurring perylenequinones<sup>8</sup>, we have investigated the pigments of two bright red species of the aphid genus *Dactynotus*, since it seemed possible that their colour might be due to preformed perylenequinones. Earlier, Todd *et al.*<sup>9</sup> had observed the occurrence of very small amounts of aphid pigments in *D. jaceas*; *D. cirsii*, however, was found to contain instead a red pigment which has not been described in detail but which, from its uncharacteristic absorption spectrum, is not a perylenequinone.

We have now found that the striking red colour of *D. rudbeckiae* (Fitch) and *D. ambrosiae* (Thomas) is likewise due to pigments which do not contain the chromophore of the erythroaphins. We have isolated the main pigment of *D. rudbeckiae* in pure, crystalline form; two very minor constituents of the pigment mixture were likewise obtained crystalline, although not yet quite pure. We propose the name rhododactynaphins *A*, *B*, and *C* for these substances (see 'Addendum'). They do not seem to be identical with the pigments of *D. cirsii*; nor are they anthocyanins, as has been claimed<sup>4</sup> on the basis of a very cursory examination of *D. rudbeckiae* (syn. *Tritogenaphis rudbeckiae*).

For the isolation of the rhododactynaphins, *D. rudbeckiae* was collected locally from *Rudbeckia laciniata*; the aphids were killed by brief exposure to dry-ice temperature, removed from the stalks of the plant, and triturated with acetone. The red, benzene-soluble portion of the deep-brown acetone extracts was chromatographed on a column of silica-gel *H* deactivated with 5 per cent water. Washing with benzene removed the  $\beta$ -carotene present<sup>4</sup>, together with a large amount of fat (or wax). Rhododactynaphin *A* was next eluted with 2 per cent methanol in chloroform; yield, ~0.2–0.4 per cent of the fresh weight of the aphids. It was followed closely by pigment *B*, while pigment *C* was eluted by methanol/chloroform 1:3. Recrystallization of rhododactynaphin *A* from benzene removed small amounts of a colourless contaminant with strong blue ultra-violet fluorescence, yielding the pure pigment. Comparison, by thin-layer chromatography, of the benzene-soluble part of acetone extracts of *D. rudbeckiae* and *D. ambrosiae* showed that the latter species likewise contains  $\beta$ -carotene and the three rhododactynaphins.

Since neither species of aphid is common, only very limited amounts of these pigments have been available thus far. They have enabled us to make the following observations, which may serve to characterize the compounds: pure rhododactynaphin *A* forms glistening deep-red platelets or scarlet needles. On heating, it gradually decomposes above about 240°. It is homogeneous on thin-layer chromatograms (silica-gel *H*, 2 per cent methanol in chloroform). Rhododactynaphin *A* is insoluble in water and hexane, more or less soluble in the common organic solvents, especially readily in acetone. The solutions are bluish-red. It is not extracted into concentrated aqueous hydrochloric acid from its solution in benzene, thus differing from the pigment of *D. cirsii*, which is extracted<sup>9</sup> from its hexane solutions. Concentrated sulphuric acid gives a crimson solution; the pigment dissolves with stable, yellowish-green colour in acetic anhydride containing one drop of concentrated sulphuric acid. The alcoholic solution of rhododactynaphin *A* turns greenish-brown with ferric chloride, purple with magnesium acetate. Aqueous or alcoholic alkali produces an emerald-green colour which changes to yellow within 1–2 seconds; afterwards, a purplish-red colour develops. These colour changes are reminiscent of those given by the mould pigment purpogenone<sup>8</sup>. Aqueous ammonia or sodium carbonate produces similar effects. The red colour of solutions in aqueous alkali or carbonate fades on further standing; that in ammonia is stable, and acidification and extraction with benzene yield a crystalline purple pigment with a characteristic four-banded absorption spectrum. The red solution of rhododactynaphin *A* in sodium carbonate is reversibly changed to yellow by sodium hydrosulphite; the resulting product is not fluorescent. Reductive acetylation of rhododactynaphin *A* gave a colourless, crystalline product.

The visible spectrum of rhododactynaphin *A* shows one band, without fine-structure;  $\lambda_{\max}$  500 m $\mu$ . IR (KBr disk):  $\nu(\text{CO})$  1,633, 1,610  $\text{cm}^{-1}$ ; other bands: 3,400, 2,910, 2,840, 1,440, 1,400, 1,280  $\text{cm}^{-1}$ , etc.

Mass-spectrometry, carried out by Mr. D. W. Thomas in Prof. Biemann's laboratory at the Massachusetts Institute of Technology, indicated a molecular weight of 274, and loss of  $\text{CH}_3$ ,  $\text{C}_2\text{H}_5$ ,  $\text{CH}_3\text{CO}$ , and  $\text{CO}$ . High-resolution mass-spectrometry, due to Dr. R. J. Highest (National Heart Institute), showed that 274 corresponds to  $\text{C}_{18}\text{H}_{14}\text{O}_8$ . However, osmometric determination gave a value of 548, making it appear very probable that rhododactynaphin *A* is a  $\text{C}_{56}$  compound, a finding which suggests that it may be related to the pigments of the aphid series. This hypothesis is further supported by the presence, in the NMR spectrum of the pigment, of a quartet centred at  $\delta \approx 1.65$  p.p.m. [ $\delta(\text{TMS}) = 0$ ] which is quite similar, in shape and location, to quartets occurring in the spectra of erythroaphin *al*<sup>4</sup> and of such related compounds<sup>10</sup> as isoeleutherin and the naphthoquinones formed on reductive cleavage of the protoaphins *fb* and *sl*. This quartet is produced by the two methyl groups of the dihydropyran ring present in these compounds. The NMR spectrum, and the mass-spectrometric cleavage into a  $\text{C}_{18}\text{H}_{14}\text{O}_8$  fragment representing (not necessarily precisely) one half of the molecule, could be interpreted on the assumption that rhododactynaphin *A* is a quinone containing at least one component with a carbon skeleton related to those present in the protoaphins. The dihydropyran ring of this component may resemble that of isoeleutherin (Fig. 1) ( $\text{CH}_3$  at position 12) rather than that of the protoaphins ( $\text{CHOH}$  at 12), since the NMR spectrum of rhododactynaphin *A* contains a group of weak bands at  $\delta \approx 2.3$  p.p.m. which is similar to the multiplet caused by the methylene protons in the spectrum of isoeleutherin<sup>11</sup>.

Those properties (spectra, etc.) of rhododactynaphins *B* and *C* which could be examined with the available trace amounts are very similar to those of *A*.

Evaporation of the benzene extracts from column chromatography gave a crystalline fat; the free fatty acids obtained from it, and their methyl esters, were likewise crystallized. Gas-chromatographic investigation of the latter, essentially as described by Barlow<sup>12</sup>, showed that the preponderant fatty acid of this species is myristic acid, in agreement with the findings<sup>7,8</sup> on other aphids. In addition, small amounts of lauric and palmitic acids and of unsaturated  $\text{C}_{18}$ ,  $\text{C}_{14}$ , and  $\text{C}_{12}$  acids were present.

It is hoped to continue the investigation of the rhododactynaphins when additional material becomes available.

We thank Dr. Harvey Souder for suggesting this investigation, Dr. Louise M. Russell for the taxonomic identification of the aphids, and Dr. Norman E. Sharpless for the gas-chromatographic study of the fatty acids. Part of the work was carried out in the Chemistry Department of Gettysburg College, Pennsylvania.

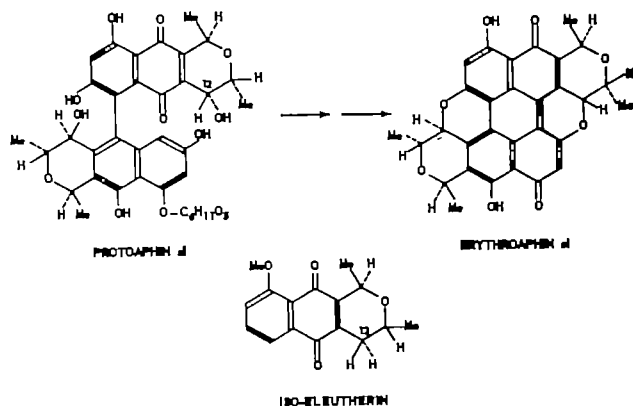


Fig. 1

**Addendum.** After completion of this manuscript, we learned from Lord Todd that Dr. D. W. Cameron and associates in his laboratory have for some time been engaged in a detailed study of the pigments from such aphid species as *D. citri* and *D. jaceae*. Our preliminary findings on the pigments of the two American species appear to agree well with the more advanced ones on *D. jaceae* made at Cambridge. Lord Todd has also informed us that the generic name 'daetynaphin' will be proposed for the pigments of *Dactynotus* spp., and has suggested the name 'rhododaetynaphin' for the pigments isolated by us. This suggestion has been adopted in the foregoing account.

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### Actomyosin and Myosin of Vascular Smooth Muscle

VARIOUS procedures have been used for extracting contractile protein from vascular smooth muscle<sup>1-3</sup>. In the course of preparing extracts from the cow carotid artery using a medium of 0.6 M KCl, the actomyosin content was estimated by measuring the ATP-sensitivity and viscosity number ( $Z_\eta$ ). In this report it will be shown that the extract from cow carotid artery consists of actomyosin and an actin-combining component, probably myosin, firmly associated with one another. It is believed that this firm association is a manifestation of unusual solubility properties of vascular smooth muscle myosin.

Extracts containing contractile protein from cow carotid artery were prepared as follows: 50 g artery were stripped of adventitia and minced, and three volumes of Weber-Eddsall solution and 0.5 ml.  $5 \times 10^{-3}$  M ATP at pH 7.0 were added. The mixture was homogenized in a Waring blender in the cold for 30 sec, shaken for 18 h at 4° C, and centrifuged. The supernatant was collected and diluted 14-fold with ice-cold water. After standing for several hours at 4° C, a precipitate formed which was collected and redissolved in 0.6 M KCl, and then reprecipitated by dilution as before. This precipitation was repeated for 6 cycles. The final yield was generally 15–20 mg of contractile protein which showed ATP-ase activity, ATP-induced syneresis, and a viscosity in 0.6 M KCl which was reversibly lowered by the addition of ATP. ATP-ase assays and syneresis tests were carried out using the protein in a solution containing 0.05 M KCl, 0.02 M imidazole buffer pH 7.2, 0.005 M of either  $\text{CaCl}_2$  or  $\text{MgCl}_2$ , and 0.0025 M ATP (Disodium, Pabst). The method of Fiske and Subba Row was used for measuring inorganic phosphate<sup>4</sup>. Protein was measured using the Lowry method<sup>5</sup>. Viscosity determinations were made at 25° C using an Ostwald viscometer containing the contractile protein dissolved in 3.0 ml. of a solution of 0.6 M KCl, 0.1 M *tris*, pH 7.2. The final concentration of ATP, when added, was  $1.67 \times 10^{-3}$  M. The rabbit skeletal muscle proteins, myosin A, F-actin, and actomyosin, were prepared according to the procedures outlined by Mommaerts<sup>6</sup>.

According to Szent-Györgyi<sup>7</sup>, the addition of one part actin to a solution containing 4–5 parts of myosin gives a viscosity drop, when ATP is added, which corresponds

to a change in the logarithm of the relative viscosity of 0.28 per 2.2 mg protein/ml. Needham and Williams<sup>8</sup> showed that 1 mg uterus actomyosin/ml. gives a change in the logarithm of the relative viscosity of 0.10–0.11. In our experiments, in order to estimate the amount of actomyosin in the extracts, we have assumed that a drop of 0.1 of the logarithm of the relative viscosity corresponds to 1 mg actomyosin/ml. The average actomyosin content in 12 extracts was found to be 27 per cent, the range being 19.4–37.5 per cent. In addition, it was found that a significant amount of the protein in the extract reacted with skeletal muscle F-actin to give an increase in viscosity which could then be lowered by the addition of ATP (Tables 1 and 2). The addition of actin caused the  $\Delta \log \eta_{rel}$  per mg protein per ml. to rise from 0.04 to 0.14 (Table 1). This value of 0.14 shows good agreement with values obtained using actomyosin from skeletal and uterine muscles<sup>7,8</sup>.

The data in Tables 1 and 2 indicate the presence of a myosin-like component. From these data it has been estimated that the composition of protein in the artery extract is roughly 1/4 actomyosin and 3/4 myosin or an actin-combining component. When the artery extract was dissolved in 0.6 M KCl and precipitated at 0.28 M KCl, pH 6.7, the resulting precipitate still reacted with added F-actin. When the artery extract was brought to 32 per cent ammonium sulphate saturation at pH 7.0, the resulting precipitate still reacted with F-actin. Both these methods are accepted procedures for separating skeletal myosin from actomyosin<sup>9</sup>.

Thus, because the actin-combining component is not readily separated from the actomyosin, it is possible that the solubility characteristics of myosin from cow artery smooth muscle differ from those of skeletal muscle myosin. Indeed, Hamoir and Laszt<sup>10</sup> observed that skeletal muscle myosin and the 47–60 per cent ammonium sulphate fraction of cow carotid artery, which they believed to be myosin-like, had different properties such as extractability at low ionic strengths, salting out ranges, amino-acid composition, net charge at neutral pH, and thermostability. Also, it is pertinent to note that smooth muscle

Table 1. EFFECT OF F-ACTIN ON THE ATP-INDUCED LOWERING OF VISCOSITY OF CONTRACTILE PROTEIN FROM BOVINE CAROTID ARTERY

Experiment 1*				Experiment 2*			
$\Delta \log \eta_{rel}^\dagger$		$\Delta \log \eta_{rel}/\text{mg total protein/ml.}$		$\Delta \log \eta_{rel}^\dagger$		$\Delta \log \eta_{rel}/\text{mg total protein/ml.}$	
Before actin	After actin	Before actin	After actin	Before actin	After actin	Before actin	After actin
0.5103	0.7960	0.06	0.14	0.2706	0.8009	0.04	0.14

\* The total protein, after the addition of F-actin which was 1/4 that of the contractile protein, was 5.8 and 6.1 mg/ml. for Experiments 1 and 2, respectively.

$^\dagger \Delta \log \eta_{rel}$  = the lowering of the logarithm of the relative viscosity induced by the addition of ATP.

Table 2. EFFECT OF F-ACTIN ON THE VISCOSITY NUMBER AND ATP-SENSITIVITY OF CONTRACTILE PROTEIN FROM BOVINE CAROTID ARTERY

Date	Preparation	$Z_\eta^*$		ATP-sensitivity $^\dagger$		Protein conc. mg/ml.
		Before actin	After actin	Before actin	After actin	
6/8	Artery protein	0.15	0.23	23	103	7.3
6/4	"	0.16	0.21	25	98	8.6
6/9	"	0.23	0.30	33	117	4.3
6/17	"	0.23	0.35	51	171	8.6
6/19	"	0.20	0.32	40	109	9.4
6/20	"	0.23	0.30	46	111	7.6
7/9†	"	0.19	0.40	57.5	121	5.8
7/10†	"	0.27	0.44	77.5	143	—
—	Actomyosin from rabbit skeletal muscle	0.46	—	124	—	4.0
—	Myosin from rabbit skeletal muscle	0.16	0.56	0	150	5.3

The preparation of the artery contractile protein is described in the text. F-actin when added was 1/4 that of the artery protein.

\*  $Z_\eta = 2.3 \log \eta_{rel}/\text{protein conc. (mg/ml.)}$ .

$\log \eta_{rel}$  = logarithm of the relative viscosity.

$^\dagger$  ATP-sensitivity in per cent =  $\frac{\log \eta_{rel} - \log \eta_{rel}^{\text{ATP}}}{\log \eta_{rel}^{\text{ATP}}} \times 100$

$^\dagger$  In addition to being reprecipitated and redissolved for 6 cycles, the contractile protein was dissolved in 0.6 M KCl, brought to 0.28 M KCl pH 6.8 and centrifuged for 30 min at 10,000 r.p.m.



myosin as prepared by other workers is not extracted directly from the muscle but rather is made by ATP dissociation of previously prepared actomyosin<sup>2,10</sup>.

Consideration was given to the possibility that the high levels of *F*-actin used were giving the same anomalous viscosity effects on artery actomyosin which have been observed for skeletal muscle actomyosin<sup>7</sup>. This, however, does not appear to be the case. The artery protein did not show the anomalous viscosity behaviour even when a large excess of skeletal muscle *F*-actin was added.

A different preparation of artery actomyosin, prepared according to the procedure of Laszt and Hamoir<sup>1</sup>, did not have the actin-combining moiety associated with it. The data (Table 3) show that even large amounts of *F*-actin failed to induce an elevated  $Z_{\eta}$  or ATP sensitivity when added to actomyosin prepared by the method of Laszt and Hamoir<sup>1</sup>.

Table 3. EFFECT OF *F*-ACTIN ON THE RELATIVE VISCOSITY OF ARTERY ACTOMYOSIN\*

<i>F</i> -actin/AM	Preparation 1 $Z_{\eta}$ ATP sensitivity (%)	Preparation 2 $Z_{\eta}$ ATP sensitivity (%)
No actin	0.25 36	0.18 27
1/20	0.33 35	0.11 32
1/10	0.29 45	0.13 40
1/5	0.37 39	0.26 72

\* The two preparations of artery actomyosin were made according to the method of Laszt and Hamoir<sup>1</sup>.

Viscometry conditions were as described in the text. The *F*-actin was from rabbit skeletal muscle. The *F*-actin/actomyosin was on a weight basis (mg protein). The concentrations of actomyosin in the viscometer were 1.5 mg/ml and 1.8 mg/ml for preparations 1 and 2, respectively.

The results of this study indicate that a firm association exists between cow carotid artery actomyosin and a myosin-like component. Since the association exists even after treatment to remove myosin it is suggested that artery myosin is different in solubility properties from skeletal muscle myosin.

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### Further Purification of Chick Interferon

CHICK allantoic fluid interferon has been purified to various extents by several groups of workers. Among those who prepared interferon of high specific activity, Lampeon *et al.*<sup>1</sup> achieved a purification factor of 1830 after treatment with perchloric acid, concentration with zinc acetate and two fractionations on 'OM-cellulose'. Such material still contained an inactive protein component that was only removed by electrophoresis on 'Pevikon'. The final material had been purified 4,500 times. Fantes *et al.*<sup>2</sup> purified interferon several thousand times by adsorption on 'Dowcil', elution with KSCN, precipitation of impurities in the presence of KSCN at pH 3.5, precipitation of interferon with methanol at pH 7.5 and chromatography on DEAE cellulose at pH 7.5. Merigan<sup>3</sup> purified crude interferon with perchloric acid and zinc acetate by the method of Lampeon *et al.*<sup>1</sup>, achieving a 15-fold purification. Such material, after one chromatographic fractionation on 'OM-Sephadex', using a pH gradient instead of Lampeon's stepwise process for eluting

the active ingredient, was claimed to be purer than Lampeon's material, though no evidence of electrophoretic homogeneity was presented.

We have slightly modified our original method<sup>2</sup>. Instead of neutralizing the acid KSCN supernatant fluid before precipitating with methanol, we lowered the pH further to 2.0 and added methanol (5 vol.) at this stage, precipitating some more inactive protein. The pH of the methanolic supernatant fluid was then adjusted to an apparent value of 7.5, causing precipitation of the active material. Chromatography on DEAE-cellulose was carried out as described before<sup>2</sup>. The interferon, when chromatographed on DEAE cellulose, became re-diluted and was reconcentrated on 'OM-Sephadex'. Adsorption was performed at pH 5.5 in 0.1 M phosphate buffer, with subsequent washing with 0.1 M phosphate buffer at pH 6 until the eluates contained no more than about 2 µg/ml. protein, as determined by the method of Lowry *et al.*<sup>4</sup>. Elution was carried out with a 0.1 M phosphate buffer gradient of rising pH.

Three such preparations were subjected to disc electrophoresis in acrylamide gel columns by Dr. J. Williams (personal communication) at the Laboratory of Molecular Biology in Cambridge. Two of the samples, after staining with amido schwarz, exhibited only one protein band. Dissection and assay of two control columns showed that the biological activity resided in those bands. The third sample contained some minor protein contaminants, estimated to amount to less than 5 per cent of the total.

The purity of the most potent DEAE-eluate fractions was sometimes of the same order as that of the post 'OM-Sephadex' fractions, but the 'OM-Sephadex' process enabled the less pure off-peak DEAE cellulose fractions to be purified to the same extent, thus increasing the overall yield of highly purified material.

The post-DEAE material—a mixture of highly and moderately purified fractions—applied to 'OM-Sephadex' had usually been purified about 500-fold. When, on the other hand, pre-DEAE material (purified about 80 times on average) was fractionated on 'OM-Sephadex' in a similar way, it was not found possible to free the active material from an inert protein with an elution peak at a pH only just above that of interferon.

The best fractions were purified well over 10,000 times and contained about one million interferon units per mg protein. As the specific activities of crude interferons and also assay methods vary from one laboratory to another, these figures do not necessarily mean that interferon prepared by the process described here was of a higher degree of purity than that obtained by other workers.

The iso-electric point of partly purified interferon was determined electrophoretically in a sucrose gradient by Dr. A. Polson (personal communication) at the University of Cape Town and found to be about pH 6.8-6.9.

Purified interferon was, as mentioned in our previous publication<sup>2</sup>, highly species specific. This has since also been reported by Merigan<sup>3</sup> and Baron *et al.*<sup>5</sup>. Contrary to the claims of a patent for 'non-specific interferon', we were unable to overcome the species specificity of crude or purified chick interferon by treatment with carboxypeptidase.

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## PHYSIOLOGY

Stimulation by Aldosterone of Active Sodium Transport by the Isolated Colon of the Toad, *Bufo marinus*

DESPITE the large volume and sodium concentration of digestive secretions, the losses of sodium incurred with faeces are negligible in normal circumstances<sup>1,2</sup>. Return to the organism of sodium present in the intestinal lumen depends in all likelihood on active transport process(es); in the case of the toad colon, this had been established by Ussing and Andersen<sup>3</sup>, who showed that sodium moves from the mucosal to the serosal surface in the absence of electrochemical potential gradient; furthermore, this movement was expressed quantitatively by the current required to annihilate the electrical potential difference which can be recorded across the membrane.

It has been reported previously that aldosterone is a physiological hormone for the toad, *Bufo marinus*, and that its concentration in plasma increased when the animals were transferred from a habitat rich in sodium to distilled water<sup>4,5</sup>. This transfer resulted in augmented active sodium transport by their ventral skin and urinary bladder. Injection of aldosterone into the toads or incubation in the presence of the hormone also brought about a stimulation of sodium transport activity by bladder<sup>4,6</sup> and skin<sup>7</sup>.

The influence of aldosterone on active sodium transport by the toad colon was examined because, in mammals, the adrenal cortical secretion, especially aldosterone, has been shown to exert an influence on faecal sodium<sup>8,9,10</sup>.

After pithing the toad, the colon was opened longitudinally and gently cleaned. Suitable unstripped fragments were mounted as diaphragms between conical 'Lucite' chambers (inner diameter of 16 or 20 mm according to the size of the tissue pieces), both surfaces being bathed at room temperature in aerated Ringer's fluid the composition of which was (in mM/l.), Na, 117.5; K, 2.0; Ca, 1.0; HCO<sub>3</sub>, 2.5; Cl, 119.0; osmolality, 0.225. The membranes were incubated according to the method devised by Ussing and Zerahn<sup>11</sup>; they were maintained short-circuited with interruptions at 10- to 15-min intervals for transmembrane potential readings. One hour of incubation was allowed before the results were considered meaningful.

In the first series of experiments, colons were used of toads kept for a few days in shallow water or in 50 per cent diluted Ringer's solution. An additional group of toads maintained in water received subcutaneously 10 µg *d*-aldosterone dissolved in 0.25 ml. Ringer's, the evening preceding the incubation. As shown in Table 1, the short-circuit current, averaged for the second hour of incubation, was one and a half times as high for the colon of toads kept in water as for that of toads kept in Ringer's. This increased activity was ascribed to the elevated aldosteronaemia in toads of the former group<sup>4</sup> since treatment of the animals with aldosterone resulted in a further increase in sodium transport (Table 1). The transmembrane potential rose more markedly than the short-circuit current, for an unknown reason.

As a next step, in ten instances, the colon of toads maintained in Ringer was divided in two, both pieces being incubated simultaneously as described. After 1 h, enough *d*-aldosterone was introduced on the serosal side of one piece of each pair to achieve a final concentration of 10<sup>-6</sup> M, and incubation was allowed to proceed further for 3 h. During the 30-min period which followed addition of the steroid hormone, the mean short-circuit current was (in µamp/2 cm<sup>2</sup>) 41.8 ± 4.2 (*S.E.*); it started to increase, after about 90 min, reaching 46.0 ± 5.5 over the last 30-min period. The corresponding values for the matched, untreated fragments decreased instead, from 40.0 ± 5.1 at the beginning to 30.9 ± 2.8 at the end of the 3-h experimental period.

Table 1. EFFECTS OF HABITAT AND OF ALDOSTERONE ON ACTIVE SODIUM TRANSPORT BY TOAD COLON (MEAN ± *S.E.M.*)

		Short-circuit current (µamp/cm <sup>2</sup> )	Potential difference (mV)
Toads maintained in saline	(20)*	21.5 ± 1.4	58 ± 0.3
Toads maintained in water	(20)*	32.8 ± 1.9	107 ± 0.4
Toads injected with aldosterone	(15)*	42.1 ± 3.0	28.6 ± 1.2

\* No. of animals per group.

Table 2. REPRODUCTIONS ON SHORT-CIRCUIT CURRENT AND SODIUM MOVEMENT OF DILUTION OF SODIUM PRESENT ON THE MUCOAL SIDE OF TOAD COLON

Exp. No.	Sodium concentration on mucosal side (in mM/L)	Short-circuit current, after dilution of sodium on mucosal side* (in % of reference value)	Sodium flux (in µequiv./h) computed from short-circuit current	Sodium flux (in µequiv./h) computed from isotopic flux	(2)/(1) × 100 (%)
10	7.1	55	0.906	0.784	87
5	7.3	70	0.397	0.520	133
9	9.5	63	2.611	2.374	91
13	9.9	74	2.945	2.332	79
11	10.4	76	1.511	1.563	106
3	16.0	82	1.056	1.204	114
1	16.3	74	0.708	0.764	108
19	24.7	86	2.229	2.202	99
8	27.0	70	1.677	2.234	133
4	40.2	90	0.659	1.228	186

\* The current averaged 53.0 µamp immediately before dilution, and 51.7 µamp after replacement of the diluted solution with unmodified Ringer's.

The influence of aldosterone was analysed as was done for studies with the toad bladder<sup>12</sup>, that is, the change of membrane activity occurring in the absence of treatment during the period of time considered (−9.1 µamp as a mean, in the present case) was subtracted from the change noted when the matched membrane was exposed to aldosterone (+3.2 µamp as a mean, in the present case). Stimulation of short-circuit current exerted by aldosterone on the toad colon thus averaged 13.3 µamp ± 5.3 (*S.E.* mean difference) (*P* < 0.05).

These results on the responsiveness of the colon of the toad to aldosterone, endogenous and exogenous, *in vivo* and *in vitro*, are similar in quality and amplitude to previous results obtained with toad skin and bladder. The significance of the resemblance is enhanced, it seems to us, by the fact that also in the case of the toad colon the sodium transport capacity is relatively independent of sodium concentration in the outside bathing solution. This was investigated as follows: after 2 h of incubation of the colon in unmodified Ringer's, the solution on the mucosal side was diluted to a variable extent with a solution in which Mg<sup>2+</sup> replaced Na<sup>+</sup>, sufficient sucrose being added to bring osmolality back to 0.225. The concentration of sodium in this modified solution was checked by flame photometry. Furthermore, 2 µc. <sup>22</sup>Na were introduced on the same side; sampling for measurement of radioactivity flux was performed during the ensuing second and third 30-min periods. The short-circuit current during exposure to this modified Ringer's was expressed relative to the baseline value.

The results of 10 such incubations are presented in Table 2. The short-circuit current decreased only slightly unless mucosal sodium concentration was markedly reduced. Current remained a valid reflexion of net sodium transport despite the asymmetry of the system since a parallelism between mucosa-to-serosa sodium flux and short-circuit current was found in these experimental conditions; the isotopic flux exceeded the flux computed from current by 13.5 per cent ± 9.8 (*S.E.*) (*P* > 0.2).

The reaction of the toad colon exposed on its mucosal side to dilute sodium solutions is thus reminiscent of what is observed with the frog skin<sup>13</sup> and toad bladder<sup>14</sup>; for both preparations, a limited, saturable permeability for sodium of the outer cell membrane(s) seems to represent an important regulating factor of their sodium transport activity. In the case of the toad bladder, there are arguments pointing at aldosterone stimulating active sodium transport by increasing the permeability at the outer cell

membrane(s) rather than by direct action on the sodium 'pump'<sup>12</sup>.

It is therefore possible that only those membranes which allow sodium in on account of a process complying with saturation kinetics are capable of response to aldosterone which would somehow weaken this diffusion barrier.

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### Adenosine Triphosphate and Muscular Contraction and Relaxation

THERE are three observations which suggest that diminution of the adenosine triphosphate (ATP) content of muscle leads to contraction: (a) the ATP and creatine phosphate (CP) content of isolated striated muscle is about 5 times that of smooth muscle<sup>1</sup>, and the latter always shows a state of partial contraction or tonus; (b) diminution of the ATP content of smooth muscle leads to contraction, and increase to relaxation<sup>2</sup>; (c) adrenaline, which causes relaxation of smooth muscle, increases the ATP content of muscle by about 70 per cent in the first 15 sec<sup>3</sup>.

It may be argued that the contraction of muscle when its ATP content diminishes is not physiological, but is some sort of rigor; the action of adrenaline, however, and the presence of tone, suggests that increase in the ATP content of the muscle leads to a physiological relaxation, and decrease in the ATP content to a physiological contraction. It is therefore tempting to assume that the normal contraction of muscle is due to sudden diminution of its ATP content.

It may be assumed that the primary event in muscular contraction is the breakdown of ATP. The protein-ATP complex in relaxed muscle breaks down into protein-ADP complex and phosphate, resulting in the contraction of muscle and release of energy. The energy which is released by the breakdown of ATP may be considered as a waste in the first instance for the purpose of contraction. It may be assumed that this energy is stored in some way and is utilized later to regenerate the lost ATP and CP; the energy may be stored by raising the energy level of some organic or inorganic ion such as magnesium. Sodium and potassium are not important for contraction, as shown by the fact that when frog stomach muscle is

frequently washed with half isotonic (0.112 M) solution of sucrose, it loses all its sodium in 1 h (ref. 4), and retains only 50 per cent of its potassium (30–35 mM/kg of wet muscle)<sup>5</sup>, but continues to contract spontaneously for about 24 h. Frog heart, when perfused with half-isotonic solution of sucrose, loses all sodium in 1 h and retains only 22 per cent of its potassium (16 mM/kg)<sup>6</sup>, but continues to contract spontaneously for 2–7 h<sup>7</sup>. The foregoing hypothesis reconciles the findings that the heat production in muscle precedes contraction<sup>8</sup> with the view that relaxation of muscle is active<sup>9,10</sup>.

It has been proposed that adrenaline causes relaxation of smooth muscle by producing lactic acid<sup>11</sup>. As adrenaline causes increase in oxygen consumption<sup>12</sup>, the lactic acid production is probably a manifestation of increased metabolism and not the cause of relaxation. It is very likely that adrenaline causes relaxation by increasing the ATP content of smooth muscle.

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### Inhibition of Aortic Calcification by means of Pyrophosphate and Polyphosphates

SOME years ago we showed that inorganic pyrophosphate and other longer-chain condensed phosphates could inhibit calcium phosphate precipitation *in vitro* at concentrations as low as 10<sup>-4</sup> M (ref. 1). Later we found that pyrophosphate is a normal constituent of both urine<sup>2</sup> and plasma<sup>3</sup>, and we suggested that it might therefore be important *in vivo* in preventing collagen and other nucleating substances from calcifying<sup>4,5</sup>; calcification would then proceed only after the inhibitor had been destroyed by pyrophosphatase, an enzyme present in high concentrations at mineralizing sites<sup>6,7</sup>. Indirect support for the inhibitory role of pyrophosphate *in vivo* has emerged from studies on urolithiasis. Patients with renal stones excrete less pyrophosphate than normal<sup>8,9</sup> and the excretion of pyrophosphate can be raised by feeding orthophosphate<sup>10</sup>, a procedure claimed to prevent stone formation<sup>11</sup>.

We have tried to gain more direct evidence that condensed phosphates can inhibit calcification *in vivo*. As our test system we have used the calcification of the aorta in rats treated with large doses of vitamin D. Rats were given daily doses of 75,000 units/kg vitamin D<sub>3</sub> orally for 5 days, an amount known to induce heterotopic calcification<sup>12</sup>. When the animals were killed 13 days after beginning the treatment, the aorta was nearly always heavily calcified.

When we gave subcutaneous injections of pyrophosphate or Graham salt (a long-chain polyphosphate,  $n \approx 20$ ) to groups of similarly treated animals from the day before the first dose of vitamin D<sub>3</sub> until they were killed, the calcification was, with the exception of the 160-g group receiving 1 mg phosphorus/kg of Graham salt, either reduced or completely prevented. Equivalent doses of orthophosphate were ineffective. The results of the calcium analysis expressed in mM calcium/g dry wt. of aorta are shown in Fig. 1. Histological analysis of the calcification using silver nitrate correlated well with the

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Blood Levels of Drug at the Equilibrium State after Multiple Dosing

THERE is ample evidence<sup>1-3</sup> that when a fixed dose of drug is administered in a fixed multiple-dose regimen, the blood levels of drug eventually reach a steady state in which the blood level time curve during any dosage interval is the same as it is in the preceding and in the following dosage interval. One may define the equilibrium state as that in which input of drug to the 'body' is equal to output of drug from the 'body' in a given dosage interval. The same equilibrium state will be reached when the initial dose is higher than the maintenance dose<sup>4</sup>.

These concepts were studied with an analogue computer applied to analysis of compartment models. The analogue computer studies led to an equation which can be used to predict the average asymptotic blood levels during a multiple-dose regimen from basic parameters estimated from single-dose studies. The equation also has been derived mathematically.

The equation is:

$$\bar{O} = FD/VK\tau \quad (1)$$

where  $\bar{O}$  is the average asymptotic blood (serum or plasma) concentration,  $D$  is the dose (weight) given at the beginning of each dose interval,  $\tau$  is the length of the dosage interval,  $K$  is the first-order rate constant for overall loss of drug from the blood,  $F$  is the fraction of each dose which is absorbed, and  $V$  is the apparent volume of distribution of the drug.

$\bar{O}$  is defined as follows:

$$\bar{O} = \frac{1}{\tau} \int_{t_1}^{t_1+\tau} O_{\infty}(t) dt \quad (2)$$

where  $t_1 - t_1 = \tau$  and  $O_{\infty}(t)$  is the blood level at time  $t$  during the equilibrium state.

Equation (1) will hold under the following conditions: (a) transfer from the blood is first order; (b)  $F$ ,  $D$ ,  $V$ ,  $K$  and  $\tau$  are constants for each dose of the multiple-dose regimen in a given subject or patient; and (c) input to the blood and transfer from the blood to any number of possible compartments and back to the blood may be described by a system of simultaneous linear differential equations.

Under these conditions, it can be shown that, following a single dose of drug:

$$FD = VK \int_0^{\infty} O(t) dt \quad (3)$$

This equation is implicit in the work of Teorell<sup>11</sup> and Dominguez<sup>12</sup> and implied by many authors, for example, in clearance studies<sup>13,14</sup>.

Combining equations (1), (2) and (3) gives:

$$\int_{t_1}^{t_1+\tau} O_{\infty}(t) dt = \int_0^{\infty} O(t) dt \quad (4)$$

Hence, under the conditions stated here, the area under the blood-level curve during a dosage interval at the steady state will be equal to the area under the single-dose blood-level curve.

The  $\bar{O}$  level expected on a given multiple-dose regimen of dose,  $D$ , every  $\tau$  hours may be predicted from single-dose blood-level data by obtaining  $FD/V$  and  $K$  from the single-dose data, then using equation (1).  $K$  may be estimated from the terminal linear segment of a semilogarithmic plot of blood (serum or plasma) concentrations versus time. Under the assumptions stated by Wagner and Nelson<sup>15</sup>,  $FD/V$  may be estimated by the equation:

$$\frac{FD}{V} = \frac{1}{n} \sum_{i=1}^n \left\{ O(T_i) + K \int_0^{T_i} O(t) dt \right\} \quad (5)$$

where  $T_i$  and  $O(T_i)$  represent the time and observed blood levels for the  $n$  points used to estimate  $K$  and the integral

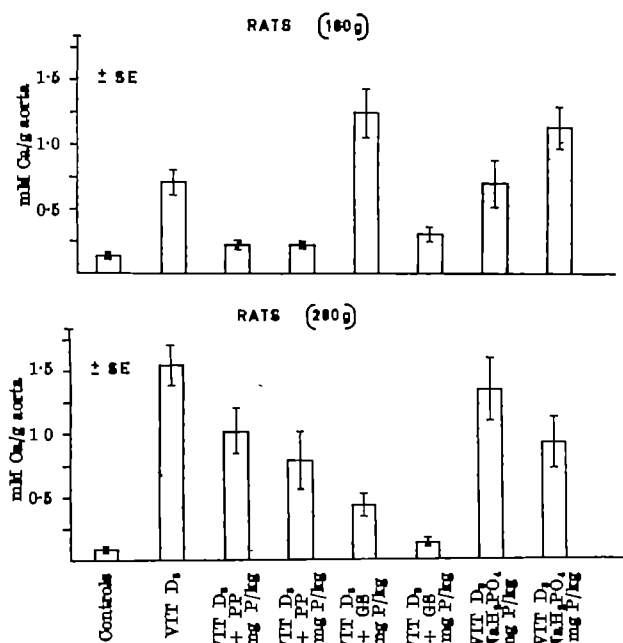


Fig. 1. Effect of pyrophosphate (PP), Graham salt (GS) and orthophosphate on the aortic calcification produced by vitamin D<sub>3</sub> in young and old rats. Each group contained 10 animals.

calcium analysis. Aortas from animals successfully treated with polyphosphates also showed no abnormalities in morphological appearance after periodic acid-Schiff and alcian blue staining.

Interesting differences seem to exist in the responses of the animals at different ages. Pyrophosphate was more effective than Graham salt in the young animals, but the reverse was true in the older animals in whom the response to pyrophosphate was only slight. It would be interesting to know whether this apparent resistance of the older animals to the physiological inhibitor pyrophosphate is of any relevance in the development of pathological calcifications in the aged.

The experiments show that condensed phosphates can prevent calcification in the aorta, and are therefore effective *in vivo* as well as *in vitro*. This property might perhaps have a place in the future in the prevention of heterotopic calcification.

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Table 1. PHENOMENA WHICH WILL INVALIDATE EQUATION (1)

Phenomenon	Expected or known result
1. Plasma level of drug exceeds protein binding capacity	$K$ increases and $V$ decreases at high blood levels
2. Distribution increases at higher blood levels	$K$ may decrease or increase, and $V$ increases at high blood levels.
3. An enzyme system metabolizing the drug becomes saturated at high blood levels	Order of elimination from the 'body' may change from apparent first order to apparent zero order.
4. The drug's metabolism is stimulated by the drug itself or by another agent	$K$ increases and $V$ remains constant.
5. The drug's metabolism is inhibited by another agent	$K$ decreases and $V$ remains constant.
6. Diffusion equilibrium is not maintained between drug in blood and drug in other fluids of distribution	
7. The fraction of the dose absorbed decreases as the dose is raised due to a low rate of dissolution and relatively fixed transit time in the gastrointestinal tract	$F$ will decrease as $D$ is increased.

is the cumulative area under the blood-level time-curve from time zero to time  $T_1$ .

There are several known phenomena which invalidate the use of equation (1). These are listed in Table 1.

Equation (1) predicts that  $\bar{O}$  will be directly proportional to the dose on a multiple-dose regimen where the doses are given at regular intervals of  $\tau$  h. If the observed  $\bar{O}$  levels are not directly proportional to the dose administered, then one or more of  $F$ ,  $K$ , and  $V$  are suspect as not being constant. One, two or all three of these may change with increasing dose and/or blood level. Then additional studies are indicated to determine which factor or factors cause the lack of proportionality.

If equation (1) is applicable, the slope of the  $\bar{O}$ ,  $D$  linear plot will be  $F/VK\tau$ . If the drug is given intravenously, then  $F=1$  and the slope will be  $1/VK\tau$  from which the metabolic clearance rate,  $VK$ , may be obtained. The ratio of the slope obtained by the non-intravenous route to the slope obtained by the intravenous route will yield the fraction,  $F$ , of the dose absorbed following the non-intravenous route. With drugs that are excreted in the bile and which also yield measurable blood levels, a material balance obtained by measuring 'unchanged' drug in the blood, urine and faeces does not provide data to assess the extent of drug absorption. This is obvious since drug measured in the faeces may have been totally or partly absorbed and excreted in the bile, to appear in the faeces if not reabsorbed. Hence, one cannot distinguish drug in the faeces which has been absorbed from drug which has passed directly through the gastrointestinal tract. The method outlined above circumvents these pitfalls but, of course, it depends on the validity of equation (1).

Equation (1) is also useful, if shown to be valid for a particular drug, to predict change in  $\bar{O}$  with change in dosage regimen. One can insert various pairs of  $D$  and  $\tau$  values, and, with  $F/VK$  calculated from the slope of the  $\bar{O}$ ,  $D$  plot originally investigated, calculate the predicted  $\bar{O}$  level for each pair of  $D$  and  $\tau$  with equation (1). Such estimations may constitute a great saving in time and money compared with a trial and error empirical method.

The average asymptotic blood level for a given dose is independent of the rate of absorption in those cases where equation (1) applies. The analogue computer studies indicated that the more rapid the rate of absorption, the greater the variation of the blood levels about  $\bar{O}$  during a dosage interval. Equation (1) also indicates that  $\bar{O}$  is inversely proportional to  $K$  or directly proportional to the biological half-life of the drug,  $t_{1/2}$ , in a particular individual. Since there is considerable variation in biological half-life from subject to subject with a given drug<sup>18</sup>, one can expect a similar variation in  $\bar{O}$  levels. These considerations may be of importance in comparing blood-level results following 'sustained release' or prolonged action preparations of a drug with those following conventional dosage forms. No clear-cut distinction may exist in a multiple-dose study even when one has been observed in a single-dose study.

Since  $t_{1/2}=0.693/K$ , one can re-write equation (1) as follows:

$$VO = \frac{1.44 F D t_{1/2}}{\tau} \quad (6)$$

where  $VO$  is the average amount of drug in the 'body' during a dosage interval at the steady state. When equation (1) is shown to apply for a given drug, some interesting rules are evident from equation (6). If  $\tau=t_{1/2}$ , then  $VO$  is 1.44 times  $FD$  or that amount of drug which reaches the circulation after a single dose. Corresponding values of  $VO/FD$  for selected  $\tau/t_{1/2}$  ratios are:

Dose interval $\tau$ in multiples of $t_{1/2}$ ( $\tau/t_{1/2}$ )	Ratio of asymptotic amount in body to amount absorbed from each dose ( $VO/FD$ )
1	1.44
2	0.72
4	0.36

The extent of accumulation in the 'body' of a drug having a long half-life and administered frequently can be readily gauged by use of equation (6). If a drug with a half-life of 72 h is given twice a day, then  $VO=8.64 FD$  provided the above conditions hold. Once the biological half-life of a drug in a panel of human subjects is known, then equation (6) may be utilized to provide initial estimates of drug accumulation with different projected dosage regimens.

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### Altered Cardiac Retention of Exogenous Noradrenaline produced by Stress in Young Rabbits

An impressive body of evidence indicates that cardiovascular reactivity either to sympathetic nerve stimulation or to injected catecholamines is considerably decreased in

adrenalectomized animals<sup>1</sup>. The administration of adrenocortical hormones to such animals either improves or restores to normal the ability of the cardiovascular system to respond either to sympathetic nerve activity or to the injection of adrenaline or noradrenaline<sup>1</sup>. These considerations suggest that one result of stress-induced adrenocortical secretion in normal animals might be an enhanced response of the cardiovascular system to the catecholamines. In support of this idea, it has been reported<sup>2</sup> that hydrocortisone increased the response of rabbit aorta to noradrenaline *in vitro*. Such potentiation could be caused by a decreased tissue-binding (that is, functional inactivation) of administered noradrenaline produced by the steroid. This suggestion was investigated by examining the influence of administered hydrocortisone on the sub-cellular distribution of tritiated noradrenaline in the rabbit heart.

Rabbits received four intraperitoneal injections, each of 10 mg of hydrocortisone sodium succinate/kg body-wt. (in a volume of 0.1 ml./kg) at hourly intervals. Fifteen min after the last injection, each animal was given an intravenous injection of 2 µg of *DL*-noradrenaline-7-<sup>3</sup>H per kg (specific activity 4.72 c./mmole). The hearts were removed 30 min later, homogenized in six volumes of 0.3 M sucrose and the homogenate was centrifuged at 135,000*g* for 30 min. The noradrenaline-<sup>3</sup>H contents of the resulting supernatant fluid and the crude particulate fractions were estimated, after extraction of each with 0.4 M perchloric acid, using methods described previously<sup>3</sup>.

Preliminary experiments revealed a significant decrease in both the supernatant and particulate noradrenaline-<sup>3</sup>H of heart homogenates after hydrocortisone treatment. An identical effect was seen in another group of animals that received intraperitoneal injections of saline (0.1 ml./kg), instead of the hydrocortisone. Furthermore, it was noted in these early experiments that this effect was only seen when young (approximately 500 g) animals were used. Accordingly, subsequent studies (those forming the basis for this communication) were conducted using only young (age 3-4 weeks) rabbits, the weight-range of which was 470-700 g. Four such animals were used in each of the nine experiments forming series 1 (Fig. 1). A random selection procedure determined the treatment given each animal. In these experiments (Fig. 1), the particulate noradrenaline-<sup>3</sup>H was significantly ( $P < 0.01$ ) reduced by each of the treatments used; the supernatant amine content also was significantly lowered after the previous injection of either hydrocortisone ( $P < 0.01$ ) or saline ( $P < 0.05$ ), but not by that of ACTH (included in this series to mimic the effect of a possible stress-induced endogenous secretion of this hormone). It was apparent that neither hydrocortisone nor ACTH administration had a greater effect than successive intraperitoneal injections of saline.

Experiments similar to those of series 1, carried out with older rabbits (age 11-13 weeks; weight 1.1-1.7 kg), revealed no alteration of the sub-cellular distribution of cardiac norepinephrine after any of the treatments.

The fact that, in young animals, the intraperitoneal injection of either saline or the stress and handling associated with its administration caused the altered distribution of noradrenaline-<sup>3</sup>H suggested that the mechanism might involve stimulation of either the pituitary-adrenal axis or the activation of the sympatho-adrenal system. In either event, the effect should be reduced or abolished by anaesthesia. Experiments were carried out (series 2, Fig. 1), therefore, using rabbits anaesthetized with dialurethane (0.5 ml./kg). Unfortunately, it was impossible to maintain young rabbits under anaesthesia for the total experimental period (4 h 45 min) used in series 1. Accordingly, intraperitoneal injections of either saline (0.1 ml./kg) or hydrocortisone sodium succinate (10 mg/kg) were given four times at intervals of 30 min. The remaining experimental procedure was identical with that described for series 1. It can be seen (series 2, Fig. 1) that in anaesthetized animals hydrocortisone appeared to reduce the amount of particulate-bound noradrenaline-<sup>3</sup>H, when compared with that of saline-treated rabbits, although the decrease was not statistically significant. The supernatant noradrenaline-<sup>3</sup>H was uninfluenced by hydrocortisone. Anaesthesia *per se* had no observable effect on amine retention (using the mean figure for the control, conscious animals of series 3). The latter control figures were used to judge the influence of anaesthesia, since the experiments comprising series 2 and 3 were carried out simultaneously.

Large volumes (50 ml. and above) of saline, injected intraperitoneally, are known to cause adrenocortical activation<sup>4</sup>. It seemed unlikely that only 0.4 ml./kg injected in the work recorded here could be responsible, *per se*, for the observed effect on the retention of noradrenaline-<sup>3</sup>H. This possibility was examined in experiments (series 3, Fig. 1), however, in which a group of animals each received 4 mock injections (consisting of the passage of a hypodermic needle through the abdominal wall) at hourly intervals, prior to the administration of noradrenaline-<sup>3</sup>H as already described. Such mock intraperitoneal injections significantly ( $P < 0.01$ ) reduced the noradrenaline-<sup>3</sup>H of both supernatant and particulate fractions of heart homogenates. The fact that mock injections are just as effective in this regard as intraperitoneal injections of saline (series 3, Fig. 1) establishes unequivocally that the mechanism responsible for the effect is initiated by the stress and handling attendant on intraperitoneal injection in young rabbits.

Table 1. PERCENTAGE DECREASE IN THE MEAN NOREPINEPHRINE-<sup>3</sup>H OF SUPERNATANT AND PARTICULATE FRACTIONS OF SUCROSE HOMOGENATES OF HEART FOLLOWING VARIOUS TREATMENTS OF YOUNG RABBITS

Series	Treatment	Interval between injections (h)	Anaesthesia	% Reduction appropriate control = 100% Super- natant	Particulate
1	4 i.p. injections of saline	1	None	30	44
	4 i.p. injections of hydrocortisone	1	None	39	51
	4 i.p. injections of ACTH	1	None	11	37
2	4 i.p. injections of hydrocortisone	0.5	Yes	4	22
3	4 i.p. injections of saline	1	None	45	60
	4 mock injections (i.p.)	1	None	58	57

Table 1 indicates that, with the exception of the experiments where 4 mock injections were given, all treatments reduced the mean particulate noradrenaline-<sup>3</sup>H content more than that of the supernatant fractions.

These studies serve to draw attention to the importance of stress as a determinant of the retention of noradrenaline by the heart of the young rabbit; they do not, however, indicate the precise mechanism or mechanisms that may be responsible for the effect. The fact that intraperitoneally administered ACTH was no more effective than saline (series 1, Fig. 1) suggests that if pituitary-adrenocortical activation mediated the depressed noradrenaline-<sup>3</sup>H retention, then such activation may be

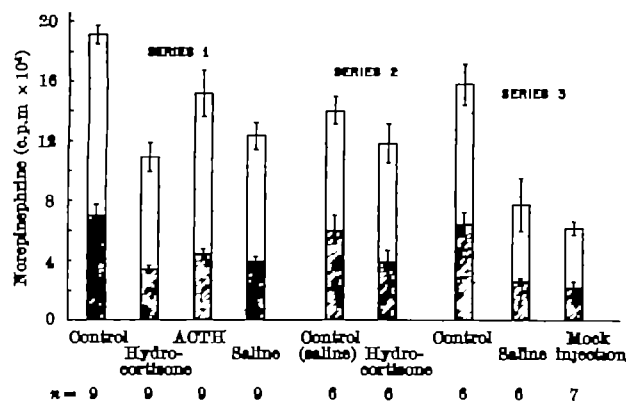


Fig. 1. The retention of noradrenaline-<sup>3</sup>H by supernatant and particulate fractions of sucrose homogenates of rabbit heart. The vertical bars describe the standard error of the mean values for retention. The figure (n), below each series, refers to the number of animals subjected to each of the treatments used. White, supernatant; hatched, particulate

maximal with the stress alone (that is, saline or mock injections). A second factor which may be involved in producing the observed effect is a stress-induced sympatho-adrenal activation. If the blood levels of catecholamine during the stress reached sufficiently high levels and were maintained until the injection of noradrenaline- $^3\text{H}$ , it is conceivable that competition for uptake sites took place, since both adrenaline and noradrenaline are bound by the same sites in heart<sup>5</sup>.

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## PATHOLOGY

### Serum Protein Changes associated with *Oesophagostomum columbianum* Infections in Sheep

THE separation of serum proteins by paper electrophoresis has been used widely in human medicine and for diagnostic purposes by veterinarians<sup>1</sup>. Parasitologists are also finding increasing use for electrophoretic techniques in the analysis of helminth infections<sup>2</sup>. In the following study the distribution of serum proteins of non-infected sheep maintained worm-free from birth, and worm-free sheep experimentally infected with 2,000 *Oesophagostomum* larvae, was analysed electrophoretically.

Blood samples were obtained from all the control and infected animals at intervals of between four and seven days by jugular puncture. The serum from these samples was stored at  $-20^{\circ}\text{C}$  until used. Total protein estimations were made using the Biuret method and a Beckman 'DU' spectrophotometer<sup>3</sup>. Electrophoretic separation of the individual proteins was obtained using Oxoid cellulose acetate strips and a barbiturate buffer, pH 8.6, 0.07 M at 0.5 m.amp/cm width of strip for 1.5 h runs<sup>4</sup>. The dried strips were stained using bromo phenol blue in 5 per cent acetic acid; the stained strips were washed in four successive changes of 5 per cent acetic acid, dried and cleared in white oil (Shell) and scanned using an 'EEL' scanner. The proportion of the individual proteins was ascertained from the scanner drawings. The form of these patterns is shown in Fig. 1.

Campbell<sup>5</sup> obtained four serum fractions from normal sheep using paper electrophoresis, namely, albumin,  $\alpha$ ,  $\beta$ , and  $\gamma$  globulins. By the use of cellulose acetate the  $\beta$  fraction was split into  $\beta_1$  and  $\beta_2$  components; each of the other separated proteins and the  $\beta_1$  and  $\beta_2$  together agree closely with the proportion allotted by Campbell<sup>5</sup> (Table 1).

In the control animals the serum patterns can be noted to change provided the observations are spread over a long enough period. There is, however, little change in the albumin and  $\alpha$  globulin content as expressed in g per cent, more notably the  $\beta_1$ ,  $\beta_2$  and  $\gamma$  globulins increase gradually, particularly the  $\beta_2$  and  $\gamma$  globulins (Table 1 shows the average values seven weeks apart).

After infection with *O. columbianum* the total protein value generally begins a slow decline. This slow decline is

due to a complex of changes, occurring all at once, involving a loss of serum albumin,  $\alpha$  and  $\beta_1$  globulins, and an increase in the  $\beta_2$  and  $\gamma$  globulin content of the serum. The actual loss of serum albumin is generally quite massive, involving as it does a loss of up to 1 g per cent. This loss is related to the dehydration caused by the infection through water loss in the faeces, which may increase by as much as 30 per cent over the normal value. Loss of water is important since albumin is re-cycled through the lumen of the gut<sup>6</sup>; the inability of the infected sheep to re-absorb water results in the loss of a great deal of the re-cycling albumin.

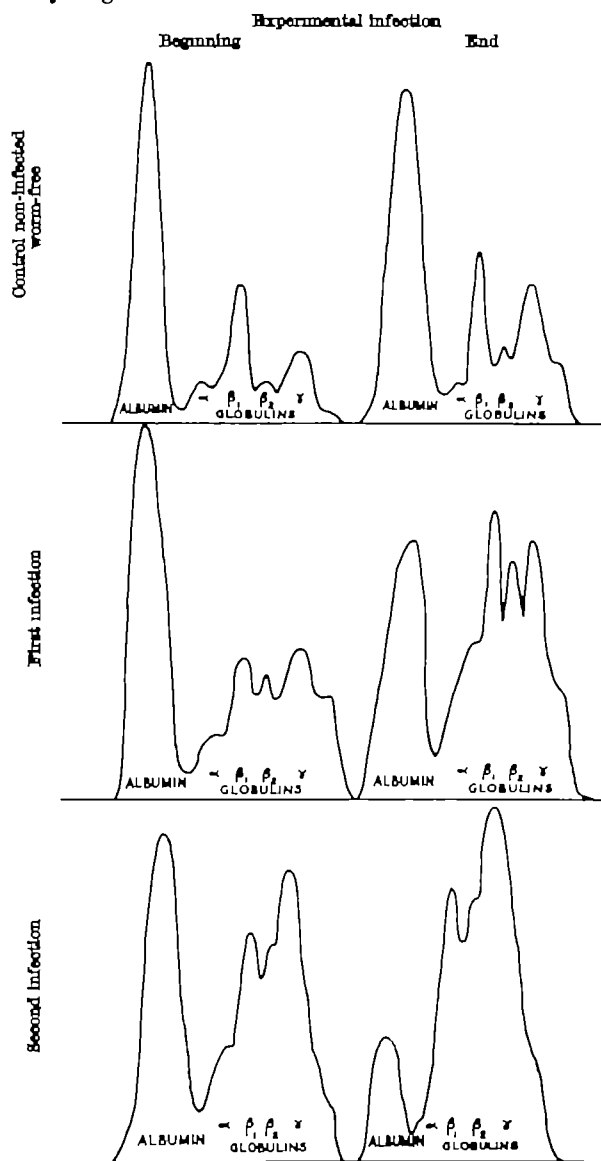
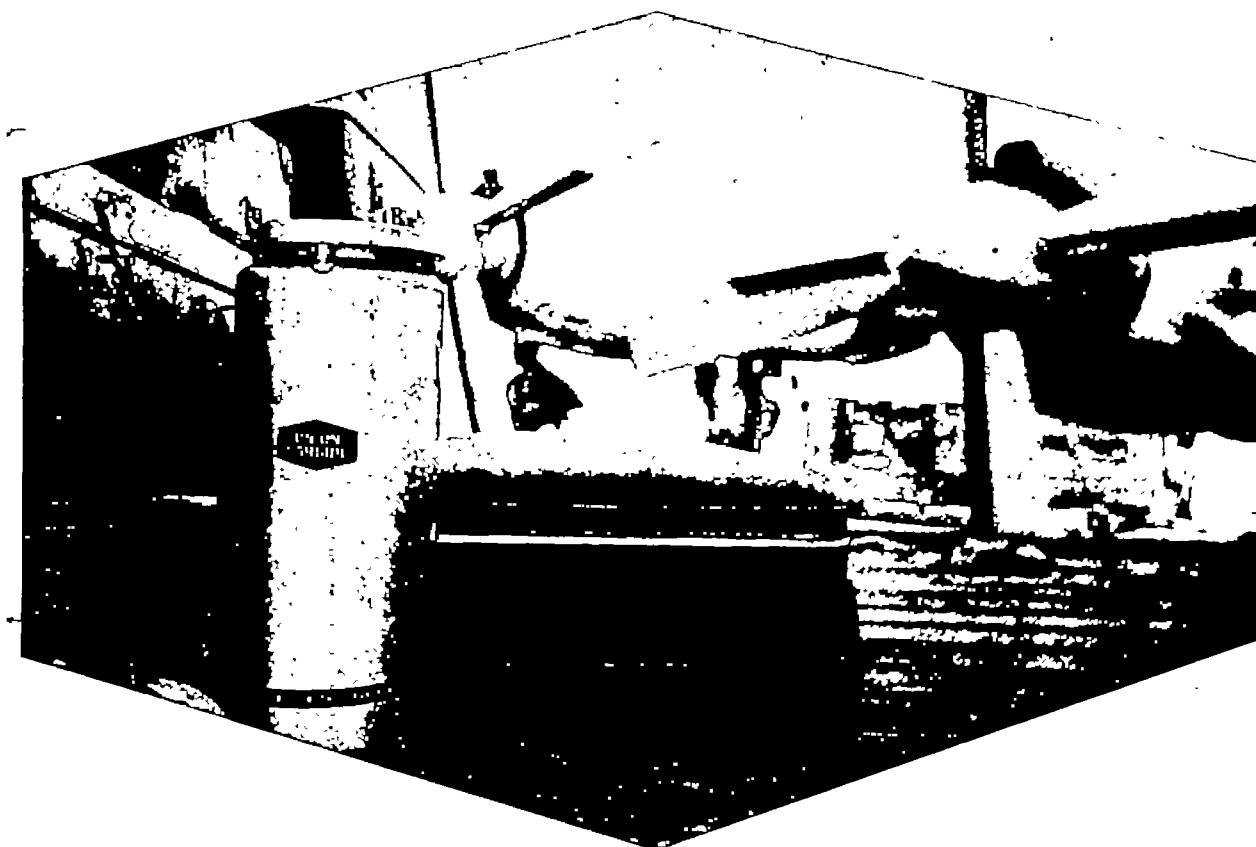


Fig. 1

Immunological activity by the host is indicated because there is a large increase in the globulin fractions, particularly  $\beta_2$  and  $\gamma$  globulins, both of which are known to contain antibody<sup>7</sup>. Proportionately there is a greater percentage increase in the  $\beta_2$  globulin fraction than the  $\gamma$  globulin. This was more evident after primary than after secondary infection. This may indicate an inordinate involvement of  $\beta_2$  substances in the resistance of the host to new nematode infections (Table 1). An anamnestic response was observed after secondary infection when the  $\gamma$  globulin fraction increased by 1 g per cent within one week. The  $\beta$  globulins were not affected in this way although there was a large but gradual increase. Comple-





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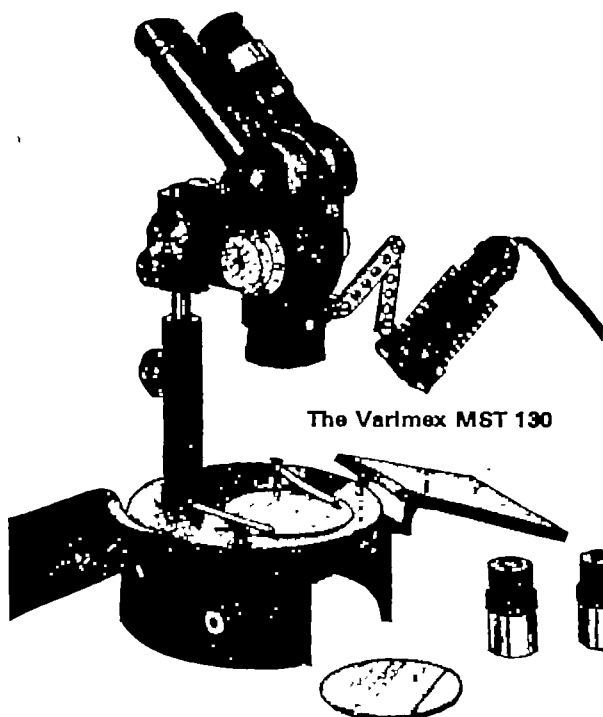
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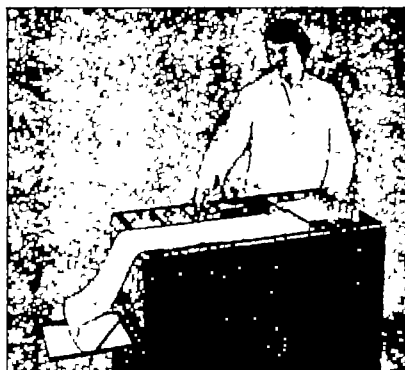
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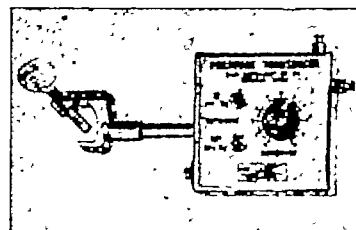
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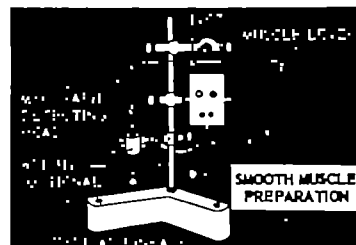
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Table 1. SERUM PROTEIN FRACTIONS FROM CONTROL, WORM-FREE, NON-INFECTED SHEEP AND WORM-FREE SHEEP EXPERIMENTALLY INFECTED WITH 2,000 *Oesophagostomum columbianum* INFECTIVE LARVAE

Group	No. sheep used	State of experiment	Serum proteins												Total protein ± error	r
			Albumin ± error	r	Globulins											
					α ± error	r	β ± error	r	β ± error	r	γ ± error	r				
Control	16	Beginning	3.53 ± 0.030	0.18	0.25 ± 0.006	1.76	0.91 ± 0.009	10.25	0.31 ± 0.002	1.72	1.06 ± 0.017	2.03	6.06 ± 0.04	1.02		
		End	3.60 ± 0.026		0.28 ± 0.001		1.04 ± 0.002		0.37 ± 0.002		1.28 ± 0.013		6.57 ± 0.026			
First infection	5	Beginning of infection	2.73 ± 0.018	12.68	0.45 ± 0.023	12.86	1.35 ± 0.037	0.27	0.49 ± 0.022	31.43	2.14 ± 0.065	3.78	7.06 ± 0.091	2.25		
		End of infection	1.79 ± 0.012		0.36 ± 0.010		1.33 ± 0.027		0.71 ± 0.012		2.55 ± 0.040		6.72 ± 0.041			
Second infection	6	Beginning of infection	2.78 ± 0.003	4.21	0.40 ± 0.002	0.38	1.25 ± 0.004	1.29	0.56 ± 0.004	13.16	2.06 ± 0.036	3.69	7.03 ± 0.041	1.96		
		End of infection	2.19 ± 0.045		0.42 ± 0.017		1.07 ± 0.043		0.61 ± 0.41		2.99 ± 0.075		7.49 ± 0.061			

ment-fixation titres of these sera show that the peak of γ globulin content of the serum occurred at the same time as the highest complement-fixation titre on both first and second infection.

Preliminary analysis of the mucus from immune animals indicated that there are large amounts of β1 and β2 globulin in these intestinal exudates and that the migration of the β2 fraction may be inhibited by the addition of specific somatic antigens prepared from the disintegrated third-stage larvae of *O. columbianum*.

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### Effect of Treatment with 2-Mercaptoethanol on Tanned Red Cell Agglutinating Antibodies In Allergic Encephalomyelitis

It has been shown that guinea-pigs and other animals injected with brain and Freund's complete adjuvant produce antibodies, both of complement-fixing and agglutinating type<sup>1,2</sup>. These antibodies react with homogenates or extracts of brain and also with purified encephalitogenic agents of the basic protein type; in this study only the anti-basic protein response has been evaluated.

Harwin and Paterson<sup>3</sup> found that in rats with allergic encephalomyelitis, complement-fixing activity of the serum was associated with 19S globulins and was destroyed by treatment with 2-mercaptoethanol. Serum with high complement-fixing activity when passively administered showed suppressive effects against the disease.

Table 1. AGGLUTINATING ANTIBODY IN SERA OF GUINEA-PIGS WITH ALLERGIC ENCEPHALOMYELITIS BEFORE AND AFTER TREATMENT OF SERA WITH 2-MERCAPTOETHANOL (BAL)

GP	Titres (tubes of doubling dilution)			
	HF antigen		BNBP antigen	
	Initial	After treatment with BAL	Initial	After treatment with BAL
2428	8	8	12	12
2429	8	8	14	14
2430	8	8	15	13
2432	7	8	14	12
2439	NT	NT	6	8
2665	0	0	8	8
2431	4	7	8	7
2237	1	2	6	8
2234	2	1	6	8
2434	4	6	8	8

NT: not tested.

It was thought of interest to investigate the presence of antibodies to a purified encephalitogenic basic protein (EF) of human brain<sup>4</sup> and also to a similar, but inactive, product derived from human sciatic nerve (SNBP) before and after treatment with 2-mercaptoethanol since this treatment is reported to inactivate 19S-type antibodies<sup>5,6</sup>. Sera were mixed with an equal volume of 0.2 M 2-mercaptoethanol left at 18° C for 24 h and the reagent removed by dialysis against normal saline for a minimum of 3 days at 4° C. Results of the antibody titrations before and after treatment are shown in Table 1. Antibody titre was not markedly reduced in any specimen; on the contrary, higher titres were obtained in a number of cases.

These results suggest that direct agglutinating antibody is of the 7S type. Because of the increase in titre shown in some cases it may be that treatment with 2-mercaptoethanol can liberate direct agglutinating fragments from non-agglutinating 19S antibodies present. Treatment with 2-mercaptoethanol did not influence the course of allergic encephalomyelitis in the 'guinea-pig'. Protection experiments carried out by pre-immunizing guinea-pigs with encephalitogen either in saline or in incomplete Freund's adjuvant gave high titre antibody of both types in each case but high protection only by immunization using the antigen in incomplete adjuvant. This suggests that either the 7S or 19S antibody to the active agent is directly associated with protective activity or that the protecting antibody may be directed against an inactive contaminant associated with the encephalitogen, enabling this to be cleared from the system before it can produce disease.

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### Stimulation of Mast Cell Production in Lymph Nodes of the Rat by Pyrrolizidine Alkaloids

MAST cells are known to increase in some tissues under certain conditions often associated with an immunological component. Well-known examples are in the skin in the vicinity of induced epitheliomas in mice<sup>1</sup> and associated

with proliferative changes in the thymus of *NZB* mice<sup>3</sup>. In lymph nodes, where under normal conditions mast cells occur in small numbers only, Miller<sup>4</sup> observed an increase in mast cell numbers after injection of *Salmonella* antigens, and Takeoka *et al.*<sup>5</sup> noted a significant increase in mast cells in the lungs and mediastinal lymph nodes in rats being fed seeds of *Crotalaria spectabilis* or the pyrrolizidine alkaloid, monocrotaline, isolated from this plant.

Because we have been investigating for some time the wide range of biological activities displayed by this group of alkaloids<sup>6</sup>, we have examined the effects on mast cells in the lymph nodes of rats given repeated small intraperitoneal doses of the three alkaloids heliotrine, lasiocarpine, and supinine which occur in *Heliotropium europaeum*<sup>6,7</sup>. Solutions of the hydrochlorides of these bases were used for all injections. The acute 72-h  $LD_{50}$  doses are 296, 77 and 405 mg/kg respectively<sup>8</sup>. In the current experiment groups of rats of a hooded strain<sup>9</sup> were given 0.1  $LD_{50}$  of heliotrine or lasiocarpine or 0.2  $LD_{50}$  supinine by intraperitoneal injection three times per week for 14 weeks. At necropsy, para-aortic lymph nodes and thymus were taken into Zenker fixative. Histological examination of paraffin sections stained with toluidine-blue and Unna-Pappenheim stain shows that there is a high mast cell count in the lymph nodes in 9 of 10 rats in the lasiocarpine group. Two only of 9 rats given heliotrine and none of 5 rats given supinine had a higher-than-normal mast-cell count in these lymph nodes. A comparison of the proportions of 'high' counts shown in Table 1 for the three alkaloids was made by computing chi-squared for the table and testing its significance. The value was significant at the 1 per cent probability level and it is clear that the frequency is highest for lasiocarpine. Mast cells were not found in the thymus with any of the alkaloids.

From a comparison of sections stained with haematoxylin-eosin, Unna-Pappenheim stain or toluidine-blue, it is clear that the mast cells appearing within the lymph nodes of animals given lasiocarpine are significantly different in appearance from those seen in connective tissue at the periphery of the nodes or in subperitoneal tissue.

In general the mast cells in the lymph node substance are larger, cuboidal rather than elongate in shape and stain less heavily with toluidine-blue. The mast cells are mostly in the usual areas where they are found after antigen stimulation<sup>10</sup> and, although they are close to medullary cords containing many plasma cells, they are in the intervening lymph spaces among the large number of reticulo-endothelial cells which often contain blood pigment. There is an impression that mast cells are especially

Table 1. MAST CELL COUNTS IN AORTIC LYMPH NODES OF RATS GIVEN REPEATED SMALL DOSES OF PYRROLIZIDINE ALKALOIDS

Alkaloid	No. of rats	Mast cell counts*	
		Individual	Proportion high
Lasiocarpine	10	0, 90, 100, 112, 125, 150, 165, 175, 200, 220	9/10
Heliotrine	9	0, 0, 0, 0, 0, 0, 8, 140, 160	2/9
Supinine	5	0, 0, 0, 4, 12	0/5

\* Each number is the mean of counts of two high-power fields of the same section from one rat. High-power field = 0.07 mm<sup>2</sup>.

abundant where the lymph channels are blocked by reticulo-endothelial cells. At these points there are plasma cells and lymphocytes in the channels and the possibility of origin of mast cells from lymphocytes via pyroninophil cells cannot be excluded. It is equally possible, however, that they may be derived from local reticulo-endothelial cells or have reached their characteristic situation from somewhere else in the body. In all the lymph nodes examined, all primary and secondary follicles are devoid of mast cells.

We thank Sir Macfarlane Burnet, who suggested this investigation.

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## IMMUNOLOGY

### Antibody Production in Mice with Isologous Tumours

THIS communication presents the results from experiments on the production of humoral antibodies by mice growing isologous transplantable tumours. The ability to produce antibodies by human cancer patients has been extensively investigated in recent years, and it has been shown that this ability is significantly depressed in patients with malignant disease<sup>1</sup>. Fewer observations have been made on the antibody production by experimental animals with tumours. Bogden and Aptekman<sup>2</sup> have demonstrated a reduction in the naturally occurring heteroagglutinins in mice with tumours, and Parfentjev *et al.*<sup>3</sup> have shown that the *proteus* agglutination titre is reduced in chickens with the Rous sarcoma. In an attempt to find an experimental model similar to the human situation, the production of humoral antibodies by two strains of mice growing transplantable isologous tumours has been investigated.

Groups of *A* strain mice with a transplantable mammary tumour (*AMT3*) and *O67* strain mice with a transplantable epithelioma (*Ep6*) were used. Tumours were transplanted by the subcutaneous injection on the back of a standard dose of tumour suspension. After 9 days the mice were immunized with 0.2 ml. *Salmonella typhi* 'H' antigen intraperitoneally and were bled 9 days later. Equal volumes of sera from two or three mice were pooled and the titre of antibody present in the pooled sera determined by the Vidal agglutination technique. Tumours were approximately 10 × 10 mm when antigen was given and 22 × 22 mm at the time of bleeding. Groups of normal



Fig. 1. Extensive mast cell proliferation in an aortic lymph node of a rat treated with 0.1  $LD_{50}$  of heliotrine (stained with toluidine-blue, × 684)

Table 1. ANTIBODY TITRE IN TUMOUR MICE AND CONTROLS (I)

Mice	No.	Titre	Mice	No.	Titre
A Tumour	69	6.3	O57 Tumour	20	5.6
A Control	63	6.4	O57 Control	20	5.9

Table 2. ANTIBODY TITRE IN TUMOUR MICE AND CONTROLS (II)

Mice	No.	Titre	Mice	No.	Titre
A Tumour	15	7.0	O57 Tumour	15	7.4
A Control	15	7.4	O57 Control	11	7.25

Table 3. ANTIBODY RESPONSE IN BCG-TREATED MICE

Mice	No.	Titre
Tumour	12	5.7
Control	12	6.0

mice immunized and bled at similar times served as controls. Titres of antibody are expressed in this report as log<sub>10</sub> reciprocals of the end-point dilution.

Results in A and O57 mice with tumours and control mice are given in Table 1.

These results show no significant difference in antibody production between the control group and tumour mice.

Two groups of A strain mice were immunized with 0.2 ml. H antigen intraperitoneally and 6 weeks later tumour suspensions were transplanted to one group. A second injection of 0.2 ml. antigen was given to both groups intraperitoneally 9 days later. After 9 days the mice were bled and antibody titre determined. A similar experiment was carried out with O57 mice and the results are shown in Table 2.

It can be seen from these results that the tumour-bearing mice responded as well as the control mice.

A study was made to observe the effect of vaccination with BCG on antibody production by groups of control and tumour mice. BCG has been shown to increase both antibody production and phagocytic activity in normal mice<sup>4</sup>. Two groups of mice were pre-treated with three daily injections of 1 vial BCG vaccine and 2 weeks later one group was injected with tumour suspension. Nine days later the mice received 0.2 ml. antigen intraperitoneally and were bled 8 days later, and antibody titres determined. The results are shown in Table 3.

Again there is no difference in antibody titre between the two groups.

The experimental system used is based on the procedure used by Berenbaum<sup>5</sup> for investigating the effect of various immunodepressant agents on antibody production. A single antigen has been used in these experiments instead of the TAB vaccine in which the 'H' antigenic potency of different samples is variable. Primary immunization was examined as it is believed to be more easily depressed than secondary immunization, delayed hypersensitivity reactions or homograft rejection. In certain human tumours there is evidence for the presence of cancer-specific antigens<sup>6</sup>. An attempt has been made to demonstrate possible cancer-specific antigens in the tumour AMT3. Antisera were prepared in rabbits by immunization on two occasions with a saline extract of AMT3 tumour with Freund's complete adjuvant. The antisera were tested by the Ouchterlony double-diffusion technique for reactions with a saline extract of tumour tissue and a saline extract of normal breast obtained from a lactating isologous mouse. After staining, no extra line was present opposite the tumour cells.

There is therefore no evidence of a soluble cancer-specific antigen in this tumour to give rise to antigenic competition, and this may explain the normal immunological activity of the tumour mice. Part of the depression of antibody formation seen in human cancer is believed to be due to the action of a specific depressing agent rather than secondary to the effects of cachexia and starvation, and a tolerance agent has been isolated from certain tumours<sup>7</sup>.

Although splenomegaly occurs in these mice with tumours<sup>8</sup>, Perovsky<sup>9</sup> has shown changes in the serum  $\gamma$ -globulins with isologous tumours of mice; the groups of mice studied with a well-developed tumour showed no evidence of an inability to respond to a foreign antigen,

and this may be due to a lack of antigenic competition with a cancer antigen in the tumour.

This work was carried out during the tenure of a grant from the British Empire Cancer Campaign for Research. I thank Prof. J. G. Murray for his advice and Messrs. Burroughs Wellcome for a supply of *Salmonella typhi* 'H' antigen.

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### Haemagglutinating, Precipitating and Lymphocyte-stimulating Factors of Phytohaemagglutinin

EXTRACTS from certain seeds of the Leguminosae are known to possess interesting biological properties. An extract of *Phaseola vulgaris*, phytohaemagglutinin (PHA), agglutinates red and white blood cells<sup>1,2</sup> and tumour cells<sup>3</sup>, and indiscriminately stimulates blood lymphocytes cultured *in vitro* to undergo blastie transformation and increased mitogenic activity<sup>4</sup>. Since lymphocytes of a sensitized donor are known to be stimulated in the same manner when exposed to a previously encountered antigen<sup>5,6</sup>, the possibility of PHA being a universal antigen has been considered<sup>7</sup>. Recently it has been noted by ourselves and others<sup>8,9</sup> that PHA likewise forms a precipitate indiscriminately with serum. Our demonstration of this ability of PHA to form a precipitin line with human serum and numerous animal sera appeared to support the concept of its being a ubiquitous antigen. If this were a valid concept, however, treatment of PHA to remove its precipitating activity would entail loss of antigenicity and might be expected to impair the lymphocyte-stimulating factor as well.

The purpose of this report is to describe the effect of heat and absorption with red blood cells on the precipitating and lymphocyte stimulating activity of PHA and to present evidence that PHA functions as a precipitating agent rather than an antigen when reacted with protein by diffusion in solid media.

Commercially available PHA (Burroughs Wellcome 'K 4597' and 'K 4912') was used throughout the experiment. PHA was absorbed three times with 0.5 ml. of washed, packed human red blood cells for 30 min. Heat inactivation of PHA was by heating at 85° C for 5 min as described by Tunnis<sup>10</sup>. Washed, packed normal rabbit red blood cells were used in the haemagglutination tests. The method for haemagglutination was essentially that described by Boyden<sup>11</sup> except that the tanned cells were not sensitized. The micro-Ouchterlony technique of Crowle<sup>12</sup> was used for the precipitin test. Lymphocyte cultures were prepared with media containing 'TC 199', foetal calf serum, penicillin and streptomycin. Cultures were maintained at 37° for 72 h in a tightly closed screw-top bottle and the percentage of blast forms was obtained by counting 1,000 lymphoid cells.

All fifty-nine human sera tested, and normal bovine, rabbit and rat sera, formed precipitates when reacted with PHA by double diffusion technique. Absorption of PHA with human red cells or heating at 85° C for 5 min destroyed this precipitating property and decreased the haemagglutinating activity. However, treatment of PHA by either technique did not affect the degree of blood lymphocyte transformation into blast forms (Table 1). Thus there appears to be no association between the

Table 1. RESULTS OF HEAT AND HAEMAGGLUTININ AND LYMOPHOYTE-STIMULATING ACTIVITY OF PHA\*

Unreated PHA			Absorbed PHA			Heated PHA		
PPTNS	HA	LS	PPTNS	HA	LS	PPTNS	HA	LS
++	46,687	81%	0	1,306	84%	---	---	---
++	7,796	64%	0	216	58%	0	216	62%
+	1,306	81%	0	216	88%	0	36	78%
+	1,306	76%	0	216	83%	0	36	74%
+	216	80%	0	36	90%	0	36	86%

The symbol HA refers to the reciprocal of the haemagglutinating titre and PHA refers to phytohaemagglutinin. LS refers to lymphocyte stimulation *in vitro* and is expressed as blast cells per 1,000 lymphoid cells in the culture.

precipitating activity and the lymphocyte-stimulating factor of PHA.

Concomitantly a large number of precipitin tests in which PHA is reacted with various sera and serum fractions indicates that the serum protein reacting with PHA is definitely not an antibody. Positive precipitin bands were formed when PHA was reacted with Cohn human serum fractions II, III, IV, VI, human albumin, serum from a child with classic primary agammaglobulinaemia and pure bovine serum albumin (BSA). The pure BSA was obtained by starch-block electrophoresis of commercial bovine serum albumin and formed a single line when reacted with rabbit anti-serum to commercial BSA on immunoelectrophoresis. Human serum albumin and Cohn fractions II, III and IV were found to contain no gamma<sub>2</sub>M, gamma<sub>2</sub>A or gamma<sub>3</sub> globulins when reacted with goat anti-human serum on immunoelectrophoresis. Demonstration that PHA forms precipitins with pure bovine serum albumin and fractions of human serum containing no immune globulins eliminates the possibility that PHA acts as an antigen in the precipitin reaction.

From these results it appears that the action of PHA in precipitating normal serum protein is not that of an antigen and thus does not support the concept that PHA stimulates lymphocyte transformation by virtue of being a universal antigen. The haemagglutinating and precipitating activity does not appear to be associated with the lymphocyte-stimulating factor in that loss of precipitating ability and marked decrease in haemagglutinin titre are not associated with a change in the ability of PHA to stimulate blood lymphocytes, cultured *in vitro*.

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### An Immunogenic Fraction extracted from *Brucella abortus* Cell Walls by Phenol

AN insoluble fraction capable of absorbing the agglutinating and mouse-protecting antibodies from *Brucella suis* antisera was isolated in this laboratory from sonically disintegrated *Br. suis*<sup>1</sup>. This fraction was assumed to be part of the bacterial cell wall. Further experiments<sup>2,3</sup> showed that cell walls and cell wall fragments from *Br. abortus* and *Br. suis* protected mice against challenge with *Br. abortus* (strain '2308') more effectively than intact cells. The immunizing activity of *Brucella* cell walls was confirmed in several other laboratories<sup>4-6</sup>.

The present communication reports the preparation and activity of a purified immunogenic fraction, extracted from *Br. abortus* cell walls by phenol. Cell walls were prepared from *Br. abortus* (strain '2308'). The bacteria

were grown on trypticase soya agar<sup>7</sup> for 4 days, gathered with physiological saline and killed by ethanol 75 per cent. The killed bacteria were washed twice with distilled water and digested for 2 h with pepsin (2 mg/ml. at 52° C, pH 2.0). The insoluble residue was washed with distilled water and resuspended in water. The pH of the suspension was brought to 8.5 following which the suspension was treated with trypsin (10 mg/ml.) at 52° C for 1 h. Cell walls were isolated by centrifuging the suspension at 12,000g for 30 min. Cell walls prepared by this method showed residues of nuclear material in the electron microscope. The residues could be removed by treatment with DNase. The nuclear material was also identified by the blue colour obtained with the diphenylamine reagent for DNA<sup>8</sup>.

To 120 ml. of cell wall suspension (60 mg/ml.), 550 ml. of 90 per cent phenol solution were added in small portions, over a period of 1 h, with continuous shaking. The suspension was allowed to stand at room temperature for 48 h, then centrifuged at 27,000g for 30 min. A heavy sediment resulted. The clear supernate was dialysed against tap water for 3 days and against distilled water for 2 days. A white fluffy precipitate was obtained, which remained in suspension. In addition, a brown-coloured precipitate settled at the bottom of the dialysis bag. The suspension containing the white material was separated and centrifuged at 27,000g for 10 min. The sediment obtained was washed once with distilled water (fraction-W, white fraction). The yield was approximately 2 per cent of the total weight of intact bacteria.

The white fraction was agglutinated by anti-*Brucella* serum and could absorb completely the agglutinating antibodies from *Br. suis* antiserum. On injection into rabbits, it produced antibodies which agglutinated whole bacteria. The immunizing activity of the white fraction was compared in mice with that of cell walls and of whole ethanol-killed bacteria, against challenge with *Br. abortus* (strain '2308') as follows: groups of 10 mice were immunized intraperitoneally with 40.0, 4.0, 0.4 and 0.04 µg of the immunizing antigen, intact or disintegrated ultrasonically. At 28 days after immunization the immunized mice together with control untreated groups were challenged with 30,000 virulent *Br. abortus* (strain '2308') cells administered intraperitoneally. Spleens were counted individually at 28 days after challenge by plating on trypticase soya agar<sup>9</sup>. The geometric mean counts (GMC) and the geometric protection index (GPI) were calculated. GPI was defined as the ratio:

$$\frac{\text{Geometric mean bacterial count in the spleens of control mice}}{\text{Geometric mean count in the spleens of immunized mice}}$$

Table 1 shows that the cell walls were the most effective immunizing agent (GPI: 6,304), but fraction-W also gave a high degree of protection (GPI: 2,406). It is noteworthy that even an amount as small as 0.4 µg was still capable of giving a significant degree of protection (GPI: 75). The data obtained so far do not allow precise comparisons of

Table 1. IMMUNIZING ACTIVITY OF FRACTION-W COMPARED WITH THAT OF CELL WALLS AND OF ETHANOL-KILLED *Brucella* CELLS

Immunizing antigen	Quantity injected (µg)	Intact*		Disintegrated ultrasonically†	
		GMC	GPI	GMC	GPI
Ethanol-killed bacteria	40.0	—	—	1.9 × 10 <sup>3</sup>	4,846
	4.0	7.9 × 10 <sup>3</sup>	697	1.8 × 10 <sup>3</sup>	736
	0.4	4.3 × 10 <sup>4</sup>	1.3	3.9 × 10 <sup>3</sup>	24
	0.04	4.6 × 10 <sup>4</sup>	1.1	—	—
Cell walls containing nuclear remnants	40.0	—	—	1.5 × 10 <sup>3</sup>	6,304
	4.0	6.8 × 10 <sup>3</sup>	808	5.2 × 10 <sup>3</sup>	1,798
	0.4	1.1 × 10 <sup>4</sup>	490	1.6 × 10 <sup>3</sup>	59
	0.04	2.2 × 10 <sup>4</sup>	24.5	—	—
Fraction-W	40.0	—	—	3.8 × 10 <sup>3</sup>	2,406
	4.0	3.1 × 10 <sup>3</sup>	1,740	2.1 × 10 <sup>3</sup>	433
	0.4	1.1 × 10 <sup>4</sup>	48	1.2 × 10 <sup>3</sup>	75
	0.04	1.2 × 10 <sup>4</sup>	4	—	—
Control untreated mice	—	5.5 × 10 <sup>4</sup>	—	9.3 × 10 <sup>4</sup>	—

—, Not done.

GMC, geometric mean count.

GPI, geometric mean bacterial count in the spleens of control mice

geometric mean bacterial count in the spleens of immunized mice

\* † The preparations were used with or without prior disintegration in an MSE ultrasonic disintegrator 18–20 kc/s 1.75 amp.

immunizing potency, but it is obvious that the activity of the white fraction was of the same order of magnitude as that of the other preparations. Further investigations are being carried out in this laboratory to determine the biological properties (toxicity, allergenicity) of this fraction. It is felt that an immunizing preparation of this type, with most of the soluble protein material removed, might be useful as a prophylactic antigen in man.

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### Lysozyme and Immune Bacteriolysis

It has long been accepted that the lysis of Gram-negative bacteria by fresh serum is due to the antibody-complement system. Over the past ten years three separate groups of workers have suggested, for different reasons, that lysozyme also plays a part in immune lysis of bacteria.

Amano *et al.*<sup>1</sup> found that lysis of *Vibrio comma* by serum is accelerated by leucocyte extracts in which the active factor proved to be lysozyme. They showed further<sup>2</sup> that removal of lysozyme from fresh serum by absorption with bentonite reduces lysis but not killing of *Escherichia coli* B. Restoration of the initial level of serum lysozyme by adding egg-white lysozyme restores lytic activity. Excess egg-white lysozyme increases also the rate and extent of killing.

Knowing that both properdin and lysozyme are absorbed by zymosan<sup>3</sup>, Muschel, Carey and Baron<sup>4</sup> examined their authentic properdin and found that it contained lysozyme. Moreover, inactive properdin deficient serum (RP) could be made bacteriolytic by egg-white lysozyme.

Wardlaw<sup>5</sup>, while investigating complement bacteriolytic activity, noticed that absorbing specific antibody with large amounts of bacteria also removed lysozyme.

There is yet another way in which the role of lysozyme in the complement system may be approached. As anti-human lysozyme serum was available<sup>6</sup>, it was thought worthwhile to compare the action of serum from which lysozyme had been removed by bentonite with serum in which lysozyme activity had been inhibited by specific antibody.

Experiments were performed on log phase cultures of *E. coli* 'Lilly', a rough strain used by Wardlaw, and *E. coli* 'WF2', a smooth strain isolated in the routine diagnostic laboratory. 'WF2' has now become rough, but identical results are given by other smooth strains. Lysis was measured by the decrease in optical density of a heavy inoculum ( $8 \times 10^8$  bacteria/ml.) using a Spekker absorptiometer. Killing was measured by repeated viable counts using small inocula (5,000 bacteria/ml.).

The complement source was human serum diluted as necessary with Tris buffer pH 8.4 to give a final ionic strength of 0.06 (Wardlaw<sup>5</sup>). Anti-human lysozyme was prepared as described by Glynn and Parkman<sup>6</sup>. The rabbit lysozyme normally present in such sera was removed by bentonite and the complement inactivated by heat.

Test systems for lysis or killing were set up with the following: (1) Normal human serum (NHS). (2) Heated

human serum (RO'). (3) Normal human serum with the lysozyme removed by bentonite (RL<sub>bent</sub>). (4) Normal human serum with the lysozyme inhibited by antibody (RL<sub>Ab</sub>). (5) Heated human serum plus anti-lysozyme (RO'RL<sub>Ab</sub>). (6) 'RL<sub>Ab</sub>' plus egg-white lysozyme (EWL). (Anti-human lysozyme does not inhibit egg-white lysozyme so that this preparation had lysozyme activity.)

'Lilly' and 'WF2' give very similar results. Fig. 1 shows lysis of 'Lilly'. Fig. 2 shows killing of 'WF2'.

Normal human serum produces rapid and extensive lysis and killing. Removal of complement by heating allows rapid growth. Sera with lysozyme removed by bentonite (RL<sub>bent</sub>) or inhibited by antibody (RL<sub>Ab</sub>) produce similar results. There is initial growth, then delayed and incomplete lysis. Later experiments not shown here suggest that RL<sub>Ab</sub> is less effective in inhibiting killing than is RL<sub>bent</sub>. Addition of egg-white lysozyme (EWL) to lysozyme deficient serum restores normal killing and lysis.

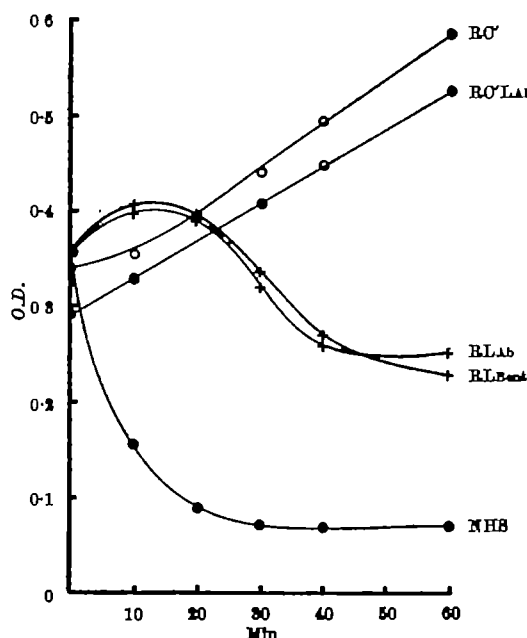


Fig. 1. Lysis of *E. coli* ('Lilly') by serum  $\pm$  lysozyme

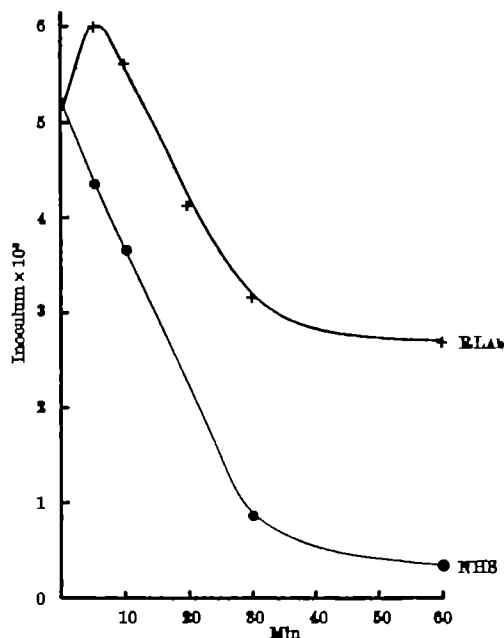


Fig. 2. Killing of *E. coli* ('WF2') by serum  $\pm$  lysozyme



In some experiments viable counts and opacity were measured in the same system, but the high inoculum needed to get a measurable opacity involves very great dilution factors in order to carry out viable counts. However, the lysis and killing curves with  $RL_{anti}$  were approximately parallel with the killing in advance of lysis.  $RL_{Ab}$  again inhibits killing to a lesser extent than does  $RL_{anti}$ .

These results, like those of previous workers, show that lysozyme is involved in serum lysis of *E. coli*. They differ from previous work in demonstrating a role for serum lysozyme in serum killing, and also in demonstrating that delayed lysis and killing occur in the absence of lysozyme or lysozyme activity. A possible explanation would be that lysozyme is released from the bacteria themselves, but none could be demonstrated. The present-day theory is that complement attached by means of antibody and antigen damages the lipoprotein and lipopolysaccharide 'layers' in the bacterial cell wall enabling lysozyme to attack the susceptible rigid mucopeptide layer underneath. All this must occur rapidly as with fresh whole serum lysis, and killing may be extensive in a few minutes. If lysozyme is removed or inactivated, presumably complement damages the outer wall as before. Possibly this sets in train some other lytic or lethal process, slower in action than lysozyme and which shows up in our curves as delayed lysis or death when the rapid lysozyme component is removed.

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## RADIOBIOLOGY

### An Apparatus for Studying the Retention of Inhaled Radioactive Vapours by Human Subjects

THE retention of radioactive substances by man is a subject of major importance in the field of atomic energy both from the point of view of deriving permissible exposure-levels and also for assessing potential hazards under accident conditions. Of the limited experimental investigations carried out on man, however, the greater proportion have involved oral or intravenous administration of the radioactive substance, notwithstanding that inhalation is the commonest accidental route of entry of most radioactive substances into the human body.

In the present investigation an apparatus has been designed for the accurate determination of percentage retention of radioactive vapours when inhaled by human subjects. The first compound chosen for investigation was methyl iodide, which is of interest because it could constitute the major hazard in the release of fission products in a reactor accident<sup>1,2</sup>. The nuclide chosen for labelling was iodine-132 which is a  $\gamma$ -emitter suitable for measurement by scintillation counters outside the body; iodine-132 was used in preference to iodine-131 because the radioactive dose delivered to the thyroid by the former is about two orders of magnitude smaller than that delivered by the same amount of the latter.

The apparatus<sup>3</sup> consists essentially of six parts, as shown in Fig. 1; of these, the humidifier, mixing vessel and chimney are each self-explanatory and will not be described further.

The evaporator (b) is a small glass U-tube which is connected to the main body of the apparatus by means of a glass-glass coupling block. It can thus be easily

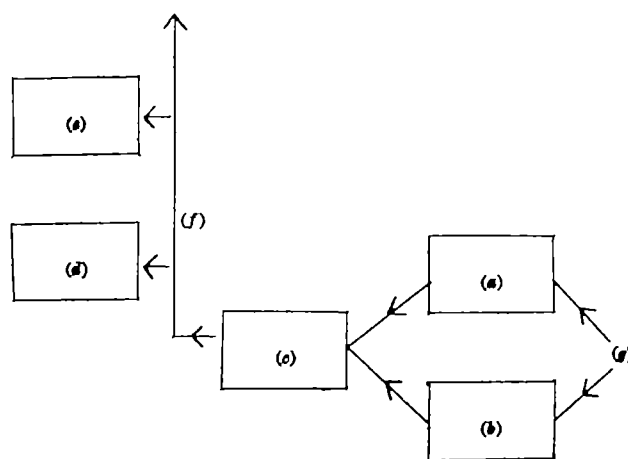


Fig. 1. Block diagram of apparatus. (a) Humidifier; (b) evaporator; (c) mixing vessel; (d) analysis; (e) inhalation-exhalation unit; (f) chimney leading to fume cupboard; (g) air supply

disconnected and it can also be connected in a similar manner to the exit of a gas chromatograph. The latter is used to isolate the requisite amount ( $\sim 1 \mu\text{c}$ ) of methyl iodide-132, which is prepared by a conventional exchange reaction between methyl iodide and sodium iodide-132.

It was initially intended to volatilize the methyl iodide over a period of about 15 min, but preliminary experiments showed that in order to prevent departures from normal breathing, a shorter period was desirable and the iodide was finally volatilized over 5 min only. To achieve a uniform and reproducible slow rate of release of methyl iodide-132 from the U-tube, when using very small amounts of carrier, necessitated surrounding the U-tube with a low-temperature bath. Even then, however, it was found that carrier levels of about 5 mg were required, and although these gave rise to concentrations of methyl iodide of about only 5 p.p.m., they still corresponded to a carrier uptake of twenty to thirty times the normal dietary uptake of iodine, and would have significantly affected the uptake and metabolism of the methyl iodide.

Because of this, the replacement of carrier methyl iodide by non-iodinated compounds of similar vapour pressure was investigated. Although on theoretical grounds, a diluent of this sort should have no effect on the rate of volatilization of the methyl iodide if a simple vaporization phenomenon was occurring, it was found in practice that the presence of the diluent greatly reduced the rate of loss of activity from the U-tube (this does not, however, render the method impracticable). This indicated that other phenomena such as desorption are probably predominating, but the practical implication is that the inactive methyl iodide can be largely replaced by a non-iodinated compound. In this way, iodine metabolism can be investigated under normal conditions; a second subsidiary benefit is that the somewhat toxic methyl iodide can be replaced by a relatively non-toxic compound such as methylene dichloride. Further experiments showed that if 4  $\mu\text{l}$ . of this were added to the U-tube prior to charging with methyl iodide-132, the activity was volatilized at a uniform and reproducible rate over a period of 5 min when the U-tube was surrounded by an ice/salt bath. This was verified by monitoring the U-tube with a sodium iodide crystal detector, the output from which was displayed on a recorder chart.

The concentration of the methyl iodide-132 in the main air stream is determined by drawing known volumes through small glass towers filled with charcoal, which have previously been calibrated with a solution of <sup>132</sup>I-labelled ammonium iodide of known activity.

The inhalation-exhalation assembly (e) consists basically of a large Y-piece of thick-walled glass containing a one-way valve in each of the arms. One of the arms is

connected to a ground-glass socket on the chimney and the other to another glass tower (of low resistance to breathing) containing charcoal. The subject then inhales from the chimney only and exhales to the tower only; to prevent condensation, a heating element is clamped below the exhalation arm and tower. The far side of the tower is connected to a 100 ft.<sup>3</sup> balloon, from which the volume of air exhaled, and therefore that inhaled, can be determined.

Preliminary experiments showed that there was no measurable decomposition of the methyl iodide, or adsorption on to particles, before inhalation occurred. During an inhalation experiment, the subject inhales from the apparatus during the 5 min in which the methyl iodide-132 is being volatilized and exhales during that time and a further 5 min through the tower and into the previously evacuated balloon. The amount of methyl iodide-132 which has been inhaled can then be calculated from a knowledge of its concentration in the main air stream and the volume of air inhaled, while the amount exhaled is determined by measuring the activity in the exhalation tower. The percentage retention is thus easily determined from the difference of these two quantities.

Because of the influence of inactive methyl iodide on the uptake of methyl iodide-132, the amounts of the former used as carrier in the U-tube are only of the order of 100 µg, that is, the amount inhaled is about 30–40 µg, which is considerably smaller than the normal daily dietary intake of iodides. Preliminary results on four volunteer subjects indicate values for the retention varying between 55 and 80 per cent. The metabolism of the retained methyl iodide is also being investigated and will be reported in detail in later papers.

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### Tracking Cobalt Project

THIS communication describes a new and accurate method of treating malignant disease by radiation. The radiotherapeutic world is convinced that accuracy in dose delivery in adequate amounts to minimum volumes has largely been responsible for improved results in limited disease. Just as surgery has its complications and rare fatal accidents, so also does a proportion of radiation injuries have to be accepted at curative dose-levels. A high standard of accuracy in treatment does much to reduce injuries by limiting the volume of tissue exposed. This new method has been the logical sequence of development over the past twenty-five years, passing through four phases.

(1) Beam directioning, by accurate 'sighting', enabled smaller radiation fields to be used without missing the primary tumour. This was first adopted for radium beam therapy<sup>1</sup>, and soon after applied to X-rays<sup>2</sup>.

(2) Further developments of methods led to the practice of uniform isodose zones enclosing the tumour, that is, the use of a pre-determined shape<sup>3</sup>.

(3) Moving-beam therapy apparatus enabled consecutive or continuous settings of radiation fields to be given with a high degree of accuracy to small zones<sup>4</sup>. This method produced mainly spheres or cylinders of pre-determined shape into which the tumour could be fitted. To extend its versatility, it was from this method that moving-beam therapy with tracking was conceived<sup>5</sup>.

(4) The cobalt tracking project will enable the spread of malignant disease from the primary growth along the

lymph node chain to be irradiated with accuracy<sup>6</sup>. A prototype apparatus at conventional voltage<sup>7</sup>, which has now been working for some years, has proved very encouraging in its use: the principal problems were solved, but owing to the distortion of the isodose pattern by bone, its future was limited. A public appeal has enabled a high quality apparatus to be made by T.E.M. Instruments, Ltd., of Crawley. The uniform dose zone of moving-beam therapy irradiates the sinuous track of the lymph node spread which is fed through it by moving the patient on a treatment couch which is guided along the track—its up-and-down and side-to-side movements being governed by electric motors, much as in the prototype design. In this instance, however, the movements are controlled by a black-on-white pattern giving the profile views of the track. Speed is shown by another series of black-and-white cut-outs which are followed by a series of photo-electric cells, that is, we have automation in radiotherapy.

The shape of the dose pattern is changed from a circle to an oval in cross-section at any point by slowing the speed of the arc movement. This allows more irradiation to that part. This is followed by acceleration of the speed which allows less irradiation; consequently an oval is produced.

Preliminary computer calculations and measurements arranged by Dr. W. A. Jennings have shown that a field of approximately 8 cm × 3 cm with reasonable definition can readily be obtained, hence the basis of attaining the principle of restricting the radiation to the diseased area while sparing the normal tissues around appears to be well within sight.

We are awaiting the construction of a room to house this apparatus so that the project may be commenced in earnest.

The expected spread of malignancy in lymph nodes, that is, the 'track', will be part of an exhibit at the International Radiological Congress in Rome in September 1965. This spread is based on information from surgeons of great operative experience and agreed by six senior United Kingdom radiotherapists. Such spread is in contrast to the post-mortem findings, when the disease is spread far and wide.

Work done at the Royal Northern Hospital has suggested that this lymph node track may be related to bony structures with a reasonable degree of accuracy and that repeat treatments may show a variation of only 0.5 cm. It is particularly fortunate that the fatty tissue which is largely responsible for the considerable variations of shape beyond the bones is, in the main, concentrated in the abdominal cavity and the superficial sites. These have no gross effect on the relation of blood and lymph vessels to the bones lying deeply in the body, hence the tracking method may prove to be valid and accurate.

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### BIOLOGY

#### A Phytochrome Mediated Effect of Light on the Hydroxylation Pattern of Flavonoids In *Plum sativum* var. 'Alaska'

THE presence of a kaempferol triglucoide (KG) and a quercetin triglucoide (QG), and of their *p*-coumaric acid esters (KGC and QGC respectively), in white-light-grown 'Alaska' peas has been reported<sup>1</sup>. Evidence has also been put forward that etiolated peas contain only the

kaempferol derivatives (KG and KGC), and that low irradiances of red light cause an increased synthesis of KGC in the terminal bud<sup>1,2</sup>—this effect being reversible by subsequent irradiation with far-red light<sup>3</sup>. A role for the phytochrome system in the light-mediated synthesis of flavonoids has been proposed for several other plants<sup>4-6</sup>. In a continuation of the work of Furuya and Thomas<sup>2</sup>, we have obtained evidence that the variations in flavonoid pigments with red light are due to increases in the rate of synthesis of the quercetin derivative (QGC) while the KGC concentration, in fact, remains relatively constant. This effect on QGC synthesis is reversible by far red light and is consequently thought to be a phytochrome effect.

Initial investigations showed that good separation of the four flavonoids using the previously reported paper chromatographic method<sup>2</sup> was difficult to achieve and a method of separation based on chromatography on a silica gel column using butanol-boric acid has been developed. The amounts of KGC and QGC in the various fractions were determined by spectrophotometric assay of the coumaric acid liberated by alkaline hydrolysis.

Recovery experiments have shown that 90-98 per cent of added flavonoid is recovered by this method. Attempts to estimate the KG and QG present by an analogous technique using acid hydrolysis and estimation of the flavonoid aglycones have not so far been successful. This method of separating the kaempferol derivatives from the quercetin derivatives has the advantage of allowing the whole extract to be used, in contrast with two-dimensional paper chromatography where only a small fraction of the extract can be used. However, it has not been found possible, as yet, to separate KG from KGC, and QG from QGC, by this method.

Plants were grown in complete darkness and subjected to low irradiances of red light (1 to 1,000 kergs cm<sup>-2</sup> at 660 mμ) or far-red light (845 kergs cm<sup>-2</sup> at 740 mμ), 6 days after sowing. The terminal buds were excised under dim green light and the KGC and QGC content determined.

Table 1. KINETICS OF THE EFFECTS OF RED LIGHT IRRADIATION (327 KERG CM<sup>-2</sup>) ON FRESH WEIGHT, KGC CONTENT AND QGC CONTENT OF TERMINAL BUDS OF 6-DAY-OLD, DARK-GROWN PEAS

Time after irradiation (h)	Fresh weight		KGC		QGC	
	mg/bud	% increase	μM/g fresh wt.	% increase	μM/g fresh wt.	% increase
0	4.4	0	0.85	0	0.10	0
4	4.9	11	1.02	20	0.17	70
8	7.3	66	1.00	18	0.20	100
12	9.1	107	0.94	11	0.25	150
16	10.2	132	1.23	56	0.20	100
20	11.4	159	0.91	7	0.20	200
24	15.3	245	0.99	5	0.45	350

Table 2. EFFECT OF VARYING ENERGIES OF RED LIGHT IRRADIATION ON THE FRESH WEIGHT, KGC CONTENT AND QGC CONTENT OF TERMINAL BUDS OF 6-DAY-OLD PEAS GATHERED 23 H AFTER IRRADIATION

Irradiation energy (kergs cm <sup>-2</sup> )	Fresh weight		KGC		QGC	
	mg/bud	% increase over D.F.*	μM/g fresh wt.	% increase over D.F.*	μM/g fresh wt.	% increase over D.F.*
Dark initial*	6.19	48	0.88	100	0.18	106
Dark final†	12.77	100	0.88	100	0.17	100
3	16.87	122	0.81	92	0.26	212
8	15.84	124	0.83	94	0.40	225
30	19.20	150	0.79	90	0.46	270
100	18.50	145	0.96	108	0.45	264
300	20.10	157	0.87	99	0.48	282
1,000	18.60	146	0.95	108	0.54	317

\* Dark initial = values for untreated buds at beginning of experiment.

† D.F. = dark final = dark-grown controls collected at end of experimental period.

Table 3. PHOTOREVERSIBILITY OF EFFECTS OF LIGHT ON THE FRESH WEIGHT AND QGC CONTENT OF TERMINAL BUDS OF 6-DAY-OLD PEAS GATHERED 23 H AFTER IRRADIATION

Treatment	Fresh weight		QGC	
	mg/bud	% increase over D.F.	μM/g fresh wt.	% increase over D.F.
Dark initial	5.35	67	0.11	85
Dark final	7.95	100	0.13	100
Red (98 kergs cm <sup>-2</sup> )	14.65	184	0.26	276
Far red (845 kergs cm <sup>-2</sup> )	9.85	118	0.17	131
Red-far red (energies as above)	9.19	116	0.19	146
Far red-red (energies as above)	12.12	165	0.23	254

Using the foregoing technique, the presence of KGC in totally etiolated terminal buds was confirmed (0.5-0.8 μM/g fresh weight). On irradiation with 327 kergs cm<sup>-2</sup> of red light, the content of QGC increased markedly during 24 h, while KGC content was not changed during this period (Table 1). When increasing dosages of red light were given, QGC synthesis increased with increasing energy with no obvious saturation-level (Table 2). KGC content, on the other hand, was not increased by irradiances of up to 1,000 kerg cm<sup>-2</sup>. These data, which are representative of several experiments, indicate that low-energy red light causes a marked increase in the synthesis of the quercetin derivative, with no effect on the kaempferol derivative. Evidence that this was indeed a phytochrome effect was obtained by carrying out a red-far red reversibility test (Table 3). It is thought likely that incomplete separation of KGC and QGC using paper chromatography is responsible for the conflict between these findings and those reported earlier<sup>1,2</sup>.

It would thus appear that in 'Alaska' peas, the presence of phytochrome in the far-red absorbing form (*P<sub>FR</sub>*) is essential for appreciable synthesis of the quercetin derivative, but is not required for the synthesis of the kaempferol derivative. The significance of this finding is perhaps heightened by the fact that the two substances, although quite complex, differ only in the presence or absence of a single hydroxyl group (kaempferol is 3,4',5,7-tetrahydroxyflavone; quercetin is 3,3',4',5,7-pentahydroxyflavone). These results, therefore, indicate that *P<sub>FR</sub>* functions, in one of its presumably more remote roles, in some way to determine the hydroxylation pattern of flavonoids. Our findings are in general agreement with the tentative conclusions recently reported by Stafford<sup>7</sup> of an involvement of phytochrome in 3'-hydroxylation of *Sorghum* flavonoids.

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### Seed-borne Primary Infection in Downy Mildew, *Sclerospora maydis* (Racib.) Butler

Downy mildew, *Sclerospora maydis* (Racib.) Butler, is a very destructive disease of corn in Indonesia and can wipe out whole fields. In 1964, the disease caused an average 90 per cent infection in Tjiandjur, West Java, causing a total crop failure in that area.

Rutgers<sup>1</sup>, while studying the mode of primary infection, reported that in one of his tests he got 4 diseased seedlings from 5 kernels obtained from diseased plants. In a second test with 50 seeds, only healthy plants were obtained. In another lot of kernels, even after hot water seed treatment at 60° C he got diseased seedlings. However, his investigations were not on an extensive scale and he could not reach any definite conclusion on the mode of primary phase of the transmission of the disease.

Palm<sup>3</sup> reported that oospores are not produced under Java conditions and that the only mode of transmission of the disease was through conidia.

Weeton<sup>2</sup> found out that in *S. philippinensis* the mycelium in badly affected ears could be traced in the cob along the funiculus of attachment in to the undeveloped parts of the abortive kernels and sometimes even in to the seed coat and the endosperm, but not in to the embryo itself.

Gattani<sup>4</sup> reported the presence of hyphae of *S. philippinensis* in the growing points of severely infected plants but did not report on the production of diseased seedlings from kernels obtained from such plants.

The present investigations were undertaken to determine the nature of primary infection of the disease. For this purpose, cobs of 'Metro' variety of corn were obtained from a seriously infected field in Purwokerto, Central Java. The kernels from these cobs were planted in the screen house at Bogor, West Java, and after 5 days of planting, diseased seedlings appeared. The percentage of infection after 5 days of planting was 10, but rose to 36.5 on the ninth day of planting. All the diseased plants produced conidia in the screen house test.

Further tests were made in a well-lit room with closed doors at 26°-29° C and 55 per cent relative humidity. Kernels of 'Metro' variety from plants which had been naturally infected in the seedling stage in the experimental field at Bogor were planted in sterile soil. Infected seedlings showing chlorosis appeared after 5 days. Such chlorotic plants did not produce any conidia while in the room but produced normal conidia in the morning, having been transferred to the outdoor screen house over-night. The percentage of infected seedlings obtained in this test, repeated 3 times, was 20.

In another experiment, plants which showed infection after 5 days were cut at the base to remove all the infected leaves. The new leaves which were later produced again showed the typical disease symptoms, indicating that such plants had systemic infection.

Four- to 9-day-old diseased plants were removed from the pots with intact roots. Such plants were dissected for their growing points. When stained with cotton blue, fungal hyphae could be detected in such growing points.

It would therefore seem that the primary infection of *S. maydis* is caused by fungal mycelium lodged in the embryo of the kernels of severely infected plants.

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## Influence of Carbon Dioxide on the Uptake of Water by Asparagus

Excised shoots of asparagus (*Asparagus officinalis* L.) with cut ends immersed in tap water undergo a marked gain in fresh weight during storage in atmospheres containing more than normal amounts of carbon dioxide<sup>1</sup>. Shoots, 16 cm in length, were stored in chambers at 1.7° C in darkness for one week. Modified atmospheres were obtained and maintained within 1.5 per cent of the desired levels by intermittent flushing with gases from pressure cylinders. When elimination of carbon dioxide was desired the carbon dioxide of respiration was absorbed in dry slaked lime.

The weight gain which was attributed to water uptake coincided with an increase in length. Typical results are shown in Table 1. Levels of oxygen lower than that of air had little effect. A level of carbon dioxide as low as 3 per cent showed some effect and the optimum level for these responses was in the 12-15 per cent range. Uptake of

Table 1. GAIN IN FRESH WEIGHT AND ELONGATION OF EXCISED ASPARAGUS SHOOTS IN RESPONSE TO CARBON-DIOXIDE-ENRICHED ATMOSPHERES

Atmospheric composition (%)		Gain in fresh weight (%)	Elongation (%)
O <sub>2</sub>	CO <sub>2</sub>		
Air (control)	0	8.1	6.2
20.5	0	8.8	6.5
16	0	9.1	6.8
15	15	25.4	17.0
15	30	18.8	12.1

water was not diminished by decapitation of the shoots; no attempt was made to determine changes in length of these shoots.

The increases in length, and in uptake of water, were accompanied by increases in pH of the expressed juice as previously reported by Fife and Frampton<sup>2</sup>, by softening of the tissue as measured by the 'tenderometer', and by apparent decreases in soluble solids. However, the percentages of soluble solids corrected for dilution, assuming the weight gain was due entirely to water uptake, were higher in asparagus exposed to higher levels of carbon dioxide. The information, as well as indications of a higher diffusion pressure deficit within the asparagus treated with carbon dioxide, as measured by a gradient of external osmotic solutions, suggests water uptake based on osmosis.

Carbon dioxide here seems to fulfil the role of a growth factor. Mer and Causton<sup>4</sup> have indicated that carbon dioxide has the ability to promote cell division in the dark. It would appear that carbon dioxide may not only promote cell division but also cell enlargement through water uptake mediated by plasticity of the tissue and changes in osmotic properties. However, it must be realized that the uptake of water by excised plant parts may not be strictly analogous to water uptake by plants with intact roots.

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## Water Economy of the Dorcas Gazelle

ALTHOUGH the physiology of the camel, kangaroo-rat, jerboa and various domestic animals of desert regions has been examined in some detail<sup>1,2</sup>, nothing is known of the heat and water relations of that most typical desert mammal, the gazelle. It has occasionally been suggested that gazelles may be able to live indefinitely on dry food without water, like desert rodents, but no experiments have been made to determine if this is true or whether, like camels, they need to drink. For this reason an investigation of the water economy of the Dorcas gazelle, *Gazella dorcas* (L.) is now being pursued here.

Preliminary results indicate clearly that gazelles must drink, even in winter, for they lose weight steadily on dry food when deprived of water. After five days desiccation a maximum of 1.5 l. fresh water can be ingested at one time; and smaller quantities of saline water are taken. With increasing dehydration, body temperature tends to lose homeostasis and there is some degree of hyperthermia, the urine becomes concentrated, faecal pellets smaller and drier and food intake is reduced.

In the Sudan, gazelles appear to inhabit desert and semi-arid regions where some water, fresh or saline, or dew and succulent food are available, even if considerable distances have to be travelled in order to obtain them.

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## ENTOMOLOGY

Laying Worker Honey Bee:  
Similarities to the Queen

It is known that in the honey bee community the queen has an influence on the behaviour and the physiology of worker bees<sup>1-4</sup>. It has been shown that the presence of a queen in a group of worker bees inhibits the development of the ovaries in the workers<sup>5</sup>. The workers, showing a special behaviour (retinue behaviour) towards their queen, recognize her by special substances. This recognition leads to a 'queenright behaviour', which in turn influences the development of the ovaries. When we take the queen away from such a group, ovarian development in the workers starts, and may result in the so-called laying workers. It seemed to be an interesting point that only a few bees out of a group reached this final stage and it was supposed<sup>6</sup> that perhaps these bees in turn would have an influence on the other bees.

It has been reported that, in a queenless colony, worker bees can evoke a retinue behaviour in other workers similar to that evoked by the queen<sup>6</sup>.

These considerations induced us to study the laying workers more closely. In 6 experimental cages, each containing 50 newly emerged worker bees, a laying worker taken from a queenless observation hive was introduced. This laying worker could be recognized by a paint mark. The bees were provided with water and a feeder containing a sugar-candy and pollen mixture. At regular intervals, during the renewal of the food and the drinking water, the behaviour of the workers towards the marked bee was studied. In all cases the laying worker evoked a distinct retinue behaviour in the workers.

When after 14 days the bees were killed and examined for ovarian development, the cages with a laying worker showed an inhibition of ovarian development in comparison with controls (Table 1).

Laying worker added to the cage		Controls	
Cage No.	Ovarial development (%)	Cage No.	Ovarial development (%)
1	13.0	1	25.1
2	16.9	2	27.0
3	17.6	3	27.6
4	19.9	4	28.0
5	20.0	5	30.3
6	25.2*	6	30.8
mean	18.6	mean	28.1

\* Laying worker died after 4 days.

In a second experiment we compared the inhibitory action of an extract of bees with highly developed ovaries with an extract of bees with undeveloped ones. For this purpose the ovaries of bees of a queenless group were examined. After division into an undeveloped, a poorly and medium developed, and a highly developed group, the first and the last group of bees were extracted in acetone by Soxhlet extraction for 6 hours. Fifty bees were used for each of the extracts.

Extracted dead young worker bees were impregnated with these extracts. A bee impregnated with the 'highly developed' extract was placed in each of 6 cages containing 50 young bees and a bee impregnated with the 'undeveloped' extract in each of 6 control cages. Every 2 days we removed the impregnated object and replaced it with a new one.

At the moment of removal of the impregnated bees observations were made about retinue behaviour. In all cases the 'highly developed' extract evoked retinue behaviour, whereas this was never observed in the control cages.

After 14 days the bees were killed and the ovarian development was determined. Table 2 shows that the 'highly developed' extract inhibited ovarian development.

Table 2

Extract of bees with highly developed ovaries		Extract of bees with undeveloped ovaries	
Cage No.	Ovarial development (%)	Cage No.	Ovarial development (%)
1	17.1	1	37.8
2	14.0	2	34.2
3	14.0	3	24.7
4	13.0	4	38.9
5	15.0	5	28.0
6	9.6	6	34.2
mean	13.6	mean	33.0

Apart from the production of eggs a laying worker obviously resembles the queen in releasing retinue behaviour and in inhibiting ovarian development in young bees under experimental conditions. Just as in the queen, this influence of the laying worker is based on the production of substances that are perceived by the other bees.

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Metabolic Effects of the Corpus Allatum  
Hormone in the Desert Locust, *Schistocerca gregaria*

SOME thirty years ago, Wigglesworth<sup>1</sup> first demonstrated that yolk deposition in insect oocytes is controlled by the corpus allatum hormone. Since then, the hormonal control of vitellogenesis has been complicated by some experiments, which suggests that the corpus allatum hormone also has metabolic effects. Allatectomy leads to the accumulation of lipid and the hypertrophy of the fat body in a number of insects<sup>2-4</sup>. From such evidence, Pfeiffer<sup>5</sup> advanced the hypothesis that the corpus allatum controls vitellogenesis by releasing a 'metabolic hormone' that in some way regulates the mobilization of precursor materials from the fat body. This hypothesis has always suffered from the serious objection that ovariectomy does not lead to the expected accumulation of yolk precursor materials in the fat body<sup>2,4</sup>.

A second hypothesis has been put forward by Doane<sup>6</sup>, based on her observations of the hypertrophied fat body of female-sterile mutants of *Drosophila melanogaster*. In such mutants, the corpus allatum is normal in function, since wild-type ovaries implanted into mutant female flies develop eggs normally; the lethality in the mutant forms resides autonomously in the ovaries themselves. Doane therefore suggested: (a) that an 'ovarian hormone' is released by active ovaries in a normal fly, the function of the hormone being to regulate the utilization of stored lipids in the fat body; (b) that the action of the ovarian hormone on the fat body is independent of any analogous action of the metabolic hormone of Pfeiffer, although its release may be indirectly controlled by the gonadotrophic activity of the corpus allatum.

A fresh approach to this problem was attempted when preliminary observations indicated that allatectomized male desert locusts were inactive.

An electron microscope study of the fat body of allatectomized male desert locusts has shown that large deposits of glycogen and lipid accumulate in the fat body. The accumulation of lipid was confirmed by chloroform-extraction of whole locusts. Implantation of active corpora allata into previously allatectomized locusts alleviated this condition.

Spontaneous locomotor activity of adult male locusts was determined by the method of Brown and Unwin<sup>7</sup>. It was found that after allatectomy there was persistent

inactivity of operated locusts, however long they survived. Implantation of active corpora allata led to the resumption of the normal, high level of locomotor activity. Extirpation of the testis and accessory reproductive glands, on the other hand, had no effect either on spontaneous locomotor activity or on glycogen and lipid utilization.

The hypothesis is therefore advanced that the corpus allatum hormone regulates the intensity of locomotor activity by a direct effect on the central nervous system. Since fat and glycogen form the chief energy reserves of locusts<sup>1</sup>, it is suggested that the accumulation of these substances after allatectomy is due to the persistent inactivity of the operated insects.

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## MICROBIOLOGY

### *Salmonella* in Bird Faeces

THE contamination of our rivers and beaches with bacteria related to *Salmonella*, caused by the disposal of domestic sewage, is well known. Domestic sewage, collected in the main drainage of the city of Hamburg, has been continually tested for *Salmonella* during the past twenty years. *Salmonella* occurs at a density equivalent to 5,000 organisms in 1,000 ml. of sewage.

After flowing into the Elbe, these organisms are dispersed, but survive, so that some 50–100 organisms can be found in 1,000 ml. of river water.

Steiniger<sup>1</sup> concluded, from his examination of faecal material from birds, that possibly these animals are the origin of the wide spread of *Salmonella* in surface water. In other words, it may not be sewage disposal that contaminates the rivers and beaches; there may exist a sort of *Salmonella* saprophytism in the gut of seabirds, forming an important factor in the spread of *Salmonella* in our environment.

We collected more than 1,000 samples of faecal material from gulls in the area of the biggest sewage disposal works of Hamburg, on bridges and pontoons in the port of Hamburg, and in the streets of the city. 78 per cent of the samples collected at the sewage disposal works were positive for *Salmonella* as were 66 per cent of the samples collected in the port and 28 per cent of the samples collected in the city. The most frequently isolated species, as in water samples of the Hamburg sewers, was *S. paratyphi* B. The faeces of gulls collected in those regions which were free from the influence of sewage never contained *Salmonellae*. As, on the other hand, the faeces of birds collected near the sewage disposal works were positive, one must conclude that the presence of *Salmonella* organisms in these birds is related to a source of infection, and does not present *visu generis* the normal bacterial flora in the gut of birds. Furthermore, this conclusion is confirmed by the range of species isolated, which exactly corresponds to the species isolated from the water of Hamburg rivers. The most frequently isolated species was *S. paratyphi* B, followed in order of frequency by *S. typhimurium*, *S. manchester*, *S. montevideo*, *S. infantis*, *S.*

*senftenberg*, *S. anatum*, *S. stanley*, *S. newport*, *S. braenderup*, *S. san diego*, *S. dubisburg*, *S. muenchen*, *S. blockley*, *S. bovis morbillicans*, and *S. panama*.

In examining the faecal material of other birds, the most striking fact was the great proportion of pigeons (30 per cent) and ducks (16 per cent) which contained *Salmonella*, while the faecal matters of thrushes (0.15 per cent) and sparrows (0.2 per cent) only occasionally showed a positive result. Birds kept indoors, such as canaries or parrots, were always negative for *Salmonella*. It is thus clear that a lack of source of infection results in a lack of *Salmonella* in the faeces. Faeces of pigeons from Tanganyika and Venice were *Salmonella* positive in 30 and 29 per cent of cases respectively. While the faeces of gulls collected in Hamburg showed a preponderance of the species *S. paratyphi* B, samples of pigeons and ducks frequently contained *S. typhimurium*: samples of pigeon-dirt collected in Hamburg 80 per cent, samples of duck-dirt 80 per cent, samples of foreign pigeon-dirt 90 per cent. The other species isolated were, in order of frequency: *S. manchester*, *S. paratyphi* B, *S. anatum*, *S. newport*, *S. panama*, *S. makumira*. This last mentioned genus was a new species with the antigen-formula 4, 12: ex: 1, 7, first isolated from the faeces of an African pigeon (Rohde and Müller<sup>2</sup>).

The occurrence of *S. paratyphi* B in considerable amounts in the faeces of healthy city-birds leads to the conclusion that this organism, which is important for the epidemiology of man, has a wide distribution. On the other hand, *S. typhi* could not be isolated in any of 3,000 faecal samples from different native birds.

Consequently there must be an epidemiological difference between these two species, although the clinical picture of the infectious disease of man, expressed as septicæmia, is the same.

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### Transfer of RNA Extracts from Immune Donor Spleen Cells to *Shigella*-tolerant Recipient Mice

INJECTION of relatively large quantities of non-living antigens into either neonatal or adult animals often results in the establishment of immunological tolerance characterized by specific suppression of antibody formation on subsequent challenge immunization<sup>1</sup>. Immune tolerance to bacteria has been demonstrated most often in mice injected with polysaccharide antigen derived from pneumococci<sup>2,3</sup>. Somewhat similar states of immunological tolerance to antigens derived from other micro-organisms have also been investigated. For example, tolerance to *Shigella paradyserteriae* antigens has been observed in mice after a single administration of a relatively large concentration of soluble *Shigella* antigen within 12–24 h after birth<sup>4</sup>. The resulting tolerance persisted for at least 8–12 weeks, depending on the dose and route of antigen administered at birth. Tolerance to *Shigella* has been terminated in unresponsive mice by transfer of spleen cell suspensions from normal or *Shigella* immune donors<sup>5</sup>. Administration of hyperimmune anti-*Shigella* serum to tolerant mice, however, has not restored agglutinin forming ability<sup>6</sup>.

There have been several recent reports concerning acquisition of antibody formation by non-immune normal animals or cell suspensions following treatment with RNA extracted from immune donors<sup>7–11</sup>. Since it is not known whether tolerance to an antigen may be due to selective removal of possible 'clones' of cells potentially

capable of forming antibody<sup>12</sup>, or due to direct suppression of antibody synthesis by 'excess' antigen in direct contact with lymphoid cells, it was thought of interest to determine whether or not RNA-rich extracts from immune donor lymphoid cells could restore immune responsiveness in specifically tolerant animals. This report is concerned with agglutinin formation to *Shigella* antigen in *Shigella*-tolerant mice treated with RNA extracts from *Shigella*-immune or normal donor mice.

Young adult NIH albino mice 8-10 weeks of age were used as donors for this study. They were injected 7-10 days before being killed with 20-50 µg *Shigella*-soluble antigen (SSA) obtained by trypsin digestion of an alcohol-killed suspension of *Shigella* organisms as described previously<sup>4-6</sup>. Groups of 10 or more immunized donor mice, as well as non-immune control donor mice, were bled from the retro-orbital plexus to obtain blood for determination of serum agglutinins to *Shigella*. The mice were then killed and spleens removed and immediately frozen with dry ice. The frozen spleens were homogenized with a 'Teflon'-tipped tissue homogenizer at 0°C, and RNA was extracted by the cold phenol method<sup>13,14</sup>. The extracted RNA was standardized by the orcinol reaction and by ultra-violet absorption so that 1.0 ml. contained 300-400 µg RNA. There was no serologically detectable antigen or antibody in the extracts.

The RNA-rich extracts from *Shigella*-immune donors were administered intraperitoneally in 1.0-ml. quantities to groups of 6-8-week-old recipient NIH mice which had not been treated previously or had been injected at birth with sufficient SSA to induce immune tolerance to the antigen. Similar groups of recipients were injected with 1.0-ml. quantities of RNA extracts prepared from the normal non-immune control donor mice. A third group of normal or *Shigella*-tolerant control recipients was injected with saline instead of RNA preparations. All recipients, in all groups, were then challenged with an intraperitoneal injection of 0.5 ml. of SSA containing 20 µg N to test their ability to form anti-*Shigella* agglutinins. Blood samples were obtained from the retro-orbital plexus from each recipient at close periodic intervals for two to three weeks and the resulting serum specimens tested for anti-*Shigella* agglutinins by standard tube agglutination procedures.

Anti-*Shigella* agglutinin titres of normal and *Shigella*-tolerant recipient mice receiving RNA and a challenge injection with *Shigella* antigen are indicated in Table 1. Most non-tolerant control mice, receiving saline or RNA from non-immune donors before challenge immunization with SSA, responded with mean peak agglutinin titres of 1:300 or greater. Nearly all mice which had received a tolerance-inducing injection of SSA at birth and saline only before *Shigella* challenge had post-challenge anti-*Shigella* titres of 1:40 or less, indicative of continued immunological tolerance. Transfer of RNA from non-immune donors similarly had no effect on tolerance. *Shigella*-tolerant recipient mice injected with RNA obtained from the immune donor mice also had markedly depressed anti-*Shigella* agglutinin titres when challenged with *Shigella* antigen at 6-8 weeks of life. Control non-tolerant mice receiving RNA from immune donors often responded with higher agglutinin titres than control mice receiving saline only or RNA from non-immune donors

Table 1. ANTI-*Shigella* AGGLUTININ TITRES OF NORMAL AND *Shigella*-TOLERANT RECIPIENT MICE AFTER TRANSFER OF RNA FROM NORMAL OR *Shigella*-IMMUNE DONOR MICE AND CHALLENGED WITH *Shigella* ANTIGEN\*

RNA donors	Normal		<i>Shigella</i> -tolerant†	
	No. positive‡	Mean peak titres	No. positive	Mean peak titres
None (saline)	18/21	1:372	5/23	1:88
Normal	17/19	1:507	2/17	1:27
<i>Shigella</i> -immune§	20/23	1:400	3/19	1:33

\* All recipients injected with *Shigella*-soluble antigen (20 µg N) at 6-8 weeks of age.

† Tolerant mice injected at birth with *Shigella* antigen.

‡ Number of mice with peak post-challenge titre of 1:64 or more.

§ Immunized 7-10 days before killing by i.p. inoculation of *Shigella* antigen.

Table 2. ANTI-*Shigella* AGGLUTININ TITRES OF MICE RECEIVING RNA FROM NORMAL OR *Shigella*-IMMUNE DONORS AND EITHER UNCHALLENGED OR CHALLENGED WITH *Shigella* ANTIGEN\*

RNA donors	Recipient mice	Challenge immunization	
		None	<i>Shigella</i> antigen†
Normal	Normal	<1:10	1:343
<i>Shigella</i> -immune‡	Normal	<1:10	1:23
	Normal	1:63	1:376
	<i>Shigella</i> -tolerant‡	1:20	1:34

\* Mean peak titres of at least 10 mice per group.

† Recipients challenged at 6-8 weeks of life with *Shigella* antigen (intraperitoneal) (20 µg N).

‡ Tolerant recipients injected at birth with *Shigella* antigen.

§ Immune donors injected with *Shigella* 7-10 days before killing.

immediately before challenge immunization. Administration of RNA extracts from immune donors to normal mice not receiving *Shigella* immunization also resulted in some level of agglutinin formation (Table 2), suggesting transfer of either *Shigella* antigens or a possible agglutinin-forming mechanism to recipient mice.

These results indicate that RNA-rich extracts from *Shigella*-immune donor mice are not capable of restoring specific agglutinin forming capacity to *Shigella*-tolerant recipient mice under the conditions of the investigation. This is in contrast to previous results in which it was found that RNA-rich subcellular fractions from *Shigella*-immune donors were capable of inducing a measurable degree of immunity in either normal or X-irradiated non-immune recipient animals<sup>15,16</sup>. The results presented here are also in contrast with the findings by Feldman *et al.*<sup>17</sup> that nucleic acid preparations from normal rabbit spleens are capable of specifically restoring the immune response to human serum albumin in rabbits rendered tolerant to that antigen following treatment with 6-mercaptopurine.

The inactivation of RNA by serum or tissue RNases in recipient animals in this study may be an important factor in the failure of restoration of immune competence in tolerant animals. However, similar levels of RNase should also be present in normal animals injected with RNA. Transfer of less purified RNA preparations, especially ribonucleoprotein extracts or subcellular fractions, might be more effective agents for biological restoration of immunity since they are less likely to be inactivated by RNase<sup>18</sup>. They are also more likely to contain persisting antigenic determinants and thus actively stimulate agglutinin formation. However, if tolerance is due to persistence of 'excess' antigen, presumably at an intracellular site, it is unlikely that administration of nucleic acid extracts containing additional antigen would restore agglutinin formation in a tolerant recipient.

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<sup>1</sup> Smith, R. T., *Adv. Immunol.*, edit. by Taliaferro, W. H., and Humphrey, J. W., 1, 67 (1961).

<sup>2</sup> Felton, L. D., and Ottinger, B., *J. Biol.*, 43, 94 (1942).

<sup>3</sup> Skidnd, G. W., Paterson, P. Y., and Thomas, L., *J. Immunol.*, 50, 220 (1963).

<sup>4</sup> Friedman, H., and Gabry, W. L., *J. Immunol.*, 85, 478 (1960).

<sup>5</sup> Friedman, H., *J. Immunol.*, 93, 201 (1964).

<sup>6</sup> Friedman, H., *J. Immunol.* (in the press).

<sup>7</sup> Mannick, J. A., *Ann. Surg.*, 156, 356 (1962).

<sup>8</sup> Cohen, R. P., and Parks, J. J., *Science*, 144, 1012 (1964).

<sup>9</sup> Fishman, M., *J. Exp. Med.*, 114, 837 (1961).

<sup>10</sup> Vredevoe, D. L., and Nelson, R. L., *Biochem. Biophys. Res. Commun.*, 18, 231 (1963).

<sup>11</sup> Friedman, H., *Science*, 145, 934 (1964).

<sup>12</sup> Kossel, G. J. V., *Adv. Immunol.*, 2, 163 (1962).

<sup>13</sup> Ghera, A., and Sohrmann, G., *Nature*, 177, 703 (1966).

<sup>14</sup> Moloney, J. B., *Adv. Intern. Contr. Cancer*, 12, 250 (1963).

<sup>15</sup> Friedman, H., *Experientia*, 19, 537 (1963).

<sup>16</sup> Friedman, H., *Ann. N.Y. Acad. Sci.*, 114, 444 (1964).

<sup>17</sup> Feldman, M., Gohenson, A., and Nachtigal, D., in *Conceptual Adv. in Immunol. and Oncology*, 424 (Harper and Row, N.Y., 1963).



## FORTHCOMING EVENTS

Wednesday, September 22

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at the School of Pharmacy, University of London, Brunswick Square, London, W.C.1), at 9.30 a.m.—Symposium on "Food Science Research in the United Kingdom".

SOCIETY OF ENVIRONMENTAL ENGINEERS (In the Mechanical Engineering Department, Imperial College, Exhibition Road, London, S.W.7), at 6 p.m.—Squadron Leader L. F. Gillard: "The Correlation of Service Defects and Laboratory Experience".

Thursday, September 23

BRITISH INTERPLANETARY SOCIETY (In the Large Physics Theatre, University College, Gower Street, London, W.C.1), from 10 a.m. to 4.30 p.m.—Symposium on "Modern Trends in Space Physics".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

SENIOR LECTURER (honours graduate with good teaching experience) IN GRAP HUBBARDY—The Principal, Essex Institute of Agriculture, Writtle, Chelmsford, Essex (September 23).

DEPUTY DIRECTOR (Research) (with considerable experience of biological research, particularly in the field of ecology, and its applications, and experience of scientific administration) OF THE NATURAL CONSERVANCY at the London Headquarters, Belgrave Square, S.W.1.—The Natural Environment Research Council, Establishments Division, State House, High Holborn, London, W.C.1 (September 24).

HEAD (with high academic qualifications with appropriate experience in industry, research and/or teaching at university level) OF THE DEPARTMENT OF MATHEMATICS AND PHYSICS, Grade IV, College of Technology, Sheffield—The Director of Education, P.O. Box 67, Sheffield (September 24).

LECTURER OR ASSISTANT LECTURER in one of the following: (a) SOCIOLOGY or (b) SOCIAL ADMINISTRATION—The Registrar, The University, Keele, Staffs (September 24).

SENIOR RESEARCH ASSOCIATE (with a good honours degree in engineering or physics and some research experience) IN THE DEPARTMENT OF MECHANICAL ENGINEERING, for work in the fields of mechanisms and gyroscopes—Prof. L. Maunders, Department of Mechanical Engineering, The University, Newcastle upon Tyne (September 24).

SENIOR RESEARCH FELLOW (graduate in science with substantial post-graduate experimental research experience in the study of ionization phenomena in gases), to hold a responsible position in a group engaged in the study of ionization phenomena in low-pressure flames—The Secretary, Department of Physics, The University, Bedford (September 24).

RESEARCH ASSISTANT (with a good honours degree, preferably with some training or specialization in marine biology) IN THE MARINE SCIENCE LABORATORIES to carry out a programme on zooplankton succession—The Registrar, University College of North Wales, Bangor, North Wales (September 25).

TEMPORARY LECTURER/ASSISTANT LECTURER (able to lecture on North America) IN GEOGRAPHY—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (September 25).

SENIOR RESEARCH FELLOW (graduate in science or engineering) IN THE DEPARTMENT OF EDUCATION to be concerned primarily with the devising of a course in programmed learning form for the Higher National Certificate in Electrical and Electronic Engineering—The Academic Registrar, Brunel College, Woodlands Avenue, London, W.3 (September 26).

RESEARCH ASSISTANT IN THE SCHOOL OF PHYSICS to work in the field of experimental geophysics or solid state physics—The Registrar, The University, Newcastle upon Tyne (September 27).

POSTDOCTORAL FELLOW (with experience in plasma physics, microwave techniques or electromagnetic theory and use of a computer) in a plasma physics group which is studying the scattering of microwaves by various plasma configurations—The Registrar, University College of Wales, Aberystwyth (September 28).

RESEARCH STUDENT (suitably qualified electronic or electrical engineer, or physicist interested in research in the field of digital frequency modulation) IN THE DEPARTMENT OF ELECTRONICS—Prof. G. D. Sims, University of Southampton, Southampton (September 30).

LECTURERS (medical practitioners registered with the South African Medical and Dental Council with special training and interest in morbid anatomy and histopathology) IN THE DEPARTMENT OF PATHOLOGY AND MICROBIOLOGY, University of the Witwatersrand, Johannesburg, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa, September 30).

SENIOR LECTURER AND LECTURER (with an honours degree in mathematics and preferably a higher degree and some experience of teaching and research) IN MATHEMATICS; a LECTURER (with a Ph.D. degree and some post-doctoral research experience) IN PHYSICAL CHEMISTRY; and a LECTURER (with a Ph.D. degree and some post-doctoral research experience) IN PHYSICAL CHEMISTRY (Theoretical) at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (September 30).

SENIOR TUTOR/TUTOR IN THE DEPARTMENT OF ZOOLOGY, School of Biological Sciences, University of New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 30).

ASSOCIATE PROFESSOR IN THE DEPARTMENT OF GEOLOGY AND GEOPHYSICS, University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 1).

LECTURER OR ASSISTANT LECTURER IN THE DEPARTMENT OF ELECTRONIC COMPUTING—The Secretary, The University, Aberdeen (October 1).

LECTURER OR SENIOR LECTURER IN INORGANIC AND PHYSICAL CHEMISTRY, or in ORGANIC CHEMISTRY at the University of New England, Armidale, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 4).

LECTURER OR ASSISTANT LECTURER IN PHYSICS at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (October 8).

ENTOMOLOGIST (with at least a B.Sc. (Agriculture), or equivalent qualification) IN THE DEPARTMENT OF AGRICULTURE, New South Wales, Australia, to carry out research on insect pests affecting crops—The New South Wales Government Office, 56 Strand, London, W.C.2 (October 6).

READER IN BIOCHEMISTRY at Guy's Hospital Medical School—The Academic Registrar, University of London, Senate House, London, W.C.1 (October 8).

ASSISTANT EXPERIMENTAL OFFICER (graduate in a biological science, pure or applied, with an interest in field work) IN THE NEMATODE DEPARTMENT, to assist in work on nematodes injurious to cereals and their control—The Secretary, Rothamsted Experimental Station, Harpenden, Herts, quoting Ref. 1052/80 (October 15).

CHAIR OF PHARMACOLOGY AT THE UNIVERSITY OF SINGAPORE—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (October 15).

LECTURER/SENIOR LECTURER (with research experience on some branch of either pure or applied mathematics) IN MATHEMATICS at the University of New England, Armidale, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 15).

RESEARCH ASSISTANT (preferably with some experience with piezoelectric materials, wave propagation, or an allied subject) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING, to design and conduct experimental investigations to clarify the principles of electromechanical filters for operation at megacycle frequencies—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (October 15).

SENIOR LECTURER IN BIOCHEMISTRY at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (October 15).

TEACHING FELLOW (with an honours degree in geophysics or physics or equivalent qualifications) IN THE SCHOOL OF APPLIED GEOLOGY, University of New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 15).

THIRD CHAIR OF MEDICINE at the University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 15).

LECTURER IN THE DEPARTMENT OF ORGANIC CHEMISTRY, University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 16).

CHAIR OF VETERINARY SURGERY at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 25).

ASSISTANT PROFESSOR IN BIOCHEMISTRY—Prof. G. H. Connell, Chairman, Department of Biochemistry, University of Toronto, Toronto 5, Ontario, Canada (October 30).

SENIOR LECTURER (with general experience in zoology and special interests and qualifications in one or more of the major divisions of zoological science) IN ZOOLOGY at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 31).

LECTURER (with an honours degree or equivalent) IN THE DEPARTMENT OF PHILOSOPHY, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 1).

SENIOR LECTURER IN THE SCHOOL OF BOTANY, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 4).

UNIVERSITY LECTURER OR UNIVERSITY DEMONSTRATOR IN THE DEPARTMENT OF PHARMACOLOGY—T. D. Kellaway, Department of Pathology, The University, Tennis Court Road, Cambridge (November 4).

LECTURER (with a distinguished academic record in ceramic engineering, chemical engineering or mining engineering, and preferably several years professional experience) IN CERAMIC ENGINEERING at the University of New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 30).

READER IN THE DEPARTMENT OF MECHANICAL ENGINEERING, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 30).

ASSISTANT PROFESSOR OF SOCIAL ANTHROPOLOGY—Dr. R. K. Crook, Dalhousie University, Halifax, Nova Scotia, Canada.

CHAIR OF BUILDING SCIENCES IN THE SCHOOL OF ARCHITECTURE—The Registrar, University of Strathclyde, George Street, Glasgow, G.1.

GRADUATE HISTOLOGIST (with an interest in mammalian histology and some knowledge of histological technique) to amplify and extend a very important and promising project—The Director, Tobacco Research Council Laboratories, Harlow Hill, Harrogate, Yorkshire.

LABORATORY TECHNICIAN, Grade II, IN THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, chiefly for administration, including ordering of stock and supervision of junior staff, in a series of laboratories concerned with chemistry, biochemistry and physiology—The Assistant Bursar (Personnel), University of Reading, Reading, Berkshire.

LECTURER IN BOTANY; a LECTURER IN CHEMISTRY; and a LECTURER IN PHYSICS at the University of Basildon, Basildon and Procter and Swadlow—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1.

LECTURER IN INORGANIC CHEMISTRY—Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N.7.

LECTURER OR ASSISTANT LECTURER IN PHYSICS at the Royal University of Malta—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1.

LECTURER OR ASSISTANT LECTURER IN THE DEPARTMENT OF PHYSICS, University of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1.

LECTURER (preferably with an interest in relativity or statistics) IN MATHEMATICS—Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N.7.

LECTURER (with a number of years' post-doctoral experience, ideally in the fields of crystallography, deformation, or electron microscopy, together with suitable teaching experience) IN THE DEPARTMENT OF METALLURGY in the field of physical metallurgy—Prof. J. G. Ball, Metallurgy Department, Imperial College of Science and Technology, London, S.W.7.

LECTURERS AND ASSISTANT LECTURERS IN MATHEMATICS, COMPUTING AND STATISTICS—The Academic Registrar, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

POSTDOCTORAL RESEARCH FELLOW IN THE PHYSICS DEPARTMENT to work on an investigation into solid state theory under the direction of Prof. K. W. H. Stevens—The Registrar, The University, Nottingham.

POSTGRADUATE RESEARCH ASSISTANT (with a good degree in chemistry or physics) IN THE DEPARTMENT OF CHEMISTRY, for work in collaboration with Dr. K. B. Jennings on the structural significance of metastable ions in the

mass spectra of organic compounds—The Registrar, The University, Sheffield, 10.

PROFESSOR (with a wide experience of education and a particular interest in the field of university education where the emphasis is on technology and applied science) OF EDUCATION—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 45/G.

RESEARCH ASSISTANT (with a good honours degree in pharmacy, microbiology or biochemistry) IN PHARMACOLOGICAL MICROBIOLOGY, to work upon a project forming part of a major work in the field of resistance of micro-organisms to antimicrobial agents—The Registrar, Bristol College of Science and Technology, Ashley Down, Bristol 7, quoting Ref. OST 65/86.

RESEARCH ASSOCIATE OR RESEARCH STUDENT (young chemist, physicist or metallurgist, with at least an honours degree or its equivalent) IN THE METALLURGY DEPARTMENT, to work on stress corrosion in high carbon steel wire (the project will involve advanced electron microscopy as well as electro-chemistry)—Dr. J. D. Ghoshal, Metallurgy Department, The University of Newcastle upon Tyne, Newcastle upon Tyne, 1.

SENIOR SCIENTIFIC OFFICER (young graduate physicist or physical chemist, preferably with several years' research experience) IN THE PHYSICS LABORATORY for work mainly concerned with an important research project supported by an earmarked grant from the Ministry of Technology—The Secretary, British Baking Industries Research Association, Chocleywood, Rickmansworth, Hertfordshire.

TECHNICIAN (with some background of laboratory work) IN THE DEPARTMENT OF PHYSICS, to assist in a programme of research on the growth of certain semiconducting crystals—The Registrar, University College of North Wales, Bangor, North Wales.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

An International Bibliography of Non-Periodical Literature on Documentation and Information. Compiled and Edited by Hans Zell and Robert Machensmy. Pp. vi+394. (Oxford: Robert Maxwell and Co., Ltd.; Long Island City: Maxwell Scientific International, Inc., 1965.) 30s.; 4 dollars. [58]

The British Non-Ferrous Metals Research Association. Annual Report 1964. Pp. 41. (London: The British Non-Ferrous Metals Research Association, 1965.) [58]

European Journal of Cancer, Vol. 1, No. 1 (June 1965). Pp. 1-74. Published quarterly. Subscription rates: (A) 280s. (40 dollars) per annum for libraries, government establishments and research institutions; (B) 105s. (15 dollars) per annum for individuals who write directly to the publisher and certify that the journal is for their personal use. (Oxford: Pergamon Press, Ltd., 1965.) [58]

Northern Ireland: Ministry of Agriculture. Leaflet No. 16: Feeding Dairy Cows. Pp. 12. (Belfast: Ministry of Agriculture, 1965.) [58]

The Royal Society. United Kingdom Volcanological Research 1965. (A summary of current volcanological studies by British universities and scientific institutions with provisional plans for future years.) Pp. 55. (London: The Royal Society, 1965.) [58]

Royal Observatory Bulletin. No. 91: Investigation of Proper Motions in the Field of the Cluster M 67. I: The Central Region. By O. A. Murray, P. M. Corbin and Mary B. Alborn. Pp. B377-B360+2 plates. 5s. 6d. net. No. 92: Results Obtained with a Danjon Astrolabe at Hicksonwood. By D. V. Thomas. Pp. B383-B340+2 plates. 10s. net. (London: H.M. Stationery Office, 1965.) [58]

Proceedings of the Dorset Natural History and Archaeological Society for 1964. Vol. 86. Edited by J. Stevens Cox. Pp. 240. (Dorchester: Dorset Natural History and Archaeological Society, c/o The County Museum, 1965.) 30s. [118]

Regional Advisory Council for Technological Education for London and the Home Counties. Bulletin of Special Courses in Higher Technology. Management Studies and Commerce, 1965-66. Part 1: Autumn Term. Pp. 188. (London: Regional Advisory Council for Technological Education for London and the Home Counties, 1965.) 5s. [118]

Ambassade de France. Service de Presse et d'Information. French Foreign Policy: Statement made by the Minister of Foreign Affairs in the National Assembly, 16th June 1965. Pp. 18. (London: Ambassade de France, Service de Presse et d'Information, 1965.) [118]

Planning, Vol. 31, No. 490 (August 1965): Housing in Britain, France and Western Germany. Pp. 215-270. (London: Political and Economic Planning, 1965.) 7s. 6d. [118]

Paper at Work No. 4: International Paper Sizes. (A Series of Spoken Guides.) Pp. 8. (London: Spoken, Ltd., 1965.) [118]

BDH Laboratory Chemicals Catalogue 1965. Pp. xxiv+413. (Poole: The British Drug Houses, Ltd., 1965.) [118]

Royal Observatory Bulletin. No. 93. Measurements of Radial Velocity from Double Plates. By Sir Richard Woolley and G. A. Harding. Pp. B363-B380. 8s. net. No. 94: Time and Latitude Service 1964, April-June. Pp. B383-B367. 8s. net. (London: H.M. Stationery Office, 1965.) [118]

### Other Countries

Memoirs of the American Philosophical Society. Vol. 63: Three Decades of British Art. By Prof. Ellis Kirkham Waterhouse. (Jayne Lectures for 1964.) Pp. xii+77. (Philadelphia: American Philosophical Society, 1965.) 2 dollars. [98]

United States Naval Observatory. Circular No. 106: Rectangular Coordinates of Mercury 1800-2000. By B. L. Duncombe, Z. Tufakoglu, and G. Larson. Pp. 218. Circular No. 107: Sunlight, Moonlight, and Twilight for Antares 1960-1968. By Louis B. Weston. Pp. 19. (Washington, D.C.: United States Naval Observatory, 1965.) [98]

United States Department of Agriculture. Leaflet No. 432: The Spotted Alfalfa Aphid: How to Control It. Pp. 8. (Washington, D.C.: Government Printing Office, 1965.) 10 cents. [98]

National Academy of Sciences—National Research Council. Subcommittee on Radiochemistry. NAS-RS 2008 (Rev.). Radiochemistry of Rhodium. By James C. Armstrong, Jr., and Gregory R. Choppin. (Nuclear Science Series.) Pp. vi+75. (Springfield, Virginia: Clearinghouse for Federal Scientific and Technical Information, National Bureau of Standards, U.S. Department of Commerce, 1965.) 1 dollar. [98]

Bulletin of the Museum of Comparative Zoology, Harvard University. Vol. 134, No. 2: The Distribution of the Oceanic Fish *Brama brama*. By Giles W. Mead and Richard L. Haedrich. Pp. 29-68. Vol. 134, No. 3: Evolution of the Tapirid Skeleton from *Haploides* to *Tapirus*. By Leonard B. Radinsky. Pp. 69-106 (3 plates). (Cambridge, Mass.: Museum of Comparative Zoology, Harvard University, 1965.) [98]

The Carlsberg Foundation's Oceanographic Expedition Round the World 1923-30 and Previous "Dana" Expeditions. "Dana" Report No. 68: The Hydromedusae of the Pacific and Indian Oceans. By P. L. Kämp. Pp. 162. (Copenhagen: Andr. Fr. Hest and Søn, 1965.) 48 D.kr. [98]

Astra Annual Report 1964. Pp. 40. (Södertälje: Astra, 1965.) [98]

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## UNIVERSITY EXPANSION IN BRITAIN

THE fifth report from the Estimates Committee for the session 1964-65\*, dealing with grants to universities and colleges, is a document of outstanding public importance. Although the publication of the report within a few days of the announcement of the Government's decision to curtail capital expenditure has focused attention, as in the debate on education in the House of Commons on August 3, on the last two paragraphs of the report, its significance is much deeper and more permanent. It presents a penetrating and thorough examination of the whole working of the University Grants Committee system in the context of the present structure of Government in Britain. The voluminous evidence throws light on the nature of the particular problems which arise in the development of new universities and the change in status of the colleges of advanced technology. Taken with the memoranda from the University Grants Committee itself, the Committee of Vice-Chancellors and Principals, the Treasury, the Department of Scientific and Industrial Research, and the Secretary of State for Education and Science, for example, it is likely to constitute for years to come a standard reference on the functioning of the whole system.

Probably the key-passage in the report is the paragraph in the introduction which notes that since the Select Committee on Estimates enquired into university grants in its fifth report for the Session 1951-52, the grant-in-aid to universities has risen from £23.5 million to £132.9 million for 1964-65; for 1965-66, when it will include the colleges of advanced technology, it is expected to reach £193 million. This increase was a main factor which led the Estimates Committee to undertake a fresh examination. However, in view of the debate on August 3 and of what was said in the House of Lords on August 4, it may be as well to dispose first of the question of the proposed restriction of the building programme. On August 4, the Minister of State for Education and Science, Lord Bowden, told the House of Lords that university building projects for which contracts had not yet been signed would be postponed for six months except in development districts and areas of high employment. The Secretary of State for Education and Science, he said, would consider urgently, in consultation with the University Grants Committee, how best to minimize the effects of this decision on the growth in student numbers. Since then, in replying to questions, Lord Bowden has stated that the restriction was being applied to universities rather than to schools, and that it would fall in some measure on technical colleges. In the House of Commons on August 3 the Minister of State, Department of Education and Science, confirmed that the minor works programme for schools would go forward this year as planned and that the school building programmes to the middle of 1967-68 would stand as previously announced. In replying on the debate, Mr. R. E. Prentice made it clear that capital improvements in the education service as a whole, except for schools, would be postponed for six months.

Mr. Prentice admitted that this was a serious matter for the universities and, while declining to estimate the

total cost to the education service of these measures, appeared to accept Dr. J. Bray's suggestion that the number of students admitted might be decreased by 10,000. He was, however, clearly hoping that the universities might find means of offsetting this, for example, by planning the more intensive use of accommodation and equipment. Concerning the technical colleges, for which the building programme had risen from £17 million in 1964-65 to £26 million in 1965-66, he thought the effect would be slight, and although he admitted that the postponement would be a disappointment to the colleges of education he told the House that entrants to the training colleges in September 1965 were expected to reach 28,000 compared with 24,000 in 1964, giving a student population of 70,000; the target of 122,000 places by 1973 remained.

Sir Edward Boyle was doubtful whether, in fact, this figure was now likely to be achieved, but he thought Dr. Bray was unduly pessimistic as to the fall in the number of university students and he did not think that it would be any answer to increase the number of students studying at home. Mr. Prentice assured him that the basic assumptions of educational policy, which visualized expenditure on education as rising annually in real terms at a little less than 6 per cent per annum, had not been changed in any way. Sir Edward also referred to the negotiations in 1964 between the Government and the universities through the University Grants Committee: these eventually led the Committee to agree that the universities should attempt to achieve the short-term expansion target of the Robbins Report for an additional capital expenditure of about £40 million instead of the £80 million originally regarded as a minimum.

This debate took place in the light not only of the Government's announcement about postponement of capital expenditure but also of the Estimate Committee's reference to evidence of widespread disquiet about the relation between the present estimates of grants to universities and the student numbers expected as a result of the Robbins Report. The universities were unanimous in their evidence that the scope of capital grants was insufficient to provide properly for the rise in numbers after 1967. While the University Grants Committee agreed that the present scale of grants would enable the targets set for 1967 to be attained, it confirmed the view of the universities that the provisional allocation for the following years would not support or sustain those numbers. The Department of Education and Science expressed the opinion that in the past the numbers of students had in practice been almost exactly those for which the capital programme was supposed to provide. In May the chairman of the University Grants Committee had already expressed the opinion that, without addition to these provisional figures, students would be very cramped and crowded.

Accordingly, the Estimates Committee records in the fifth report its fear that a conflict between desired numbers and desirable standards may be inevitable and that pressures within the universities may undermine the plan of the University Grants Committee to use the period 1967-72 to repair obsolescence in buildings and equipment. Its enquiry did not extend to the possibility of a more intensive use of university buildings and facilities by such

\* Fifth Report from the Estimates Committee together with the Minutes of the Evidence taken before Sub-Committee B and Appendices, Session 1964-65—Grants to Universities and Colleges. Pp. xiv+1+288. (London: H.M.S.O.) 28s. net.

measures as changes in the university terms, nor was it for the Committee to recommend a reduction in student numbers. The report is emphatic, however, that if the target set by the Robbins Report is to be achieved without prejudice to accepted standards of university education, a further large increase in capital grants is unavoidable.

Neither the target of numbers nor accepted standards are necessarily sacrosanct. For all the Robbins Report, it should be remembered that that target is still regarded by some responsible opinion as too high, at least so far as universities are concerned. Nor are questions of standards to be considered without reference to that of the whole purpose of university, as distinct from other forms of higher education. But while the Estimates Committee at this point rightly reminds us that we have not yet willed the means for the end we have accepted, the pertinence of the Report in this connexion lies in its further reminder that by failing to do so—still more by the six-month postponement now proposed—we are in danger of gross waste of public resources.

Appended to the report is a memorandum from the University of Kent, which makes this point very effectively by citing specific instances of loss of efficiency through inability to commit capital except at short range. This effect of uncertainty on efficiency was confirmed in evidence from Sir Robert Matthew, a past president of the Royal Institute of British Architects. Such inefficiency and even waste is accentuated by the postponement of building projects now proposed by the Government, and independent of any effect on the eventual achievement of the target set for university expansion, the one certain result is considerable waste of public money. If achieved at all, the target will require even greater financial expenditure and possibly also more skilled manpower.

It is entirely relevant to the Government's action to point out that the whole purpose of this enquiry by the Estimates Committee into the greatly increased expenditure on the universities, which over the past decade has risen more than six-fold, was to satisfy Parliament that these large sums were wisely expended and that the Nation was obtaining a reasonable return on its investment. A start-and-stop policy makes nonsense of such recommendations as the Estimates Committee was able to make or of the steps which the University Grants Committee or the universities have already taken. Furthermore, it accentuates a major and inherent defect of the quinquennial system—that of the transition from one quinquennium to the next, which imposes a stress which can amount almost to a standstill. The Estimates Committee considered, in fact, that for the last two years of a quinquennium the consequent uncertainties already had a serious and depressing effect within universities, particularly with teams of research workers. This, of course, is in accordance with the observations and advice of the Plowden Committee on the Control of Public Expenditure, which unreservedly condemned a start-and-stop policy so far as research was concerned.

In the scientific field, a start-and-stop policy also accentuates a difficulty which is already acute in regard to equipment. The rate of obsolescence is rising rapidly and such equipment is also liable to a 5 per cent per annum rise in costs solely on the factor of increasing complexity. Moreover, the existing practice is criticized both by the Treasury and the universities. On a rising building programme it is difficult to estimate how much equipment will be required for a particular building or a particular year, and the rule governing capital grants favours those obtaining grants in contrast to those who have to re-equip

out of recurrent grant. The Estimates Committee recognizes that this is a serious problem and it recommends that the University Grants Committee should undertake a survey, on the lines of the obsolescent buildings schedule, of at least all major scientific departments to determine the degree of obsolescence in equipment. Moreover, it suggests that the Treasury Working Party which is considering budgetary control should examine, as a matter of urgency, the idea of an annual recurrent capital grant for bringing up to standard some of the worst equipment in the older buildings. When a reasonable standard has been achieved, the possibility should be considered of a process of amortization, writing off and depreciation of equipment over a period of time.

Otherwise this report of the Estimates Committee does not bear greatly on the difficulties into which the Government's latest decision has put the universities. It attempted to discover ways of getting value for money and also ways in which the structure of Government could be altered to provide greater efficiency and greater incentives towards economy in the universities. By and large, the universities and the University Grants Committee come out well from this examination, as does the Treasury, but that can scarcely be said for the Department of Education and Science. The evidence, however, indicates a response to its efforts to enlist the universities both in increased co-operation with the University Grants Committee and in individual and collective efforts to demonstrate publicly that they are making the best use of public money and the facilities they enjoy.

It might be noted here that the Estimates Committee fully supports the recommendation of the Robbins Committee that the level of fees should be raised from the present 10 per cent of the average cost of their education to 20 per cent. Treasury evidence indicated that a Government which was prepared to halt building projects for six months was not ready to take a step which the universities themselves would seem to welcome and which would also help to bring home to students that payment of fees does not meet costs. In the Estimates Committee's view, raising fees to a fairer level would have the advantages of involving local authorities to a greater extent in university education and also recouping a higher proportion of the cost of education from overseas students. The present rules should be modified to prevent the increased contribution causing parental hardship.

Evidence was taken from the Department of Scientific and Industrial Research on the provision of grants to universities for research and equipment for research, which in 1964-65 amounted to £8 million. On this the Estimates Committee makes two comments only. Such equipment is now consigned completely to the university concerned because the Department does not want the responsibility of dealing with obsolete equipment. The Estimates Committee makes the sensible comment that while this may be generally sound, on occasion the conclusions of a particular piece of research or the break-up of a special research team may leave a university with serviceable equipment for which there is no definite use. Secondly, while the Department of Scientific and Industrial Research thought it important that as soon as possible research projects should be fully assimilated by the university into its control, such transfer occurs at the end of the quinquennium. The Estimates Committee thinks that this practice might be a deterrent and suggests that universities should be encouraged to take over projects at any time during the quinquennium, even with the use of private funds.

For the remainder, the report is concerned with the University Grants Committee and its functioning rather than with the actual expenditure of the grant-in-aid of universities. The earlier controversy over the principle of academic freedom in this matter is not reopened. It is accepted that the system of allocation and control of the various grants to universities is intended: (1) to give the universities, as institutions responsible for their own academic planning, management and development, an assurance of the amount of Government support they would obtain; (2) to keep university development responsive to national needs, while maintaining the principle of academic freedom; (3) to ensure incentives and safeguards for economical use of Government money. The first of these is, of course, violated absolutely by the Government's decision, but it is with the third that the report, apart from its scrutiny of the University Grants Committee, is essentially concerned.

There are now four parties to the present system: the Treasury, the Department of Education and Science, the University Grants Committee and the universities themselves. While the Estimates Committee is sometimes critical of the Department of Education and Science, and the universities, in general, are afraid that their claims may not be put with the same energy, *vis-à-vis* the rest of the education, as when the University Grants Committee approached the Treasury direct, this was vigorously denied by the Department, which, like the Treasury, puts the main responsibility for financial control on the University Grants Committee. It continues to be the view that a proper regard to the academic independence of the universities should be the counterpart of a responsible attitude in the universities towards Government money. The Estimates Committee agrees with the Robbins Committee in maintaining the principle of a Committee independent of politics and not subject to ministerial directions—yet maintaining close contact with the organization of Government—which advises on the magnitude of the amounts needed and which distributes the funds available.

The report shows that it is open to question whether the University Grants Committee, as at present constituted, can adequately fulfil these functions. It points to the heavy responsibilities of the Chairman and his growing number of largely routine duties. The routine work of members of the Committee is also so onerous that it could prevent them from giving to the universities as a whole, and to the newer universities in particular, the sort of guidance they need to frame their building programmes in line with national needs and financial possibilities. The Estimates Committee received considerable evidence that the University Grants Committee is understaffed: while paying tribute to the devotion and public spirit of past and present members of the Committee, it considers that growing responsibilities of the Committee call for much more full-time responsibility than at present. It recommends, therefore, that the Secretary of State for Education and Science should review the composition and responsibility of the University Grants Committee to discover whether it is advisable to increase the number of members and to require more of them to undertake full-time duties.

There are other specific recommendations. The Deputy Chairmanship should be made a full-time appointment, and it is recommended that the time-table of visitations should be rearranged so that they are spread over the whole quinquennium in order to facilitate two visits to each university. The Committee's Finance Division should be strengthened by full-time services of the statistician, with, if necessary, further statistical help for

the other Divisions, and a full-time cost accountant attached to the Committee as a whole. It is also recommended that an immediate review should be made of the Architect's Division so that a better career structure can be set up, and, to secure an immediate increase, that full attention should be paid to the secondment of additional staff from other Government departments. In reviewing the Architect's Division, the authorization of additional posts (one to be filled by a consulting engineer) should be considered.

By strengthening in this way the technical expertise available to the Committee, particularly in accountancy techniques, the Estimates Committee hopes that the University Grants Committee will be able to develop cost control more effectively and also to give more constructive and effective guidance to the universities in planning university buildings. As to informing universities of national needs, the Estimates Committee considers that this is primarily a responsibility of the Department of Education and Science. It recommends that the Department should enlarge the functions of its officers responsible for collecting and processing information on national needs for graduates in industry and elsewhere. Jointly with the University Grants Committee and the Treasury, the Department should undertake urgently a review of the quinquennial system in the light of the requirements suggested by the Treasury, and any changes seen to be necessary should be put into effect in the early part of the next quinquennium.

Two of the Treasury suggestions are worth noting. The system should provide ample time for the University Grants Committee to assess the requirements and efficiency of new universities and, moreover, it should provide the universities with a reasonable assurance that the necessary funds will be available so that they can proceed in accordance with approved targets. It should also provide the Government with as good a forecast as possible of the Government recurrent finance which would be needed over a period—realistically not more than five years—and it should be capable of review and being brought up to date at intervals. The system should not involve too detailed a control of expenditure by the universities, and also provide Parliament with an assurance that the sums provided are adequate to finance universities in accordance with Government policy and yet provide proper incentives to efficiency in university administration.

Five other recommendations are addressed to the University Grants Committee itself. The procedure for bringing up and dealing with claims by universities for a supplement to the recurrent grant is subject to exercises of judgment to an extent which the Estimates Committee regards as unfair to the universities. Accordingly, it recommends that the University Grants Committee should inform the Committee of Vice-Chancellors that if the claims of universities are co-ordinated and submitted by them at any time, such claims will be transmitted as soon as possible for the Government's decision. On the control of building programmes it recommends that the University Grants Committee should invariably receive confirmation of the Government allocation of building starts not less than four years in advance and, as soon as possible after this has been done, the Committee should make its allocation to individual universities. It also recommends that the University Grants Committee should inaugurate an exercise to work out standard requirements for university buildings and that the more general requirements should be given effect as soon as possible.

This suggestion originated with the Royal Institute of British Architects, whose evidence was that the University Grants Committee was relating costs not to standards but the money available and the bulk of building. The Estimates Committee appreciates the difficulty of calculating maintenance costs and recognizes that the Department of Education and Science could help by making available all its information on maintenance costs. None the less, the Committee recommends that the University Grants Committee should also examine the proposal that contractors tendering for university buildings should be asked to tender for their maintenance as well, although the proposal at first sight appears to cut across demarcation lines between capital and recurrent expenditure.

Finally, the Estimates Committee hopes that if increased staff can be attracted to the Architects' Branch of the University Grants Committee they will undertake research into university building work on a larger scale. Only a few universities have yet made use of industrial building techniques, and the evidence submitted to the Committee suggested that the lack of research at national level to discover what systems are suited to different universities was the main factor. Other points to which the Committee directs attention are the tendency for savings to be in time and future maintenance rather than immediate costs and the impracticability of using any system to full advantage without a capital programme of several years. The fifth recommendation to the University Grants Committee is thus that the Committee should invite the National Building Agency and experienced contractors to pool all available information on industrial building techniques, and to combine with them to discover which systems are suitable for the different regions, categories and conditions of individual universities.

While, as already noted, the Estimates Committee's scrutiny did not extend to the scale of university expansion or to the purpose of a university education, it nevertheless gave the whole system a thorough examination, from which the University Grants Committee and the system as a whole emerge with much more credit than some critics have been disposed to allow. Its recommendations, if adopted, should strengthen the Committee and the system, where weaknesses have for some time been widely recognized, but there seems little reason to anticipate drastic modification of the Committee or the quinquennial system as a result of the further enquiries recommended. That is subject to the observance of the fundamental conditions on which the whole system rests. It is here that the Government's own action causes the gravest anxiety and the most serious doubts. University finance and university expansion are far too complex and technical for political prejudices to operate if waste of national resources is to be avoided and if the nation is to receive a due return from the large expenditure now involved. The scale of expansion and its rate must obviously be determined by political considerations, but those considerations must be limited to the extent of national resources available and to national needs in the light of other conflicting claims. They cannot be determined by considerations of party advantage.

Once the scale and rate of expansion are agreed the resources committed should be beyond interference. A policy of start-and-stop or even of go-slow destroys the whole basis of efficiency. It should be clear that the latest Government decision not only ignores the responsible advice of the Plowden Committee, but also the supporting

evidence adduced in the Estimates Committee's enquiry. It makes nonsense of at least three of the recommendations advanced for strengthening the present system and removing certain weaknesses which have been detrimental to full efficiency and led to certain waste of resources. Reasons advanced in the House of Commons debate on August 3 by Mr. R. E. Prentice are quite untenable in the light of this report, and to make exceptions in development areas makes nonsense of university policy. If the Government and the nation wish to expand the universities at a certain scale and rate they must will the means, even if it involves curtailing available resources elsewhere. If they are not prepared to will the means, the universities themselves must decide on the scale and rate of expansion which they can achieve without undermining either the standards of their work or the purpose of their education.

## THE CARNEGIE UNITED KINGDOM TRUST

### Welfare In Trust

A History of The Carnegie United Kingdom Trust 1913-1968. By W. Robertson. Pp. 282. (Dunfermline: The Carnegie United Kingdom Trust, 1964.) n.p.

THIS book was commissioned by the Carnegie United Kingdom Trust to commemorate its jubilee. It is not for sale but can be borrowed from any public library.

It is a faithful record of the way in which the Trust, founded by Andrew Carnegie in 1913, has been interpreted and administered by the original Trustees and their successors. Though the author has succeeded on the whole in presenting his subject against the background of changing social conditions, which was essential if the Trust is to be seen in perspective, his story is not always easy to follow. This is because it has not been consistently handled either chronologically or by subject. The variety of subject-matter and the length of the story may have made this unavoidable; but it is a drawback for the reader.

The Trust, with an original capital of ten million dollars, was less rich than some of the great American Trusts and indeed than some of those more recently formed in Great Britain, but it was the first of its kind in the United Kingdom and it has been able both to exert considerable influence on social policy and to make possible and encourage a very large number of individual schemes of varying size and importance.

Its activities have from the first been largely determined by two factors—the terms of the trust and the methods of grant allocation.

The terms of the Deed, "the improvement of the well-being of the masses of the people of Great Britain and Ireland", were sufficiently wide to allow the trustees to support a number of different kinds of project at any one time and also to change their overall policy to meet changing social needs and conditions.

The method of grant allocation adopted at the outset, and unchanged in essence since, has been to leave the initiation not only in decision making but even in planning almost exclusively in the hands of individual trustees rather than in those of a professional secretariat. The Trustees over the years have been well served by their secretaries so far as the collection of data and detailed management are concerned, but they do not appear, by and large, to have looked to them for a dynamic lead. Policy has not been thought out from first principles but has tended to grow step by step in a somewhat haphazard way dependent on the particular interests and strength of character of individual trustees and their friends. Projects that have been put up to the Trust have been examined, and their acceptance determined, in relation to the kind



of things that the Trustees happened to be supporting at the time, provided in a general way that they did not conflict too obviously with certain criteria that have remained constant: first that money should not be provided for what was a statutory obligation (and even here exceptions were made when a trust contribution was held, often rightly, to provide a necessary spur to statutory action), and secondly that money should only be given to projects which included some element of pioneering and experiment—and here too exceptions were made.

Thus much has depended on the interests and personalities of individual trustees, and these have undoubtedly been a remarkable and devoted band of men and women with a wide knowledge of educational and social problems. The projects which they have brought forward for consideration and which have won support have been varied in nature, in size and in importance, and any estimate of their relative value is bound to be largely subjective, since it is a matter of opinion whether it is better to spend money on, for example, libraries or youth hostels, child welfare or the handicapped. It is, however, possible to make some judgments regarding the success of the different projects, and this the author has attempted to do. While paying tribute to the large number of movements where the support of the Trust played an important part, such as libraries, rural development, help for the handicapped, he has not hesitated to mention less-successful ventures such as the Bureau of Current Affairs and the Land Settlement Scheme.

Success may be judged in relation both to the satisfactory outcome of the particular project and to the effect that project has on the development of social policy. A community centre or a youth hostel may be a success in itself for the opportunities it offers its members; but it will be a greater success if it shows the way for other centres and hostels which will serve a wider public. This the Trust has always recognized; but at the same time it has never lost sight of the value of supporting even quite small groups in their efforts on behalf of the community. Its support of pioneering work and of voluntary effort has been among its main contributions to social progress.

Whether or not the projects supported by the Trust have been successful in the double sense mentioned above has depended to a great extent on its co-operation with voluntary bodies which are in close touch with contemporary social needs and problems. Perhaps the most outstanding example of such co-operation has been that with the National Council of Social Service, particularly in the sphere of rural work. Started in the early days of Prof. W. G. S. Adams, this has continued to be a growing partnership of great value to both sides and to the causes which they sponsored. Village halls, of which more than two thousand have been assisted by the Trust, are the visible sign of work in the countryside which covers a wide variety of interests and has enriched the lives of countless country men and women. The Trust provided the money and the National Council the expertise—not only knowledge of how village life could best be enriched, but also detailed information connected with applications for grant.

*Welfare in Trust* is a worthy title for this record of fifty years' service to the community. Five million pounds have been distributed to good causes during this period and the Trustees have faithfully carried out the wishes of the founder. They have contributed to "the well-being of the masses" by supporting libraries, village halls, adult education, research, music and the arts, playing fields and youth work and by welfare schemes for family and community. They have made it possible for national organizations concerned with these things greatly to expand their work and have at the same time encouraged small groups and even individuals to pioneer and to experiment. Andrew Carnegie could well be proud of the extent and value of the work made possible by his foundation.

MARY MORRIS

## SPACE RESEARCH

### Space Research V

Proceedings of the Fifth International Space Science Symposium, Florence, May 12–16, 1964. Edited by D. G. King-Hele, P. Muller and G. Righini. Pp. xix+1248. (Amsterdam: North-Holland Publishing Company, 1965.) 300s.

THE annual symposium organized by the Committee for Space Research is an event of primary importance for all participants in space research. *Space Research V* is concerned with the proceedings of the fifth such international symposium. It contains papers dealing with the physical side of the subject only. Papers dealing with life sciences in relation to space research are to be published in another volume. Even with this limitation on its contents it is a tremendous tome. It is made up of 1,248 pages and contains 154 papers grouped under twelve headings. It weighs nearly 6 lb. and costs £15. It is virtually impossible to give anything approaching an adequate summary of its contents as the contributions range over many fields. Many of them are not directly related, other than through the fact that they refer to results obtained by the use of satellites, rockets or balloons. The twelve sections give some idea of the range of matter. They are: (1) "Interaction of Energetic Particles with the Atmosphere"; (2) "Ionospheric Processes and Anomalies"; (3) "Precipitation and Effects of High Energy Particles"; (4) "Radiation Belts"; (5) "Solar Radiation and Interplanetary Medium"; (6) "Ionosphere"; (7) "Atmospheric Structure and Composition"; (8) "Galactic X-ray Astronomy"; (9) "Tracking of Satellites"; (10) "Dynamics of Satellites"; (11) "IQSY Programmes and Results"; (12) "Upper Atmosphere Studies with Rockets and Satellites".

Most of the papers are in English, a few in French. The majority have been abstracted in Russian. We are informed in the editorial preface that there were more than 450 participants representing thirty-three countries. Of the contents it is difficult to say more than that they form an invaluable compilation of results both experimental and theoretical and give an excellent cross-section of work in progress at the time of the symposium. Most of the topics included are now well-established fields of activity, but a recent and very welcome newcomer to the scene is that labelled "Galactic X-ray Astronomy"—a tantalizingly short section of less than five pages made up of three papers in the form of abstracts. This is a subject likely to develop much in the years ahead and indeed has already made appreciable progress since the symposium was held.

One is filled with admiration for the way the editors have faced up very successfully to an obviously difficult task. The dividing line between some of the sections is rather shadowy and there is naturally in such cases some overlap of content, but on the whole they are fairly clearly defined and self-contained. While the contributions vary in importance, none is unimportant, and all groups of workers in the field of space research will surely find it necessary to possess, or have access to, a copy of the *Proceedings*. The very high price, however, is likely to deter many individual workers from buying it for themselves, and this raises the sole serious criticism of the book—one wonders whether it was necessary to reproduce the *Proceedings* on the scale in which they have been reproduced, or alternatively whether it was necessary for all the sections concerned with the physical sciences to be collected together in one volume. There must be many space research workers who would gladly have on their shelves copies of the sections concerned with their field of interest but who nevertheless cannot afford to pay the very high price demanded for the complete set of sections, and it occurs to me that if it could have been split up into two or three volumes each containing three or four cognate sections it would have met the needs of individuals



better. As it is, it seems that only research institutions and universities can afford to purchase it. While many of the papers are full-scale papers of twenty pages or more, others are much shorter, and some are very short and take the form of abstracts. In so much as it is typical of papers presented at conferences generally that they are published afterwards in modified form in other journals, one wonders whether as an alternative a good proportion of the papers reproduced in this set of *Proceedings* could not also have been reduced in size or even published as abstracts, so reducing both size and cost of the volume. All this sounds like carping criticism of a very important and excellent book, but it would seem that one consequence of this wholesale publication has been that very nearly a year elapsed between the holding of the symposium and the publication of its *Proceedings*. In fact, publication occurred only a short while before the sixth symposium was held in May 1965 so that shortly after publication a number of the papers were already superseded by papers presented at the sixth symposium.

The production of the book is up to the usual high standard of the North-Holland Publishing Company, and misprints, so far as I have been able to observe, are few in number and trivial in nature. E. A. STEWARDSON

## THE CONTEXT OF LIFE

### Handbook of Physiology

A Critical, Comprehensive Presentation of Physiological Knowledge and Concepts. Edited by D. B. Dill, E. F. Adolph and C. G. Wilber. Section 4: Adaptation to the Environment. Pp. ix+1056. (Washington, D.C.: American Physiological Society; London: Baillière, Tindall and Cox, Ltd., 1964.) 288s.

SEVERAL volumes of the *Handbook of Physiology* are now on the library shelves, if not permanently and irritatingly out on loan. Those earlier doubts as to whether it is still possible to produce a literature both so contemporary and so permanent as to deserve the rank of 'handbook' have largely faded into the background. Since few besides a reviewer will have the time or the occasion to study a volume of the *Handbook* in its entirety, and private possession will be exceptional, these volumes must be enjoyed in small doses and referred to time and again by many individuals. It is quite likely, then, that their physical lives will expire before the subject-matter has lost its relevance, accuracy and appeal. Surely few librarians would complain of that fate for these expensive, but not unreasonably expensive, volumes.

This volume on adaptation to the environment is essentially an American production. Although valuable contributions are made by authors from ten other countries with representatives from every continent, 46 of the 64 contributions emanate from laboratories in North America. The broad biological approach to the subject, and the depth of the appreciation of the biological significance of adaptation owe much to those exciting schools of physiological zoology which are particularly active and successful in the United States.

After three excellent opening chapters on perspectives concerning the history, theory and general properties of adaptation, which serve to determine the dimensions of the subject, subsequent contributors settle in to the immense task of filling in the details so far as they are known. Following a chapter on the cellular level of adaptation, the adaptive capacities of the nervous, muscular, special sensory, cutaneous, skeletal, cardiovascular, respiratory, digestive, excretory, osmoregulatory and thermoregulatory systems are considered in turn. There are chapters which consider the geophysical environment itself, and laboratory facilities for the provision of controlled experimental changes in the environment. The effects of cold, dry heat and humid heat on

arthropods, reptiles, birds, mammals and man are then discussed together with chapters on the hibernators and aestivators. The next group of papers considers the adaptations of animals in aquatic environments, and is followed by four chapters on animals in toxic environments which include the effects of insecticides on insects and why some individuals survive to breed resistant strains. The effects of polluted air, industrial chemicals and narcotic analgesics on man and other animals are also discussed. The effects of and responses to altitudes range from those of man permanently resident high on the Andes to those of man temporarily resident in a space capsule. Finally, there is a group of papers dealing with the adaptation of man to a miscellany of circumstances, both natural and not so natural, in which he may find himself, or in which he is wont to place himself. These include his responses to caloric deficiency, repeated injury, solar and ionizing radiations, motion and noise.

Life and the environment in which it thrives must be considered together once the physiologist concerns himself with the functions of whole and integrated organisms, or seeks to make comparisons between individuals or species. Gehlne (p. 259) says "In whatever region of the Earth they have settled, animals have adjusted their lives to the environment", and Thomas (p. 210) points out that "all truly physiological reactions of an organism to changes in its environment are basically adaptive in character". The realization that an organism is, in a sense, the product of its own environmental history has grown clearer over more than 2,000 years since Hippocrates recognized the role of adaptive factors in relation to health and disease. The properties common to most organisms such as the use of high-energy phosphate bonds for energy transfer, genetic coding by nucleotides, specificity of proteins as catalysts, and the selective permeability of cell surfaces, must have become established at the very dawn of life before the creation of a stable reproducing cell. All subsequent evolution in organizational complexity and in diversity of both form and function must exist because of the survival of chance mutations which afforded some advantage to the organism in relation to its environment. Adaptation to the environment, then, is no peripheral aspect of physiology of interest only to those who are concerned with the reactions of man and other animals when exposed to both natural and unnatural variations in ambient conditions. On the contrary, adaptation to the environment is the very *raison d'être* of the integrative complexities of all organisms.

This volume is concerned with two distinct levels at which adaptation can be considered. One relates to the genetically fixed characteristics which permit one species to occupy a particular ecological niche, or which increase the chances of survival of an individual in the face of existing hazards, or of a change in the environment which is otherwise disastrous to the species. These characteristics are at all levels of biological organization from the sub-cellular to that of gross morphology. The other is that complex of limited physiological and behavioural adjustments which all organisms can make in the face of limited changes in the environmental conditions, and referred to as 'acclimatizations'. Here, again, many tissues and systems may be involved, and the extent to which individuals can acclimatize may also be genetically determined.

Adaptation to the environment as the determining factor in evolution has depended essentially on mutations which affect the chances of survival of the individual up to the time of its own reproductive activity, since survival beyond this phase in life is of little or no evolutionary consequence. However, modern man now demands 100 per cent survival into adult life and seeks to give every individual the chance to procreate irrespective of genetic make-up. When man becomes the dominating interest in this volume, genetic differences between individuals become of less importance than the general ability of

most individuals to adjust themselves to changing environmental circumstances. By learning to control both the environment in which he lives, and the influence of the environment on himself, man has created an unparalleled change in the course of Nature.

"This was the law of the Yukon,  
that only the strong shall thrive,  
That surely the weak shall perish  
and only the fit survive".

While this shift in the principles and practice of adaptation to the environment becomes abundantly clear as one peruses this volume, there is, wisely perhaps, no direct comment on it, still less any speculation on its possible consequences.

The *Handbook of Physiology* is described as a "critical, comprehensive presentation of physiological knowledge and concepts". It is unlikely that any other volume of the *Handbook* will attempt to do just this for so vast and relatively unexplored a field, but this aspiration is largely achieved. One can only express admiration for a remarkable compilation. The contributors, in general, are quick to point out the inadequacy of their knowledge, and the young research worker who has not yet been persuaded to direct his whole attention to a more confined aspect of biological function will find the inspiration here for studies which could fill many lifetimes.

Each contributor has been given ample space to state his thesis, and the volume avoids that brittle economy of words which sometimes mars the pleasure of reading scientific literature. There are repetitions, here and there, of matters already discussed by earlier contributors, but no one should cavil at this since sections are referred to in isolation from each other. Each chapter should be, and is, self-contained.

Whatever the policy may be for other volumes in this series, this particular volume should be within the reach of every teacher and research worker in the field of biology.

J. BLIGH

## HUMAN GENETICS AND CYTOLOGY

### Les Chromosomes Humains

(Caryotype Normal et Variations Pathologiques.) Par Prof. Raymond Turpin et Prof. Jérôme Lejeune. Pp. viii+535. (Paris: Gauthier-Villars, 1965.) 54 francs.

### Human Genetics

Cold Spring Harbor Symposia on Quantitative Biology, Vol. XXIX. Pp. xiv+492. (Cold Spring Harbor, L.I., New York: The Laboratory, 1965.) 15.00 dollars.

A JOINT review of these two recent volumes can be justified by considering them as complementary. Both publications are mainly summaries of the work of many research workers during the past five years, but the book by Turpin and Lejeune, having only one additional contributor and a single subject-matter, the chromosomes, is more homogeneous and rounded in presentation than the symposium volume which contains 44 separate contributions by different authors.

*Les Chromosomes Humains* starts with a history of our knowledge of the human chromosomes, touches on the techniques for their investigation and then describes the normal human karyotypes. Three chapters deal with the numerical anomalies of autosomes and one chapter with the anomalies of their structure, followed by a chapter on chromosomal studies in leukaemias and cancer. Three further chapters are devoted to the numerical and structural aberrations of the sex chromosomes, and one is concerned with hermaphroditism and pseudo-hermaphroditism. Chromosome differences between twins and the mechanisms and effects of abnormal sex chromosomes are also separately treated. Finally some biochemical pecu-

liarities which are associated with chromosomal abnormalities are discussed by H. Jerome. The approach to most problems presented in this book is both cytological and clinical. The origins of mosaicism and the various hypothetical mechanisms which may result in abnormal karyotypes are discussed at some length, and it is probably from these parts of the book that some controversy may arise. There are numerous graphs, illustrations and tables. The bibliography covers publications well into 1964 and is invaluable as a source of information. The rapidity of discovery and development in this field is, however, such that already, a few months after the publication of the book, new important information is available which could not be included.

*Human Genetics* deals with three active fields of research, namely population studies, the genetics of somatic cells and cells in culture, and human proteins. In it, chromosome anomalies play only a minor part in the second section, which deals with cell culture. There the main emphasis is on the sex-linked genes, and the state of the X chromosome is discussed in conjunction with the Lyon hypothesis. Otherwise, this part is mostly concerned with cell selection, cell antigens and the localization of biochemical mutants. Interesting structural effects of irradiating chromosomes in cultures and of radiomimetic substances are reported, but the analysis of gene mutations by such means is still in its infancy. Other techniques may prove more successful in this field. The impression gained from reading this section of the symposium is one of rapid growth in separate specialized groups without, as yet, much regard to the possibilities of integration or generalization. On the other hand, the last section of the monograph, which deals with the genetics of human proteins, covers the considerably more homogeneous and well-established subject of the polymorphisms of haemoglobins, haptoglobins, gammaglobulins and certain enzymes as well as immunogenetics. There the mutual understanding of the several participants is also obvious from the quality of their discussions.

The first part of the book contains studies of many different kinds of human population; this subject seems to be in a state of flux so that a few contributions appear a little repetitive and perhaps tired, while others hint at novel approaches. General problems such as the effects of drift, of the genetical load, of lethal equivalents, polymorphism, and the effects of pleiotropism are once more discussed. Perhaps more rewarding are specific genetical studies on populations in Brazil, India, the American Indians as well as the 'Amish'. Most promising is the study of the nature and consequences of ethnic mixtures among the immigrant communities of Israel; the information summarized in this paper will provide an important and unique base line for future research. A lively contribution by Gajdusek describes some very unexpected consequences of the highly lethal Kuru disease in New Guinea which has led to a considerable numerical increase in the affected Fore population. His vivid account of other vagaries of 'selection' and the general unpredictability of events which control the genetical fate of small primitive people provides a most impressive and healthy contrast to the traditional unquestioning assumptions of gene equilibria, or at best the slow action of persistent selective forces in all circumstances. These have operated in the large populations of modern man, and some of their effects are described by the other contributors.

In the framework of a short review it is unfortunately impossible to do justice to all the contributions in the symposium, nor is it possible to offer detailed criticism. While most papers individually come up to the high standards of the Cold Spring Harbor Symposia, it appears to me that as a synthesis this particular volume does not reach the excellence of its best predecessors. This is borne out by the last concluding chapter, where Bentley Glass more or less despairs of summarizing the heterogeneous subject-matter.

H. KALMUS

**Egypt to the End of the Old Kingdom**

By Cyril Aldred. (The Library of Early Civilizations.) Pp. 143 (illustrations). (London: Thames and Hudson, 1965.) 30s.

**Early Mesopotamia and Iran**

By M. E. L. Mallowan. (The Library of Early Civilizations.) Pp. 142 (illustrations). (London: Thames and Hudson, 1965.) 30s.

**B**OTH these authors are front rank authorities on their subjects, and both are able to write clearly and simply. In neither case is it the intention to offer a book for the specialist; what is intended is to provide a short, clear, up-to-date account of the story of both these regions, so vital to the development of human civilization. Both volumes are profusely illustrated, not a few examples being in colour.

The Egypt volume starts with a detailed chronology and a map. Chapter one deals with the beginnings of human settlement, and rightly mentions finds of early palaeolithic date. An actual date of 200,000 years ago can only be guess-work; they may well be older! The change-over from hunting to farming is next dealt with. This was one of the great stages in human development. For the first time community life was possible and village life evolved. There follows a brief account of the early pre-dynastic period, mention being made of such early cultures as those from the Fayum, Merimde and Badari. Illustrations of the typical tools and pottery are given. In the same way the later predynastic period is dealt with in the next chapter, and following this there is an account of the transition to the Dynastic Age. The Dynasties I-VI next pass under review, and there is a concluding chapter on the sculpture of the Old Kingdom. For anyone wishing to know the essentials of early Egyptian archaeology, and what has been unearthed, this book can be strongly recommended.

Much of what has been said about Cyril Aldred's book on Egypt can be said about M. E. L. Mallowan's *Early Mesopotamia and Iran*. Once again the volume is profusely illustrated, not infrequently in colour. There are chronological charts and a map. The first chapter deals with the urban development in Uruk and Iran somewhere round about 3500 B.C. Many examples of the painted pottery from this early period are indeed lovely. We pass on to an account of the temples in the plains and their associated finds and sculptures. At this time we can note the invention of writing, which has had so profound an influence on civilisation. There is an interesting account of the daily life at Uruk. Next we pass to the early Sumerians and their culture, followed by the early dynastic period when we note a considerable improvement in architecture and ornaments of all kinds. For example, the well-known Royal Standard of Ur, which illustrates the activities of the King and his court both in peace and in war, is an object of great beauty. Some of the sculptures, too, show an amazing control over the medium when the early date is remembered. Once again this is a volume to be read by anyone wishing to know something of the fundamentals of the early civilizations in Mesopotamia, an area which has played such an important part in the growth of civilization as we know it to-day.

M. C. BURKITT

**Tensors in Mechanics and Elasticity**

By Prof. Leon Brillouin. Translated from the French by Robert O. Brennan, S.J. (Engineering Physics: an International Series of Monographs, Vol. 2.) Pp. xviii + 478. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd. 1964.) 89s. 6d.

**TENSORS in Mechanics and Elasticity** is a translation from the French of the 1938 edition of Prof. L. Brillouin's well-known text-book. It is, of course, a classical book in this field and nowadays forms an excellent introductory text. Although, to-day, there are many

text-books (in English) which describe the use of tensors in physics in great detail, there is still room for the broad introductory treatment. The clarity of Prof. Brillouin's writing has been carried through the translation. On the whole the text is given in good clear English, although there are places where it is slightly stylized.

Unfortunately it is inevitable that this book is dated both in the subject material and in the reference list, neither of which has been brought up to date during the translation. However, because of the basic work covered, this is not too great a disadvantage. The material covered runs from a description of vector geometry, affine geometry and Riemann space to wave mechanics and elastic waves in solids. The idea of a tensor is introduced before proceeding to a discussion of the role of tensors in physics. The reader is led through the mathematical analysis step by step. This part of the book occupies the first seven chapters. A discussion of problems in mechanics and elasticity then fills the remaining five chapters. This discussion demonstrates how tensors form a natural basis for, and so simplify, the treatment of these subjects. Altogether it is a useful addition to the English booklist.

The production is good and the print is clear and easy to read; unfortunately this seems to have kept the price high.

P. A. EGGLESTAFF

**Radioactive Isotopes in Instrumentation and Control** By N. N. Shumilovskii and L. V. Mel'tser. Translated by R. F. Kelleher. Translation edited by P. J. Blaetius and G. A. Young. (International Series of Monographs on Nuclear Energy. Division 4: Isotopes and Radiation, Vol. 3.) Pp. xiv + 198. (London and New York: Pergamon Press, 1964.) 70s. net.

**U**NTIL recently the application of radioactive isotopes to control engineering was mainly confined to a few simple techniques such as the use of tracers, and the measurement of thickness or level. At present there is an increasing interest in more sophisticated techniques which permit the measurement of fluid flow, pressure, humidity, and chemical composition. Descriptions of single techniques, or brief reviews of specialized applications, are scattered in the literature, making the subject difficult to include in formal studies. The present monograph, by authors who have an international reputation in the field, not only rectifies this but will also probably do much to stimulate the imagination of instrument designers and manufacturers.

The introduction, which occupies a quarter of the book, is unsatisfactory since it assumes no previous knowledge and yet attempts too briefly to cover nuclear physics and statistics up to the point of practical application. However, for a reader with previous knowledge of conventional nuclear instrumentation the introduction may prove useful for its formulae, arranged in convenient form for numerical interpretation.

Other chapters are concerned with measurements of thickness and density by absorption and backscattering methods, the measurement of level and flow of liquids, and the measurement of flow and pressure of gases. There is also a chapter on composition control by a variety of interesting techniques, including ionization methods, absorption and scattering, radiation spectrometry, and neutron activation. Two chapters are devoted to system analysis. One of them gives a most useful analysis enabling the radioactivity of the source to be minimized for a particular dynamic response. The other gives an analysis of electromagnetic relay operation.

The misprints are few, and most of them are obvious. However, a few could be misleading to a reader without previous nuclear knowledge (for example, "neutron" for "neutrino" on p. 3). The uncustomary symbols in the equations were found stimulating rather than annoying, and the book can be recommended for the inspiration it will give to instrument designers in industry.

B. M. WHITLEY

## A BIOLOGICAL RETROSPECT\*

By SIR PETER MEDAWAR, F.R.S.

Director, National Institute for Medical Research

THE title of my presidential address, you will have discerned, is "A Biological Retrospect", and on the whole it has not been well received. 'Why a biological retrospect?', I have been asked; would it not be more in keeping with the spirit of the occasion if I were to speak of the future of biology rather than of its past? It would indeed be, if only it were possible, but unfortunately it is not. What we want to know about the science of the future is the content and character of new scientific theories and ideas. Unfortunately, it is impossible to predict new ideas—the ideas people are going to have in ten years' or in ten minutes' time—and we are caught in a logical paradox the moment we try to do so. For to predict an idea is to have an idea, and if we have an idea it can no longer be the subject of a prediction. Try completing the sentence 'I predict that at the next meeting of the British Association someone will propound the following new theory of the relationships of elementary particles, namely . . .'. If I complete the sentence, the theory will not be new next year; if I fail, then I am not making a prediction.

Most people feel more confident in denying that certain things will come to pass than in declaring that they can or will do so. Many a golden opportunity to remain silent has been squandered by anti-prophets who do not realize that the grounds for declaring something impossible or inconceivable may be undermined by new ideas which cannot be foreseen. Here is an instructive passage from the philosophic writings of a great British physiologist, J. S. Haldane (father of J. B. S.). It comes from *The Philosophy of a Biologist* of 1931, and its subject is the nature of memory in a very general sense that includes 'genetic memory', for example, the faculty or endowment which ensures that a frog's egg develops into a frog and, indeed, into a particular kind of frog.

Haldane is very critical of the theories of memory propounded by Ewald Hering and Richard Semon, who "assume that memory in general is dependent on protoplasmic 'engrams', and that germ-cells are furnished with a system of engrams, functioning as guide-posts to all the normal stages of development". ('Engrams', I should explain, are more or less permanent physical memory traces or memory imprints that act as directive agencies in development<sup>1</sup>). "This theory", Haldane goes on to say, "has quite evidently all the defects of other attempts at mechanistic explanations of development. How such an amazingly complicated system of signposts could function by any physico-chemical process or reproduce itself indefinitely often is inconceivable<sup>2</sup>."

What Haldane found himself unable even to conceive is to-day a commonplace. Only twelve years after the publication of the passage I quote, Avery and his colleagues had determined the class of chemical compound to which genetic engrams belong. In the meantime our entire conception of 'the gene' has undergone a revolution. Genes are not, as at one time or another people have thought them, samples or models; they are not enzymes or hormones or prosthetic groups or catalysts or, in the ordinary sense, agents of any kind. Genes are messages. I think Kalmus<sup>3</sup> was the first to use this form of words, but the idea that a chromosome is a molecular code

script containing a specification of development is Schrödinger's<sup>4</sup>.

My purpose in this address is to identify some of the great conceptual advances that have taken place during the past twenty-five or thirty years on four different planes of biological analysis. As I have pointed out elsewhere<sup>5</sup>, working biologists tend nowadays to classify themselves less by 'subjects' than by the analytical levels at which they work—a horizontal classification where the older was vertical. So we have molecular biologists, whose ambition is to interpret biological performances explicitly in terms of molecular structure; we have cellular biologists, biologists who work at the level of whole organisms (the domain of classical physiology), and biologists who study communities or societies of organisms. We can discern each of these four strata within each 'subject' of the traditional, that is, the vertical, classification. There are molecular and cellular geneticists, geneticists in Mendel's sense, and population geneticists. So also in endocrinology or immunology: each is now studied at the molecular and cellular level as well as at the level of whole organisms. They abut into the population level, too: we study the effects of crowding and fighting on the adrenals and so indirectly on reproductive performance, and we study the epidemiological consequences of natural or artificial immunization and the evolutionary consequences of epidemics. I have noticed that a biologist's interests and understanding, and also, in a curious way, his loyalties, tend to spread horizontally, along strata, rather than up and down. Our instinct is to try to master what belongs to our chosen plane of analysis and to leave to others the research that belongs above that level or below. An ecologist in the modern style, a man working to understand the agencies that govern the structure of natural populations in space and time, needs much more than a knowledge of natural history and a map. He must have a good understanding of population genetics and population dynamics generally, and certainly of animal behaviour; more than that, he must grasp climatic physiology and have a feeling for whatever may concern him among the other conventional disciplines in biology (I have already mentioned immunology and endocrinology). There is no compelling reason why he should be able to talk with relaxed fluency about messenger-RNA, and it is not essential that he should ever have heard of it—though an unreasonable feeling that he 'ought' to know something about it is more likely to be found in a good ecologist than in an indifferent one.

I shall now take one example from each of these four planes of biological analysis and try to show how our ideas have changed since the last Cambridge meeting of the British Association in 1938—a period that corresponds roughly with my own professional lifetime.

## Population Genetics and Evolution Theory

Biologists of my generation were still brought up in what I call the 'dynastic' concept of evolution. The course of evolution was unfolded to us in the form of pedigrees or family trees, and we used the old language of universals in speaking of the evolution of *the* dogfish, *the* horse, *the* elephant and, needless to say, of Man.

The dynastic conception coloured our thoughts long after the revival of Darwinism had made it altogether

\* Presidential address delivered to Section D (Zoology) on September 2, 1965, at the Cambridge Meeting of the British Association for the Advancement of Science.

inappropriate. By the 'revival of Darwinism' I mean the reformulation of Darwinism in the language of Mendelian genetics—the work, as we all so very well know, of Fisher, J. B. S. Haldane, Wright, Norton and, in a rather qualified sense, of Lotka and Volterra. The subject of evolutionary change, we now learned, was a population, not a lineage or pedigree: evolution was a systematic secular change in the genetical structure of a population, and natural selection was overwhelmingly its most important agent. But to those brought up in the dynastic style of thinking about evolution it seemed only natural to suppose that the outcome of an evolutionary episode was the devising of a new genotype—of that new genetical formula which conferred the greatest degree of adaptedness in the prevailing circumstances. This improved genetic formula, a new solution of the problem of remaining alive in a hostile environment, would be shared by the great majority of the members of the population, and would be stable except in so far as it might be modified by further evolution. The members of the population were predominantly uniform and homozygous in genetic make-up, and, to whatever degree they were so, would necessarily breed true. Genetic diversity was maintained by an undercurrent of mutation, but most mutants upset the hard-won formula for adaptedness and natural selection forced them into recessive expression, where they could do little harm. When evolution was not in progress natural selection made on the whole for uniformity. Polymorphism, the occurrence of a stable pattern of genetic inequality, was recognized as an interesting but somewhat unusual phenomenon, each example of which required a special explanation, that is, an explanation peculiar to itself.

These ideas have now been superseded, mainly through the empirical discovery that natural populations are highly diverse. Chemical polymorphism (allotypy\*) is found wherever it is looked for intently enough by methods competent to reveal it. The molecular variants known in human blood alone provide combinations that far outnumber the human race—variants of haemoglobin, non-haemoglobin proteins, and red-cell enzymes; of red-cell antigens and white-cell antigens; and of haptoglobins, transferrins and gamma globulins. To-day it is no longer possible to think of the evolutionary process as the formulation of a new genotype or the inauguration of a new type of organism enjoying the possession of that formula. The 'product' of evolution is itself a population—a population with a certain newly devised and well adapted pattern of genetic inequality. This pattern of genetic differentiation is determined and actively maintained by selective forces: it is the population as a whole that breeds true, not its individual members. We can no longer draw a distinction between an active process of evolution and a more or less stationary end-product: evolution is constantly in progress, and the genetical structure of a population is actively, that is dynamically, sustained.

These newer ideas have important practical consequences. The older outlook was embodied in that older, almost immemorial ambition of the livestock breeder, to produce by artificial selection a true breeding stock with uniform, and uniformly desirable characteristics; and this was also the ambition—sometimes kindly, but always mistaken—of old-fashioned 'positive' eugenics. It now seems doubtful if, with free-living and naturally outbreeding organisms, such a goal can ever be achieved. Modern stockbreeders tend to adopt a very nicely calculated regimen of cross-breeding which, abandoning the goal of a single self-perpetuating stock, achieves a uniform marketable product of hybrid composition. The genetical theory underlying this scheme of breeding embodies, and was indeed partly responsible for, the newer ideas of population structure I have just outlined.

I cannot predict what new ideas will illuminate the theory of evolution in future, but it is not difficult to

guess the contexts of thought in which they are likely to appear. The main weakness of modern evolutionary theory is its lack of a fully worked out theory of variation, that is, of *candidature* for evolution, of the forms in which genetic variants are proffered for selection. We have therefore no convincing account of evolutionary progress—of the otherwise inexplicable tendency of organisms to adopt ever more complicated solutions of the problems of remaining alive. This is a 'molecular' problem, in the newer biological usage of that word, because its working out depends on a deeper understanding of how the physicochemical properties and behaviour of chromosomes and nucleoproteins generally qualify them to enrich the candidature for evolution; and this reflection is my cue to turn to conceptual advances in biology at the molecular level.

### Physical Basis of Life

In the early 1930's no one knew what to make of the nucleic acids. Bawden and Pirie had not yet shown that nucleic acid was an integral part of the structure of tobacco mosaic virus, and we were still a decade from the astonishing discovery by Avery and his colleagues, in the Rockefeller Institute, that the agent responsible for pneumococcal transformations was a deoxyribonucleic acid.

Since there was nothing very much to say about nucleic acids you may well wonder what everybody *did* talk about. One topic of conversation was the crystallization of enzymes. Sumner had crystallized urease in 1926 and Northrop pepsin in 1930; soon Stanley would crystallize tobacco mosaic virus, at that time still thought to be a pure protein. But the most exciting and, as it seemed to us, portentous discoveries were those of W. T. Astbury, whose X-ray diffraction pictures of silk fibroin and hair and feather keratins had revealed an essentially crystalline orderliness in ordinary biological structures. For some purposes, however, X-ray analysis was too powerful. The occasion called for resolving powers between those of the optical microscope and the X-ray tube, and this need was fulfilled by electron microscopy. I saw my first electron-photomicrograph in *Nature* in 1933; its resolving power was then one micron.

Electron microscopy has shown that cells contain sheets, tubes, bags and, indeed, micro-organs—real anatomical structures in the sense that they have firm and definite shapes and look as if only their size prevented our picking them up and handling them. Moreover, there is no dividing line between structures in the molecular and in the anatomical sense: macromolecules have structures in a sense intelligible to the anatomist and small anatomical structures are molecular in a sense intelligible to the chemist. (Intelligible *now*, I should add: as Pirie<sup>1</sup> has told us, the idea that molecules have literally, that is, spatially, a structure was resisted by orthodox chemists, and the credentials of molecules with weights above 5,000 were long in doubt.) In short, the orderliness of cells is a structural or crystalline orderliness—a 'solid' orderliness, indeed, for 'the so-called amorphous solids are either not really amorphous or not really solid'<sup>4</sup>.

This newer conception represents a genuine upheaval of biological thought, and it marks the disappearance of what may be called the *colloidal* conception of vital organization, itself a sophisticated variant of the older doctrine of 'protoplasm'. The idea of protoplasm as a fragile colloidal slime, a sort of biological ether permeating otherwise inanimate structures, was already obsolete in the 'thirties; even then no one could profess to be studying 'protoplasm' without being thought facetious or slightly mad. But we still clung to the colloidal conception in its more sophisticated versions, which allowed for heterogeneity and for the existence of liquid crystalline states, and it was still possible to applaud Hopkins's famous aphorism from the British Association meeting of 1913,

that the life of the cell is "the expression of a particular dynamic equilibrium in a polyphasic system". For inadequate though the colloidal conception was seen to be, there was nothing to take its place. Peters's idea of the existence of a 'cytoskeleton' to account for the orderly unfolding of cellular metabolism in time and place now seems wonderfully prescient, but there was precious little direct evidence for the existence of anything of the kind, and much that seemed incompatible with it.

The substitution of the structural for the colloidal conception of 'the physical basis of life' was one of the great revolutions of modern biology; but it was a quiet revolution, for no one opposed it, and for that reason, I suppose, no one thought to read a funeral oration over protoplasm itself.

### Cellular Differentiation in Embryonic Development

Embryology is in some ways a model science. It has always been distinguished by the exactitude, even punctilio, of its anatomical descriptions. An experiment by one of the grand masters of embryology could be made the text of a discourse on scientific method. But something is wrong; or has been wrong. There is no *theory* of development, in the sense in which Mendelism is a theory that accounts for the results of breeding experiments. There has therefore been little sense of progression or timeliness about embryological research. Of many papers delivered at embryological meetings, however good they may be in themselves—in themselves they are sometimes marvels of analysis, and complete and satisfying within their own limits—one too often feels that they might have been delivered five years beforehand without making anyone much the wiser, or deferred for five years without making anyone conscious of great loss.

It was not always so. In the 1930's experimental embryology had much the same appeal as molecular biology has to-day: students felt it to be the most rapidly advancing front of biological research. This was partly due to the work of Vogt, who had shown that the mobilization and deployment of cellular envelopes, tubes and sheets was the fundamental stratagem of early vertebrate development (thus relaying the foundations of comparative vertebrate embryology); but it was mainly due to the 'organizer theory' of Hans Spemann, the theory that differentiation in development is the outcome of an orderly sequence of specific inductive stimuli. The underlying assumption of the theory (though not then so expressed) was that we should look to the chemical properties of the inductive agent to find out why the amino-acid sequence of one enzyme or organ-specific protein should differ from the amino-acid sequence of another. The reactive capabilities of the responding tissue were emphasized repeatedly, but only at a theoretical level, for 'competence' did not lend itself to experimental analysis, and the centre of gravity of actual research lay in the chemical definition of inductive agents.

Wise after the event, we can now see that embryology simply did not have, and could not have created, the background of genetical reasoning which would have made it possible to formulate a theory of development. It is not now generally believed that a stimulus external to the system on which it acts can specify the primary structure of a protein, that is, convey instructions that amino-acids shall be assembled in a given order. The 'instructive' stimulus has gone the way of the philosopher's stone, an agent dimly akin to it in certain ways. Embryonic development at the level of molecular differentiation must therefore be an unfolding of pre-existing capabilities, an acting-out of genetically encoded instructions; the inductive stimulus is the agent that selects or activates one set of instructions rather than another. It is just possible to see how something of the kind happens in the induction of adaptive enzymes in bacteria—a

phenomenon of which the older description, the 'training' of bacteria, reminds us that it too, at one time, was thought to be 'instructive' in nature. All this applies only to biological order at the level of the amino-acid sequences of proteins or the nucleotide sequences of nucleic acids. Nothing is yet known about the genetic specification of order at levels above the molecular level.

The function performed by the hierarchy of inductive stimuli as it occurs in vertebrate development is to determine the specificities of time and place: it is an inductive stimulus which determines that a lens shall form just here and just now—not elsewhere, and at no other time. As I see it, it is the inductive process that allows vertebrate eggs and embryos before gastrulation to indulge in the prodigious range of adaptive radiation to be seen in germs as disparate as a dogfish's egg and a human being's—a case I have argued elsewhere and need not go over here again\*.

### Biology of the Organism: Animal Behaviour

If experimental embryology was the subject that seemed most exciting to students of the 'thirties, that most nearly on the threshold of a grand revelation, the study of animal behaviour (in the sense in which we now tend to use the word 'ethology'), seemed just as clearly the most frustrating and unrewarding. Twenty years later it was the other way about: embryology had lost much of its fascination and many of the ablest students were recruited into research on behaviour instead. What had happened in the meantime?

In the early 1930's we had one new behavioural concept to ponder on: the idea that an animal might in some way apprehend a sensory pattern or a behavioural situation as a whole and not by a piecing together of its sensory or motor parts. That was the lesson of *Gestalt* theory. We had also learnt finally, and I hope for ever, the methodological lesson of behaviourism: that statements about what an animal feels or is conscious of, and what its motives are, belong to an altogether different class from statements about what it does and how it acts. I say the 'methodological' lesson of behaviourism, because that word also stands for a certain psychological theory, namely, that the phenomenology of behaviour is the whole of behaviour—a theory of which I shall only say that, in my opinion, it is not nearly as silly as it sounds. Even the methodology of behaviourism seemed cruelly austere to a generation not yet weaned from the doctrine of privileged insight through introspection. But what comparable revolution of thought ushered in the study of animal behaviour in the style of Lorenz and Tinbergen and led to the foundation of flourishing schools of behaviour in Oxford, here in Cambridge, and throughout the world?

I believe the following extremely simple answer to be the right one. In the 'thirties it did not seem to us that there was any way of studying behaviour 'scientifically' except through some kind of experimental intervention—except by confronting the subject of our observations with a 'situation' or with a nicely contrived stimulus and then recording what the animal did. The situation would then be varied in some way that seemed appropriate, whereupon the animal's behaviour would also vary. Even poking an animal would surely be better than just looking at it: that would lead to anecdotalism: that was what bird-watchers did.

Yet it was also what the pioneers of ethology did. They studied natural behaviour instead of contrived behaviour, and were thus able for the first time to discern natural behaviour structures or episodes—a style of analysis helped very greatly by the comparative approach, for the occurrence of the same or similar behavioural sequences in members of related species reinforced the idea that there was a certain natural connectedness between its various terms, as if they represented the



playing out of a certain instinctual programme. Then, and only then, was it possible to start to obtain significant information from the study of contrived behaviour—from the application or withholding of stimuli—for it is not informative to study variations in behaviour unless we know beforehand the norm from which the variants depart.

The form of address 'project and development' belongs to the four levels of hierarchy in the service of the major dev- In the recent growth of 'beliefs' belonging to the 'no opportunity' of modern 'of bodily con- of the material' anything except 'functions of' ough of the 'some morals, 'behaviour—in par- 'mental approach—' 'ments in the Baconian' of contriving 'experiences' 'our general store of empirical' to sustain or confute a specific 'opposition. The history of embryology' 'ars of an imagined self-sufficiency, for' 'an inviolable fragment of knowledge without' 'often wonder what academics mean when

they say of a certain subject that it is a 'disc own right'; for what science is entire of its may think our recent history entitles us to pleased with ourselves. Perhaps: but then we pleased with ourselves twenty-five years in twenty-five years time people will look back wonder at our obtuseness. However, if compl be deplored, so also is humility. Humility is of mind conducive to the advancement of le one formula will satisfy that purpose, for the kind of scientist; but a certain mixture of cor restless dissatisfaction will be an ingredien formulae. Confident we may surely be, fo twenty-five years will throw up several ne profound and astonishing as any I have ye namely . . . but I have no space left to tell you are.

<sup>1</sup>See *The Mimesis* by R. Semon, London, 1921, particularly p 211; and Hering's paper "On Memory, a Universal Aesthetic Matter" in *Ann. Acad. Wiss. Wien.*, 20, 253 (1870).

<sup>2</sup>*The Philosophy of a Biologist*, 162 (London, 1931).

<sup>3</sup>"A Cybernetical Aspect of Genetics", *J. Hered.*, 41, 19 (1950).

<sup>4</sup>Schrödinger, E., *What is Life?*, especially pp. 19-20, 61-62, 1944). For Weymann's far-sighted views on the matter, *Lectures of Matter* by Toulmin, S., and Goodfield, J. (London).

<sup>5</sup>In my Tizard Memorial Lecture, *Encounter* (August 1965).

<sup>6</sup>A term coined by J. Oudin to describe  $\gamma$ -globulin variants: I generalized to include all molecular polymorphism.

<sup>7</sup>Pirie, K. W., "Patterns of Assumption about Large Mo *Biochem. Biophys.*, Suppl. 1, 21 (1962).

<sup>8</sup>*J. Embryol. Exp. Morph.*, 8, 172 (1964).

## BONE METABOLISM INFERRED FROM FALL-OUT INVESTIGATION

By J. RIVERA

Health and Safety Laboratory, U.S. Atomic Energy Commission, New York

From nuclear weapons tests has contaminated human diet and resulted in the accumulation of  $^{90}\text{Sr}$  in the skeleton. Measurements of  $^{90}\text{Sr}$  levels in the diet made over several years enable us to draw inferences as to the level of strontium and to predict changes in concentrations resulting from changes in diet variations.

Monthly measurements of the  $^{90}\text{Sr}$  content of food in the New York City area have been made at the Health and Safety Laboratory. In addition to measurements, starting in 1960 analyses for  $^{90}\text{Sr}$  in other composite foods purchased in New York City, such as meat, canned vegetables, etc., have been made at this laboratory<sup>1</sup>. Based on the results of more than 400 analyses, estimates have been made of the yearly  $^{90}\text{Sr}$  contamination of the diet in New York since 1954. These estimates are shown in Fig. 1. The  $^{90}\text{Sr}$  concentration of bone due to dietary  $^{90}\text{Sr}$  levels can be expected, since each year of bone mineral is replaced by minerals from the diet. In adult bone there is essentially no net loss or diminution in bone, hence the amount of  $^{90}\text{Sr}$  at each year must be equal to that gained. As the total calcium in bone does not change from year to year in adults, an equation expressing the changes in concentrations in bone with changes in  $^{90}\text{Sr}$  intake

$$B_n = 0.975B_{n-1} - f \cdot 0.975B_{n-1} + f K Z_{n-1} \quad (1)$$

is the  $^{90}\text{Sr}/\text{Ca}$  ratio of bone at the beginning of year 'n',  $B_{n-1}$  is the  $^{90}\text{Sr}/\text{Ca}$  ratio of the bone at the beginning of year 'n-1',  $f$  is the fraction of the bone turned over during the year 'n-1',  $K$  is the ratio of  $^{90}\text{Sr}/\text{Ca}$  in the bone to that in the diet (observed ratio)<sup>2</sup>,  $Z_{n-1}$  is the  $^{90}\text{Sr}/\text{Ca}$  ratio of the diet during year 'n-1'. The factor 0.975 is introduced to account for the radioactive decay of  $^{90}\text{Sr}$  in one year.

Various investigations have shown that the observed ratio ( $K$ ) for adults is about 0.25 (ref. 3). Rates of turn-over rates for different bones have been made. Liniecki<sup>4</sup>, using the data of Kulp and his co-workers<sup>5</sup>, found that the  $^{90}\text{Sr}$  concentrations in total adult skeleton in New York City (1954-58), estimated the turn-over rate of the skeleton to be about 3.5 per cent per annum by the method which is independent of the observed rate. Bryant and Loutit<sup>6</sup> have estimated that the rate of vertebrae is about 8 per cent per annum and the mid-shafts of femora is about 1.5 per cent per annum. Using the same method, involving specific activity ( $^{90}\text{Sr}/\text{mg Sr}$ ) measurements in diet and bone, we have found substantially these same turn-over rates for vertebrae and femur shafts<sup>7</sup>.

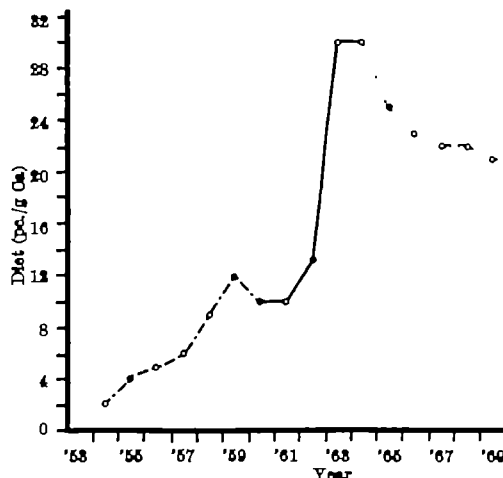


Fig. 1. The yearly strontium-90 content of the diet in New York. —, Estimated; —, measured; . . . , predicted.



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With the co-operation of the New York Medical Examiners Office, this laboratory has obtained over the past four years for  $^{87}\text{Sr}$  analyses more than 400 specimens of human vertebrae from victims of accidents. In addition, about 60 specimens of long bone shafts from osteogenic sarcoma victims obtained through the co-operation of Dr. H. Q. Woodard of the Sloan Kettering Institute were analysed for  $^{87}\text{Sr}$  (ref. 8). The results of the analyses of these bone specimens provide data to evaluate the accuracy of previously estimated bone turn-over rates.

Equation 1 was used to calculate  $^{87}\text{Sr}$  concentrations in vertebrae ( $V$ ) and femur shafts ( $F$ ) assuming the turn-over rates for these bones were 8 and 1.5 per cent per annum respectively. In addition, calculations of the  $^{87}\text{Sr}$  concentrations in the skeleton ( $S$ ) for New York City residents using a turn-over rate of 3.5 per cent per annum were made for the period 1954-64. The results of these calculations together with observed values are shown in Table 1.

Table 1. CALCULATED AND OBSERVED PROPORTIONS OF STRONTIUM-90 PER GRAM OF CALCIUM IN VERTEBRAE, FEMUR SHAFTS, AND SKELETONS OF NEW YORK CITY ADULTS

Year (a)	Diet ( $\bar{D}$ )	Vertebrae ( $V$ )		Femur shafts ( $F$ )		Skeletons ( $S$ )		
		Calc.	Obs.	Calc.	Obs.	Calc.*	Calc.	Obs.
1954	2	0.02		0		0.01	0.01	0.02
1955	4	0.03		0.02		0.04	0.04	0.04
1956	5	0.17		0.04		0.08	0.07	0.06
1957	6	0.25		0.06		0.12	0.12	0.10
1958	9	0.39	0.22	0.08	0.07	0.17	0.18	0.15
1959	12	0.56		0.12		0.25	0.25	0.19
1960	10	0.82		0.16		0.36	0.35	
1961	10	1.19	0.83	0.19		0.49	0.43	0.43†
1962	13	1.45	1.00	0.22	0.23	0.59	0.49	0.50†
1963	20	1.75	1.55	0.30	0.31	0.73	0.65	0.73†
1964	30	2.15	2.02	0.41	0.36	0.93	0.69	1.01†

\* Calculated using equation 1.

† Calculated from  $V$  observed divided by 2.

The agreement between calculated and observed results indicates that equation 1 can be used to predict changes in  $^{87}\text{Sr}$  concentrations in bone and that the turn-over rates of vertebrae, femur shafts, and whole skeletons previously estimated are quite reasonable.

However, equation 1 implies that there is a single exponential law of excretion of strontium from bone. Since the skeleton comprises bones of different turn-over rates the assumption of a single turn-over rate for it must be only approximately valid.

From the work of Bryant and Loutit it would appear that the skeletal components with fastest and slowest turn-over rates are vertebrae and femur shafts respectively. It seemed reasonable, therefore, to attempt to describe the excretion of strontium from the skeleton as a combination of excretion from a vertebrae-like component and a femur-shaft-like component.

If  $W$  is the total amount of mineral in the skeleton,  $X$  is the amount of mineral in the vertebrae-like component, and  $Y$  the amount in the femur-shaft-like component, then:

$$W = X + Y$$

The amounts of mineral excreted each year from each component are  $f$  times the amount initially present. Hence:

$$0.035 W = 0.080 X + 0.015 Y$$

Solving these equations simultaneously, we find:

$$X/W = 0.3 \quad Y/W = 0.7$$

The skeleton can, therefore, be thought of as consisting of three-tenths vertebrae-like bone and seven-tenths femur-shaft-like bone. If this is the case, then:

$$S = 0.3 V + 0.7 F \quad (2)$$

where  $S$  is the  $^{87}\text{Sr}/\text{Ca}$  ratio of the skeleton and  $V$  and  $F$  are the  $^{87}\text{Sr}/\text{Ca}$  ratios of vertebrae and femur shafts, respectively. Calculations of  $S$  using equation 2 are listed in Table 1.

By comparing observed and calculated results in Table 1 it is evident that equation 1 has adequately described the

variation of vertebrae, femur shaft and skeletal  $^{87}\text{Sr}$  concentrations with time in the past and, therefore, probably provides a basis for predicting future  $^{87}\text{Sr}$  concentration in these bones. The skeletal  $^{87}\text{Sr}$  concentrations calculated by using equation 1 or 2 are similar. But, as can be seen, this similarity does not persist. The ratios of  $^{87}\text{Sr}$  concentrations in vertebrae to total skeleton have been about two and those of femur shafts to total skeletons have been about one-half. These ratios have remained remarkably constant. As Lindecki has indicated, these ratios should change only very slowly with time and, therefore, future estimates of skeletal  $^{87}\text{Sr}$  concentrations can be made by determining the  $^{87}\text{Sr}/\text{Ca}$  ratio of vertebrae and dividing by two ( $V/S$ ) or by measuring the  $^{87}\text{Sr}/\text{Ca}$  ratio of femur shafts and dividing by  $F/S$ , that is, one-half.

The average  $^{87}\text{Sr}/\text{Ca}$  ratio of the diet in New York City ( $Z$ ) has been related to the cumulative deposit of  $^{87}\text{Sr}$  in the soil ( $D$ ) and to the fall-out rate experienced during the year ( $R$ ) in the following way:

$$Z = 0.1 D + 0.2 R$$

where  $Z$  is in  $\mu\text{g}/\text{g}$  Ca,  $D$  is in  $\text{mc./mile}^2$  and  $R$  is in  $\text{mc./mile}^2/\text{yr.}$

From estimates of expected fall-out rates for the next nine years provided by Volchok<sup>9</sup>, values of  $Z$  were calculated and used with equations 1 and 2 to predict vertebrae, femur shaft, and total skeletal  $^{87}\text{Sr}$  concentrations. These results are shown in Table 2. The estimated average dose to the skeletons of New York City residents by the end of 1964 from  $^{87}\text{Sr}$  was 8.6 millirads. By 1974 the average accumulated dose will be about 60 millirads. Doses received by vertebrae will be about twice this amount while those to femur shafts will be about half as great. These estimates are probably high since no account has been taken of the lesser availability of  $^{87}\text{Sr}$  in the soil to plants with time, which would cause the diet (and bone-levels) to decrease somewhat faster than has been assumed.

Table 2. PREDICTED PROPORTIONS OF STRONTIUM-90 PER GRAM OF CALCIUM IN VERTEBRAE, FEMUR SHAFTS, AND SKELETONS OF NEW YORK CITY ADULTS

Year (a)	Diet ( $\bar{D}$ )	Vertebrae ( $V$ )		Femur shafts ( $F$ )		Skeletons ( $S$ )	
		( $\bar{V}$ )	( $\bar{F}$ )	( $\bar{V}$ )	( $\bar{F}$ )	( $\bar{S}$ )†	( $\bar{S}$ )
1965	25	2.48	0.50	1.09	1.03	1.22	1.23
1966	25	2.70	0.58	1.22	1.21	1.31	1.37
1967	25	2.88	0.64	1.31	1.27	1.40	1.43
1968	25	3.02	0.70	1.40	1.43	1.47	1.53
1969	21	3.14	0.75	1.53	1.57	1.59	1.67
1970	21	3.24	0.80	1.60	1.75	1.64	1.82
1971	20	3.31	0.85	1.64	1.82	1.68	1.89
1972	20	3.37	0.90	1.68	1.89		
1973	19	3.42	0.94				

\* Assuming no further atmospheric tests of nuclear weapons.

† Calculated using equation 2.

The dose rate to bone from natural background radiation has been estimated to be about 130 mrad/yr<sup>10</sup>; in the period 1954-64 the dose to bone from background would be 1,300 mrad and from 1954-74 the dose would be 2,600 mrad. It is evident, therefore, that the radiation dose to bone from  $^{87}\text{Sr}$  has been negligible compared with that from natural background.

$^{87}\text{Sr}$  from nuclear weapons tests has provided a tracer material which can be used to examine mineral metabolism for individuals of all ages. In the adult it appears that the turn-over rate of the vertebrae-like component of the skeleton is 8 per cent per annum while that for the femur-shaft-like component is 1.5 per cent per annum. The total skeleton can be thought of as consisting of 30 per cent vertebrae-like bone and 70 per cent femur-shaft-like bone. In the adult these turn-over rates as well as the bone diet  $^{87}\text{Sr}/\text{Ca}$  observed ratios do not appear to change. In children, however, it is known that this is not the case. For example, in the very young (< two years old) the diet bone observed ratio  $\bar{X}$  is greater than 0.25 and may approach 1.0 during the first month of life and the turn-over rate may also approach 1.0 during early infancy<sup>11</sup>. Investigations, now in progress in this laboratory, of the changes in  $^{87}\text{Sr}$ -levels in bone with changes in dietary

may provide new information on the rate of bone mineral metabolism of individuals twenty years of age. Kletzing for help in the calculations and J. S. E. Kelley for the preparation and plates.

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# AL OF PUMICE, SUPPOSEDLY FROM THE 1962 SOUTH SANDWICH ISLANDS ERUPTION, ON SOUTHERN AUSTRALIAN SHORES

By F. L. SUTHERLAND  
Queen Victoria Museum, Launceston, Tasmania

Strandings from external eruptions are fairly common along Australian shores. One of the first was that of Clarke<sup>1</sup> in 1842, who remarked: "The range of this drift pumice along the coast of Australia and Tasmania would not be an employment". Generally, however, there have been little work done in this connexion. Considerable quantities of pumice were washed ashore on the coasts of Australia. The arrival of the first pumice on the Tasmanian coast was recorded by me<sup>2</sup>. It was noted that the pumice was a remnant of the large eruption from a submarine eruption near the South Sandwich Islands in March 1962 (ref. 3). Strandings of pumice along the coast of the South Sandwich Islands at time-intervals consistent with the drift of pumice towards the West Wind Drift have been recorded from Bouvet Island<sup>4</sup>, Heard Island<sup>5</sup>, and Macquarie Island<sup>6</sup> (K. G. Simpson, personal communication). The geological and oceanographical aspects of the South Sandwich Islands eruption are being examined by Adie, University of Birmingham. This article reports the results of further examination of the pumice washed up on southern Australian shores during 1964-65, and its dispersal. The specimens referred to are collections of the Queen Victoria Museum.

## The Pumice

The pumice, as found washed up, is commonly rounded and encrusted with marine growth, mainly algae (*Lepas*) and algae. The pieces vary in size, rarely greater than 1 ft. across. Most of the pieces are uniform whitish-grey in colour, but some, particularly from Western Australia, show colour variations from whitish to dark grey. A number of specimens of small xenoliths of olivine basalt, the largest being 7 cm x 6 cm x 4 cm in size.

A general petrological description of the pumice washed up on Tasmania has already been given<sup>7</sup>. Macroscopically the pumice from Australian strandings is closely similar in appearance to a sample from the original South Sandwich Islands raft and to samples stranded on Heard and Macquarie Islands in 1963.

In thin sections the pumice consists of vesicular glass containing crystals of plagioclase, hypersthene, clinopyroxene; lesser amounts of quartz, magnetite, hornblende; a little biotite, and rare small crystals of apatite and sphene. The plagioclase is andesine-labradorite in composition and the crystals are mainly euhedral. A few enclose a core pitted and riddled with generally elongate inclusions of glass (?). The hypersthene is pleochroic with X yellowish brown, Y pale yellow, Z pale green, and has a 2V<sub>a</sub> of about 50°-60°. The clinopyroxene shows a ZAC extinction angle of 40°, a 2V<sub>γ</sub> of about 60°, and is weakly pleochroic from pale brown to pale green. It is ferroan diopside or augite. The proportions of the two pyroxenes vary and they occur both as separate crystals and in association, mainly in parallel intergrowth. The hornblende and biotite appear to have formed from late magmatic alteration of the pyroxenes. The quartz occurs mainly as sporadic clusters of deeply embayed phenocrysts, up to 1.5 mm across.

The pumice shows petrological similarities with the description of the South Sandwich Islands material<sup>8</sup> in the size and relative abundances of the plagioclase and pyroxene phenocrysts and in the twinning, zoning, and composition of the plagioclase. It differs in the presence of ferroan diopside, corroded quartz, hornblende and biotite. However, I was able to observe both the first two features in thin sections of pumice samples from the South Sandwich Islands raft and from Heard Island. A comparison of the mineralogy of pumice samples from Australian localities, from the South Sandwich Islands, and from Heard Island is given in Table 1.

COMPARISON OF THE MINERALOGY OF THE CRYSTALS CONTAINED IN PUMICE SAMPLES FROM AUSTRALIA, HEARD ISLAND, AND THE SOUTH SANDWICH ISLANDS

Locality	Trial Harbour, W. Tasmania	Maatsuyker Is. S. Tasmania	D'Entrecasteau Pt. W. Australia	City Beach, Perth, W. Australia	Heard Is.	South Sandwich Islands
Plagioclase	Andesine-labradorite	Ditto	Ditto	Ditto	Ditto	Ditto
Quartz	Present	Ditto	Ditto	Ditto	Ditto	Ditto
Hypersthene	Carlsbad, Albite	Ditto	Ditto	Ditto	Ditto	Ditto
Pyroxene	Some crystals with cores riddled with glass inclusions	Ditto	Ditto	Ditto	Ditto	Ditto
Pyroxene	Hypersthene, pleochroic	Ditto	Ditto	Ditto	Ditto	Ditto
Pyroxene	Ferroan diopside, weakly pleochroic	Ditto	Ditto	Ditto	Ditto	Ditto
Pyroxene	Present, corroded	Ditto	Ditto	Ditto	Ditto	Ditto
Biotite	Present	Ditto	Ditto	Ditto	Ditto	Ditto
Apatite	Present	Present	Present	Absent	Absent	Absent
Sphene	Present	Present	Absent	Present	Absent	Absent

The glass in Australian samples has largely altered to palagonite and anisotropic breakdown products, thus making accurate determinations of its refractive index difficult. Chemically, the pumice shows the same low potash content and low potash/soda ratio of the South Sandwich Islands material (Table 2).

### Source of the Pumice

The close match, both petrologically and chemically, of the pumice with that of the South Sandwich Islands eruption indicates it is a remnant of the original raft ejected in 1962. Data, given later, on the dispersal of the pumice in Australian waters indicate a south-westerly derivation, also supporting this correlation. A submarine eruption to the south-west of Australia, distinct from the 1962 South Sandwich Islands eruption, but which ejected a similar type of pumice, is a further possibility. I am unaware of any reports of such an eruption, and this is considered to be unlikely. Another possible source which must be considered, however, is the eruption of Mt. Agung, Bali, on March 17, 1963. In her eye-witness account of the eruption, Matthews<sup>4</sup> describes the ejection of ash, sand and volcanic bombs, but not pumice specifically. Although not a scientific document, her writings make it clear that she was well acquainted with the nature of pumice. M. M. Purbo Hadiwidjojo, Geological Survey of Indonesia, has also informed me that no pumice was found following the eruption of Mt. Agung. There remains the remote possibility that pumice was ejected during the explosive eruption with sufficient violence to carry it out to sea without any falling on land. Chemical analyses of lapilli and ash deposits from Mt. Agung (supplied by M. M. Purbo Hadiwidjojo) are similar to analyses of the Australian pumice (Table 2) in having low potash contents and low potash/soda ratios, but in all cases the soda contents are somewhat lower. An analysis showing the highest soda content determined for the eruptive products of the Mt. Agung eruption is shown in Table 2. It would seem, then, that it is unlikely this eruption was the source of the pumice. This is further substantiated by the recovery of small balls of pumice apparently identical with that of the Australian strandings, from the stomachs of fledgling mutton birds on March 29, 1963, in Bass Strait<sup>5</sup>. It is highly improbable that pumice erupted from Bali on March 17, 1963, could have drifted far enough in 12 days to be picked up by mutton birds nesting almost 4,000 miles to the south-west.

### Dispersal of the Pumice

The following account of the dispersal of the pumice in southern Australian waters is based on numerous reports of strandings received by me during 1964 and early 1965. In nearly every case a specimen of pumice was forwarded enabling its identity to be checked. All the samples of pumice, with one exception from the coast of New South Wales, are of similar nature and were presumably derived from the one source, considered to be the South Sandwich Islands raft. It must be pointed out that the reported date of finding of stranded pumice is not necessarily the date of arrival.

From all available reports the first pumice strandings appear to have occurred on the west coast of Tasmania in late December 1963 or at the beginning of January 1964. Pumice was found on the south-west coast of King

Island in mid-January, showing it had reached western Bass Strait by this time. It was first reported from the south coast of Tasmania in February, from the east coast in May, and from the north coast in late August. No pumice strandings were observed by me during a visit to Flinders Island, eastern Bass Strait, in mid-April. The earliest record of the pumice on the mainland of Australia is late April at Port Campbell, Victoria. These reports suggest the pumice arrived from west, rather than east of Tasmania.

The current flows in Tasmania differ in summer and winter (Fig. 1). This has been demonstrated by a recent drift bottle programme<sup>6</sup> and is supported by a scrutiny of the earlier drift bottle data of Russell<sup>8-14</sup>. The winter flow is eastwards and southwards, and reverses in the summer, setting westerly and northerly. The arrival of the pumice on the west coast of Tasmania by January in terms of the summer flow suggests the pumice was carried up from the south-west. This is compatible with the pumice being part of the South Sandwich Islands raft. Weather conditions experienced at this time also would greatly assist the currents in driving the pumice ashore on Tasmania. The local meteorological report for January<sup>15</sup> states: "Rarely has a mid-summer blasted Tasmania with the westerlies like the period December 25, 1963-January 28, 1964. The scale and duration of low pressure systems affecting the State was extreme—only occasionally in some winters does this occur". Reported sighting of pumice washing ashore during the later half of January and early February suggest a southward drift down the west coast of Tasmania. It is possible that the abnormal

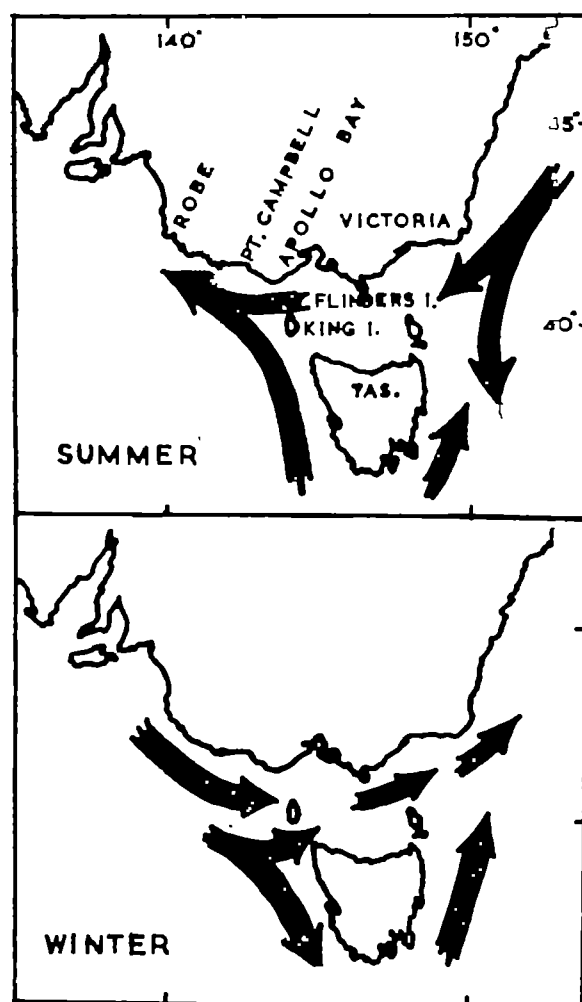


Fig. 1. Drift of surface water in Tasmanian and Victorian waters in summer and winter (after ref. 7)

Table 2. SODA AND POTASH CONTENTS OF PUMICE SAMPLES FROM AUSTRALIA AND THE 1962 SOUTH SANDWICH ISLANDS ERUPTION, AND OF LAPILLI FROM THE 1963 MT. AGUNG ERUPTION, BALI

Locality	South Sandwich Islands*	Port Davey, W. Tasmania†	Maatsuyker Island, S. Tasmania†	Mutton birds, Bass Strait†	Lapilli, Bendang, Bali‡
Na <sub>2</sub> O	4.66	3.88	3.82	3.69	2.33
K <sub>2</sub> O	0.67	0.63	0.60	0.63	1.56

\* Gamm, Harris and Holdgate (ref. 2).

† Tas. Dept. Mines, G. J. Penman, analyst.

‡ M. M. Purbo Hadiwidjojo, Geol. Surv. Indonesia.

; January caused a brief anomalous reversal in flow to winter flow as occurred in the 59-60 (ref. 7).

current flows and prevailing weather explain the absence of any pumice strandings until the beginning of 1964, although the South Sandwich Islands raft would be to the south, probably in April or May 1963. Migration of the pumice would be inhibited by the current flow then operating. Furthermore, the weather systems exercised a dominant influence on the weather during the autumn from late July to early August, greatly reducing the severity of the 19-20. Most of August was also quieter than the marked absence of strong west winds<sup>10</sup>.

to compare the arrival of the pumice on a like stranding on New Zealand. Here, as the first strandings appear to have occurred after the passage of the front of the South Sandwich Islands raft to the south. The pumice was on Stewart Island off the south coast in 1963. Large quantities of pumice came ashore at Mason Bay, which faces due west, and a washing up at the end of March 1964 (Watters, personal communication). Dr. Watters informed me that Stewart Island experienced gale-force south-westerly winds over the winter of 1963. The front of the South Sandwich Islands raft reached Macquarie Island by June 1963, so that during the winter and spring 1963 it was positioned less than 500 miles to the south. The marked effect of pressure on the drift of floating objects in the Southern Ocean is a fact that the pumice was not blown ashore until November suggests a mitigating effect.

The arrival of the pumice on Stewart Island all have resulted from a reversal in October of current flow, south-west of New Zealand, been shown to occur about this time in Tasmania. There are comparatively few data at this point, but it is perhaps significant that the survey of the coastal currents of New Zealand in summer and autumn releases off the south coast recovered but no winter and spring releases. A later date of the pumice strandings compared to Stewart Island can be accounted for by the northerly latitude of

number of pieces of pumice covered with recent marine growth, washed up on the beaches during a visit to the west coast of Tasmania in August. These observations indicate a further influx of pumice into southern Australian waters associated with the winter current flows. It is considered that the pumice was brought in on the strong north-easterly indraft of the West Wind Drift which impinges on the south-western corner of Australia<sup>11-14</sup>: the main body of the South Sandwich Islands raft having by this time dispersed sufficiently far northwards to be tapped. This current breaks against the Australian continent, forming branches that sweep the west and south coasts, and these would carry the pumice northwards and eastwards respectively. The extent of the northward drift is not well known, but eastwards the pumice was passing through Bass Strait and also washing up on the west coast of Tasmania by July. The influx of pumice on these currents was probably checked somewhat with the establishment of the summer flows, as the beaches near Fremantle, Western Australia, were bare of pumice in November (G. Smith, personal communication).

During January 1965 pumice gravel was observed washing up on the southern coast of Western Australia at Denmark. By March, strand lines on the beaches between Denmark and Cape Leeuwin were well marked with fine granular pumice with only an occasional larger piece, and there were quite large banks of this material west of D'Entrecasteau Point (G. Kendrick, personal communication). Small pumice pieces were also being stranded on the east coast of Tasmania in early March 1965 above Cape Bougainville where there was no pumice washing ashore in late January 1965 (M. Olsen, personal communication). These strandings are interpreted as resulting from the passage of the dispersed 'tail' of the South Sandwich Islands raft. In the eastward drift of the raft across the Southern Ocean the larger pieces of pumice would be more subject to assisting wind pressures and the finer material would tend to lag behind at the tail end. Calculations based on the date of the arrival of pumice on Macquarie Island indicate the front of the raft covered the 8,000-mile journey at an average speed of approximately 18 miles/day. Such a speed has been recorded for a drift bottle that covered 1,250 miles in the Southern Ocean<sup>15</sup>, but the maximum average speed recorded by bottles that have covered 8,000 miles is about 12 miles/day<sup>16</sup>. This suggests that the larger pumice pieces were more responsive to wind assistance than drift bottles. With the foregoing speed it would seem that the front

strandings on the mainland were reported from many points along the southern coast (Fig. 2). Presumably some of the pumice also drifted up on to the east coast of the continent as the data of Russell<sup>17-19</sup> show a number of cases of northward drift of cases of northward drift of the southerly sweep of the Australian Current. Strandings reported from the west coast north as Wedge Island. Noticeable amounts of pumice were reported washed up on the beaches of Western Australia on the onset of the weather about the middle of the most northerly stranding in August, but had arrived a bit earlier. Counts of pumice were also being taken up on the Southern Ocean coast near Robe in June; Sydney, Victoria, in late June; Furneaux Islands, eastern Tasmania in mid-July. I also noted a

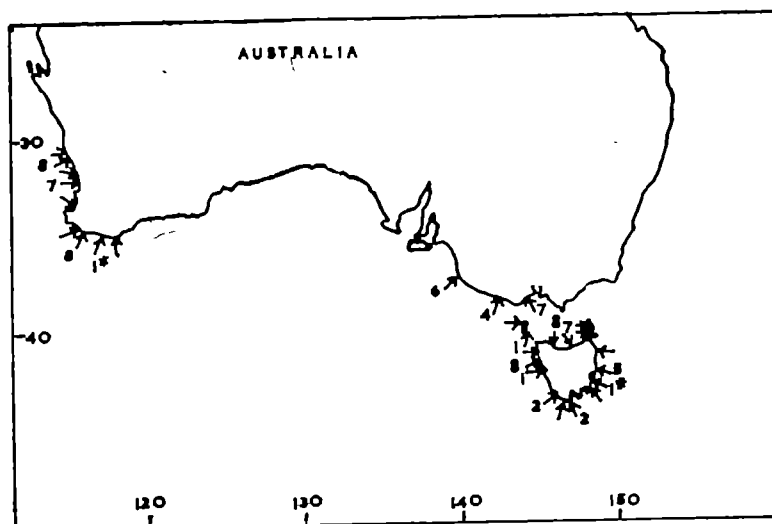


Fig. 2. Reported localities of the pumice strandings on the southern Australian coast, 1964-65. The figures indicate the month in which the strandings were first reported or the month of later fresh arrivals. For strandings taking place in 1965 the monthly figure is marked with an asterisk.

of the raft, provided there had not been complete dispersion or sinking due to water logging, circumnavigated the Southern Ocean by the time the 'tail' of the raft passed Tasmania in early 1965. This gives an idea of the extent of the longitudinal dispersion of the raft under the influence of the West Wind Drift from the time of its first sighting in March 1962, when it covered a degree and a quarter of longitude\*. The average drift speed of the fine pumice gravel which appears to form the 'tail' of the raft, based on its arrival on Australian shores, is about 6-7 miles/day. This agrees quite well with the speed of 8 miles/day quoted by Deacon<sup>17</sup> for the drift of objects in the West Wind Drift lying more or less awash and presumably not greatly assisted by wind pressures.

### Ornithological Implications

The recovery of pumiceous balls, mentioned earlier, from mutton bird (*Puffinus tenuirostris*) fledgelings in Bass Strait in late March 1963 (ref. 2) is of ornithological interest. The balls were found in the stomachs of fledgelings on Big Dog Island in the Furneaux Group on March 29. On examination the pumice balls appear to be petrologically identical with the pumice stranded on southern Australian coasts in 1964, and chemically show the same potash and soda content (Table 2). The Australian strandings are correlated with the South Sandwich Island raft, and knowledge of the position of the raft at the time of recovery of the pumiceous balls from the fledgelings should give information on the feeding range of the parent birds.

The front of the South Sandwich Islands raft apparently reached Macquarie Island in June 1963. Barber, Dedswell and Ingle<sup>22</sup> record that: "A message bottle dropped about 1,250 miles to the east of Macquarie Island was recovered from that Island 10 weeks later". This gives a surface drift speed of at least 18 miles/day, which is also the average drift speed previously determined for the front of the South Sandwich Islands raft. Calculations based on these data suggest that at the time of the recovery of the pumiceous balls from the fledgelings in Bass Strait, the front of the raft was, at least, about 1,200 miles west of Macquarie Island. The raft was presumably not farther north than latitude 47° S., as shown by the absence of strandings on Tasmania and Stewart Island until several months after the front of the raft crossed these longitudes.

On this basis the parent mutton birds must have flown about 1,000 miles to the south-west of their breeding ground to have picked up the pumice. Even if allowances are made for a possible over-estimation of the drift speed of the pumice raft in the milder than normal westerly spring weather of 1963, a conservative estimate suggests a south-westerly flight of at least 500 miles. Pumiceous

balls were again recovered from mutton bird fledgelings on Bass Strait Islands in the following breeding season in 1964 (M. Olsen, personal communication), but by this time pumice had drifted into Tasmanian waters. Other cases of sea-birds, notably the albatross, picking up floating pumice were observed at Macquarie Island in 1963 (K. G. Simpson, personal communication) and at Heard Island (P. J. Stephenson, personal communication) in 1933.

I thank the numerous persons who sent in reports and specimens of the drift pumice and generally assisted in making this compilation possible. In particular I acknowledge the assistance of Dr. W. A. Watters, New Zealand Geological Survey, for a sample of the original South Sandwich Islands pumice and information of the New Zealand strandings; of Dr. N. H. Fisher, Bureau of Mineral Resources, Australia, for specimens and information on pumice strandings on Heard Island; of Mr. M. M. Purbo Hadidwidjojo, Geological Survey of Indonesia, for information on the eruption of Mt. Agung, Bali; of Mr. K. G. Simpson, C.S.I.R.O., Australia, for information and pumice samples from Macquarie Island; of Mr. M. Olsen for oceanographical information; of Mr. W. St. C. Manson, Tasmanian Department of Mines, in regard to chemical analyses; of Messrs. D. Merilees and G. Kendrick, Western Australian Museum, for samples and information of Western Australian strandings; of Mr. R. Both, Tasmanian Museum, for thin sectioning of pumice samples; and of Mr. W. F. Ellis, director, Queen Victoria Museum, for his advice.

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## NEWS and VIEWS

### Electronic and Electrical Engineering in the University of Birmingham

E. D. R. SHEARMAN, senior lecturer in the Department of Electronic and Electrical Engineering in the University of Birmingham, has been appointed to an additional chair in that Department. The new chair is being established from October 1, 1965, as a result of the continued growth of the Department of Electronic and Electrical Engineering. There will thus be four professors in this Department; the others are Prof. D. G. Tucker (head of Department), Prof. J. T. Allanson and Prof. H. A. Prime. The Department will soon be moving to a new building and already has nearly 120 graduate workers and staff in addition to the undergraduate students.

Prof. E. D. R. Shearman

MR. SHEARMAN graduated with first-class honours in electrical engineering from the Imperial College of Science and Technology in 1945. He was awarded four Premiums from the Institution of Electrical Engineers for outstanding merit in his publications. From 1945 until 1947 he worked with the Admiralty on naval communications, and from 1947 until 1961 at the Radio Research Station, Slough, on radio propagation and ionospheric research. During 1960 he spent six months in Canada assisting in the design of the *Alouette* topside-sounder satellite. Since 1961 he has been senior lecturer in electromagnetism in the University of Birmingham, where he has built up an active research group in communication systems. He



number of Government and professional

Mathematics in the University of Newcastle  
Prof. A. Jeffrey

Dr. J. H. Jeffery, senior mathematician in the Advanced Laboratories at Rolls-Royce Ltd., has been appointed to the newly established chair of engineering at the University of Newcastle upon Tyne. Dr. Jeffery is thirty-six years of age, and was educated at the Grammar School, Croydon Polytechnic, and the University of London, from which he received his Ph.D. degree in 1953. From 1953 until 1956 he was engaged in guided-missile problems in the research department of the General Electric Co. In 1956 he joined Rolls-Royce Ltd., and was engaged initially on mathematical problems which arose in the design of nuclear reactors for submarine propulsion. Later on, he became a senior mathematician in the Company's Advanced Laboratories, and during this period he spent a year in the Magneto-Fluid Dynamics Division, of the University of the State of New York, working under the direction of Prof. H. Grad. Dr. Jeffery also co-operated with T. Taniuti from the University of Tokyo, in the preparation of a book on *Nonlinear Wave Propagation*. Dr. Jeffery's research has been the study of nonlinear partial differential equations and, in particular, a detailed study of the appearance and propagation of discontinuities in solutions. He has applied this work to a wide variety of different physical problems to illustrate the physical interpretation of the mathematical phenomena. He took up his new appointment in 1958.

3 In the University of Aberdeen :  
Prof. J. R. Mallard

FALLARD has been appointed to the newly  
r of medical physics in the University of  
will take up his duties on October 1. Dr.  
up in 1944 from Northampton Grammar  
iversity College, Nottingham, where he  
30. He stayed on at Nottingham as a  
ent and demonstrator, working on the  
erties of uranium and its alloys. After  
J., he went in 1951 to the Liverpool Radium  
1953, he took a post at the Hammersmith  
the Postgraduate Medical School. Rapid  
owed and, when the Department of Medical  
reated in the Postgraduate School in 1958,  
deputy director and was appointed reader  
ity of London. Then, in 1964, he moved  
iversity to St. Thomas's Hospital Medical  
up a new Department of Biophysics. Dr.  
-doctoral work has covered many topics in  
35. He has been particularly interested in  
pplications of radioactive isotopes, usually  
one collaboration with clinical colleagues;  
is on scanning are well known. In addition,  
ctively engaged on electron spin resonance  
, especially as applied to wet tissue speci-  
earch on scanning methods and on electron  
e will be continued, along with other work,  
rity of Aberdeen. The arrival there of  
and his associates seems sure to give that  
l aspects of medical physics in the North  
and which the creation of the chair was  
rovide.

the University of Zambia :  
Prof. C. T. Mortimer

MOBTINER has been appointed to the chair at the University of Zambia, on secondment from the University of Keele, for a three-year period from

January 1, 1966. In addition, a Special Commonwealth Award has also been made to him by the Ministry of Overseas Development. Educated at Askworth School, Yorkshire, he took a B.Sc. degree at the University of Manchester in 1950 and the M.Sc. and Ph.D. degrees in 1951 and 1953, respectively. After two years at the University of Kharrourm, Dr. Mortimer took an appointment at the University of Keele in 1955, where he is now a senior lecturer. A Commonwealth travel grant enabled him to visit the University of Ibadan, Nigeria, for three months in 1960. Dr. Mortimer is the author of some forty papers and a monograph on thermochemistry, and a D.Sc. degree was recently conferred on him by the University of Manchester for this research. He has also published a number of papers on higher education in Africa.

Social Studies in the University of Newcastle upon Tyne : Prof. P. Collison

DR. P. COLLISON has been appointed to the chair of social studies in the University of Newcastle upon Tyne with effect from September 1. Dr. Collison was born in May 1925. After service in H.M. Forces he entered the University of Birmingham in 1947 and was awarded the degree of B.Com.(Hons.) in 1950. After a period of research he was awarded a Ph.D. degree in 1953 when he also became a research student at Nuffield College, Oxford. In 1954 he started to undertake part-time teaching in the Department of Social and Administrative Studies at Oxford, and since 1955 he has had a full-time post in that department. He is now university lecturer in sociology. His main research work has been in the field of urban studies, but he has had a subsidiary interest in institutions of higher education. He has published a large number of papers, and his book, *The Outer-School Walls: a Study in Social Class*, was published in 1963.

### Grants to Universities and Colleges

THE recommendations of the Estimates Committee in its fifth report for the session 1964-65 on grants to universities and colleges are discussed on p. 1319 of this issue of *Nature*. Nevertheless, the report contains such voluminous information on the functioning of the university grants system that a brief note on its contents seems desirable. Included with the report of the Estimates Committee itself and the minutes of evidence taken before its sub-committee are a number of memoranda. These include a memorandum submitted by the Department of Education and Science, which describes the system, including the constitution and the functioning of the University Grants Committee. Also presented are memoranda from the Committee of Vice-Chancellors and Principals describing its constitution and functions, and one from the Department of Scientific and Industrial Research on its Research Grants Committee, as well as on its policy in regard to computing science. Furthermore, there are memoranda from the Ministry of Health on its relations with the University Grants Committee, and from the National Health Service on the responsibility for the cost of building teaching hospitals. One submitted by the Royal Institute of British Architects deals with architects' fees for university buildings, and another, by Mr. W. E. Parker on university grants, considers the scope of the audit made of university accounts; the latter memorandum comments on the possibilities of using *post facto* examinations to make a comparative assessment of efficiency in regard to the expenditure of non-recurrent grants for buildings. A note by the Vice-Chancellor of the University of Birmingham deals with the control of expenditure and incentives to economy in university financial administration. A memorandum by the Department of Education and Science describes the *Trees* index used for measuring the change in university recurrent costs other than those on academic and related salaries.

Two particularly interesting tables from the University Grants Committee itself set out staff/student ratios on two different bases for each university, except Oxford and Cambridge, and university recurrent costs per student; while a note from the University of Kent instances losses of efficiency through inability to commit capital except at short range. It may be noted here that the Estimates Committee thought that it was time for the Vice-Chancellors' Committee to become more effectively representative, and that there was room for more intimate co-operation between the universities and the regional economic councils which have recently been set up. It also thought that the colleges of advanced technology might be unduly optimistic as to their prospects in the larger world of university finance, and was concerned that these colleges as a whole possessed no cushion against financial rigours such as the newly founded universities possessed in their large appeals. The Estimates Committee was critical of the lengthy process of negotiation between the Ministry of Health, the Department of Education and Science and the University Grants Committee involved in the development of medical schools and would welcome a simplification of the present procedure.

#### Production of Phosphorus and Its Compounds in Canada

The largest subsidiary overseas of the well-known Albright and Wilson Chemical Group of the United Kingdom is the Electric Reduction Company of Canada, Ltd., the main interest of which is in the manufacture of phosphorus, phosphoric acid, industrial and agricultural phosphates, and chlorates. 'ERCO', as it is known, operates in four factories: at Buckingham and Varennes, Quebec; at Port Maitland, Ontario; at Vancouver, British Columbia; and maintains its research laboratories and executive offices in Toronto, Ontario. A newly-issued and copiously illustrated brochure entitled *Ercos Today* vividly tells the growth story of this chemical manufacturing company and of its place as a supplier of these products to world markets (Pp. 48. Toronto: Electric Reduction Company of Canada, Ltd., 1965). The functions of each main plant and the processes involved are clearly described. At Varennes the primary concern is elemental phosphorus; the source is phosphate rock from Florida, whence it is shipped in 20,000-ton cargoes up the Atlantic seaboard and by way of the St. Lawrence Seaway to a wharf near the plant. Each shipload is unloaded and transported to the Varennes stock-piles within 30 h to cope with the daily capacity of two giant electric furnaces (more than 750 tons) to which are fed precisely sized and mixed quantities of coke, silica, and phosphate rock (tri-calcium phosphate) every 24 h. "Inside the steel-clad, carbon-walled furnaces, 35,000 kilowatts of Quebec's abundant hydro-electric power flow through huge carbon-based electrodes to convert these raw materials into more than 20,000 tons of yellow, wax-like elemental phosphorus each year." From Varennes phosphorus in water is transported to the Buckingham plant for further processing. Fundamental at Buckingham is the production of phosphoric acid; much of this is reacted with sodium carbonate or hydroxide to form eight different kinds of soda phosphate; some, with lime, goes to manufacture of mono-calcium phosphate, also, with appropriate chemicals, to organic phosphates; Buckingham also produces red amorphous phosphorus and phosphorus sesquioxide used in the match industry. Products of the Chlorate and Agricultural Chemicals Divisions are described; the role of the latest Port Maitland plant in manufacture of superphosphates and phosphatic fertilizers is particularly impressive.

#### World-wide Radio Noise Measurements

Since July 1967, measurements of the level of atmospheric noise received on eight radio frequencies between

0.013 and 20 Mc/s have been made at eighteen stations throughout the world in a co-operative programme coordinated by the U.S. National Bureau of Standards. The results of these measurements are published in a series of *Technical Notes* entitled *Quarterly Radio Noise Data*, the latest of which is No. 18-21 (Pp. vi+98. Washington, D.C.: Government Printing Office, 1965. 50 cents). This tabulates the results obtained during the three-month period December 1964-February 1965. These *Technical Notes* are prepared by W. Q. Crichtow, R. T. Disney and M. A. Jenkins of the Central Radio Propagation Laboratory, Boulder, Colorado. The present publication contains photographs of the standard type of atmospheric radio noise recorder and of the aerial system used at all the stations. An accompanying map shows the distribution of the 17 land stations, and a typical position of the floating Antarctic research vessel, U.S.N.S. *Ellanor*, which operates as a mobile station in the South Pacific. Following an introductory description of the evaluation of the three basic parameters of the noise—mean power, mean envelope voltage and mean logarithm of the envelope voltage—the values measured at every hour of each day during the three-month period are tabulated. A separate set of tables summarizes the observed values of radio noise for each of the frequencies and in 4-h time blocks throughout the three-month period, in accordance with the provisions of Report No. 322 of the International Radio Consultative Committee (CCIR). The values presented in the tables reflect the actual measured values of radio noise. The only editing for man-made noise or other contamination of the records has been done by the station operators, and no statistical study has been made in order not to delay the dissemination of the data in their present preliminary form. A short bibliography is attached to the report. This refers not only to individual scientific papers, but also to the publications of the CCIR and of the International Scientific Radio Union.

#### Advance In Knowledge of a Fossil Fern

Almost a century ago, Carruthers described a British Tertiary fern stem as *Osmundites douckeri*; he realized it was like *Osmunda* but he felt he knew too little to identify it with *Osmunda*. Recently, the stems of the Recent *Osmundaceae* have been investigated and we know how to distinguish the genera and even the sub-genera from a transverse section of the stem. Recently, also, a second British specimen was found, very carefully sectioned by the late W. N. Croft, and now described by Miss Marjorie E. J. Chandler (*Bulletin of the British Museum (Natural History)*. Geology. Vol. 10, No. 6: *The Generic Position of Osmundites douckeri Carruthers*. Pp. 139-161+12 plates. London: British Museum (Natural History), 1965. 42s.). She finds that it is a true *Osmunda* but belongs to the section *Plenasium* and not to the section which includes the British *O. regalis*. The ferns of *Plenasium* grow in South-east Asia. This then is another item in which our Eocene flora agreed with the living plants of Malaysia. It is interesting, too, that fragments of an *Osmundaceous* fern leaf from the British Tertiary (*O. lignitum*) also probably belongs to the sub-genus *Plenasium*—perhaps the two kinds of fossil represent a single species.

#### Soil Moisture

ALTHOUGH an estimate of moisture content is one of the most common exercises in soil analysis, it is realized that the methods used have various disadvantages. A useful review, with a comprehensive bibliography, on "Measuring Soil Moisture", by F. Cope and E. S. Trickett, appears in *Soils and Fertilizers* (28, No. 3, 201; 1965). As in other analytical techniques, the sampling error imposes limits on the reliability and reproducibility of the results, and the need for a quick and accurate method for measuring soil moisture *in situ* is emphasized. The authors deal with the estimation of the amount of soil moisture by

netric, lysimetric, penetration, chemical, methods. The most successful nuclear probe containing a source of fast neutrons, slowed down by the hydrogen of the soil, is a slow neutron detector. The energy of neutrons may be measured by tensimetric-pressure, resistive, thermal and electrical methods. The neutron probe is cheap and can give values over a wide range, but its accuracy is not very precise. Other methods await investigation or improvement, and some system of recording is required to reduce the labour needed because of soil variability.

#### In Tasmania

Archaeological research in Tasmania has been initiated, and one of the first reports appears in *Man* ("Excavations on a Stone in Tasmania", by Rhys Jones; 65, No. 62, 65). This is a short interim report of an excavation of the Bay of Fires on the north-east coast of which resulted in the excavation of a shell two stratified stone alignments. The stone alignments of Australia have for long been known to be excavations demonstrated that at least some arrangements must be of aboriginal origin. With certain stone settings of the Australian is possible that the Bay of Fires alignments are ceremonial centres, and the nature of the site is the first time that the tradition of such stone alignments in Tasmania must have existed for an unknown length of time.

#### The Shipwreck

The wreck off the island of Antikythera, first discovered in 1900, is well known not only because it was the earliest wreck found but also because of the wide range of objects recovered, including the papyrus. Some controversy has developed over the date of the wreck, and, using a recent use of the computer as a basis, a team of specialists has examined in detail the glass vessels, the Greek Roman pottery and amphorae, in an attempt to determine precisely the date of the disaster (*The Shipwreck Reconsidered*. By G. D. Weintraub, G. R. Edwards, H. S. Robinson, Morton and E. K. Ralph. Pp. 48. *Trans. Soc.*, New Series, 55, Part 3, 1965). All the evidence suggests a time between 80 and 50 B.C., a fragment of wood from the ship yielded a date of  $220 \pm 43$  B.C. (T-846, half-life of  $^{14}\text{C}$  represents the time of the cutting of the plank). The main cargo of the vessel, sculpture, have been on its way to Rome as spoils from the Mediterranean shores, although its exact place is unknown. Fragments of the actual ship were discovered in 1900, and P. Throckmorton contributes a statement of their value towards a reconstruction of the ship. This has been augmented by the 1953 examination of the wreck by men from the island which suggests that a substantial part of the ship still await recovery from the great depth of the sea.

#### Human Affairs

Years ago biology was little studied in the British Isles. In the junior schools the teaching consisted almost entirely of nature study or botany; in secondary schools little but botany and that largely in girls' schools. In 1935 the *British Biology* was founded by the then British Biological Council with the general aim of developing biology in schools. The particular aim was to encourage growth up with a better understanding of nature and working of their own bodies and

acquire a greater sense of responsibility for their conduct in personal and social life. With the spread of biology teaching, the Council broadened its aims to focus greater attention on human biology and, in 1942, the name of the journal was changed to *Biology and Human Affairs*. From the beginning it has appeared once a term and, largely through voluntary effort, has maintained a high standard of contributions which have done much to bring about greater awareness of social biology among those who teach biology in schools and colleges. For nearly the whole of its existence the editor of the journal has been R. Weatherall, late of Eton College, who must be congratulated on maintaining such a high standard in a wide range of contributions over so many years. Further information about the journal can be obtained from the Editor, British Social Biology Council, 69 Eccleston Square, London, S.W.1.

#### Experiments on Living Animals

The Universities Federation for Animal Welfare (UFAW), 7a Lamb's Conduit Passage, London, W.C.1, has issued the following statement: "The Home Office report on the above subject for the past year, which was published on August 17, shows that of the 4.5 million animals affected, 4 million were used for the testing of drugs, for experimental inoculations, and for like purposes. A large proportion of such procedures are concerned with toxicity-testing, which has greatly increased since the thalidomide tragedy was so dramatized as to give rise to a panic. In our view, which seems to be shared by most scientists who have a knowledge of the subject, many of the tests are necessitated by what may be called 'political' rather than scientific considerations and are, in fact, valueless, entailing a wasteful use of animals; moreover, in other cases it may well become possible to improve the technique from a humane point of view. UFAW is accordingly about to set on foot, under the guidance of its expert Scientific Advisory Committee, a three-year investigation of toxicity-testing, centred in the Medical School in the University of Birmingham and directed by one of the most eminent authorities on the subject. This undertaking will cost at least £30,000, but while its primary object is to save animals from useless suffering it is almost certain to lead also to results beneficial to human medicine, if the requisite financial support can be obtained".

#### Field Equipment for Naturalists

Much useful advice for naturalists contemplating the purchase of binoculars, telescopes, tape-recorders, tents, clothing, transport, compasses, thermometers, barometers, light meters, maps or cameras is contained in a special supplement of *Animals* (6, No. 14). The issue also contains articles on "Marine Life of North America's Pacific Coast", by Prof. C. M. Yonge; "Bird Watching in Jamaica", by M. S. Curtler; "Pets", by Dr. Maurice Burton; "The Ngorongoro Crater", and "Lake Manyara National Park"; and "Britain's Bats", by S. L. Bissacrot.

#### Lincolnshire Naturalists

In his presidential address to the Lincolnshire Naturalists' Union, Frank Norris reviewed present knowledge about the snakes of Lincolnshire, and appealed for an extension of the reporting system, with emphasis on observations of behaviour (*Transactions of the Lincolnshire Naturalists' Union*, 16, No. 1; December 1964). Besides the usual sectional reports, the *Transactions* also contain articles on the "Basal Lias near Long Bennington", by Dr. P. E. Kent; "Lincolnshire Natural History Recording Units", by Mark R. D. Seaward; and "Biological Surveys of the Humber Estuary", by E. Hinton Clifton.

#### The Beilby Medal and Prize, 1966

Awards from the Sir George Beilby Memorial Fund are made by the Administrators of the Fund, representing

the Royal Institute of Chemistry, the Society of Chemical Industry and the Institute of Metals. Sir George Beilby was president of each of these three bodies, and they jointly sponsored the appeal for subscriptions whereby the Fund was raised as a memorial to him after his death in 1925. The Beilby Medal and Prize, which consists of a gold medal and a substantial sum of money, is specified as being "For Advancement in Science and Practice". Such an award is now being offered annually. The awards are made to British scientists in recognition of independent original work of exceptional merit, carried out continuously over a period of years and involving the development and application of scientific principles in any field related to the special interests of Sir George Beilby, namely chemical engineering, fuel technology or metallurgy, in their modern interpretations. The awards are intended as an encouragement to younger men and women (preferably under the age of 40) who have done distinguished work of practical significance in any of these fields. The award for 1966 is shortly to come up for consideration. Outstanding work of the nature indicated may be brought to the notice of the Administrators, either by persons who desire to recommend the candidate or by the candidate himself, not later than December 31, 1965, by letter addressed to the Convener of the Administrators, Sir George Beilby Memorial Fund, the Royal Institute of Chemistry, 30 Russell Square, London, W.C.1.

#### Meldola Medal, 1965

THE next award of the Meldola Medal, the gift of the Society of Macrobasians, will be made early in 1966 to the chemist who, being a British subject and less than 30 years of age on December 31, 1965, shows the most promise as indicated by his or her published chemical work brought to the notice of the Council of the Royal Institute of Chemistry before December 31, 1965. Further information can be obtained from the President, the Royal Institute of Chemistry, 30 Russell Square, London, W.C.1, the envelope being marked "Meldola Medal".

#### Announcements

DR. M. C. WILLIAMS has been appointed to the directorship of the Virus Research Institute at Entebbe, and has already taken over his duties.

THE 1965 congress of the International Federation for Documentation will be held in Washington during October 10-16. Further information can be obtained from the

Secretariat of the Congress, 9650 Wisconsin Avenue, Washington, D.C.

A SYMPOSIUM on "Computers in Medicine" will be held in Queen's University, Kingston, Ontario, during October 7-8. Further information can be obtained from the Director of Continuing Education, Faculty of Medicine, Queen's University, Kingston, Ontario.

THE national conference of the Institute of Personnel Management on "Making the Most of Manpower" will be held at Harrogate during October 7-9. Further information can be obtained from the Institute of Personnel Management, 80 Fetter Lane, London, E.C.4.

A MEETING on "Oxidation of Metals", organized by the Société d'Etudes, de Recherches et d'Applications pour l'Industrie, will be held in Brussels during October 6-8. Further information can be obtained from Mr. C. Sonnen, o/o S.E.R.A.L., 1091 Chaussée d'Alsemberg, Brussels 18.

A SYMPOSIUM on "Industrial Organic Analysis" sponsored by the Analytical Chemistry Division of the Chemical Institute of Canada, will be held at Sarnia during October 4-6. Further information can be obtained from R. M. Small, Research Department, Polymer Corporation, Ltd., Sarnia, Ontario.

AN informal meeting of the Physical Biochemistry Group of the British Biophysical Society on "Light Scattering" will be held at the School of Pharmacy, University of London, on October 4. Further information can be obtained from Prof. L. Saunders, School of Pharmacy, Brunswick Square, London, W.C.1.

A CONFERENCE on "Mathematics for Engineers", sponsored jointly by the Engineering Institutions Joint Council, the Institute of Mathematics and its Applications and the Joint Mathematical Council of the United Kingdom, will be held at Queen Mary College, London, during September 30-October 1. Further information can be obtained from the Registrar, Institute of Mathematics and its Applications, o/o Imperial College of Science and Technology, London, S.W.7.

CORRECTION. Prof. A. H. Glasser has written to the Editor stating that the chemical compound described in his joint communication with Dr. C. Beretta and Dr. R. Ferrini, which appeared on p. 421 of the July 24, 1965, issue of *Nature*, was in fact "1,6-Dimethyl-8 $\beta$ -carbobenzylxyaminomethyl-10 $\alpha$ -ergoline" and not "1-Methyl-8 $\beta$ -carbobenzylxyaminomethyl-10 $\alpha$ -ergoline".

## THE NIGHT SKY IN OCTOBER

All times are in Universal Time

MOON		CONJUNCTIONS WITH THE MOON	
New Moon	24d 14h	Venus	28d 00h, 2° S.
Full Moon	10d 14h	Mars	28d 02h, 0.01° N.
		Jupiter	16d 04h, 3° S.
		Saturn	7d 16h, 3° N.

#### PLANETS

Times of Rising (R) and Setting (S) during the month							
Name	R/S	Beginning	Middle	End	Mag.	D <sub>g</sub> (10 <sup>6</sup> miles)	Zodiacal position
Mercury	—	Unfavourably placed for observation			—	120	—
Venus	S	18h 45m	18h 26m	18h 25m	-3.8	84	—
Mars	S	19h 10m	18h 45m	18h 30m	+1.4	180	Scorpio
Jupiter	R	21h 00m	20h 05m	19h 05m	-2.1	484	Gemini
Saturn	S	2h 30m	2h 25m	1h 30m	+0.9	836	Aquarius

D<sub>g</sub> is the distance of planet from the Earth on the 15th of the month

#### OCCULTATIONS OF STARS BRIGHTER THAN MAGNITUDE +6 AT GREENWICH

Star	R/D	Time	Mag.
27 Cap	D	5d 21h 58.4m	+5.6

(D, disappearance; R, reappearance)

#### MEMOIRS

Name	Active period	Date of maximum	Radiant	Remarks
Glaucobids	9d-10d	9d 18h	262° R.A. + 54° Dec.	Unfavourable as minor activity expected
Orionids	15d-25d	21d	96° R.A. + 15° Dec.	Fairly favourable

#### OTHER PHENOMENA

16d 12 h, Mars 4° N. of Antares.  
17d 15h, Venus 2° N. of Antares.  
19d 15h, Venus 1.6° S. of Mars.  
28d 02h, Mars occulted by Moon (visible Australasia and Pacific only).  
Taurid meteors became active after 28d.

## THE ELECTRICAL RESEARCH ASSOCIATION

fourth annual general meeting of the Research Association held on April 28 annual report for 1964\*.

rd, Sir Ronald German, president of the fers to the work of the Association having d, in the main, in relation to the electricity y, but in recent years increasing attention oted to light current problems and this most certainly be accentuated.

rrations on the year's work are made in a tory section constituting the report of the s is followed by the Director's report on ich summarizes the activities of the six ons and the Electronics Department. A s devoted to membership, public relations, reau and library. A full list is given of the ts issued during the year. The remainder mprises a list of members of the Association, members of the Council and committees. es are listed under the particular division is attached, and of particular value is the statement of the terms of reference of

of Government grant to the Association, the Department of Scientific and Industrial e to an end in December 1964, and during w application had been prepared and sub- result of this application had been the r cent grant for a three-year period, subject ion collecting at least £350,000 of grant- e.

the income of the Electrical Research s increased by 90 per cent, or, more realistic- of 1955 spending power, by 52 per cent, e annual total of some £352,000. It is, ularly noteworthy that during this period a of total income derived from sponsored supported researches has increased from ent.

of the year, the Association transferred to of Reading the Field Station at Shinfield, past twenty years work has been carried on e applications of electricity in agriculture ure. It was felt that there would be co-ordinating more closely the work at the ion with that of the Faculty of Agriculture sity and to that end a joint agreement was - the University, the Electricity Council rical Research Association.

association moved its headquarters and main o Leatherhead in 1956, the facilities for ting remained for the time being at Perivale. 64 a new switchgear laboratory was built at Leatherhead and the work transferred. ening of this new laboratory was in October

sion dealing with insulating materials, results a two programmes of fundamental investiga- tion to the stable state, work on the dielectric rogen bonding has been completed and a ed dealing with possible implications for uctures. An important fact has emerged e mechanism of degradation of ceramics ric field at high temperatures, namely, that chemical rather than purely electrical in is very dependent on the materials of the d on the ambient atmosphere. Further

investigations have been made on the trapping of surface charges in dielectrics and an apparatus has been constructed which enables charges to be deposited, either directly electrically or as a result of photo-electric emission. Theoretical work on dielectric properties has led to a generalization of the Onsager theory to the frequency-dependent case, and it has been shown that electrostatic dipole coupling plays an important part in determining the distribution of relaxation times. Research on fundamental phenomena of instability has included the development of a theory of ionic diffusion in impregnated paper capacitors and this has been found to be in satisfactory agreement with observation. Another theoretical study deals with the propagation of breakdown channels through liquids and regards the channel as a vapour bubble at high pressure. The subject of the intrinsic electric strength of plastics is being re-examined using an improved technique of measurement. By the new method, intrinsic electric strengths of about twice the value given by the old method have been obtained at a temperature of 85° C.

In the Power Plant Division work on steels for high temperature and on the properties of steam continues. The investigation of the magnetically constricted low-pressure d.c. arc has been completed. This study has provided useful information on the effects of boundary layers at electrodes immersed in a low-pressure laminar stream of plasma.

A special Research Advisory Committee and Technical Panel is directing the work on the synthetic testing of circuit breakers which comes within the Division of Switchgear, Transformers and Power System Behaviour. This represents a major co-operative undertaking in which manufacturers, the Central Electricity Generating Board and the Association are involved. It is hoped that the work will lead to the national adoption of a standard method of synthetic circuit-breaker testing. Work in progress to establish the validity of the method as applied to air-blast circuit breakers has compared the severity of the direct and the synthetic test in several hundred trials made at three of the major stations of the Association of Short-circuit Testing Authorities. The maximum power-level of these tests has been 1,200 MVamp and the maximum current =0 k.amp.

Work continues at the British Iron and Steel Research Association Laboratories on improved alloys for transformer sheet. The demonstration of a relationship between resistivity and saturation has prompted a design study among manufacturers to determine whether any reduction in saturation could be accepted in return for reduced losses from increased resistivity.

In relation to switching over-voltages, useful information on the detail of surge generation and transfer has been obtained from the analysis of data from site tests on a 100-MV generator-transformer installation. Energizing a 132/33-kV transformer, to which a 33-kV earthing transformer was connected, demonstrated magnification of the switching surges transferred to the earthing transformer resulting from resonances between the 132-kV and the 33-kV systems. An investigation in progress on tolerable levels of discharge severity for oil-impregnated, paper-insulated bushings has demonstrated that degradation can be caused by test over-voltages and by surges such as may occur in service.

In the field of power system analysis, the increased facilities of the network analyser or, as it is termed, "the Integrated Computer" have been used to study transient problems of greater complexity. In addition, feasibility studies have been made of the desirability of coupling a small digital computer to the transient analyser and of

1 Research Association. Forty-fourth Annual Report for December 31, 1964. Pp. 96. (Leatherhead: The Electrical Association, 1965.)

the potentialities of such a hybrid system in power system applications.

Experimental work on the current-carrying capacity of cables continues in relation both to steady-state and to fault conditions, and considerable information has been obtained from the co-operative field investigation on the influences of the thermal properties of soil. In relation to the calculation of cable ratings, methods are being developed for the utilization of digital computer programmes.

The behaviour of the British electricity supply system in relation to thunderstorm activity throughout the ten-year period 1950-60 is the subject of a comprehensive report. The report examines the frequency of supply interruptions in the high-voltage system, their relationship with thunderstorm activity, the effect of arc suppression coils, the rate of equipment failure and the protective effects of surge diverters and spark gaps on the rate of transformer failures. Reports have also been issued analysing the frequency and distribution of thunderstorms in Britain for the years 1962 and 1963, and a ten-year thunderstorm map of Britain for the period 1955-64 is in preparation. The collection of statistical information on the frequency of lightning discharges in different parts of the world is being facilitated by the adoption, as an interim international standard, of a modification of a form of lightning-flash counter originally developed in association with the Electrical Research Association.

Electric heating is represented, in the main, by a group of researches relating to off-peak floor heating, to ceiling heating and to thermal storage. The storage of off-peak energy by means of the lime-water reaction has been studied on a full-scale test equipment in the laboratory and, following a comprehensive theoretical assessment, experiments have been put in hand on the use of sodium hydroxide.

Within the division dealing with industrial applications, the programme of work on electrical equipment for hazardous atmospheres continues. In the same division research on methods of reducing contact erosion is being pursued by a detailed study of a novel dissimilar metal system.

It appears, from theoretical considerations, that metal transfer could be virtually eliminated for a certain range of circuit conditions by the use of suitably chosen dissimilar contact metals. This has been confirmed in a series of experiments using platinum as the positive and silver or palladium as the negative electrode. Experimental work on unbalanced magnetic pull in electrical machines is now in progress on a machine specially constructed and instrumented for these measurements.

The major part of the programme of the Electronics Department is on thin films and is supported by a group of industrial contributors and by the Ministry of Aviation. It is reported that work on the deposition of metal from an ion beam has encountered difficulties, due to a flow of neutral gas molecules from the ionization chamber through the extractor electrode. The Penning ion source has been replaced by one utilizing electron bombardment to ionize copper vapour. Experimental capacitors have been made by depositing silicon oxide dielectric films on gold electrodes. Early results, however, suggest that the silicon oxide films deposited under ultra-high vacuum conditions may be insufficiently oxidized. Work on the conduction mechanism in thin films has led to an investigation of the influence of the substrate material. It has been shown that the electrical conductivity of glasses used as substrates for microcircuits is high enough to contribute to the transport of electric charges by a thin film deposited on the surface. Two new lines of investigation have been started, one on the properties of semi-conductor materials in thin film form and the other on the resistance changes occurring in thin gold films when subjected to mechanical strain.

Some of the fundamental theoretical work which has been done in applying the theory of the electrical discharge to certain astrophysical phenomena has undergone further comparison with observation. During the past year it has been demonstrated that the maximum magnetic fields recorded by Babcock's solar magnetograph of the order of 1,000 gauss are compatible with the much larger fields of  $10^4$ - $10^5$  gauss deduced from the theory of solar flares some years ago.

J. GRING

## POWER SUPPLY IN BRITAIN

IN asking the House of Commons on August 4 to note the Report and Accounts of the Electricity Council for 1963-64 and of the Central Electricity Generating Board for 1963-64, Mr. F. Leo, the Minister of Power, said that the average cost of new conventional power stations had fallen from about £80 per kW in 1955 to about £40 to-day. Britain had by far the largest output from commercial nuclear power stations of any country, and was producing more nuclear power than the whole of the rest of the Western world, including the United States. Whatever the outcome of the Government's present review of the second nuclear power programme, the industry would continue to need coal for many years to come, and the Generating Board was now using 64 million tons of coal, compared with 61 million tons in 1963-64. Fifteen new coal-fired stations were under construction, partly to replace the older stations which were closing down, and on completion of these stations the Generating Board would have much of its capacity in the form of new coal-fired stations. The working life of the new stations would be twenty-five years or more, and they would burn coal with high efficiency in the 500-MW sets which had been adopted as standard for the next few years. Forty-seven of these sets were now on order and they would produce substantially more power than the entire capacity of the industry ten years ago.

On the transmission side, the industry was introducing transmission at 400 kV. Great efforts were being made at

considerable cost to reduce the impact of the new lines on the countryside and this research had high priority. Some research was being undertaken into the use of d.c. transmission instead of a.c., and about one-third of the total expenditure of the Board on transmission lines in 1964 was spent on underground cables. This was expected to rise to more than two-thirds by 1970, and during the three years, commencing in 1968, the Board expected to spend £39 million on 1,350 miles of overhead lines, and £107 million on 500 miles of underground cable. Rural electrification was now approaching completion, and 94 per cent of the 280,000 farms in England and Wales had an electricity supply. In distribution, as in generation and transmission, research was going ahead and a new laboratory had been set up at Copenhurst. The major effort at the new laboratory would be concentrated on research into distribution and on appliances and methods of using electricity as part of a research programme covering generation, transmission and distribution, on which the industry planned to spend £10 million this year.

The Minister's observations on research were generally welcomed in the debate, but Mr. A. F. Palmer suggested that there might have been some neglect of research into the use of direct current. Sir Richard Nugent pointed out that the Select Committee on the Nationalized Industries had been critical of expenditure on research on transmission, and especially on distribution, particularly

boards. Mr. J. H. Osborn suggested that the Central Electricity Generating Board, and Energy Authority might survey the variety of water reactors, and asked whether the virtues of water and similar types of reactor for smaller units were being considered. The achievement of the Atomic Energy Authority in developing the gas-cooled reactor was welcomed in the debate. Mr. E. Lubbock and Mr. P. Jenkin were also present. The comparative cost of the gas-cooled reactor developed in the United States at

Oyster Creek: this, it was said, could produce electricity at 0.84d. per unit. These and other speakers in the debate repeatedly stressed the importance of research. In replying on the debate, Mr. J. Morris, the Parliamentary Secretary to the Ministry of Power, said that the Minister was satisfied with the programmes to date and referred to the greater co-operation and co-ordination recently established between the gas, electricity and coal industries. As regards Oyster Creek, he said that this was not a comparison of like with like, and the Dungeness tenders were all competitive under British conditions.

## BRITISH RESEARCH AND DEVELOPMENT IN TECHNOLOGY

In an answer in the House of Commons on July 27, 1965, the Minister of Technology, said that the Research Development Corporation was making grants in support of programmes and projects of research and development by various firms in the country. His Department would continue to support research by sharing the cost of contracts under the Computer Techniques Programme, involving commitments by the Government of about £965-66 and through contracts with universities and industry to a further £500,000. It had been made with the Minister of Housing and Government to bring the Computer Advisory Committee to the notice of local authorities. Twenty-three American computers were on order for Government departments, including the General Post Office. Computers of American design and foreign origin were in use in Government departments, nationalized industries. Eighty computers in Britain were in use in Government departments and 66 in the nationalized industries. A programme between the Atomic Energy Research Establishment and industry on the desalination of sea-water was under study for a 30-Mgal./day plant, distillation techniques, had been completed in 1964 with Weir, Westgarth, Ltd.

He said that he had appointed Dr. J. B. Condon to the Ministry of Technology with effect from July 12, and that Dr. Adam would be in charge of the scientific and technological work of the industry. An chart for the Ministry of Technology was the Official Report for July 27, and the work of the Economics and Statistics Division in detail. This Division is organized in four branches: Economics Branch, responsible for advising on economic aspects of the Ministry's policies and

This Branch provides support for the industries conducted by the technological units within the Ministry. (2) Statistics Branch is responsible for collating, analysing and statistics of industries for which the Ministry has functions. These statistics are collected by the Board of Trade. (3) Manpower

Branch, which is concerned with manpower resources for science and technology and keeps under review the human and social problems resulting from technological change. (4) Engineering Branch, which has the main function of initiating or encouraging action to enhance the status of engineers and to improve the supply. It is also responsible for co-ordinating the implementation of the recommendations of the Fielden Report on Engineering Design.

Mr. Cousins also stated that the Ministry communicated technological information by personal contact, scientific and technical publications, exhibitions, conferences, press, film, radio and television, and was building up a network of regional offices and industrial liaison centres to supplement this effort. The Ministry's laboratories dealt with about 2,500 enquiries each month, mainly from industry. Much information was being received from scientific counsellors in foreign countries. The Department was undertaking a technical and economic study of the scientific industrial process-control instrument industry, and had initiated studies of engineering standards, the metric system and the industrial use of new materials. Also contained in the Official Report for July 27 was a full list of the leaders of the various scientific and technological teams studying selected industries and processes, with their date of appointment and relevant experience.

On July 27, replying to a question regarding the Advisory Council on Technology in the House of Lords, Lord Snow, the Parliamentary Secretary to the Ministry, said that the Council had met ten times; its terms of reference were to advise on the application of advanced technology in British industry and to give special attention to the industries for which the Minister had sponsorship responsibility. Among subjects considered have been the status of engineers and designers, development of British standards for export markets, the promotion of the metric system and problems of industrial structure. The relations between the Council for Scientific Policy and the Advisory Council on Technology were close and there was common membership. Lord Snow thought that there was no danger of any serious lacuna between them. Relations between the Ministry and the Department of Economic Affairs must also be close and he thought there was little danger of overlapping.

## THE MELLON INSTITUTE

The second annual report of the Mellon Institute \* for the year ended February 28, 1965, at the close of which the staff of 539 included 355 professional and technical personnel. A list of visitors' lectures at colloquia and another of members' publications during the year, and brief accounts of research investigations

during the year refer, under biochemistry and molecular biology, to improvements in instrument design and experimental procedures which have given a precision of 0.2-0.3 per cent in determining sedimentation coefficients in examining structural transformations in *Bromegrass* mosaic virus, while further electron microscopical studies of actomyosin have confirmed the identification of 'ladders' as the principal morphological form of con-

\* Fifty-second Annual Report for the Fiscal Year ended February 28, 1965. (Pittsburgh: Mellon Institute, 1965.)



tractile suspensions. Visible light shadows of a model of part of the tropocollagen macromolecule, made from foam plastic atomic models, with one or more of the three strands broken indicate that electron microscopy should detect collagenase activity on tropocollagen.

Environmental investigations have been concerned with the purification of the atmosphere, the electrokinetic properties of particles suspended in water and the presence of organic compounds in water. A revision of Volume 2 of *Systems and Specifications of the Steel Structures Painting Manual* was prepared, and the feasibility of protecting steel temporarily by depositing small amounts of metallic zinc on bare metal surfaces by shot-blasting was examined. An investigation of the damaging effects of light on art objects and of new methods for technical examination of museum objects continued; several reviews of the literature were published.

Under "Structure and Properties of Organic Materials" reference is made to a kinetic study of the nucleocarbamoylpyridinium ion and to the ring-opening reaction of dimethylcarbamoylpyridinium chloride with hydroxide ion to give a compound which afterwards decomposes to glutacondialdehyde; to the synthesis of the tetracyano-dimethane derivative of dibenzopentalenoquinone, which forms deeply coloured salts with amines; to the synthesis of turnefordene and hastanecine and their identification with the basic nitrogenous fragments obtained by cleavage of *Sesecio* alkaloids; and to the identification of hydrocarbons obtained by heating 1 : 2 : 6-cyclononatriene. Further work on the thermal degradation of wet marine muds has demonstrated the formation of benzene, thiophen, styrene and possibly methylthiophen, in addition to the toluene and *m*-xylene known to arise from the carotenoid components. Since phenylalanine yields benzene, toluene, styrene and other products, it may be a natural precursor of these aromatic hydrocarbons. Investigations of petroleum asphaltene in the bending region of the infra-red have indicated the existence of four distinct absorption bands, and an extension of the dry-filtration theory has provided a reliable means for specifying requirements of filter media based on the electrostatic contributions of both the fabric and the collected particulate matter.

Considerable information was obtained during the year on internal motions in radicals by examining details of their electron spin resonance patterns, while the Bendix Time-of-Flight mass spectrometer has proved very suitable for examining ionization reactions occurring with electrons of less than several thousand electron volts energy. A series of cyclo dienes and their diepoxides has been synthesized for cyclopolymerization investigations of dicyclic compounds; light-scattering and viscosity examinations of a series of linear polystyrenes of narrow molecular weight distribution between 10° and 110° C in dilute solutions in decalin were almost completed. Light scattering and viscosity investigations of some poly(*bis*-phenol-*A*-carbonate) fractions were undertaken, and a comparison was made of the crystallization kinetics of nylon 6 and nylon 66 in the light of recently developed concepts of crystallization. Search continued for improved weather- and corrosion-resistant coatings for building materials.

The viscoelastic behaviour of glass-forming materials is being examined and further mathematical analyses made on the theory of the free-vibration experiment for a linear viscoelastic material. The crystal structure of tetrahydro-*p*-benzoquinone dehydrate has been determined and refined from three-dimensional Weissenberg data and an electron spectrometer further developed and used to investigate inelastic electron scattering. Experimental facilities for low-frequency internal rotation spectroscopy were greatly expanded, and work on internal rotation continued vigorously, while the procedure for obtaining accurate solutions of the molecular Schrödinger equation was improved. The measurement and interpretation of the electronic spectra of transition metal cyanide complexes were extended to all the known complex cyanides, and the presence of hydrophobic bonding in the quaternary ammonium halides in aqueous solution was investigated by electrical conductance, transference and viscosity measurements. Chemical and physical properties of a set of molten quaternary ammonium salts were investigated, as well as the electrical conductivity of acidic solids in ultra-high vacuum and the silica-alumina catalysed isomerization of cyclopropane and the heat capacity below liquid-helium temperature of a series of copper-zinc alloys.

## MAGNETOSPHERE AND THE MARTIAN BLUE CLEARING

By DR. RONALD BLUM

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WHEN Mars is observed in yellow or red light, surface details of the planet can be seen; however, in blue or ultra-violet light of wave-length  $\lambda < 4550 \text{ \AA}$  one sees a uniformly hazy disk with occasional bright patches, which obscures surface details<sup>1</sup>. This blue haze, believed to be due to a 'violet layer' in the upper Martian atmosphere, has been observed to clear up for several days at a time, particularly near opposition, when the Earth is between Mars and the Sun. It has been suggested<sup>2</sup> that the blue haze may be due to the ionizing effect of solar protons on the Martian atmosphere; the protons are deflected by the Earth's magnetic field when the two planets are in opposition. This hypothesis has been rejected by Sagan<sup>3</sup> on the grounds that: (1) 2-MeV protons are required to produce molecular ions with the requisite absorption bands at an altitude of 150 km in the Martian atmosphere, whereas solar wind protons are of the order of 1 keV; (2) proton fluxes of  $5 \times 10^{14}/\text{cm}^2\text{-sec}$  are needed—large compared to solar wind fluxes of the order of  $10^8/\text{cm}^2\text{-sec}$ ; (3) the magnetosphere is too small to produce the required shadow effect at Mars; (4) the dipole field falls off too rapidly to have any effect at Mars's radius.

A possible answer to objections (3) and (4) may be found in Michel's calculation of the Mach angle of the oblique hydromagnetic shock extending into the solar wind downstream of the magnetosphere<sup>4</sup>. Typical values of the solar wind parameters yield a hydromagnetic Mach number of 6, and a Mach angle of  $8.5^\circ$ – $9.5^\circ$  (the Mach cone is slightly asymmetrical). Thus, the region behind the shock, or 'wake', would have the appearance of a circular cone with its apex at the Earth, and an apex angle of approximately  $9^\circ$ . The axis of the cone should actually lag the Earth-Sun line by about  $5^\circ$  due to the orbital motion of the Earth. This is very suggestive when compared with the observation<sup>5</sup> that the Martian blue clearing is not observed whenever Mars lies very far above or below the ecliptic at opposition. Thus it would seem that clearing is possible only when Mars passes through a cone of small angle the apex of which is at the Earth, and the axis of which is approximately along the Earth-Sun line.

If we assume that the Earth and Mars both lie in the ecliptic plane and have nearly circular orbits, we may roughly calculate how long it would take Mars to pass through the conical wake of the Earth. We let  $R_e$  be the radius of the Earth's orbit;  $R_m$  = the radius of Mars's

orbit;  $T_e$  = the period of the Earth's orbit;  $T_m$  = the period of Mars's orbit;  $\alpha$  = the Mach angle of the Earth's wake;  $T$  = the time required for Mars to pass through the wake. Since the base of the wake at Mars's orbit has a radius:

$$R = (R_m - R_e)(\tan \alpha) \quad (1)$$

it subtends an angle of  $2R/R_m$  at the Sun. If Mars's trajectory subtends an angle  $2\pi T/T_m$  at the Sun during time  $T$ , then the Earth's trajectory subtends an angle:

$$2\pi T/T_e = 2\pi T/T_m + 2R/R_m \quad (2)$$

during this time. Thus we find:

$$T/T_e = \left( \frac{R_m - R_e}{R_m} \right) \left( \frac{T_m}{T_m - T_e} \right) \left( \frac{\tan \alpha}{\pi} \right) \quad (3)$$

which yields  $T = 10.5$  days for closest opposition (34.5 million miles) and  $T = 16.1$  days for furthest opposition (63.5 million miles), for  $\alpha = 9^\circ$ . Sagan<sup>8</sup> notes: 'Most reported durations of opposition blue clearings lie in the range between 1 and 30 days, although there is a tendency for blue clearings at favourable oppositions to last nearer 30 days than 1'. W. M. Sinton<sup>9</sup> estimates the average clearing to last about ten days. Thus, the interference of the Earth's Mach cone with the solar wind yields clearing times of the right order of magnitude. This wake should not be confused with the extended tail of the magnetosphere, a cylinder of approximately forty Earth radii in diameter and far too small to account for the observed clearing times.

We may adduce further observations to favour the hypothesis that the Martian blue clearing is caused, in some way, by the magnetohydrodynamic wake due to the interaction of the geomagnetic field with the solar wind. These are: (1) clearing may lead or lag opposition by as much as 10–14 days, an effect which shows some dependence on the heliocentric latitudes of the Earth and Mars; (2) clearing durations seem to depend inversely on the difference in heliocentric latitudes of the Earth and Mars; (3) there is a statistical tendency for blue clearings to lag opposition<sup>1</sup>. The first two observations are plausible if one considers the asymmetries and fluctuations in the solar wind; the last agrees with the lag due to the Earth's orbital motion. Furthermore, preliminary *Mariner IV* results<sup>6</sup> indicate that the surface pressure of the Martian atmosphere is only about ten millibars, that its magnetic field is not greater than 0.1 per cent that of the Earth, but that it does possess an ionosphere with a density of about  $10^6$  electrons/cm<sup>3</sup>. Thus, the violet layer is much more exposed to the solar wind than previously surmised.

If we now repeat Sagan's calculations, using Chamberlain's model<sup>7</sup> of the Martian atmosphere, reduced in density by an order of magnitude to agree with the *Mariner IV* results, we find that there are approximately  $10^{-4}$  g/cm<sup>3</sup> of gas above the violet layer. If we compare this figure with the range of protons in air<sup>10</sup> we find that  $> 85$ -keV protons can penetrate to this depth in the Martian atmosphere. Such energies are commonly found in solar flares; furthermore, since the notoriously turbulent interplanetary magnetic field should be even more unstable in the vicinity of Mars, there is an enhanced possibility of local acceleration processes. Objection (2) still holds, implying that some other mechanism besides that of Urey and Brewer gives rise to the blue haze; however, my hypothesis does not stipulate the mechanism of interaction, only that such an interaction may occur.

The question arises: Will the shock attenuate appreciably as it expands downstream of the Earth, and will the energy density in the shock be a significant fraction of the energy density of the solar wind arriving at the orbit of Mars? To answer these questions we must look at the shock from the point of view of an observer moving outward from the Sun with the solar wind. As the Earth approaches him the solar wind is forced to flow around the magnetosphere, which is approximately a cylinder with a hemispherical nose some twenty to forty  $r_e$ ,

Earth radii ( $r_e = 6,400$  km), in diameter. This will compress the interplanetary magnetic field around the obstacle and form a cylindrical magnetosonic wave propagating radially outward into the solar wind from the surface of the magnetosphere. This is, in essence, the stationary shock wave as seen by a terrestrial observer.

To calculate the energy in this wave we may estimate energy density by means of the normal one-dimensional magnetohydrodynamic Rankine-Hugoniot conditions applied at the front of the magnetosphere. For typical values of the solar wind's parameters its internal (magnetic plus thermal) energy density at Mars's orbit is  $U \sim 10^{-10}$  erg/cm<sup>3</sup>, its convective energy,  $W \sim 20 \times 10^{-10}$  erg/cm<sup>3</sup>. The internal energy density behind the shock at Earth is calculated<sup>11</sup> to be  $U_{se} \sim 100 \times 10^{-10}$  erg/cm<sup>3</sup>, initially located in an annular pulse of radius  $10r_e$  about the Earth-Sun axis. For a hydromagnetic Mach number  $M = 6$ , this annular pulse will undergo an increase in radius of  $(R_m - R_e)/M$  by the time it reaches the orbit of Mars. Since the conductivity of the solar wind is  $\sigma \sim 5 \times 10^{-7}$  (E.M.U.), the characteristic decay time of hydromagnetic disturbances is  $\tau \sim 4\pi\sigma L^2$ , where  $L \sim 10r_e/M \sim 10^6$  cm; hence,  $\tau \sim 6 \times 10^{13}$  sec. Thus, neglecting turbulence or dissipation we assume that the wave propagates undistorted through the interplanetary medium; the decrease in its energy density is solely due to geometrical factors. At Mars the energy density of the pulse is  $U_{sm} \sim (10Mr_e/(R_m - R_e))U_{se} = 0.5 \times 10^{-10}$  erg/cm<sup>3</sup>, or 50 per cent of  $U$  and 2.5 per cent of  $W$ . Despite the imprecision of such a calculation, these numbers lend credence to the idea that the shock wave may persist with sufficient strength to affect significantly the flow of the solar wind at the orbit of Mars, where the weakness or absence of a Martian magnetic field leaves the violet layer vulnerable to changes in the solar wind.

Although the shock does not attenuate, one would expect its energy density to be degraded in the form of a turbulent wake as it propagates outward; in such a case it has been suggested<sup>12</sup> that the passage of the shock may serve to trigger an instability in the wind capable of causing the blue clearing. Another possibility is that the tail of the Earth's magnetosphere is kept open, and even broadened, by the radiation pressure of hydromagnetic waves propagating into the tail from regions of higher magnetic field<sup>13</sup>. It should be mentioned that preliminary *Mariner IV* results from measurements made over a period of 5 days at  $3,000r_e$  downstream of the Earth showed no evidence of a geomagnetic tail; this does not, however, preclude the possibility of the shock, through which the spacecraft may have already passed.

In my calculations I have assumed the duration of the pulse to be determined by the radius of the magnetosphere, not by the length of the magnetospheric tail.

In conclusion, I should emphasize that this article was written in a speculative vein in the hope of stimulating further discussion of this topic. There is, at present, only one competing theory<sup>14</sup>, namely, that the blue haze is caused by dust particles from meteor streams passing Mars, and that the blue clearing is a purely random effect, unrelated to opposition.

I thank Drs. E. N. Parker, A. Palm and W. M. Sinton for their advice.

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<sup>3</sup> Sagan, C., *Icarus*, **1**, 70 (1962).

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<sup>7</sup> Sinton, W. M., *App. Optics*, **3**, 175 (1964).

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<sup>10</sup> Bates, D. R., in *The Earth as a Planet*, edit. by Kuiper, G. P. (Univ. Chicago Press, 1964).

<sup>11</sup> Blum, R., *Icarus*, **1**, 459 (1963).

<sup>12</sup> Parker, E. N. (personal communication).

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<sup>14</sup> Palm, A., and Basu, B., *Icarus*, **4**, 111 (1966).

# MAGNETOTURBULENT ENERGY AND STABILITY OF SUPERMASSIVE STARS

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THE discovery and subsequent investigation of the quasi-stellar radio objects, or 'quasars', have aroused great interest by many investigators<sup>1-3</sup>. The observed luminosities are of the order of  $10^{44}$  ergs sec<sup>-1</sup> and their lifetimes fall in the range of  $10^4$ - $10^6$  years. Hoyle and Fowler<sup>4</sup> proposed that a mass of the order of  $10^6 M_\odot$  has condensed in the galactic nucleus into a single massive star in which nuclear energy generation occurs. Later on they suggested the release of gravitational energy during general relativistic collapse after the exhaustion of nuclear matter by the ONO bi-cycle. Fowler<sup>5</sup> showed that these massive stars become unstable before the central temperature has risen to  $5.8 \times 10^7$  °K for  $10^6 M_\odot$ . This discussion was based on the binding energy of massive stars in hydrostatic equilibrium which he derived from general relativistic equations in the post-Newtonian approximation of general relativity. Chandrasekhar<sup>6-11</sup> used the exact dynamical equations of general relativity and obtained the same result. Fowler showed that general relativistic instability sets in for masses greater than  $10^6 M_\odot$ .

Recently, Fowler<sup>12</sup> suggested that this general relativistic instability can be removed by a small amount of rotation. On assuming that these massive stars are uniformly rotating gaseous systems, he showed that masses up to  $10^7 M_\odot$  are possible, consistent with the maximum angular momentum. If one takes a non-uniformly rotating system in which the central regions rotate faster than the outer regions, then stable masses of  $10^6 M_\odot$ - $10^{10} M_\odot$  are also possible. In the case of uniform rotation, Roxburgh<sup>13</sup> also obtained the same result.

Layzer<sup>14</sup> suggested that magnetoturbulent energy can also be effective in stabilizing these massive stars. In this article, I shall show, using Fowler's binding energy method, that the turbulent kinetic energy associated with the internal magnetic disturbances (with conservation of magnetic flux) is also an effective physical mechanism to remove the general relativistic instability. It is found that stable masses of  $10^6 M_\odot$ - $10^{10} M_\odot$  are possible depending on the parameter  $\alpha$ , which will be defined later.

The binding energy  $E_b$  of a star is equal but opposite in sign to the total energy  $E$  due to rest mass energy and 'bulk motions' throughout the star and is given by:

$$-E_b = E = (M - M_0) c^2 + E_{\text{dyn}} \quad (1)$$

where  $E_{\text{dyn}}$  is the dynamical energy due to 'bulk motions'. Here:

$$M = \int_0^R dM_r = \int_0^R 4\pi r^2 \rho \, dr \quad (2)$$

where  $R$  is the radius of the star,  $M_r$  is the mass interior to radius  $r$ , and  $\rho$  is the mass-energy density measured by a local observer and is given by:

$$\rho = \rho_0 + \frac{u}{c^2} \quad (3)$$

where  $\rho_0$  is the rest mass density and  $u$  is the internal energy due to gas and radiation. The rest mass ( $M_0$ ) of the star is given by:

$$M_0 = \int_0^R \rho_0 \left(1 - \frac{2GM_r}{rc^2}\right)^{-1} dV \quad (4)$$

where  $dV$  is the co-ordinate element of volume  $V$ . Using equations (2)-(4) in equation (1), we get  $E$  in the post-Newtonian approximation as follows:

$$E = \int u dV - \int \frac{GM_r}{r} \rho \, dV + \int \frac{GM_r}{rc^2} u dV - \frac{3}{2} \int \frac{G^2 M_r^2}{r^2 c^2} \rho \, dV + E_{\text{dyn}} \quad (5)$$

The physical interpretation of each term is already given by Fowler<sup>5,12</sup>.

In the present case, the contribution of dynamical energy which arises due to magnetic disturbances is considered. It is well accepted now that, in a medium of high electrical conductivity, turbulence will be associated with a spontaneous generation and subsequent amplification of magnetic fields since the pioneer work by Batchelor<sup>15</sup>. Also, in a state of equilibrium, the energy per unit volume in the magnetic field and the velocity fields will approach equality or equipartition<sup>16,17</sup>. Later on Chandrasekhar<sup>18</sup> showed for stationary, homogeneous and isotropic turbulent conditions that magneto-turbulent energy ( $m_e$ ) is given by:

$$m_e = \frac{1}{8\pi} \langle H^2 \rangle = \alpha \frac{1}{2} \rho \langle v^2 \rangle \quad (6)$$

where  $\alpha$  is 1.6265,  $\langle v^2 \rangle$  and  $\langle H^2 \rangle$  are the mean square velocity and magnetic intensity respectively.

Assuming conservation of flux,  $\langle H \rangle R \sim \text{constant}$ <sup>14,18</sup>, since lines of force are frozen and they are crowded together. In this case we write  $E_{\text{dyn}}$  as:

$$E_{\text{dyn}} = \frac{m_e}{\alpha} = \frac{\Phi^2}{\alpha R^2} \quad (7)$$

where  $\Phi$  is the magnetic flux and all other constants are now included in  $\Phi^2$ .

The equation of hydrostatic equilibrium in the post-Newtonian approximation of general relativity is<sup>4</sup>:

$$\frac{dp}{dr} = - \frac{GM_r \rho}{r^2} \left(1 + \frac{p}{\rho c^2} + \frac{2GM_r}{rc^2} + \frac{4\pi p r^2}{M_r c^2}\right) \quad (8)$$

To obtain the virial theorem, we multiply equation (8) by  $4\pi r^2 dr$  and integrate, we get:

$$3 \int p dV - \int \rho \frac{GM_r}{r} dV + \int \frac{GM_r}{rc^2} p dV - 3 \int \frac{G^2 M_r^2}{r^2 c^2} \rho dV = 0 \quad (9)$$

Combining equations (5), (7) and (9), we get:

$$E = - \frac{(3\bar{\Gamma}_4 - 4)}{3(\bar{\Gamma}_4 - 1)} \int \frac{GM_r}{r} \rho dV + \frac{2}{3} \int \frac{GM_r}{rc^2} u dV + \frac{3}{2} \frac{5 - 3\bar{\Gamma}_4}{3(\bar{\Gamma}_4 - 1)} \int \frac{G^2 M_r^2}{r^2 c^2} \rho dV - \frac{1}{\alpha} \frac{\Phi^2}{R^2} \quad (10)$$

where we have put<sup>12</sup>:

$$p = (\Gamma_1 - 1) u, \quad \Gamma_1 - 1 = \frac{\gamma - 1}{3(\gamma - 1) - \beta(3\gamma - 4)} \quad (11)$$

where  $\beta$  is the ratio of gas pressure to total pressure due to gas and radiation. For massive stars  $\beta < 1$ , then:

$$\Gamma_1 \sim \frac{4}{3} + \frac{1}{\beta} \quad (12)$$

It should be noted that if we include Newtonian non-linear terms due to velocity (as Fowler<sup>12</sup> included centrifugal acceleration) in equation (8), then the coefficient of  $1/\alpha \Phi^2/R^2$  in equation (10) will become  $(5 - 3\Gamma_1)/3(\Gamma_1 - 1)$ , which, for massive stars, is of order one. I shall discuss this case more elaborately in a later publication, in which the interaction of magnetic fields with rotation will be considered. Using equation (2) in (10) and evaluating the integral for polytropic index  $n = 3$ , we get<sup>13,14</sup>:

$$\frac{E}{Mc^2} \sim -\frac{3}{8}\beta\left(\frac{R_g}{R}\right) + 1.265\left(\frac{R_g}{R}\right)^2 - \frac{1}{\alpha}\left(\frac{\Phi^2}{Mc^2 R^2}\right) \quad (13)$$

where  $R_g$  is Schwarzschild's radius and it is given by:

$$R_g = \frac{2GM}{c^2} = 3 \times 10^3 (M/M_\odot) \quad (14)$$

The condition of magnetic stability of a magnetic star due to Chandrasekhar and Fermi<sup>15,16</sup> is the following:

$$(3\Gamma_1 - 4)(|\Omega| - m_*) > 0, \Gamma_1 = \beta + \frac{(4 - 3\beta)^2(\gamma - 1)}{\beta + 12(\gamma - 1)(1 - \beta)} \quad (15)$$

where  $\Gamma_1$  is Chandrasekhar's radiative adiabatic coefficient. In general relativity  $\Gamma_1 \rightarrow 4/3$ , hence magnetic instability sets in if  $|\Omega| < m_*$ . This gives the critical value of  $\Phi_{cr}$  as:

$$\Phi_{cr}^2 = \frac{3}{2} GM^2 R \quad (16)$$

for polytropes  $n = 3$ . We define a parameter 'a' given by:

$$a = \frac{\Phi^2}{\Phi_{cr}^2} \quad (17)$$

which shows that magnetically stable configurations are possible only for  $a \leq 1$ . Using equations (16) and (17) in equation (13), we get:

$$\frac{E}{Mc^2} \sim -\frac{3}{8}\beta\left(\frac{R_g}{R}\right) + 1.265\left(\frac{R_g}{R}\right)^2 - \frac{3a}{4\alpha}\left(\frac{R_g}{R}\right) \quad (18)$$

Choosing the critical value of  $R_g$  at the moment when the minimum in  $E/Mc^2$  is reached, equation (18) gives:

$$R_{cr} = \frac{5.487}{a(1 + 0.8\beta/\alpha)} R_g \quad (19)$$

Now the radius of main-sequence massive objects given from the structure of the star in Schwarzschild radius unit is given by<sup>8</sup>:

$$R_{ms} = 7.5 \times 10^3 R_g \left(\frac{M}{M_\odot}\right)^{-1}$$

Hence from equations (19) and (20) we have:

$$\frac{R_{ms}}{R_{cr}} = 1.387 \times 10^3 a_1 \left(\frac{M_\odot}{M}\right)^{\frac{1}{2}}$$

where  $a_1 = (a + 0.81\beta)$ . Equation (21) shows that stability sets in for masses greater than:

$$M = 1.88 \times 10^{10} a_1^2 M_\odot$$

It can be seen from equation (22) that the magnetically stable masses (for  $a = 1$ ) up to the order of  $1.8 \times 10^{10}$  are possible (since  $\beta < 1$ ).

I have shown in this article that magnetoturbulent energy is also very effective in removing general relativistic instability. On varying  $a_1^2$  from  $10^{-1}$  to  $10^{-4}$ , we find that stable masses of  $10^9 M_\odot$ – $10^{10} M_\odot$  are possible. How the interaction between non-uniform rotation and magnetic fields reduces the angular velocity for equatorial instability, and their maintenance in supermassive stars will be discussed in much detail in a later publication.

I thank Prof. William A. Fowler for directing my interest to this problem, and for showing me the manuscript of his paper<sup>15</sup> in advance of sending it for publication. I also thank Dr. Gerard Stephenson, jun., for suggestions and comments.

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## A NEW METHOD FOR DEUTERON STRIPPING CALCULATIONS (I)

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AS is well known, all deuteron stripping reactions are potentially capable of yielding considerable information regarding nuclear energy-levels and nuclear structure. The simple theory developed many years ago by the present author<sup>1,2</sup> has proved useful for determining spins and parities of nuclear states in many cases. It suffers the disadvantage, however, that it over-estimates absolute cross-sections, so that it may reasonably be expected to yield information only of ratios of nuclear reduced widths. Moreover, experimental angular distributions of reactions involving medium and heavy nuclei sometimes

show little relationship to the results of the simple theory and for such cases no determination of spins and parities can be made on the basis of this theory.

Attempts to improve the theory have generated several distorted wave Born approximation (DWBA) theories<sup>3</sup>. The DWBA approach, however, is based on a premise which in my opinion is unjustified<sup>4</sup>. It describes the centre of mass motion of the deuteron by an optical model wave function, but leaves the internal motion unpolarized. Yet it is likely that contributions to stripping reactions arise predominantly from deuterons which

considerably stretched, with one particle interacting on the average much more strongly with the nucleus than the other, so that deuteron distortion effects are in all probability quite crucial<sup>1</sup>. Although it may well be possible to choose optical model parameters to fit deuteron elastic scattering, this does not mean to say that the full deuteron wave function is known, particularly in those configurations relevant in stripping.

These remarks are borne out by actual results of calculation. As a general rule agreement with experimental stripping angular distributions can be obtained in the DWBA theory only by a choice of parameters different from those required in elastic scattering. Moreover, the number of parameters involved is so great that any unique assignment of spins and parities is lost.

I suggest a new approach which appears quite promising, particularly for  $(d,p)$  reactions. This was reported briefly at the 1964 Conference of the Australian and New Zealand Association for the Advancement of Science<sup>4</sup>.

The basic matrix element for a  $(d,p)$  reaction, for example, may be written in the form:

$$I(\mathbf{k}_p, \mathbf{k}_d) = \langle \psi_d^+(\mathbf{k}_d, \mathbf{r}_p, \mathbf{r}_n) | V_{np} | F(\mathbf{r}_n) \psi_p^-(\mathbf{k}_p, \mathbf{r}_p) \rangle \quad (1)$$

Here  $\mathbf{r}_p$  and  $\mathbf{r}_n$  are the neutron and proton co-ordinates respectively,  $\mathbf{k}_d$  is the incident deuteron momentum and  $\mathbf{k}_p$  is the outgoing proton momentum. The wave function  $\psi_d^+$  describes elastically scattered deuterons with outgoing spherical waves,  $\psi_p^-$  describes elastically scattered protons with incoming spherical waves and  $F$  is the wave function of the final bound neutron. The normal neutron proton interaction is represented by  $V_{np}$ . In the form of equation (1) all other nuclear co-ordinates have been integrated out, so that  $\psi_p^-$  may be considered to be a known optical model wave function.

Now we assume, instead of the usual distorted wave, that  $\psi_d^+$  satisfies the equation:

$$(T_n + T_p + V_{np} + V_n + V_p) \psi_d^+ = E \psi_d^+ \quad (2)$$

where  $T_n$  and  $T_p$  are kinetic energy operators and where  $V_n$  and  $V_p$  are optical potentials for neutron and proton, respectively, incident on the initial nucleus. Strictly these are energy dependent and should be considered as operators dependent on the square of the momenta of the particles.

We know that a plane wave deuteron has the following transform:

$$e^{i\mathbf{k}_d \cdot \mathbf{r}} \chi(\mathbf{r}) = \frac{N}{2\pi^3} \int d\mathbf{k}'_p G(\mathbf{k}'_p, \mathbf{k}_d) e^{i\mathbf{Q}' \cdot \mathbf{r}_n} e^{i\mathbf{k}'_p \cdot \mathbf{r}_p} \quad (3)$$

where  $\mathbf{Q}' = \mathbf{k}_d - \mathbf{k}'_p$ . The internal deuteron wave function is written:

$$\chi(\mathbf{r}) = N (e^{-\gamma r} / r - e^{-\zeta r} / r)$$

and:

$$G(\mathbf{k}'_p, \mathbf{k}_d) = \left\{ \frac{1}{(\mathbf{k}_d/2 - \mathbf{k}'_p)^2 + \gamma^2} - \frac{1}{(\mathbf{k}_d/2 - \mathbf{k}'_p)^2 + \zeta^2} \right\} \quad (4)$$

Equation (3) may be discussed in time-dependent terminology in a way which can be instructive. If a 'snap-shot' be taken of a moving deuteron wave packet with an 'exposure time' short compared with the natural period of the deuteron, the neutron and proton would appear to be described by independent wave packets with momenta  $\mathbf{k}'_p$  and  $\mathbf{Q}'$ . The probability for finding given momenta is described by  $G$ .

For deuterons with a sufficiently high momentum involved in a stripping reaction it would seem plausible that any interaction of the neutrons and protons with the initial nuclei might occur in a period small compared with the natural period of the deuteron; this might be particularly the case if the reaction tends to be confined to a surface volume. It could then be a reasonable approach to use a 'sudden' approximation for  $\psi_d^+$  in equation (1) and to write it:

$$\psi_d^+ = \frac{N}{2\pi^3} \int d\mathbf{k}'_p G(\mathbf{k}'_p, \mathbf{k}_d) \psi_n^+(Q', \mathbf{r}_n) \psi_p^+(\mathbf{k}'_p, \mathbf{r}_p) \quad (5)$$

where  $\psi_n^+$  and  $\psi_p^+$  are optical model wave functions for neutron and proton respectively with the appropriate momenta. Thus  $\psi_n^+$  and  $\psi_p^+$  satisfy wave equations:

$$(T_n + V_n) \psi_n^+ = E_n \psi_n^+$$

and:

$$(T_p + V_p) \psi_p^+ = E_p \psi_p^+ \quad (6)$$

with  $E_n$  and  $E_p$  being energies appropriate to the momenta  $\mathbf{k}'_p$  and  $\mathbf{Q}'$  respectively.

We use equation (2) to re-express the quantity  $V_{np} \psi_d^+$  of the matrix element (1) as follows:

$$V_{np} \psi_d^+ = \{E - (T_n + T_p + V_n + V_p)\} \psi_d^+ \quad (7)$$

On now substituting the approximation (5) for  $\psi_d^+$ , and making use of the wave equations (6), the matrix element (1) takes the form:

$$I(\mathbf{k}_p, \mathbf{k}_d) = - \frac{\lambda^2 N}{2\pi^3 m} \int d\mathbf{k}'_p g(\mathbf{k}_d, \mathbf{k}'_p) \langle \psi_p^+(\mathbf{k}'_p, \mathbf{r}_p) | \psi_p^-(\mathbf{k}_p, \mathbf{r}_p) \rangle \langle \psi_n^+(Q', \mathbf{r}_n) | F(\mathbf{r}_n) \rangle \quad (8)$$

where  $m$  is the nucleon mass and:

$$g(\mathbf{k}_d, \mathbf{k}'_p) = \{(\mathbf{k}_d - \mathbf{k}_p/2)^2 + \gamma^2\} G(\mathbf{k}_d, \mathbf{k}'_p) \quad (9)$$

The element  $\langle \psi_p^+(\mathbf{k}'_p, \mathbf{r}_p) | \psi_p^-(\mathbf{k}_p, \mathbf{r}_p) \rangle$  is determinable; it is predominated by a term  $2\pi^3 \delta(\mathbf{k}_p - \mathbf{k}'_p)$ . Thus the predominant term in equation (8) is simply:

$$I(\mathbf{k}_p, \mathbf{k}_d) = - \frac{4\pi N \lambda^2}{m} g(\mathbf{k}_d, \mathbf{k}_p) \langle \psi_n^+(Q, \mathbf{r}_n) | F(\mathbf{r}_n) \rangle \quad (10)$$

Equation 10 is much more amenable to numerical calculation than the usual DWBA approach. It involves a bound state neutron wave function  $F$ , characterized by a given orbital angular momentum  $l$ , and the neutron optical model wave function  $\psi_n^+$ . Thus the angle integrations in equation (10) may be trivially performed leaving a one-dimensional integral over the neutron radial co-ordinate  $r_n$ . Parameters of the radial neutron optical model wave function are appropriate to an energy  $\lambda^2 Q^2 / 2m$  and are therefore different for each angle of scattering. Nevertheless neutron optical model parameters have been well tabulated over large energy ranges, so that equation (10) may be considered to have no unknown parameters except the orbital angular momentum of the bound neutron.

Perhaps the main difficulty of this approach lies in the treatment of the coulomb interaction between proton and nucleus which is not of short range and for which the sudden approximation cannot be expected to apply accurately. To the extent, however, that the main coulomb deviations of the proton occur in the vicinity of the nucleus no serious coulomb error should occur within this formalism, at least for  $(d,p)$  reactions.

In a forthcoming publication<sup>5</sup> by Dr. C. A. Pearson and me it will be shown how equation (8) can be formally derived by means of standard impulse approximation techniques. Numerical calculations are being undertaken by Dr. Pearson and will also be published shortly. Preliminary results of these calculations, which include also the polarization resulting from stripping reactions, appear to be in remarkably good agreement with experiment.

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## A NEW METHOD FOR DEUTERON STRIPPING CALCULATIONS (II)

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A FEW impulse approximation method for use in deuteron stripping and pick-up calculations has recently been presented by S. T. Butler<sup>1</sup>. This method goes beyond the simple Butler-Born (B-B) approximation<sup>2</sup>, yet is free from the conceptual and methodological difficulties which beset distorted wave Born approximations.

To exemplify the method, Butler considers a  $(d,p)$  reaction. The basic matrix element may be written, in the usual notation<sup>3</sup> and with the nuclear co-ordinates integrated out, as:

$$I(\mathbf{k}_d, \mathbf{k}_p) = \langle F^*(\mathbf{r}_n) \psi_p^-(\mathbf{k}_p, \mathbf{r}_p) | V_{np} | \psi_n^+(\mathbf{k}_d, \mathbf{r}_n, \mathbf{r}_p) \rangle \quad (1)$$

Here  $\mathbf{k}_d$  and  $\mathbf{k}_p$  are respectively the incident deuteron and outgoing proton momenta, and  $F$  is the wave function of the final bound neutron with binding energy  $-E_B$  ( $E_B \equiv \hbar^2 \chi^2 / 2m$ ). The impulse approximation consists of writing the initial deuteron wave function  $\psi_n^+$ , as a momentum space convolution integral between a free deuteron wave function and the optical model wave functions for neutron and proton interacting separately with the nucleus. Then, after some manipulation<sup>1,2</sup>, one obtains the result (the final equation (10) of ref. 1):

$$I(\mathbf{k}_d, \mathbf{k}_p) = -\frac{\hbar^2}{m} g(\frac{1}{2}\mathbf{k}_d - \mathbf{k}_p) \langle F^*(\mathbf{r}) | \psi_n^+(\mathbf{Q}, \mathbf{r}) \rangle \quad (2)$$

Here  $\psi_n^+(\mathbf{Q}, \mathbf{r})$  is the optical wave function for an (outgoing) neutron elastically scattered from the nucleus, with energy  $E(\mathbf{Q}) = \hbar^2 \mathbf{Q}^2 / 2m$ ;  $\mathbf{Q}$  is defined as  $\mathbf{Q} = \mathbf{k}_d - \mathbf{k}_p$ ; and as usual the function  $g(\mathbf{p})$  is:

$$g(\mathbf{p}) = \{p^2 + \gamma^2\} G(\mathbf{p}) \quad (3)$$

where  $G$  is the Fourier transform of the deuteron internal wave function, and the deuteron binding energy is written as  $\hbar^2 \gamma^2 / m$ .

(The B-B result is obtained from (2) by writing  $\psi_n^+$  as a plane wave outside the nucleus, and as zero within.)

In the work recorded here we use a square well for the neutron-nucleus interaction to effect an exploration of the consequences of the approximation (2). The results shed light on the agreements and discrepancies between B-B theory and experiments.

Suppose the optical wave function,  $\psi_n$ , for a neutron elastically scattered by the nucleus is given by the complex potential:

$$\begin{cases} V(r) = V_0 + iV_I; & r < a \\ V(r) = 0 & ; r > a \end{cases} \quad (4)$$

Let the bound state wave function,  $F$ , be given by a real square well potential with the same radius:

$$\begin{cases} U(r) = U_0; & r < a \\ U(r) = 0 & ; r > a \end{cases} \quad (4')$$

By the argument which usually leads to the orthogonality result, we obtain:

$$(E(\mathbf{Q}) + E_B) \int F^*(\mathbf{r}) \psi_n^+(\mathbf{Q}, \mathbf{r}) d\mathbf{r} = \int F^*(\mathbf{r}) \{V(r) - U^*(\mathbf{r})\} \psi_n^+(\mathbf{Q}, \mathbf{r}) d\mathbf{r} \quad (5)$$

We now take the real parts of  $V$  and  $U$  to be equal. In practice,  $V_0 = U_0$ , to within at most a few MeV; such equality is a self-consistency check on the optical model. (The character of the subsequent results is not altered if we allow a difference  $|V_0 - U_0| \approx |V_I|$ .) Equation (5) becomes:

$$(E(\mathbf{Q}) + E_B) \int F^*(\mathbf{r}) \psi_n^+(\mathbf{Q}, \mathbf{r}) d\mathbf{r} = i V_I \int F^*(\mathbf{r}) \psi_n^+(\mathbf{Q}, \mathbf{r}) d\mathbf{r} \quad (6)$$

Rearranging equation (6), we get for the matrix element in equation (2):

$$| \langle F^* | \psi_n^+ \rangle | = \frac{|V_I|}{\{E(\mathbf{Q}) + E_B\}^2 + V_I^2} \left| \int_{r>a} F^* \psi_n^+ d\mathbf{r} \right| \quad (7)$$

For our square well, the radial parts,  $f(r, l, m)$  and  $\varphi_n(r, l, m)$ , of the wave functions  $F(r)$  and  $\psi_n^+(r)$  can be written down for  $r > a$ :

$$f(r, l, m) = A(l, m) h_l(i\chi r) \quad (8)$$

$$\varphi_n(r, l, m) = e^{i\delta_l} \{ \cos \delta_l j_l(Qr) - \sin \delta_l n_l(Qr) \}$$

Here  $h_l$  is the spherical Hankel function of the first kind,  $j_l$  and  $n_l$  are the spherical Bessel functions of the first and second kind, respectively,  $A$  is the same constant which appears in the B-B theory, and  $\delta_l(Q)$  is the (complex) phase shift<sup>4</sup> for the  $l$ -th partial wave.

If the bound state neutron can be characterized by a unique orbital angular momentum quantum number  $l$ , the angle integrals on the right-hand side of equation (7) can be readily performed. Next the properties of Bessel functions can be used to evaluate the radial integral. Choosing the vector  $\mathbf{Q}$  as the polar axis, we can finally write:

$$\int_{r>a} F^*(\mathbf{r}) \psi_n^+(\mathbf{Q}, \mathbf{r}) d\mathbf{r} = \frac{\{4\pi(2l+1)\}^{1/2} a^3}{Q^3 + \chi^2} A(l, 0) e^{i\delta_l} \cos \delta_l [W(j_l(Qa), h_l(i\chi a)) - \tan \delta_l W(n_l(Qa), h_l(i\chi a))] \quad (9)$$

Here  $W$  denotes the Wronskian defined as:

$$W(u, v) = \left\{ u \frac{\partial v}{\partial r} - \frac{\partial u}{\partial r} v \right\}_{r=a} \quad (10)$$

Equations (2) (7) and (9) thus give the matrix element for a  $(d,p)$  reaction in the new approximation. We proceed to comment on the relationship between the present results and the B-B theory, with respect to: (A) absolute magnitudes of cross-sections; (B) angular variation in differential cross-sections; (C) zeros in the differential cross-section.

(A) Returning to my earlier remarks, we observe that the B-B estimate of the matrix element in equation (2) is just the expression in equation (9) with  $\delta_l = 0$ . Thus, from equation (7) we see that in the limit when  $\delta_l = 0$ , the new approximation gives differential cross-sections which differ from the B-B theory only by a factor:

$$\frac{V_I^2}{\{E(\mathbf{Q}) + E_B\}^2 + V_I^2} \quad (11)$$

Since the angle dependence of this factor (contained in  $\mathbf{Q} = \mathbf{k}_d - \mathbf{k}_p$ ) is much weaker than that contained in the Wronskian  $W(j_l, h_l)$ , we may say that if the optical phase shift is small, the new approximation and the B-B approximation will give essentially the same angular distribution, but the new approximation will give an absolute cross-section smaller than the B-B one by the significant factor (11).

This is relevant to the notorious experimental fact that even in the many instances when B-B theory explains the angular distribution excellently, it over-estimates the absolute cross-section by as much as an order of magnitude<sup>5</sup>. Indeed, nuclear reduced widths are extracted by fitting the shape of the B-B angular distribution; such empirical reduced widths are generally smaller than those

calculated by B-B theory by factors which increase from about 2-6 at low binding energies up to about 6-20 at larger binding energies ( $E_B > 10$  MeV). This is the behaviour to be expected from equation (11); the value of  $V_1$ , taken from neutron elastic scattering<sup>4</sup>, is about 3 MeV for  $E(Q)$  around a few MeV.

In Table 1, we take  $E(Q)$  to be around 3 MeV, and display the factor (11) alongside the ratio of empirical reduced widths,  $\theta_s^2$  (exp.), to B-B reduced widths,  $\theta_s^2$  (B-B), for neutron capture into  $2s$  states and for capture into  $1d$  states<sup>5</sup>.

Table 1

Neutron binding energy (MeV)	The factor (11)	$\frac{\theta_s^2(\text{exp.})}{\theta_s^2(\text{B-B})}$ ( $2s$ )	$\frac{\theta_s^2(\text{exp.})}{\theta_s^2(\text{B-B})}$ ( $1d$ )
2	0.25	0.4 (4)	0.1 (5)
7	0.08	0.1 (6)	0.07
12	0.04	0.08	0.05

(B) For a more detailed comparison between equations (7) and (9), and the simple B-B theory, it is necessary to compute  $\delta_l(Q)$  as a function of  $Q = |\mathbf{k}_d - \mathbf{k}_p|$  for any particular ( $d, p$ ) reaction.

For this article we note that for given  $l$ , the modulus of the phase shift is generally small for small  $Q\alpha$  (for example, for  $l = 3$ ,  $\alpha = 4f$ ,  $V_0 = 50$  MeV, then  $|\tan \delta_l| < 0.1$  for  $E(Q) < 13$  MeV). Thus without going into detail we can estimate that the corrections to the B-B angular distributions, which are contained mainly in the term  $\tan \delta_l W(n, h_i)$  in equation (9), will be more significant for higher energies of the incident deuteron, and for larger angles ( $Q = |\mathbf{k}_d - \mathbf{k}_p|$  is then larger for given

$\mathbf{k}_d, \mathbf{k}_p$ ). These remarks accord with experimental tendencies<sup>6,7</sup>.

(C) Finally we note that equation (9) does not permit zeros in the differential cross-section. Such zeros (the zeros of the Wronskian  $W(j, h)$ ) are an unphysical feature of the B-B theory.

To prove this assertion, we write the complex phase shift as  $\tan \delta_l = \alpha + i\beta$ . Then apart from obviously non-vanishing terms, the differential cross-section can be written:

$$\frac{d\sigma}{d\Omega} \propto \frac{1}{(1+\beta)^2 + \alpha^2} [(W(j, h) - \alpha W(n, h))^2 + \{\beta W(n, h)\}^2] \quad (12)$$

This expression has no zeros (unless  $\beta = 0$ , that is, unless  $V_1 = 0$ ).

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## POTASSIUM-ARGON AGES OF ROCKS FROM JAN MAYEN AND AN OUTLINE OF ITS VOLCANIC HISTORY

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JAN MAYEN is the most northerly of the volcanic islands which rise from the Mid-Atlantic Ridge. It occupies an isolated position just north of the 70th parallel, some 500 km east of Scoresby Sound in East Greenland. Its maximum dimensions are 54 km  $\times$  16 km; it is elongated in a north-east-south-west direction and is widest at the north-eastern end. The present-day island is a small emergent portion of an extensive submarine volcanic accumulation rising from the crest of the mid-Oceanic ridge on the south-east flank of the central rift. The island is wholly volcanic in origin, and to-day is dominated by one great volcanic mountain, Beerenberg, 2,277 m high, forming the entire northern half of Jan Mayen. The central volcanic vent of Beerenberg has been inactive for several thousand years, but parasitic activity on the flanks has occurred more recently. Eruptions on the southern flank of the mountain were observed in 1732 and 1818, and seismic shocks have been reported frequently. Beerenberg is the most northerly of the world's large volcanic mountains, and its summit is covered by a permanent icefield drained by some 20 radial glaciers. The southern half of the island is a narrow volcanic tableland, nearly 500 m high, built by repeated fissure-controlled eruptions and characterized by strings of trachytic domes.

The first extensive geological reconnaissance of Jan Mayen was made by Wordie in 1921 (refs. 1 and 2). Between 1948 and 1963, a series of British university expeditions have gradually extended detailed geological mapping over most of the island. Their work is described

in accounts by Dollar<sup>3</sup>, Nicholls<sup>4</sup> and Fitch<sup>5-7</sup>. The Norwegian geologist, Carstens, mapped the far south of the island in 1959 (ref. 8) and has discussed the petrology of a number of the trachytic domes<sup>8</sup>. Very little is known of the composition of the submarine bank, but evidence from accidental blocks in agglomerates and tuff-breccias suggests that it is composed of both tholeiitic and alkali-basaltic rocks with related differentiates. The rocks above sea-level, both lavas and pyroclastics, are predominantly of alkali-basalt and picrite, with subordinate amounts of trachybasalt, mugearite and trachyte. Ankaramites (both ultrabasic and basaltic varieties) are very common in some lava sequences, and in this respect Jan Mayen is unique among the volcanic islands of the Atlantic Ocean. The petrology of the rocks is discussed in the papers by Nicholls, Carstens and Fitch already mentioned here, and also in papers by Holmes<sup>9</sup>, Tyrell<sup>11</sup> and Roberts and Hawkins<sup>12</sup>. Notwithstanding the presence of Beerenberg and numerous other central volcanoes, fissure volcanism, both pyroclastic and effusive, is the dominant eruptive mechanism on Jan Mayen. Accounts of the more unusual volcanic phenomena have been given by Dollar<sup>13</sup> and by Hawkins and Roberts<sup>14</sup>.

The geological history of Jan Mayen begins with the building of the submarine bank by extensive basaltic eruptions along and close to the crest of the mid-Oceanic ridge. The age range of this phase in its development is unknown; but, by analogy with Iceland, this phase most probably occurred during mid-Tertiary to Early Pleistocene times. The oldest rocks seen above sea-level are well



exposed along the base of the great sea cliffs between Sorbukta and Ullringbukta in the far south of the island. They consist predominantly of pyroclastic whalebacks and related flows of ankaramitic and trachybasaltic lavas that appear to have been erupted from a fissure swarm aligned close to N. 50° E. This early phase of emergence was followed in South Jan by a period during which an extensive plateau of trachybasaltic and less basic lavas was erupted from an intersecting network of fissure swarms, the older N. 50° E. set and a new N. 40° E. swarm. North Jan and the proto-Beerenberg began to grow where this new and powerful fissure-swarm was intersected by a major cross-fault. This, possibly rather lengthy, period was brought to a close by the intrusion of numerous trachytic plug domes sealing the principal volcanic throats. For some time after the intrusion of the trachytic domes, volcanism on Jan Mayen was much reduced in intensity. During this interval, extensive erosion occurred throughout the island and a large ice-cap formed on the proto-Beerenberg. Tillites or fluvioglacial deposits of this age are found throughout the island. As the massive tillites of this time are the only evidence of major glaciation known from Jan Mayen, it has been assumed that they were formed during the last maxima of the Pleistocene (see the arguments in ref. 6). If this is a correct assumption, then the preceding volcanism must have occurred within the Pleistocene, unless evidence of previous maxima is missing.

In the period immediately following the deposition of the tillite sheets, erosion continued to be more important than volcanism in South Jan. The principal focus of activity moved north-eastwards to the Beerenberg and the major part of the mountain was constructed in four successive stages.

First, a series of explosive fissure eruptions occurred along the N. 40° E. fissure swarm, especially along the north-east ridge of Beerenberg, extending the mountain considerably in that direction. On the south flank of the mountain, sub-glacial hyaloclastic tuffs<sup>18</sup> indicate that ice was still present on the proto-Beerenberg at the beginning of this stage. In the second stage a gigantic ash-flow eruption from the central vent deposited a thick sheet of basic pumice siller on the southern flanks of the mountain. The third stage was a prolonged period of Hawaiian-type volcanism from the central vent which resulted in the development of a large ankaramitic basalt shield volcano. Finally, after a short erosion interval, a large composite trachybasaltic lava cone was built on the summit of the lava dome, completing the major part of Beerenberg. The formation of the summit cone was followed by an important erosion interval during which extensive sea-cliffs developed all around Jan Mayen.

There have been two major phases of volcanism subsequent to the formation of the summit cone, both entirely fissure-controlled. At first volcanism was related to the N. 40° E. fissures which became active again throughout the island except across the higher parts of Beerenberg. A number of radial fissures were active, however, on the

south-west and south flanks of the mountain during this episode. This fissure volcanism was both ankaramitic and trachybasaltic in character. It frequently consists of extensive and violent pyroclastic eruption followed by quiet lava effusion. Throughout Jan Mayen great pyroclastic ridges were built along fissure lines, and lava streams flowed into the intervening depressions and spread over existing cliffs to make coastal laval platforms. During the later phase of this post-Beerenberg volcanism an additional fissure trend at N. 65° E. became apparent especially in the far north-east of the island. A study of Jan Mayen geomorphology and of the history of growth and decay of the Beerenberg icefield over the past 10,000 years suggests that the high summit cone was completed at least 5-6,000 years ago and that the most important subsequent volcanic episodes occurred between 4-5,000 and 2,500-3,500 years ago (see arguments in ref. 6 and 15-18). The coastal lava platforms of the latter episode sit on a widespread post-glacial raised beach.

The age estimates referred to here were made in 1963 after consideration of the available geological and geomorphological evidence, and are necessarily based on a number of unproved assumptions. Recent discussion of Continental Drift and of the evolution of the Atlantic Ocean by Wilson<sup>19,20</sup>, Miller and Fitch<sup>21</sup> and others has made it essential for accurate age information to be obtained from the volcanic islands of the Atlantic.

This investigation of the most northerly of the volcanic islands on the mid-Ocean ridge was undertaken as a test of the methods to be used. It is intended that it should be the first of a number of such investigations. A stratigraphically representative collection of some fifty Jan Mayen rocks was made by Fitch during the 1963 Imperial College Beerenberg Expedition. From this stratigraphical collection ten rocks were selected for potassium/argon age determination by omegatron. The instrument has recently been described by Grasty and Miller<sup>22</sup>. To minimize possible errors introduced by any serial changes in potassium content that might occur, especially in flow-aligned igneous rocks, each specimen was cut into three parallel slices using a diamond saw. The rocks were examined petrologically in thin sections cut at right angles across the direction of slicing. Potassium oxide contents were determined in the two outer slices and an average value calculated. Two independent argon determinations were then made on the inner slice.

The results of potassium/argon age measurements on the three geologically oldest rocks are listed in Table 1. As the youngest two rocks of this sequence were found to be too young to produce a satisfactory potassium/argon age, it was obviously impracticable to attempt the analysis of the even younger specimens, and, therefore, this has not been done. The oldest rock analysed was SJ 1, an olivine-basalt lava flow exposed at the base of the cliffs at the far southern end of Sorbukta. In thin section it is seen to be an unaltered, strongly flow-aligned, olivine-basalt, in which small microporphyritic groups of olivine and, much more rarely, augite (occasionally associated

Table 1. NW POTASSIUM/ARGON AGE DETERMINATIONS

Sample	Reference and method	K <sub>2</sub> O %	% Atmos. contam.	Vol. radiogenic <sup>40</sup> Ar per g sample (mm <sup>3</sup> NTP)	Apparent age and error (m.y.)
Flow-aligned microporphyritic olivine-basalt lava flow of oldest pre-tillite volcanic sequence on Jan Mayen, collected from the base of the cliffs at far south of Sorbukta, south Jan Mayen	SJ 1 K/Ar whole rock, Omegatron	1.99	95.2	$3.19 \times 10^{-4}$	$0.49 \pm 0.12$
		1.99	93.9	$2.04 \times 10^{-4}$	$0.31 \pm 0.12$
Vesicular ankaramitic basalt lava flow of oldest pre-tillite volcanic sequence on Jan Mayen, collected from the base of the cliffs in the middle of Ullringbukta, south Jan Mayen	SJ 9 K/Ar whole rock, Omegatron	1.31	> 99.6	Less than $1.065 \times 10^{-4}$	Less than 0.24
		1.31	> 99.8	Less than $1.819 \times 10^{-4}$	Less than 0.03
Ankaramitic lava flow of Krosbukta Fm., immediately below Kapp Flahburn tillite, Krosbukta, north Jan Mayen	SJ 15 K/Ar whole rock, Omegatron	1.21	94.3	$7.53 \times 10^{-4}$	$0.19 \pm 0.10$
		1.21	> 99.5	—	Less than 0.09

$$\lambda_8 = 4.72 \times 10^{-10} \text{ y}^{-1}, \quad \lambda_6 = 0.584 \times 10^{-10} \text{ y}^{-1}.$$

with plagioclase) are carried in a matrix of labradorite laths with interstitial augite, olivine and iron ores.

The next oldest rock was *SJ 9*, a vesicular lava flow of ankaramitic basalt exposed at the base of the vertical cliffs in the middle of Ullringbukta. In thin section it is seen to consist of euhedral augite and olivine phenocrysts set in a fine-grained matrix of minute plagioclase laths, pyroxene and iron ores, which encloses also numerous small microphenocrysts of augite and olivine. Widespread iddingitization of the marginal zones of olivine crystals is probably deuteric in origin and does not necessarily indicate that any significant argon loss from the feldspars or pyroxenes has taken place. The feldspars and pyroxenes are quite fresh and unaltered. Both these rocks belong to the earliest phase of emergence of Jan Mayen. The volcanic formation of which they are members is the older of the two pre-tillite volcanic sequences of South Jan. The ages obtained fall in the range 500,000 to less than 30,000 years; the better determination, that of *SJ 1*, suggests an age of  $400,000 \pm 80,000$  years for that rock. *SJ 9* must be considerably younger, certainly less than 240,000 years old, but exactly how young it is cannot be determined as yet with absolute certainty.

The same age arguments apply to *SJ 15*, an absolutely fresh ankaramite lava flow exposed immediately below the tillite in Kroesbukta, North Jan. Stratigraphically, it is at the top of the two pre-tillite sequences of Jan Mayen. In thin section the rock is seen to consist of numerous euhedral olivine and augite phenocrysts set in a fine-grained pyroxene-rich matrix of labradorite, pyroxene, olivine and iron ore. The potassium/argon evidence suggests that *SJ 15* is younger than either *SJ 1* or *SJ 9*, certainly less than 190,000 years old and possibly much younger. All these rocks are Pleistocene in age. The latest evidence from Africa and America suggests that the Pleistocene Period lasted from between 2.5 and 1.5 m.y. to about 10,000 years ago. The rocks of the oldest

part of Jan Mayen would therefore appear to belong to the upper part of the Pleistocene.

The potassium/argon age investigation reported in this article confirms the conclusions reached by geological interpretation, that is, that the emergent part of Jan Mayen began its development in late Pleistocene times, and that the majority of the visible rocks are very young, mostly post-Pleistocene in age. This conclusion is further strengthened by the absence of reversed magnetization among the post-tillite lava sequences of Beerenberg sampled in 1961 and 1963 (ref. 7, and work in preparation). It suggests that volcanism on Jan Mayen is closely related in time to the latest Pleistocene and Recent volcanism in Iceland. At present the combined evidence from Jan Mayen and Iceland appears to be compatible with the hypothesis of ocean floor spreading, for in this region the youngest rocks are certainly found closest to the central parts of the mid-Oceanic ridge.

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<sup>15</sup> Fitch, F. J., Kinsman, D. J. J., Sheard, J. W., and Thomas, D., *Internat. Assoc. Sci. Hydrol. Comm. Snow and Ice*, **58**, 201 (1963).

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<sup>17</sup> Kinsman, D. J. J., Sheard, J. W., and Fitch, F. J., *Nature*, **196**, 897 (1962).

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## TWO NEW TAXA OF THE GLOBOROTALIINAE (GLOBIGERINACEA, FORAMINIFERA) ASSISTING DETERMINATION OF THE LATE MIOCENE/MIDDLE MIOCENE BOUNDARY

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THE proposed Swansea symposium, to be held jointly by the Palaeontological Association and the Geological Society of London, will review the fossil record and provide a summary of present knowledge of the ranges of fossil organisms in geological time. In order to provide a biostratigraphical sub-division of the Neogene as a framework for this purpose, as proposed by the Comité du Néogène<sup>1</sup>, research in the Palaeontology Branch, Exploration and Production Research Division of the BP Research Centre, Sunbury-on-Thames, has been directed towards the establishment of a sequence of zones based on the ranges of planktonic foraminifera. This sequence of zones has been compiled from evidence obtained from many areas (for example, Trinidad, Jamaica, Barbados, Venezuela, Italy, Sicily, Malta, Libya, Tanganyika, West Irian, Papua and New Zealand), is being related to the succession of other fossil faunas, and is being correlated, so far as is possible, with the European standard stages. This preliminary account is intended to outline the distinction between the planktonic foraminiferal zones which are believed to permit the determination of the boundary between the Middle Miocene (Tortonian) and the "Upper Miocene" (Late Miocene, Messinian) in circum-equatorial areas of the world.

A total of twenty-three zones, based on planktonic foraminifera, will be proposed to sub-divide the geological

succession from the Early Miocene to the Holocene. Of these zones, Zone N.16 is believed to comprise the late Middle Miocene and Zone N.17 is believed to represent the early part of the Late Miocene. The base of Zone N.16 is defined by the first evolutionary appearance of *Globorotalia* (*Turborotalia*) *acostaensis* (*sensu stricto*) Blow, 1959, which occurs at virtually the same horizon as the first evolutionary appearance of *Globorotalia* (*G.*) *morotumida*, n.sp. The top of Zone N.16 is placed immediately below the first evolutionary appearance of *Globorotalia* (*G.*) *tumida pleiotumida*, n.sub-sp., which defines the base of Zone N.17. The presence of these zones may be recognized on the occurrence of other species, for the stratigraphically earliest *Candolina nitida* (*sensu lato*) appears just below the base of Zone N.16, and *Globorotalia* (*Turborotalia*) *acostaensis humerosa* (Takayanagi and Saito) and *G.(T.) crassaformis* (*sensu stricto*) (Galloway and Wisler) both first appear just below the base of Zone N.17. The boundary between the two zones is taken at the first evolutionary appearance of *Globorotalia* (*G.*) *tumida pleiotumida* from *G.(G.) morotumida* because such evolutionary lineages are believed to provide the most reliably isochronous datum-planes in biostratigraphy<sup>2,3</sup>.

The succession of Zones N.16 and N.17 has been recognized by us to occur in the Pozón section<sup>4</sup>, eastern Falcón, Venezuela, in the lower part of the type Bowden formation

(at present being re-described by E. Robinson, University of the West Indies, Mona, St. Andrew, Jamaica), Jamaica, in the Sarimi formation of the Sarimi region, West Irian, in the Muruan and Toa mudstone succession of western Papua\*, and in a sub-surface section in Somalia. The Tortonian succession at Rio Mazzapiede-Castellania, Italy, has been sampled by D. D. Bayliss (British Museum, Natural History) and the Units 2-4 of Gino\* have been found to comprise Zone N.16, the Zone N.17/N.16 boundary occurring there near the top of the section, very near the top of Unit 4. The sub-surface successions penetrated in the Cubagua wells Nos. 1 and 2, which were originally studied by J. P. Beckmann (in Kugler\*), have been re-examined, and the cored samples from well Cubagua No. 2 have provided the sequence of foraminiferal assemblages which are used as paratype for these zones. The detailed biostratigraphy of these wells, and of the other stratigraphical sections referred to here, will be described later with the full account of the complete succession of Neogene planktonic foraminiferal zones. A horizon referred to the lowest part of Zone N.16 yielded assemblages of specimens morphologically intermediate between *Globorotalia* (*Turborotalia*) *languensis* Bolli and *G.(G.) merotumida* n.sp. The morphological transition between *G.(G.) merotumida* and *G.(G.) tumida plesiotumida* n.sub-sp. occurs at the base of Zone N.17. *G.(G.) tumida tumida* first occurs at the base of Zone N.18, where it is associated with morphologically intermediate forms from *G.(G.) tumida plesiotumida*. This evolutionary sequence, from *G.(T.) languensis* to *G.(G.) tumida tumida*, parallels the analogous evolution, in the Early Miocene, of *G.(G.) lobata robusta* (Bolli) from *G.(T.) fohsi barisanensis* (LeRoy)\*; in each case, a species of carinate *Globorotalia* (s.s.) evolved from *G. (Turborotalia)* with concomitant increase in test-size, and the evolution within the forms referable to *Globorotalia* (s.s.) in each case led to the development of more tumid tests with massive carinae.

*Globorotalia* (*G.*) *merotumida* Blow and Banner n.sp. (Figs. 1a-c).

**Description of holotype**<sup>14</sup>. The test consists of three whorls of regularly enlarging chambers, six in each whorl; successive whorl heights are in the approximate ratios 1 : 2 : 5. The test is completely evolute dorsally, and the dorsal walls of all the chambers are thin, translucent and finely perforate; there is no conspicuous deposit of thick, secondary laminae to obscure the early whorls. The

dorsal surface is slightly convex, but the dorsal chamber walls are but little, if at all, inflated; the dorsal sutures are very weakly depressed, but they are marked by continuously developed, slightly raised sutural limbations of clear, imperforate shell material. The spiral suture is lobulate. The globular proloculus (c. 0.015 mm diameter) is surrounded by a whorl of reniform chambers with smoothly curved intercameral sutures; in succeeding whorls, the intercameral sutures become straighter and are, in their mid-parts, oblique to the weakly lobulate spiral suture at angles of about 45°; however, the proximal ends of the later intercameral sutures may be sharply re-curved, meeting the spiral suture virtually at right angles. The axial periphery is acute and is furnished, throughout ontogeny, with an imperforate carina, which is continuous with the dorsal sutural thickenings. In axial profile, the chambers are slightly concave immediately dorsally and ventrally to the carina. The ventral surface of the test is much more strongly convex than the dorsal surface, the ratio of ventral to dorsal convexity being about 2 : 1. The ventral side is completely involute, only six chambers being visible. The ventral intercameral sutures are narrowly and shallowly depressed and, throughout most of their length, are nearly straight (or only very slightly curved) and radially arranged, meeting the weakly lobulate periphery and the narrow, closed umbilicus at right angles. The ventral chamber walls are very finely perforate; the pores, being spaced at distances no greater than 0.005 mm, are approximately equal in size and density of distribution to those on the dorsal surface. Most of the ventral surface is smooth, but granules are developed in the immediate vicinity of the umbilicus on the earlier chambers and on the area of the wall of the first two chambers which immediately faces the aperture. The last-formed chamber possesses a very low, rounded umbilical shoulder; the umbilical shoulders of the earlier chambers are concealed by embracing overlap of the succeeding chambers. The apertural face is flattened and broad; it is broadest near the axial periphery, where it is delimited by the carina, and narrowest near the umbilicus; at its mid-point, the breadth of the apertural face is approximately equal to two-thirds of its total length. The apertural face is separated from the ventral face of the last chamber by an abrupt change in slope, but the area of the change in slope is rounded (not sharply angular), and the texture and structure of the apertural face and the ventral wall are very similar.

The narrow, interiomarginal aperture extends from the umbilicus to the ventral margin of the peripheral carina, and it is of approximately uniform breadth throughout. The aperture is furnished, through its length, by a thick, narrow lip, which is most strongly developed towards the umbilicus. The maximum diameter of the test is 0.38 mm.

**Provenance of holotype**: from a core taken at 2,700 ft. depth, well Cubagua No. 2, Island of Cubagua, Venezuela; "*Globorotalia menardii*/*Globigerina nepenthes* zone" of Blow, 1959; paratype locality and level for Zone N.16, *Globorotalia* (*Turborotalia*) *acostaensis*/*Globorotalia* (*G.*) *merotumida* Zone of Blow and Banner, MS; Middle Miocene (Tortonian).

**Depository of holotype**: British Museum (Natural History) No. P45684.

**Comparative diagnosis of species**: *Globorotalia* (*G.*) *merotumida* typically differs from *G.(G.) tumida plesiotumida* in possessing (1) a test which is smaller in size at the same growth stage as measured by the numbers of chambers present, (2) a slower increase in whorl height as seen dorsally, (3) more uniformly enlarging chambers, (4) more consistently oblique dorsal intercameral

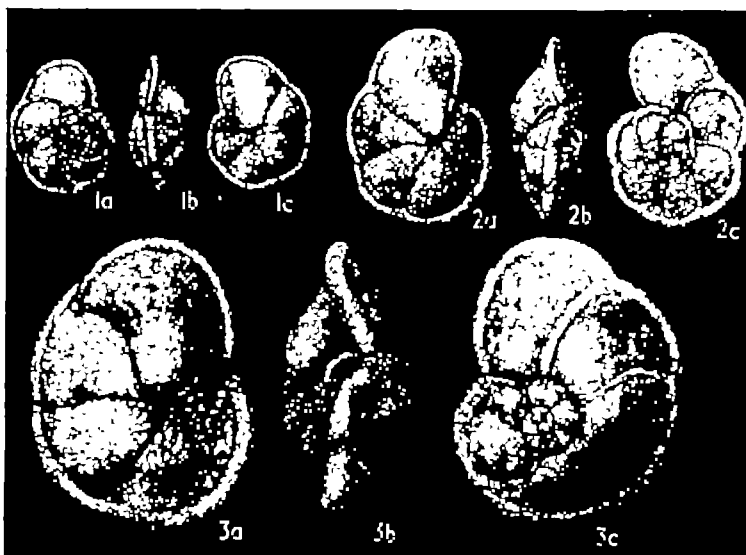


Fig. 1. a-c. *Globorotalia* (*G.*) *merotumida* n.sp., dorsal, axial and ventral views of holotype ( $\times 45$ ).

Fig. 2. a-c. *Globorotalia* (*G.*) *tumida* (Brady) *plesiotumida* n.sub-sp., ventral, axial and dorsal views of holotype ( $\times 45$ ).

Fig. 3. a-c. *Globorotalia* (*G.*) *tumida* (Brady) *sensu stricto*, ventral, axial and dorsal views of hypotype ( $\times 45$ ).

sutures, (5) a thinner and more finely perforate test wall, (6) a thinner carina, (7) a relatively greater ventral convexity, and (8) a relatively broader apertural face. *G.(G.) merotumida* differs from *G. (Turborotalia) languensis* Bolli in possessing (1) a clearly developed imperforate peripheral carina, (2) a test which has about 25 per cent greater diameter at the same growth stage, as measured by the number of chambers present, (3) a more rapidly increasing whorl height, and (4) less strongly curved dorsal intercameral sutures. *G.(G.) merotumida* has a much more convex and tumid test than any sub-species of *G.(G.)* *ultrata* (s.l.) (d'Orbigny). *G.(G.) unguata* Bermúdez, although possessing a dorso-ventral convexity comparable with that of *G.(G.) merotumida*, has a much weaker carina, circumferentially longer chambers in dorsal aspect, a shorter aperture, and a much more rapid increase in whorl height.

*Globorotalia (G.) tumida* (Brady)

*Globorotalia (G.) tumida* (Brady) *sensu stricto* (Fig. 3a-c). *Pulvinulina menardii* (d'Orbigny) var. *tumida* Brady, 1877, *Geol. Mag.*, n.s., dec. 2, vol. 4, p. 535; Brady, 1879, *Quart. Jour. Micr. Sci.*, vol. 19, n.s., p. 80.

*Pulvinulina tumida* Brady, 1884, *Rept. Voy. Challenger*, Zool., vol. 9, Pt. 22, p. 692, pl. 103, Figs. 4-6.

*Globorotalia tumida* (Brady), Cushman, 1927, *Contr. Cushman Lab.*, vol. 3, p. 91, pl. 19, Fig. 12; Bolli, Loeblich and Tappan, 1957, *U.S. Nat. Mus. Bull.*, 215, pp. 41-42, pl. 10, Figs. 2a-c; Banner and Blow, 1960, *Contr. Cushman Fdn.*, vol. 11, pp. 26-27, pl. 5, Fig. 1a-c.

*G.(G.) tumida tumida*, as here restricted by the separation of its ancestral sub-species (*G.(G.) tumida plesiotumida*), first appears at the base of Zone N.18, the zone which, in its lower part, is believed to represent the late Messinian. *G.(G.) tumida tumida* is nominally validated by its described and illustrated lectotype<sup>10</sup> and paralectotype<sup>11</sup>, both of which were originally obtained from the "post-Tertiary" (probably Pliocene or latest Miocene) of New Ireland; *G.(G.) tumida tumida* has also frequently been recorded and illustrated from recent seas<sup>12-14</sup>.

The specimen of *G.(G.) tumida tumida*, illustrated here for comparison with *G.(G.) tumida plesiotumida*, was obtained from the lower part of Zone N.18, latest Miocene, from a section in the Moencok River, West Irian (sample Su 596).

*Depository of hypotype*: British Museum (Natural History) No. P45685.

*Globorotalia (G.) tumida* (Brady) *plesiotumida* Blow and Banner n.sub-sp. (Fig. 2a-o).

*Description of holotype*<sup>14</sup>. The test consists of three whorls of chambers; the successive whorl heights are in the approximate ratios 1:4:8. The test is completely evolute dorsally and the dorsal walls of all the chambers are translucent and uniformly perforate; the dorsal surface of the early whorls is clearly thickened by secondary laminae, but the surface is smooth, not pustulate, and the perforation distribution and density is not appreciably affected, nor is the chamber arrangement obscured. The dorsal surface is smoothly convex, but the dorsal chamber walls are uninflated. The dorsal intercameral sutures are very weakly depressed between the last two or three chambers only; the sutures between the earlier chambers are flush with the surface of the test (because of the laminal thickening) but are marked by continuously developed, flush or very slightly raised, sutural limbations of imperforate, clear shell material. The globular proloculus (c. 0.010 mm diameter) is surrounded by a whorl of five reniform chambers, with smoothly curved intercameral sutures. The succeeding whorl, of six chambers, possesses straighter intercameral sutures, which are oblique to the very weakly lobulate spiral suture at angles of about 45°. The proximal ends of the later sutures become increasingly re-curved, so that in the last whorl (of 6+ chambers) the inner ends of the intercameral

sutures are approximately sub-radial, only their distal parts being curved to meet the periphery obliquely. The axial periphery is acute and is furnished, throughout ontogeny, with an imperforate carina which is continuous with the dorsal sutural limbations. In axial profile, the last-formed chamber is slightly concave immediately ventrally to the carina. The ventral surface of the test is slightly more strongly convex than the dorsal surface, the ratio of ventral to dorsal convexity being about 3:2. The ventral side is completely involute, only the six and one-half chambers which comprise the last whorl being visible. The ventral intercameral sutures are narrowly and shallowly depressed, are nearly straight or very slightly curved or sinuous, and are radially arranged, meeting the weakly lobulate periphery and the narrow, almost completely closed umbilicus approximately at right angles. The ventral chamber walls are finely and uniformly perforate, the pores, being spaced at distances no greater than 0.01 mm, being approximately equal in size and density of distribution to those of the dorsal surface. Most of the ventral surface is smooth, but granules are developed in the immediate vicinity of the umbilicus on the four earlier chambers of the last whorl as well as on areas of the walls of the first two chambers which immediately face the aperture. The last-formed chamber possesses a low, rounded umbilical shoulder; this shoulder is visible, to a decreasing extent, on the two preceding chambers, but it is concealed on earlier chambers of the last whorl by intercameral embrace and overlap. The apertural face is flattened and broad; it is broadest near the axial periphery, where it is slightly concave and is delimited by the carina, and narrowest near the umbilicus; at its mid-point, the breadth of the apertural face is approximately equal to one-half its total length. The apertural face is separated from the ventral face of the last chamber by a change in slope; the area of the change is rounded (not sharply angular), and the texture and structure of the apertural face and the ventral wall are very similar. The narrow, interiomarginal aperture extends from the umbilicus almost to the ventral margin of the peripheral carina; the aperture, which is a low arch, broadest just dorsal to its mid-point, is furnished, throughout its length, with a narrow, uniformly developed lip. The maximum diameter of the test is 0.52 mm.

*Provenance of holotype*: from a core taken at 1,400 ft. depth, well Cubagua No. 2, Island of Cubagua, Venezuela; "Sphaeroidinella seminulina Zone" of Blow, 1959; paratype locality and level for Zone N.17, the *Globorotalia (G.) tumida plesiotumida*/*Globorotalia (Turborotalia) crassaformis* Zone of Blow and Banner, MS; Late Miocene (Messinian).

*Depository of holotype*: British Museum (Natural History) No. P45683.

*Comparative diagnosis of sub-species*: *Globorotalia (G.) tumida (sensu stricto)* differs from *G. (G.) tumida plesiotumida* in developing a much larger test at the same growth stage (as determined by the number of chambers attained), with a much more rapid increase in whorl height (successive whorl heights being in the approximate ratios 1:4:12). *G.(G.) tumida tumida* also characteristically differs from *G.(G.) tumida plesiotumida* in possessing a much more massive carina, thicker test walls, a greater development of coarse granules on the earlier chambers of the ventral side, a tumid test of approximately equal dorso-ventral convexity (that is, dorsal height/ventral depth being in the approximate ratio 1:1) and often in possessing a higher aperture, furnished with a very broad, thick lip.

We thank the British Petroleum Company, Ltd., for permission to publish this account, and we thank Dr. J. D. Emeis, Bataafse Internationale Petroleum Maatschappij N.V., for the samples from West Irian, which included sample Su 596, and Dr. H. G. Kugler (Basle) for the core samples from Cubagua wells Nos. 1 and 2.

<sup>1</sup> Butsch, R. F., *Wat. Med. Geol. Microb.*, 44, 96 (1955).

<sup>2</sup> Compare with Jolefsky, J. A., *J. Paleont.*, 39, 135 (1965).

<sup>3</sup> Compare with Miller, T. G., *Palaeontology*, 8, 113 (1965).

<sup>4</sup> Originally described by Blow, W. H., *Bull. Amer. Paleont.*, 39, 67 (1969).

<sup>5</sup> Described geologically by the Australasian Petroleum Co. Pty. Ltd., *Geol. Jour. Geol. Soc. Austral.*, 8, pt. 1 (1961).

<sup>6</sup> Gino, G., di Napoli, R., Ruscotti, M., and Giannotti, A., *Riv. Ital. Paleont. Mem.* VI (1953).

<sup>7</sup> Kugler, H. G., *Bull. Geol. Soc. Amer.*, 68, 555 (1957).

<sup>8</sup> Boll, H., *Contr. Ooshman Fdn.*, 1, 82 (1950).

<sup>9</sup> Banner, F. T., and Blow, W. H., *Palaeontology*, 3, 1 (1960).

<sup>10</sup> Banner, F. T., and Blow, W. H., *Contr. Ooshman Fdn.*, 11, 1 (1960).

<sup>11</sup> Boll, H., Loeblich, A. R., and Tappan, H., *U.S. Nat. Mus. Bull.*, 215, 1 (1957).

<sup>12</sup> Brady, H. B., *Rept. Voy Challenger, Zool.*, 9 (22), 692 (1884).

<sup>13</sup> Whisman, J. D. H., and Ovey, O. D., *Proc. Geol. Assoc., Lond.*, 61, 28 (1960).

<sup>14</sup> Paratypes of the new taxa described here have been deposited in the United States National Museum, Washington, D.C.; *Globoletaria* (G.) *microtumida*, two specimens, No. 641923, G. (G.) *tumida planotumida*, three specimens, No. 641929.

## MICROBIAL AND RESIDUAL MYCOSTASIS IN SOILS

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IN 1953, Dobbs and Hinson<sup>1</sup> described a widespread inhibition of fungal spore germination and hyphal growth in soils, now commonly referred to as soil myco-stasis or fungistasis. Further work in many parts of the world has since confirmed the general occurrence of this phenomenon in surface soils, and its complex, mainly microbial, origin, although there is still much discussion about the mode of action and the identity of the organisms concerned.

Many organisms, bacteria, actinomycetes and fungi, have been shown to produce, when cultured on agar media or autoclaved soil, an inhibition of the germination of test spores or the growth of hyphae separated by agar or cellulose film from the culture, or directly in contact with it; but before any of these can be shown to produce myco-stasis in natural soils, their active growth in such soils must be demonstrated.

The restoration of myco-stasis by re-infection of autoclaved soil from natural soil provides no clear evidence, since autoclaved soil may be a relatively rich and artificial medium. Possibly the nearest approach to a demonstration of the involvement of particular micro-organisms in a naturally induced soil myco-stasis was that provided by Dobbs, Bywater and Griffiths<sup>2</sup>, who showed that myco-static properties were restored to a neutral, acid-washed dune sand, both by natural aerial infection and by inoculation from untreated sand or soil. The only organisms which could be isolated from the re-infected sand were bacteria, of which only the Gram-negative isolates were inhibitory in agar culture to the test spores.

However, despite the difficulties in identifying the organisms responsible for the phenomenon in any given soil, there can be no doubt that this type of soil myco-stasis is associated with the presence of soil micro-organisms, and may be referred to as 'microbial myco-stasis'. It is characterized by its complete removal by autoclaving and all sterilizing agents, its continued absence so long as sterile conditions are maintained, its absence also from naturally sterile or near-sterile sub-soils, and by its temporary masking by the addition or presence of certain nutrients, notably sugars, and organic soil amendments, including those liberated by partial sterilization. Though the inhibitory substance or substances concerned have not been identified, when obtained in sterile filtered soil extracts, or in sterile agar disks, they have been shown to be thermolabile.

This distinction is necessary in view of the occurrence of a more stable type of inhibition, first observed in several of a collection of about 40 soil samples from England and Wales examined at Bangor in 1957-58 (ref. 3). In these the inhibition did not react to glucose and survived autoclaving. The soils in question were all calcareous, varying from marine sands containing about 4 per cent to chalk and limestone sub-soils with more than 90 per cent calcium carbonate. Since these soils, where tested, could be shown to give rise to the microbial type of inhibition in sterile, neutral sands, they must be assumed to possess both types

of myco-stasis: the unstable microbial type, and the stable type which remains after that has been removed by sterilization. This thermostable and non-sugar-sensitive type of inhibition is therefore referred to as 'residual myco-stasis' and has been the subject of recent study at Bangor.

The marine sands of Newborough Warren, Anglesey, were chosen for the main investigation in the hope of simplifying the problem by the use of simple soils. Samples from all the ecological zones of the Warren, when tested by the cellulose film method<sup>4</sup> with spores of *Mucor ramannianus*, were strongly myco-static, as they were also to a range of other fungi, including some isolated from the sand. After autoclaving, certain samples supported growth on the test films. All these came from the fixed dunes or the *Salix* slack associates, which had accumulated an appreciable organic content. All the sands which were deficient in organic matter, that is, those from the mobile dunes and the sea strand, exhibited a strong residual inhibition after autoclaving, giving zero germination on the test films.

Fig. 1 shows the average percentage germination (based on 6 experiments with 25 replications each) of test spores of *Mucor ramannianus* on water agar disks. These were first incubated at 21° C for 48 h, with a 'Cellophane' separator to maintain sterility: A, on autoclaved dune sand; B, on acid- and water-washed, neutral, control sand, re-infected with untreated dune sand; C, on uninfected, neutral, control sand. The disks of each series were then exposed for 10 min, in air, to a range of temperatures of 20° C-85° C in a water-bath, before, finally, the spores were added and tested for germination. The marked difference between the graphs for A and B clearly illustrates the relative thermostability of the residual inhibitor

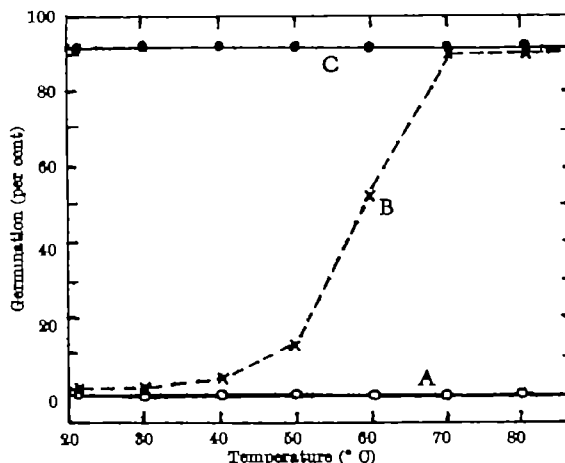


Fig. 1. Germination of spores of *Mucor ramannianus* on sterile water-agar disks pre-incubated on dune sands. Effect of temperature (5-10 min exposure) on: A, residual myco-stasis in autoclaved sand; B, microbial myco-stasis in re-infected, neutral 'control' sand; C, non-mycostatic control on acid- and water-washed, sterile, neutral sand.

and the breakdown of the microbial inhibitor when exposed to temperatures above 50° C. These results would also seem to eliminate competition for nutrients as the main causal factor in soil mycoastasis, at least as demonstrated in these sands—a suggestion which arises from time to time, and has recently been revived by Lockwood<sup>4</sup>.

The residual mycoastasis of the heat-sterilized dune sand was unaffected by extensive washing in up to 40 times its volume of hot water, or by the addition of glucose in solution to the sand. Sand sterilized with alcohol also showed a similar mycoastasis. These preliminary tests, therefore, were taken to have eliminated the autoclaving process, and also sea-salt or other simple and highly water-soluble constituents of the sand, as causal factors; while the absence of a counter-inhibitory effect from the addition of glucose suggested that the effect of organic matter is not to be attributed to its sugar content.

On the other hand, washing or leaching the sand with dilute hydrochloric acid, followed by water-washing until the pH was restored to within the range 5–8, was effective in completely removing the inhibition, leaving a neutral 'control' material, neither inhibitory nor stimulatory to spore germination, on the cellulose film.

Constituents regularly detected in the acid leachate were calcium, ferrous and ferric iron, sodium, potassium, phosphate and nitrate. The effects on spore germination were investigated of the addition of laboratory chemicals to neutral 'control' sand, these being chosen to match so far as possible those found to be present, and added both singly, and in various combinations, in the proportions naturally present.

The progressive addition of 'AnalaR' CaCO<sub>3</sub> to 'control' sand was ineffective in reducing appreciably the germination of test spores of *Mucor ramannianus* until levels of about 30 per cent w/w were reached, inhibition being complete when the mixture contained more than 70 per cent CaCO<sub>3</sub> (Fig. 2). The level of calcium carbonate in Newborough Warren sands is about 4 per cent and never exceeds 8 per cent, so that it is evident that this constituent, though capable of inducing a complete residual mycoastasis at high concentrations, could not be responsible for that found in the Warren sands.

Of the remaining constituents of the acid leachate, only iron was present in appreciable quantities (0.5–1.2 per cent). All the artificial mixtures containing inorganic ferrous or ferric iron, at levels comparable to those found, were inhibitory to some extent to spore germination, while controls containing the same acid radicals with no iron were not, the ferric iron being more active than the ferrous. None of the mixtures lacking iron (or high levels of calcium carbonate) was inhibitory.

However, the inorganic iron compounds, such as ferric chloride, oxide, hydroxide and citrate, ferrous hydroxide,

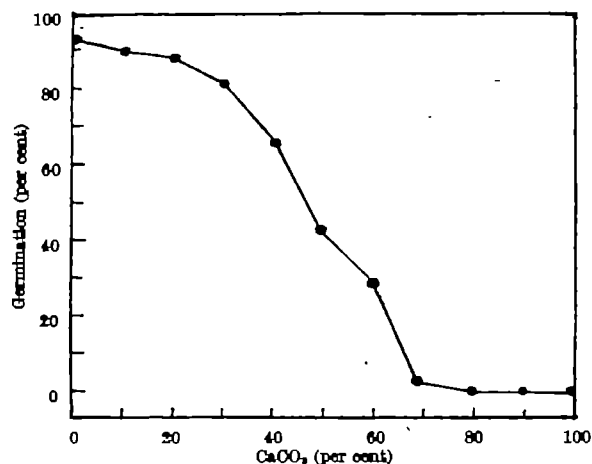


Fig. 2. Effect of calcium carbonate on germination of spores of *M. ramannianus* on water-agar disks pre-incubated on a range of mixtures of 'AnalaR' CaCO<sub>3</sub> with neutral 'control' sand.

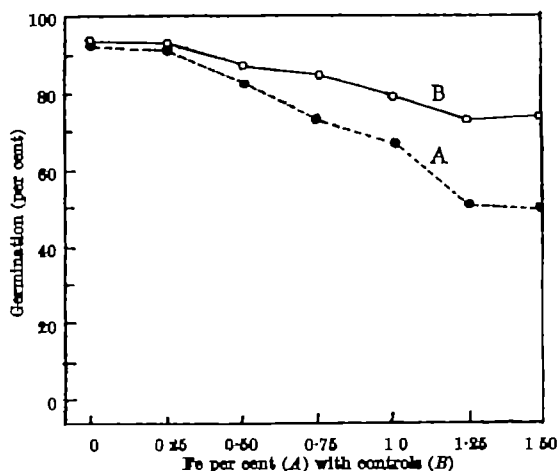


Fig. 3. Effect of chelated iron on germination of spores of *M. ramannianus* on water-agar disks pre-incubated on mixtures of 'control' sand with a chelating agent (EDTA): A, containing iron in sodium/ferric/EDTA compound expressed as per cent iron w/w added to sand; B, controls containing no iron but equivalent amounts of disodium EDTA. Both unbuffered; pH 5.9–6.0.

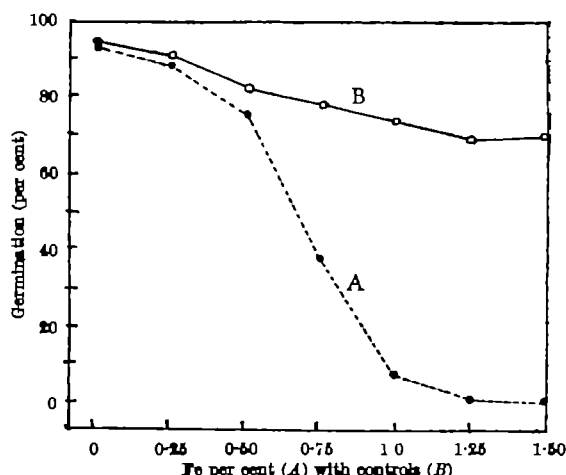


Fig. 4. As in Fig. 3, but both mixtures buffered to pH 8.5 liberating iron from the chelating bond.

sulphate and ferrous ammonium sulphate, tend strongly to alter the pH when added to 'control' sand. The effect was therefore tested of adding the iron to the 'control' sand in chelated form, as the ferric sodium salt of sequestric acid (EDTA). Fig. 3 shows in graph B the effect of adding increasing quantities of the disodium salt of EDTA to control sand, resulting in a slight fall in germination, at present unexplained. Graph A gives the corresponding curve for the ferric sodium salt, resulting in a greater fall, from more than 90 to about 50 per cent germination. In this case the ferric salt was unbuffered, resulting in a fall of pH from 5.9 to 5.0, the controls following a closely similar range of pH. At this pH the chelating bond presumably would remain intact.

Fig. 4 shows the results of a similar experiment in which the EDTA/sand mixtures were buffered with sodium hydroxide to a pH of 8.5. In graph B (disodium salt control), the germination falls slightly as in Fig. 3, graph B; but in graph A (ferric sodium salt of EDTA) the fall is much more pronounced than in Fig. 3 and germination approaches zero at all levels above 1 per cent iron (w/w). At this pH the ferric/EDTA bond will be broken, liberating free iron. This would appear to confirm the hypothesis, based on the experiments with inorganic iron compounds, that the iron content of the sand is responsible for its residual mycoastasis. Samples of a relatively pure Barton sand from the New Forest which were found to have a very low iron content have been found to possess no residual mycoastasis, or, occasionally, to reduce germination

slightly. On the other hand, an Old Red Sandstone soil which was lacking in residual mycoestasis was also found to have a considerable organic content. When this was leached with NaOH and Na<sub>2</sub>CO<sub>3</sub>, it showed a moderate degree of inhibition. A recent paper by D. A. Griffiths<sup>5</sup> reports residual mycoestasis in a red latosol in Malaya.

The addition of the chelating agent (the disodium salt of EDTA) to mobile dune sand was found to modify its residual mycoestasis, though not completely to abolish it; germination being increased from 0 to the region of 50–70 per cent, as compared with 94 per cent for neutral washed sand. A similar result was obtained by the addition of organic material, mechanically separated from the fixed sand of a wet slack, to the mobile dune sand, both when autoclaving followed and when it preceded the making of the mixture. The indication would therefore seem to be that the organic material removes the free iron by chelation, an effect which is known in other connexions. For example, the fertilizing effect of a lignite on mustard plants has been related to its chelating effect on iron, which is then rendered available for absorption by the plant<sup>6</sup>.

Work on residual mycoestasis in calcareous soils, mainly from the limestone areas near Penmon in Anglesey, indicates two potential sources of the inhibition. When the CaCO<sub>3</sub> level is high, as in the powdered limestone subsoil, this in itself would seem to account for the inhibition, as indicated by the data graphed in Fig. 2. This situation, however, may be modified by the presence of a high humus content, and further complicated by the presence of iron. In certain soils under grass, over carboniferous limestone, the removal of most of the humus by mechanical and chemical means, before sterilization, resulted in a drop in germination from 90 per cent to 35–40 per cent, with a minimum of 15 per cent. In these cases removal of the calcium carbonate did not appreciably increase germination, but subsequent leaching with a chelating agent did, raising the germination level to about 70 per cent.

Though we found it convenient to continue to use as our main test fungus *Mucor ramannianus*, a species usually found in acid soils, but with a germination rate not

appreciably affected by pH within the range found in natural soils, other fungi, including some isolated from the alkaline sands, have also been tested. These have shown broadly similar results, although, in the case of the limestone inhibition, the shape of the germination curve may vary somewhat with the fungus used.

It would thus appear that both calcium carbonate and iron may contribute to the residual mycoestasis found in these soils. There remains also a probability that other inorganic materials, either naturally present or artificially added, may later be found to be responsible for a similar condition in other soils.

There are indications also that the microbial type of inhibition may also be of several kinds; for instance, the 'toxic' surface soil of Gore Heath, near Wareham, Dorset, first described by Rayner and Neilson-Jones<sup>7</sup>, possesses a thermolabile inhibition which is less sensitive to the addition of glucose than is that responsible for the ordinary widespread soil mycoestasis<sup>8</sup>. Information from other workers, mainly unpublished, seems to show that, especially with the pathogenic fungi, more complex nutrient requirements than sugars are present; for example, Boocallis<sup>9</sup> found that, with *Helminthosporium sativum*, soil amended with potato broth gave 38–44 per cent germination, while dextrose broth gave only 0–1 per cent.

In conclusion, therefore, we would stress the necessity for distinguishing the type of inhibition being investigated, and the nutrient requirements of the test fungi used, in any further studies of this phenomenon of soil mycoestasis.

<sup>1</sup> Dobbs, O. G., and Hinson, W. H., *Nature*, **179**, 197 (1953).

<sup>2</sup> Dobbs, O. G., Bywater, J., and Griffiths, D. A., *Rep. Forest Res. for 1959*, 95 (H.M.S.O., London, 1960).

<sup>3</sup> Dobbs, O. G., and Bywater, J., *Rep. Forest Res. for 1958*, 98 (H.M.S.O., London, 1959).

<sup>4</sup> Dobbs, O. G., Hinson, W. H., and Bywater, J., in *The Ecology of Soil Fungi*, 180 (Liverpool Univ. Press, 1960).

<sup>5</sup> Lockwood, J. L., *Ann. Rev. Phytopath.*, **2**, 341 (1964).

<sup>6</sup> Griffiths, D. A., *Malayan Forester*, **27**, 318 (1964).

<sup>7</sup> DeKock, P. O., *Trans. Seventh Intern. Congress Soil Sci.*, III, 1960, 573 (1961).

<sup>8</sup> Rayner, M. O., and Neilson-Jones, W., *Problems in Tree Nutrition* (Faber, London, 1944).

<sup>9</sup> Boocallis, M. G., *Phytopath.*, **52**, 1172 (1962).

## STRUCTURE-FUNCTION RELATIONSHIPS IN THE ACTIVE C-TERMINAL TETRAPEPTIDE SEQUENCE OF GASTRIN

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AND

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THE heptadecapeptide amides gastrin I and II isolated from hog antral mucosa by Gregory and Tracy<sup>1</sup> are believed to be related to the hormone which is released from the antral mucosa during digestion and plays a major part in the stimulation of gastric secretion. The total synthesis of these peptides was accomplished by Anderson *et al.*<sup>2</sup>, following elucidation of their structures by Gregory *et al.*<sup>3</sup>. In an associated investigation, Tracy and Gregory<sup>4</sup> described the physiological properties of a series of synthetic peptides structurally related to gastrin I and showed that, of the 17 residues of the molecule, only the C-terminal tetrapeptide sequence Try-Met-Asp-Phe-NH<sub>2</sub> (which is found in both gastrins) is required for the remarkable range of physiological effects displayed by the natural hormones on the gastric and pancreatic gland-cells and on the gastro-intestinal musculature. They remarked that the simplicity of this active tetrapeptide and its wide range of distinctive actions make it a promi-

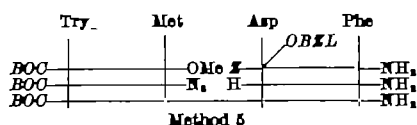
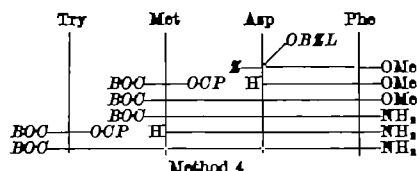
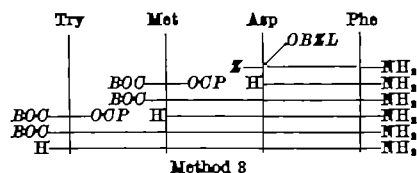
ing subject for the investigation of structure-function relationships by synthesis of analogues and exploration of their properties.

This article describes the results of such an investigation, in which 33 peptide derivatives have been synthesized and examined. Of these, 24 were analogues of the tetrapeptide in which a single substitution had been made in one of the amino-acid residues, or in the amide-group masking the C-terminal residue; in a further 4, more than one substitution was made. Two pentapeptide derivatives were also studied; one of these, *N*-*t*-butyloxycarbonyl-β-Ala-Try-Met-Asp-Phe-NH<sub>2</sub> (this and other active peptide derivatives are covered by pending patent applications in the name of Imperial Chemical Industries, Ltd.), proved to be particularly active. There were also examined: (1) the *i*-butyloxycarbonyl-tetrapeptide amide, BOC-Try-Met-Asp-Phe-NH<sub>2</sub>, which was comparable to the benzyloxycarbonyl-tetrapeptide amide in activity,



and the carbamoyl-tetrapeptide amide,  $H_2N.CO.Try.Met.Asp.Phe.NH_2$ , which was considerably more active; and (2) the *t*-butyloxycarbonyl tetrapeptide,  $BOC.Try.Met.Asp.Phe.OH$ , where the terminal amide group had been replaced by hydroxyl. Like related esters<sup>4</sup>, the latter was found to be almost inactive.

The two methods previously used in the synthesis of the C-terminal gastrin tetrapeptide amide by Anderson *et al.*<sup>3</sup> and the three additional methods (3-5) were suitably adapted in the synthesis of analogues. L- or D-Amino-acids of established purity were used in the preparation of all analogues except compounds 4-6, where DL-methyl-tryptophans were used (in method 3). Compounds 4-6 are therefore mixtures of the D-L-L-L- and L-L-L-L isomers. Compound 24 was prepared by methods 3 and 5 using aspartylphenylalanine in place of aspartylphenylalanine amide, and by saponification of  $BOC.Try.Met.Asp.Phe.OMe$ . The sulphone (compound 11) was also prepared by direct oxidation. The pentapeptide derivatives were prepared from the tetrapeptide amide by coupling with the appropriate active ester (compounds 29, 31), or by coupling with *N*<sup>α</sup>-benzyloxycarbonyl-*N*<sup>ε</sup>-*t*-butyloxycarbonyl-L-lysine 2,4,5-trichlorophenyl ester followed by hydrolysis of the product (compound 30). Compound 29 was also prepared by coupling *N*-*t*-butyloxycarbonyl-β-alanyl-L-tryptophan azide with L-methionyl-L-aspartyl-L-phenylalanine amide, and by coupling *N*-*t*-butyloxycarbonyl-β-alanyl-L-tryptophyl-L-methionine azide with L-aspartyl-L-phenylalanine amide. Compound 32 was prepared by direct carbamoylation of the tetrapeptide amide. The purity of all samples used for biological testing was established by thin-layer or paper chromatography in at least three different solvent systems, and, in some cases, by amino-acid analysis after acid or enzymatic hydrolysis, ultra-violet and p.m.r. spectroscopy and thin-layer or paper electrophoresis (after treatment of *BOC*-derivatives with cold trifluoroacetic acid). In cases where analogues were prepared by more than one method, the resulting samples had identical optical rotations; the rotations of compounds 3 and 27 and of 25 and 33 were equal and opposite to sign. In the synthetic work, details of which will be published elsewhere, we wish to acknowledge the valuable co-operation of Drs. H. Gregory, A. H. Laird and J. M. Smith.



BOC, Me<sub>3</sub>C.O.O.; Z, PhCH<sub>2</sub>; O, OCH<sub>2</sub>Ph; OCP, OC<sub>2</sub>H<sub>4</sub>Cl<sub>2</sub> (2,4,5).

The actions of the natural gastrins and the experimental conditions in which they may be demonstrated have been described in detail by Gregory and Tracy<sup>1</sup>; the virtually identical properties of the C-terminal tetrapeptide amide, which is the parent compound of the present series of analogues, have been described by Tracy and Gregory<sup>4</sup>. Briefly, in conscious dogs provided with denervated pouches of the gastric fundus and/or isolated jejunal

loops, and in conscious or anaesthetized dogs arranged for the collection of pancreatic juice, the following effects are obtained: (1) Subcutaneous injections in appropriate dosage strongly stimulate gastric acid, but not pepsin, secretion; larger doses inhibit gastric acid secretion and stimulate pepsin secretion. (2) Rapid intravenous injections inhibit a 'background' secretory response maintained by continuous administration of histamine or of gastrin (I or II) and stimulate pepsin secretion; they also stimulate pancreatic secretion (volume-rate and enzyme secretion) and gastric tone and motility. They cause a biphasic intestinal response in which strong contraction is rapidly followed by inhibition of tone and motility. The C-terminal tetrapeptide amide has a small inhibitory action on basal gastric tone and motility following stimulation; this effect is not commonly seen with gastrin itself.

In the work recorded here, the same methods and the same group of surgically prepared animals were used as in the two previous investigations; the responses of these dogs to the gastrins and to the tetrapeptide were thus well established. As before, histamine was used as the stimulus for producing a steady 'background' secretion of gastric juice, against which to examine the power of each compound to: (1) inhibit gastric acid secretion; (2) stimulate pepsin secretion. In a few instances gastrin was used as the stimulus. In acute experiments in which pancreatic secretion was studied, the possibility (not previously explored) that gastrin or the peptides might act by liberating the intestinal hormones secretin and pancreozymin was excluded by removal of the entire small intestine.

For each peptide there were nine possible actions to be examined; and for a combination of reasons, formal assays of potency in respect of each were not feasible. It was therefore decided to express the findings in a simple manner based on the dosage level required for an effective response. This minimized the number of experiments required for each compound and made it possible to study a wide range of analogues, embracing several substitutions for each of the residues. The experimental procedure adopted was to test each compound first in a dose (50 μg) which was known to give a well-defined response in respect of all the possible actions when the 'parent' tetrapeptide was used. If comparable responses were observed with the analogue, this was indicated (Table 1) as +. If little or no effect was observed, larger doses were used on subsequent occasions, up to a maximum dose of 500 μg. Well-defined responses obtained at this dosage or small responses observed at a lower dose-level (200 μg) were marked as (+). Failure to respond to 500 μg was marked as 0; a doubtful positive response at this dose-level was indicated as (? +). As in the previous investigation<sup>4</sup> a few instances were found in which even maximal doses (500 μg) augmented, instead of inhibiting, a background histamine response when injected intravenously; as before, these were recorded as 0\*. The probable interpretation of this type of result is indicated by our finding that a very small (1 μg) intravenous injection of gastrin I or II augments a background histamine response; a larger dose (5 μg or more) causes the usual inhibition.

These are set out in Table 1, in which the peptides are arranged in five groups. The first four groups correspond to substitutions in the four residues of the parent tetrapeptide amide, and the final group is a miscellaneous group containing analogues with more than one substitution and *N*-acylated derivatives of the intact tetrapeptide amide. While, in general, the power to stimulate gastric acid secretion in a peptide derivative was associated with all the other types of activities enumerated for gastrins I and II, this was not always so. Thus, several peptides possessing the power to stimulate gastric acid secretion did not uniformly affect gastrointestinal motility (gastric motility in particular) (for example, compounds 9, 11, 12-15, 20, 21). Some that stimulated gastric secretion at high dosage did not inhibit at this level (for example, compounds 2, 3, 11, 12, 21) and there was an occasional

indication that inhibitory activity could be associated with lack of, or weak, stimulating properties (compounds 13 and 18). There were no cases of marked selectivity, in a qualitative sense, in respect of any of the properties studied. The power to stimulate the secretion of pepsin was more widely observed than any of the other physiological actions.

Restricting the discussion to the power to stimulate gastric acid secretion as seen in the gastrin C-terminal tetrapeptide amide, Try.Met.Asp.Phe-NH<sub>2</sub>, the following tentative conclusions may be drawn:

(1) Activity is preserved in all *N*-acylated derivatives of the tetrapeptide amide (compounds 29-33). The level of activity may vary considerably, but when the acylating group is *t*-butyloxycarbonyl- $\beta$ -alanyl or carbamoyl, the resulting peptide derivatives (compounds 29 and 32) show the same order of activity as gastrin on a simple weight-for-weight basis (Fig. 1). This situation parallels that reported<sup>4</sup> for  $\alpha$ -MSH; derivatives of  $\alpha$ -MSH in which the *N*-terminus is acylated may equal or exceed the biological activity of counterparts possessing a free *N*-terminal amino group.

(2) Substitution of all, or part, of the L-amino-acid residues by D-amino-acid residues leads to virtually complete loss of activity (compounds 10, 18, 25, 26, 27). There are two exceptions to this generalization:

(a) In compound 3, where L-tryptophan is replaced by D-tryptophan, some activity (chiefly on gland-cells) is shown at a high dosage level. This result is regarded as real, since there are clear indications that the geometry at this position is not critical (cf. high activity of compound 7). However, the possibility of activity being due, at least in part, to the presence of a small amount of the

L-L-L-L peptide cannot entirely be discounted; 1 per cent contamination would probably account for the activity shown. (b) Compound 22, where L-phenylalanine is replaced by D-phenylalanine, shows similar activity, particularly in respect of gastrointestinal motility.

Those compounds in which one or more D-amino-acids had been substituted for the L-forms were tested for their power to inhibit secretion when injected intravenously against a background response maintained by gastrin. The results did not differ significantly from those set out in column 2 of Table 1, which were obtained using histamine as the stimulus.

The general situation in regard to the D-form substitutions closely parallels that described for bradykinin by Stewart and Woolley<sup>6</sup> and contrasts with that described for the 6-10 MSH pentapeptide, His.Phe.Arg.Try.Gly, by Hano, Koida, Kubo and Yajima<sup>7</sup>. All D-bradykinin does not show bradykinin-like activity and does not inhibit the response to bradykinin, while inversion of a single amino-acid residue may preserve bradykinin-like activity. The all D-MSH pentapeptide is believed to be a competitive inhibitor of the physiological actions of the all L-pentapeptide and of MSH.

(3) Replacement of the tryptophan by other L-amino-acid residues (compounds 1 and 2) generally lowers activity considerably. However, activity may be preserved despite removal of the amino-group (compound 7), a result to be compared with the high biological activity of 'deamino-oxytocin'; and substitution of the tryptophan nucleus (compounds 4, 5 and 6) may produce highly active compounds (Fig. 1). This, to our knowledge, is the first recorded example of the biological effects of substitution in the tryptophan nucleus of a biologically active peptide,

Table 1

No.	Peptide derivative	Gastric acid secretion		Gastric motility		Intestinal motility		Pancreatic secretion		Pepsin secretion
		Stim.	Inhib.	Stim.	Inhib.	Stim.	Inhib.	Vol.	Enzyme	
1	Z.Phe.Met.Asp.Phe-NH <sub>2</sub>	(+)	(+)	(+)	0	(+)	(+)	(+)	(+)	+
2	Z.His	(+)	0	0	(+)	(+)	(+)	(+)	(+)	+
3	BOC.D.Try	(+)	0*	(?+)	0	0	(?+)	(+)	(+)	+
4	BOC.L-Me.Try	+	+	+	0	+	+	+	+	+
5	BOC.L-Me.Try	+	+	+	0	+	+	+	+	+
6	BOC.L-Me.Try	+	(+)	+	0	+	+	+	+	(?+)
7	2-Indolyl-(CH <sub>2</sub> ) <sub>4</sub> -CO	+	+	+	0	+	+	+	+	+
8	BOC.Try.Nle.Asp.Phe-NH <sub>2</sub>	+	+	+	0	+	+	+	(+)	+
9	Z.His	+	+	0	0	+	+	+	0	+
10	BOC.D.Met	0	(?+)	0	0	0	0	0	0	(+)
11	Z.Met	(+)	0*	0	0	0	0	0	(?+)	+
12	Z.Met	(+)	0	(+)	0	+	+	+	(+)	0
13	Z.Nval	(+)	+	0	0	+	+	(+)	(+)	(+)
14	Z.Aibut	(+)	(+)	0	0	+	+	0	0	+
15	Z.Ala	(+)	(+)	0	0	0	0	(+)	(+)	+
16	Z.Try.Met.Asp	0	0*	0	0	0	0	0	0	(+)
17	Z.Try.Met.Glu.Phe-NH <sub>2</sub>	0	0	0	0	0	(?+)	(+)	(?+)	0
18	BOC.D.Asp.NH <sub>2</sub>	0	(+)	(?+)	0	0	0	0	0	(?+)
19	BOC.Asp.Phe.OH	0	0	0	0	0	0	0	0	(+)
20	Z.Try.Met.Asp.Tyr-NH <sub>2</sub>	(+)	(+)	0	0	0	0	(+)	0	+
21	BOC.Phe-NH <sub>2</sub> O <sub>2</sub> H <sub>11</sub>	(+)	0	0	0	0	0	0	0	+
22	BOC.D.Phe-NH <sub>2</sub>	(+)	(+)	+	0	+	+	(+)	+	(+)
23	BOC.Tyr-NH <sub>2</sub>	+	+	(+)	0	(+)	(+)	+	+	+
24	BOC.Phe.OH	0	0*	0	0	0	0	(+)	(+)	(?+)
25	BOC.D.Try.D.Met.D.Asp.D.Phe-NH <sub>2</sub>	0	0	0	0	0	0	0	0	0
26	BOC.D.Try.D.Met.Asp.Phe-NH <sub>2</sub>	0	0	0	0	0	0	0	0	(+)
27	BOC.Try.D.Met.D.Asp.D.Phe-NH <sub>2</sub>	0	0	0	0	0	(?+)	0	0	0
28	BOC.D.Try.Met.Asp.Phe.OH	0	0*	0	0	0	(+)	0	0	(+)
29	BOC.L-Ala.Try.Met.Asp.Phe-NH <sub>2</sub>	+	+	+	+	+	+	+	+	+
30	Z.Lys	(+)	(+)	+	(+)	+	+	(+)	+	0
31	Z.Glu	(+)	(+)	+	+	+	+	+	+	+
32	H <sub>2</sub> N.CO	+	+	+	(+)	+	+	+	+	+
33	BOC	+	+	+	+	+	+	+	+	+

For explanation of symbols see text.

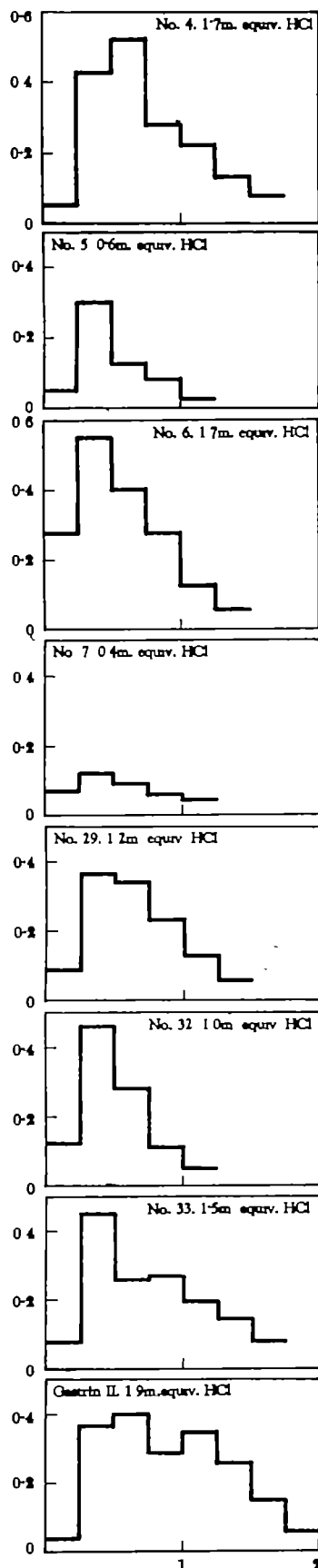


Fig. 1. Secretory responses of a conscious dog provided with a denervated fundus pouch to the subcutaneous injection (on different occasions) of 10 µg of peptides 4, 5, 6, 7, 30, 32, 33 and gastrin II. Injection in each case at time 0. Ordinates, acid output per 15-min period (m equiv. total HCl) abscissae, time (h). The total acid output in each experiment is recorded.

although substituted tryptophan residues are probably present in the rufomycin group of antibiotics<sup>1</sup>.

(4) The methionine position will tolerate the greatest amount of change. Thus, the norleucine (compound 8) and ethionine (compound 9) analogues show high activity, and low activity is still found in the norvaline,  $\alpha$ -amino-butyric acid and alanine analogues (compounds 13, 14 and 15). The sulphone and *S*-oxide (compounds 11 and 12) are only marginally active.

The situation at the methionine position is closely paralleled by the results obtained by analogous replacements of methionine in ACTH<sup>10</sup> and  $\beta$ -MSH<sup>11</sup>.

(5) All changes at the aspartic acid position lead to virtually inactive compounds. Thus, the  $\beta$ -aspartyl and glutamic acid analogues (compounds 16 and 17) show only slight effects; while compound 19, where the polarity has been preserved while switching the terminal amide and aspartic hydroxyl groups, is the least active of any in this group, or indeed of any in the series except for the multi-D analogues 25–27. It is even less active than the free acid of the parent tetrapeptide, No. 24.

(6) At the phenylalanine position, the tyrosine analogue (compound 20) is only weakly active, but the *O*-methyl-tyrosine analogue (compound 23) has considerable activity. The former result recalls the considerable loss of activity that accompanies the replacement of phenylalanine by tyrosine in eliodoin<sup>12</sup>. Replacement of tyrosine by phenylalanine in oxytocin or in lysine vasopressin likewise lowers biological activity, but *O*-methyloxycytocin is either virtually inactive<sup>13</sup> or an antagonist of oxytocin<sup>14</sup>.

(7) Substitution of the terminal amide group by a cyclohexyl group (compound 21) greatly reduces the activity (dimethylation of the terminal amide group of eliodoin has a similar effect<sup>15</sup>), and the corresponding acid (compound 24) is almost inactive. This latter result confirms the observations on the methyl ester of the same compound made by Tracy and Gregory<sup>4</sup>; it was considered desirable to substantiate their findings by an independent synthesis and re-examination.

The interpretation of the fundamental importance of these structural changes in relation to physiological activity requires caution. First, many factors may influence the apparent biological activity seen in assays with intact animals. Secondly, there are many cases in the present investigation where a structural modification has resulted in a marked decrease of potency but not total inactivation. As Hofmann has pointed out<sup>6</sup>, an analogue exhibiting low activity still appears to retain the structural prerequisites for function although higher concentrations are required to achieve the biological effect. However, the results now obtained suggest that in the tetrapeptide amide sequence, and presumably also in gastrin, the tryptophan, methionine and phenylalanine positions are binding, rather than functionally active sites, and also that the most important feature of the molecules is the aspartic carboxylic-terminal amide relationship.

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## EFFECTS OF RED AND BLUE LIGHT ON THE GROWTH AND MORPHOGENESIS OF *Acetabularia crenulata*

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**P**HOTOMORPHOGENIC responses are of great importance in the development of higher plants and have recently received much attention<sup>1</sup>. The only such response known for an alga, however, is that described by Lazaroff and Schiff for the blue-green alga, *Nostoc muscorum*<sup>2</sup>. Aseriate colonies growing in the dark on a medium containing sugar failed to differentiate unless exposed to weak illumination. Red light at 650 m $\mu$  was most effective in promoting the transition, and its effect was reversed by subsequent irradiation with wave-lengths between 500 and 600 m $\mu$ , but not by far-red. The findings of Haupt<sup>3</sup> and Haupt and Thiele<sup>4</sup>, respectively, that chloroplast orientation in the green alga *Mougeotia* and *Meoetacnium* is reversibly responsive to red and far-red light open the question of phytochrome-mediated reactions in algae. Moreover, the occurrence of photoperiodic responses in *Acetabularia* seems clear for vegetative growth, though not yet demonstrated as a direct effect for morphogenesis<sup>5</sup>.

One useful approach to the study of photomorphogenic reactions has been to explore the effects of exposing plants to light of limited spectral regions. Investigations of this kind have been carried out with several types of algae, but most of the recent work has been done with species having no clear-cut differentiation, such as *Ohlreella*. The siphonaceous green alga, *Acetabularia*, is remarkable for its large size and possession of a single nucleus which can easily be excised, leaving a nucleate and an enucleate fragment. The fact that both fragments are capable of regeneration, the former being totipotent and the latter being capable of enlargement by as much as 30–40 per cent and differentiation of a gametangium (cap), has made this organism a favourite subject for studies of nucleio-cytoplasmic relations<sup>6</sup>. The cell germinates from a zygote and grows out as a thin filament for several months until reaching a length of 20–30 mm, whereon elongation ceases and a disk-like cap forms at the apex of the stalk. This clear-cut two-stage development makes *Acetabularia* particularly well suited for studying morphogenic responses.

Investigations on the effects of light quality on *Acetabularia* have recently been undertaken independently in three laboratories. Richter<sup>7</sup> allowed 10-mm nucleate parts of *A. mediterranea* and *crenulata* to regenerate under broad-band red (570 m $\mu$  and upwards), and blue (400–600 m $\mu$ ) light sources adjusted to equal energy. In 3 months under these conditions both species had produced more than 20 mm of stalk, but those in red formed no caps while 78–94 per cent of those in blue did so. Plants of both species quickly initiated caps on being transferred to white light after 35 days in red light. Clausen, using Philips red and blue fluorescent tube sources, adjusted to equal energy, with the red passed through a 'Plexiglas' filter, obtained somewhat different results<sup>8</sup>. Nucleate parts of *A. mediterranea*, allowed to regenerate in continuous red illumination, grew less than 5 mm in 14 days and then ceased to grow entirely, but those given blue light grew at a steady rate and formed caps normally. The cells the development of which had been arrested in red light were able to resume growth and form caps when the red light régime was interrupted by 1 h or even 1 min of blue light. Far-red light was ineffective in replacing blue

light for this effect. 1 h of blue light per day in the absence of red light, however, was inadequate to support growth. In contrast to the results of Kowallik<sup>9</sup>, with synchronized cultures of *Ohlreella*, there was no differential effect of red and blue light on the protein/dry weight ratio of *Acetabularia*.

In the experiments reported here, cells or cell fragments of *A. crenulata* were exposed to various light régimes in 500-ml. Erlenmeyer flasks containing Erdschreiber medium. Control experiments used white light of several intensities from GE 'Daylight' fluorescent tubes. The blue source was a pair of 15-watt GE 'Blue' fluorescent tubes, in front of which was placed a dark blue 'Plexiglas' filter which did not transmit more than 580 m $\mu$  and gave peak transmission at 440 m $\mu$ . The red source in most of the experiments consisted of 2 or 4 15-watt GE 'Red' tubes, used with or without a Du Pont cellulose filter which had no discernible effect. This source provided a symmetrical band of radiation including wave-lengths from 590 to 720 m $\mu$  and had peak output at 640 m $\mu$ . The blue and red sources gave intensities of 1.5 and 1.6 m-watts/cm<sup>2</sup>, respectively. In one experiment tungsten bulbs were used with three thicknesses of red 'Cellophane' to provide a red source which did not transmit below 590 m $\mu$ , but was rich in wave-lengths more than 700 m $\mu$ .

Given continuous blue light, *A. crenulata* grew slightly more rapidly than when given a somewhat lower (1.2 mW/cm<sup>2</sup>) irradiance of white light (Fig. 1). The average rate of elongation of a culture was nearly linear until cap formation began; thereafter the growth registered was contributed only by those cells which had not yet initiated caps. The same pattern was observed with large capless cells (initial length 28.4 mm), and with cells initially of this size that had been cut back to 3.0 mm and allowed to regenerate. On the other hand, practically no growth occurred in red light; the large cells grew only

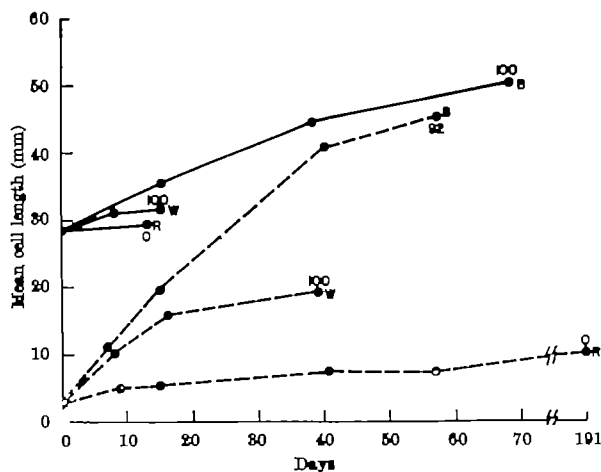


Fig. 1. Elongation of *A. crenulata* cells in white (W), blue (B) and red (R) light at 25–27° C. Sources were GE 'Daylight', 'Blue' (used with a filter which did not transmit above 580 m $\mu$ ) and 'Red' fluorescent tubes and gave energy fluxes of 1.2, 1.5 and 1.6 mW/cm<sup>2</sup>, respectively. ●—●, Intact cells, initially 28.4 mm in length. ●---●, cells of the same initial size but cut back to 3 mm and allowed to regenerate. Numbers over the terminal points refer to the percentage of cap-bearing cells in the sample at the end of the run.

0.9 mm in 13 days, while the regenerating cells grew 2.1 mm in the first 9 days but only another 5.3 mm in the following 182 days.

These and other similar experiments confirm Clause's result that growth virtually ceases in red light within two weeks. In an experiment similar to the foregoing but with the red source not carefully shielded from extraneous light, rapid growth in red light suggests that his somewhat broader source included wave-lengths that overcame the red effect, or that his cultures were occasionally exposed to white or blue light, possibly during measurements.

In comparison with white light that gave an approximately equal growth rate, blue light resulted in both quantitative and qualitative morphogenic effects. Cap formation was greatly delayed by blue light (Table 1). The time required for 50 per cent of the cells in a sample to initiate caps was much longer and the mean length of the cells in the sample at this time was considerably greater in blue light. These differences were particularly pronounced in the cells that regenerated from 3 mm bases in blue light (Fig. 2) in that they reached more than 2.5 times the length of the white light controls before forming caps. Another difference resulting from these treatments is revealed in comparing the effects of stalk amputation. It was shown by Beth that cells regenerating in white light after amputation produce caps at much shorter lengths than do intact cells<sup>10</sup>. This was clearly demonstrated in the present experiment (Table 1), where the respective lengths were 17 and 30 mm. However, the relationship was reversed in blue light, in which the generating cells, at the time of 50 per cent cap formation, were 6 mm longer on the average than the unoperated cells. Thus, the large cells that, as shown by the white light control, were on the verge of cap formation at the outset of the experiment were not delayed by blue nearly so much as those which had to regenerate.

This result implies that the (red component of) white light had had a preliminary morphogenetic effect on the intact cells before their transfer to blue light and that this effect was removed by amputation of most of the stalk.

Material	Light	Intensity of radiation (mW/cm <sup>2</sup> )	Time to 50% cap formation (days)	Mean cell length at 50% cap formation (mm)
Large cells	White	1.2	6	30
28.4 mm initially	Blue	1.5	19	37
Regenerating from 3.0 mm	Red	1.6	—	—
	White	1.2	24	17
	Blue	1.5	45	43
	Red	1.6	(> 188)	—

In addition to these quantitative effects, blue light produced a qualitative difference in cap morphology. Under laboratory conditions, in white light of moderate to high intensity, *A. oreanulata* differentiates at lengths between 20 and 30 mm, forming caps the individual chambers of which are free and without any particular orientation (at right in Fig. 2). Caps formed in blue light, however, were planar structures in which each chamber was laterally attached to the adjacent chambers (Fig. 2,



Fig. 2 Mature cap-bearing *A. oreanulata* cells grown in blue light (1.5 mW/cm<sup>2</sup>, at left) and white light (1.2 mW/cm<sup>2</sup>, at right). Note differences in cell length and cap morphology.

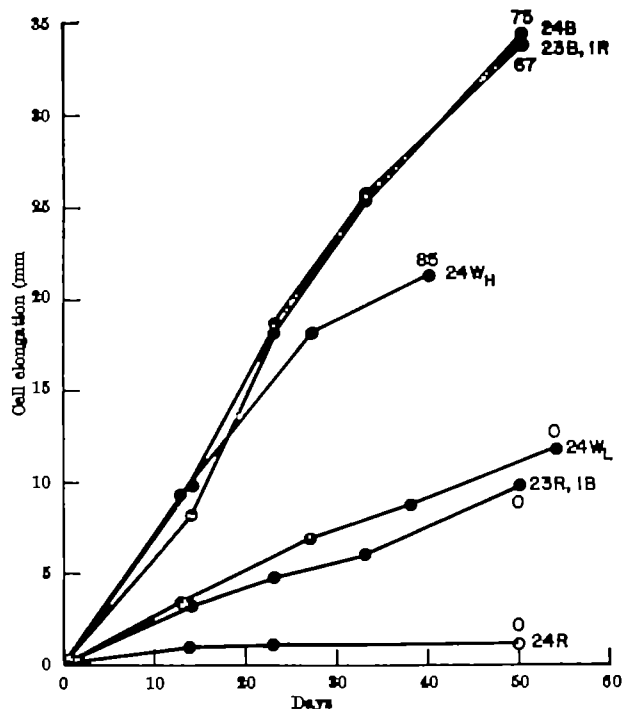


Fig. 3. Elongation of *A. oreanulata* cells in white light of two intensities ( $W_R$ , 0.2 mW/cm<sup>2</sup>;  $W_L$ , 0.05 mW/cm<sup>2</sup>) and in blue ( $B$ , 1.5 mW/cm<sup>2</sup>) and in red light ( $R$ , 1.6 mW/cm<sup>2</sup>) in differing proportions, 24  $R$ , continuous red illumination; 23  $B$ , 1  $R$ , 23 h red plus 1 h blue illumination per day, etc. Numbers over the terminal points refer to the percentage of cap-bearing cells in the sample at the end of the run. Initial mean cell length was 10.5 mm.

left). *A. oreanulata* as found in Nature differs conspicuously from cells grown in the laboratory in white light but closely resembles those that develop in blue light. A sample of 50 cap-bearing *A. oreanulata* cells from Biscayne Bay, Florida (I am greatly indebted to Dr. Stanley Burg and Ellen Burg for collecting this material), had a mean length of 45.3 mm and without exception possessed completely planar caps with mutually attached chambers. The length can be compared with a mean length of 44.7 mm for cells which formed caps in blue light in these experiments. Since the portion of the spectrum which penetrates the sea most readily lies between 450 and 550 m $\mu$ , the energy reaching a depth of a few metres at 650 m $\mu$  being only 11 per cent of that at 500 m $\mu$  (ref. 11), it seems likely that most of the morphological differences between laboratory cultures and natural populations can be accounted for by the spectral quality of the light reaching the cells. This conclusion also suggests that the red part of the spectrum, though apparently not by itself able to support growth, promotes (hastens) the initiation of functional but atypical caps when given in addition to blue.

The interaction of red and blue light has been examined in two ways. In one experiment cells were given continuous red or blue, or 23 h of either plus 1 h of the other, each day (Fig. 3). Growth performance in these régimes was compared with that in white light of two intensities, 0.2 and 0.05 mW/cm<sup>2</sup>, corresponding to energy fluxes far less than those provided by either the red or blue source. Compared with continuous blue light, 1 h of red plus 23 h of blue produced no discernible difference in growth. Caps were formed at approximately the same time and cell length in both régimes, and considerably later than in the low-intensity white light. Though in white light the cell length at morphogenesis is inversely dependent on light intensity<sup>12</sup>, caps were formed later (that is, at a greater length) in blue of a higher intensity than the white control, thus showing conclusively that the delayed morphogenesis in blue light was not due merely to an effect of intensity.

One hour of blue light inserted into a continuous red régime, however, had a very pronounced effect, as Clause also discovered<sup>8</sup>. While continuous red gave virtually no growth after the first 14 days, the insertion of 1 h of blue (that is, 23R, 1B) gave an appreciable and steady rate, roughly equal to that produced by white light of 12 ft.-candles (the latter, however, providing only 3 per cent of the daily irradiance). In another experiment it was found that the 23R, 1B régime gave 2.5 times the growth in 11 days that in sum took place in two cultures receiving separately 24R and 1B. Since, furthermore, Clause found that even 1 min of blue per day could enhance growth in red light, there can be no doubt that the two wave-length bands are synergistic.

In order to determine whether red light exerts any real inhibitory effect, rather than merely being insufficient for either growth or morphogenesis, experiments were run under light sources having widely different proportions of red and blue radiation while providing approximately the same total energy flux. These were obtained by combining GE 'Blue', 'Daylight' and 'Red' fluorescent tubes in various proportions. Blue tubes were used with a filter which entirely excluded red light. The ratio of energies received at 650 and 450 m $\mu$  was computed from spectral energy distribution curves published by the General Electric Co. Cultures grown under sources with  $I_{650}/I_{450}$  ratios of 0.0, 0.7 and 1.5 were found to elongate at the same rate over a period of 3 weeks before cap formation. The mean cell length in these cultures at the time of 50 per cent cap formation, however, was 34.8, 26.5 and 24.5 mm respectively. Thus the greater the proportion of red light received, the shorter the length at which caps are formed. The results show clearly that red light *per se* is not inhibitory, either to growth or to cap initiation, and again suggest that it hastens the latter.

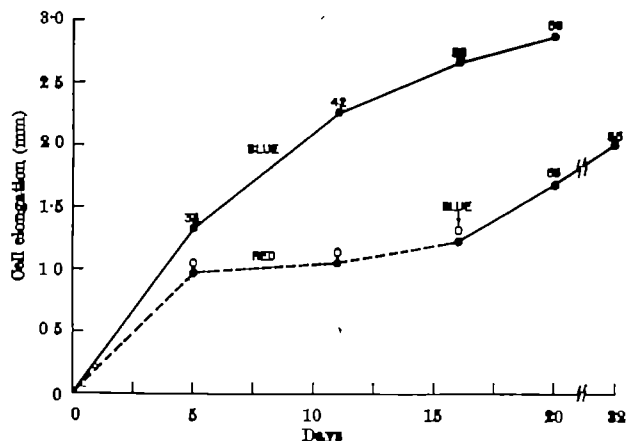


Fig. 4. Elongation of enucleate cells of *A. crinale* in blue (1.5 mW/cm<sup>2</sup>) and red (1.6 mW/cm<sup>2</sup>) light. Cells given red light were transferred to blue light after 11 days. Numbers over the points refer to the percentage of cap-bearing cells in the sample. —●—, Blue Illumination; ---●---, red Illumination.

The fact that two weeks or more may pass before growth ceases altogether in red light suggests the slow disappearance of a rate-limiting substance or reaction which can be restored, or maintained in part, by as little as 1 min of blue light per day. That the substance(s) limiting growth in red light is both stored and produced in the cytoplasm is indicated by the data in Fig. 4 from an experiment with enucleate cells. Though the total growth capacity of enucleate cells is far lower than that of intact cells (2.9 mm growth in 20 days in blue light, compared with 15–20 mm for controls) they are able to grow nearly as much in red light (1.2 mm in 16 days compared with results of 0.9, 2.0 and 2.1 mm for nucleate cells), implying that the substance or process limiting growth in red light is primarily localized in the cytoplasm. Moreover, when the enucleate cells were placed in blue light after 16 days in red light the immediately resumed growth and formed caps, showing

that blue light can stimulate growth in the absence of a nucleus. The failure of *Acetabularia* to form caps in red light cannot be considered a specific inhibitory effect on morphogenesis, since growth is greatly restricted under these conditions. Cells placed in white light of an intensity low enough to limit growth likewise failed to initiate caps (see Fig. 3).

All attempts to relate the effect of red light on *Acetabularia* to previously described phenomena have so far been unsuccessful. Haupt's discovery of a reversible red, far-red effect on chloroplast orientation in *Mougeotia*<sup>9</sup> and *Mesostemonium*<sup>10</sup> implies that phytochrome may be of widespread occurrence among the algae. However, in our experiments red light alternating with dark periods of various lengths, or with dark periods broken by 5-min periods of far-red light, has produced no more growth than continuous red light. A red source made up with tungsten flood lamps and red filters was richer in far-red light than in red and should have brought the two forms of phytochrome to a steady-state equilibrium, but this also failed to promote growth.

The possibility that Emerson enhancement of photosynthesis is involved seems excluded in view of the facts that (a) the red sources used in these experiments contained wave-lengths down to 590 m $\mu$ , and (b) several experiments with interference filters showed that photosynthesis in *A. crinale* was considerably more efficient at both 684 and 694 m $\mu$  than at 450 m $\mu$ . Furthermore, a direct short-wave-length enhancement of photosynthesis is in any case improbable because irradiation by the two wave-length regions need not be simultaneous in order to be effective.

Nitrate reduction is known to be stimulated by the blue end of the spectrum. However, replacing the NO<sub>3</sub><sup>-</sup> by ammonium salts in the normal Erdschreiber medium, or adding various amino-acids, had no stimulatory effect on growth in red light.

Red and blue light have been found to have differential effects on many plant systems; in higher plants on catalase activity<sup>12</sup>, protein and carbohydrate contents<sup>13,14</sup> and oxygen uptake<sup>15</sup>. Various green algae, red algae and diatoms have been grown under broad-band red, green and blue light sources with resulting growth rates that can be largely explained in terms of the relative intensities of the sources and/or the absorption characteristics of the species in question<sup>16,17</sup>. In no case did light which was absorbed to an appreciable extent fail to support development, although, interestingly, the diatom, *Chaetoceros*, assumed a larger average cell size in blue light than it did in red<sup>18</sup>. Kowalik, working with cultures of *Chlorella* synchronized by an alternating light-dark régime in which the light supplied was either red or blue, found differential effects of light quality on the time of division, the average size, RNA content, and protein/carbohydrate ratio of the cells in these cultures<sup>19</sup>. The photochemical mechanism responsible for these differences is still obscure, though they may possibly be related to the results with *Chlorella* of O'Leary and Emerson<sup>20</sup> and Hauschild *et al.*<sup>21</sup>, who found, using <sup>14</sup>CO<sub>2</sub> in short-term experiments, that blue light stimulated a greater incorporation of label into several amino-acids than did red light. That red light might alter the products of photosynthesis in *Acetabularia* in such a way as to halt growth remains as a possible explanation of its effect, even though several attempts to enhance the growth of *A. crinale* cells in red light by supplementing the normal inorganic medium with sugars, acetate or amino-acids were unsuccessful. The fact that small quantities of blue light can catalyse growth and even cap formation in red light suggests that blue light may stimulate a specific non-photosynthetic process or the photoconversion of a reserve substance stored in the cytoplasm.

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## METHOD FOR CHARACTERIZING SAWFLY EGG PIGMENTATION

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DURING the course of genetic investigations it was noted that, in some families of *Neodiprion swainsoni* Middleton, sister females had either green or yellow abdominal venters, resulting from similar colour differences in the contained egg masses. Observations on a number of common diprionid species showed wide inter-specific variation in the egg colour. Forsius<sup>1</sup> recorded a great variety of colours among the unlaid eggs of 124 species of European sawflies, and Scheidter<sup>2</sup> and Sturm<sup>3</sup> used the colour of laid eggs in field diagnoses of the egg clusters of central European diprionid species. In spite of these earlier observations on egg colour and the apparent usefulness of this characteristic, taxonomists and other workers have not undertaken detailed examinations of it. There are probably two main reasons for this lack of interest: taxonomists in the past seldom investigated living specimens or had such material readily available; secondly, meaningful colour descriptions were not easy to achieve. The latter problem became very evident to us in the early stages of the work. Initially, attempts were made to obtain ratings by comparisons with standard colour charts in the *Munsell Book of Color*<sup>4</sup>. Although much superior to arbitrary descriptions, this approach was of limited value because of its intrinsic subjectivity. A spectrophotometric method of assessment appeared feasible and also desirable. The opal glass transmission method of Shibata<sup>5</sup> was adapted for use with sawfly eggs in the following manner.

A full egg complement, removed from a naturally emerged female by cutting off the tip of the abdomen just anterior to the genitalia, was placed in a shallow, open-ended trough on an opal slide (Fig. 1). The opal slide consisted of a 50 × 12 mm rectangular piece cut from commercial opal glass about 3.4 mm thick, comprising a 0.4-mm opal layer bonded to a clear back about 3 mm thick. Two 20 × 2 mm pieces of No. 0 cover glass (0.085–0.13 mm thick) were cemented to the opal surface spaced about 3 mm apart, and in a position such that, when the opal slide was inserted vertically in the cuvette carrier, the beam passed through the centre of the 20 × 3 mm area between the rectangles of cover glass. The eggs were placed on the opal slide, covered with 0.15 M sodium chloride solution, and then separated from one another using fine forceps. Unwanted materials, such as ovariole tissue, immature eggs, and portions of the gut, were removed and the eggs washed with the saline, using filter paper to remove the wash solution. The eggs were arranged more or less in a single layer filling the beam

area of the trough. A slip of No. 0 cover glass was placed over the layer of eggs and saline was then run under the cover, filling the voids between eggs by capillary action. Excess saline was removed with a piece of filter paper to the extent that the cover glass was drawn tightly over the eggs, but the space under the cover around the eggs was completely filled. The edges of the cover were sealed with rubber cement, taking care to leave the beam area clear. Drying of the rubber cement tended to draw the cover slip even more tightly over the eggs, flattening them slightly and helping to lock them in position. A saline-filled reference slide was prepared in the same manner without eggs.

The sample and reference slides were placed in the cuvette carrier of a Beckman 'DU' spectrophotometer with photomultiplier so that the opal surfaces of the slides faced the monochromator. Hard cork blocks were used to ensure stability and uniform placement in the carrier. Optical density readings were taken at 10-mμ intervals, or at more closely spaced positions in regions of band maxima, over the range 360–700 mμ, using the tungsten source and a constant slit width of 0.78 mm. The beam size, normally about 6 mm high by 3 mm wide, with a slit of 0.78 mm, was reduced to less than 1 mm in diameter

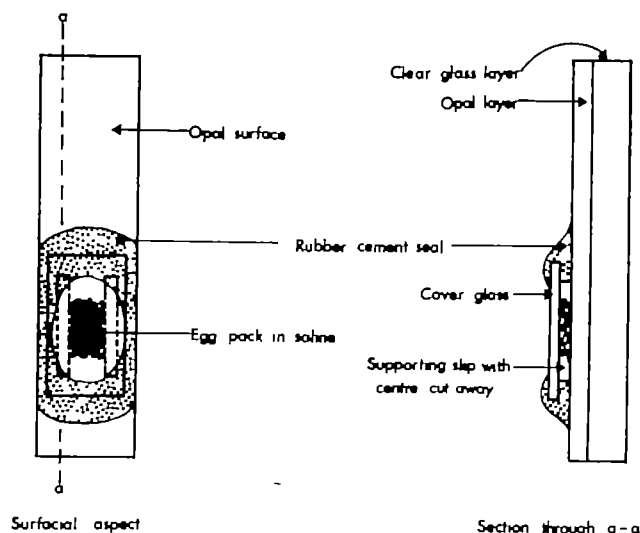


Fig. 1. Arrangement of opal slide bearing sawfly eggs



by using a microcell slide adapter with its smallest aperture (0.46 mm) in position. High electrical gain was required in the near ultra-violet range and at the red end of the visible spectrum. Each run took about three-quarters of an hour with an instrument operator and a record taker.

Little difficulty was encountered in using the foregoing method and satisfactory egg spectra were obtained (Fig. 2). The length of time required to obtain a single spectrum and the lack of a continuous record over the test range, however, caused some difficulty. It was impossible to obtain spectra of a satisfactory number of egg complements during the fairly short period that adults of any given type were available. The long observation period was particularly troublesome while determining the stability of the preparations and when instrument instability was suspected. The repeated mechanical shifting of the sample-positioning carriage, necessary to place the reference and sample slides alternately in the beam, often caused changes in the spacing of eggs during the course of a run. In most instances such shifts were detected as sudden unexpected changes in optical density, but the danger of not noticing more subtle changes was always present, and frequent re-checking of the optical density at the starting wave-length was required. Beam restriction intensified the problem of shifting eggs and introduced a sampling effect, because only a few of the eggs produced by a female were in the beam, and there is some variation in egg colour within a single complement. Subsequent investigations showed that it is undesirable and unnecessary to reduce the beam area by masking. The high electrical gain required at the near ultra-violet and red ends of the spectrum prevented accurate readings and limited the spectral range amenable to examination. In addition, the large slit width resulted in poor resolution

as the spectral slit width was 11 m $\mu$  at 360 m $\mu$  and 78 m $\mu$  at 700 m $\mu$ . In later work, most of the difficulties associated with instrumentation were overcome by using a recording spectrophotometer.

Utilization of the complete egg complement obtained by dissection of individual females avoids the time-consuming and painstaking work required to dissect eggs from needles once they have been laid. Moreover, the use of eggs dissected from females enhances repeatability, since once oviposition takes place, embryogenesis commences even in those species overwintering in the egg stage<sup>4</sup>, and it is doubtful if meaningful spectra could be obtained without recourse to uniform sample timing. On the other hand, our observations suggest that the total egg production of a diprionine female is developed by the time of emergence from the cocoon, and Smith's<sup>5</sup> work on *Gilpinia hercyniae* (Hartig) indicates that at the time of emergence the eggs are relatively quiescent, all in a single stage of development, awaiting the stimulus of oviposition to trigger the maturation divisions of the nucleus. During our investigation, neither resorption of eggs over a prolonged adult life-span nor changes in egg pigmentation during the life-span of the adults after normal emergence from the cocoon have been observed, and the pigments were found to be stable in egg preparations over the period of spectrophotometric examination.

Because it was not possible to control the thickness of the egg preparations and the spaces between eggs to a fine degree, the spectra ranged from relatively flat ones of overall low apparent density to ones with very distinctive features and high apparent density. However, by plotting the spectra on a log<sub>10</sub> ordinate scale, a common practice in the investigation of characteristic spectral form<sup>6</sup>, curves of reasonably similar shape were obtained from preparations of very different thickness and spacing (Fig. 2). This technique allowed a visual analysis of spectral homogeneity among individuals of the same as well as different populations.

Fig. 2 illustrates a number of spectra of the blue-to-turquoise eggs of *Diprion simile* (Hartig), the yellow-green eggs of *Gilpinia hercyniae* (Hartig), the orange-yellow eggs of *Gilpinia fructorum* (Fabricius), the purple-brown eggs of *Neodiprion sertifer* (Geoffroy) (all Diprionidae: Diprioninae), the pale yellow eggs of *Pitonema alaskensis* (Rohwer), and the yellow-green eggs of *Pitonema dimmocki* (Cresson) (Tenthredinidae: Nematinae). Consistent differences in spectral form are evident, which is not surprising, since the egg colour differences in the examples chosen are all readily observed by eye, but the method works equally well even when slight or no colour differences are visually discernible.

An initial investigation of the chemical basis of egg pigmentation showed that moderate-to-strong yellow colours associated with absorption shoulders in the region about 450 m $\mu$  are due to carotenoids, and that the blue, green and purple colours correlated with absorption maxima at wave-lengths longer than 570 m $\mu$  are produced by bile chromoproteins.

The application of the foregoing method to problems in the taxonomy of the conifer sawflies (Hymenoptera: Diprionidae) was demonstrated at the Centennial of Entomology in Canada, September 1963 (ref. 9). A full account of this work and its subsequent development will be published elsewhere.

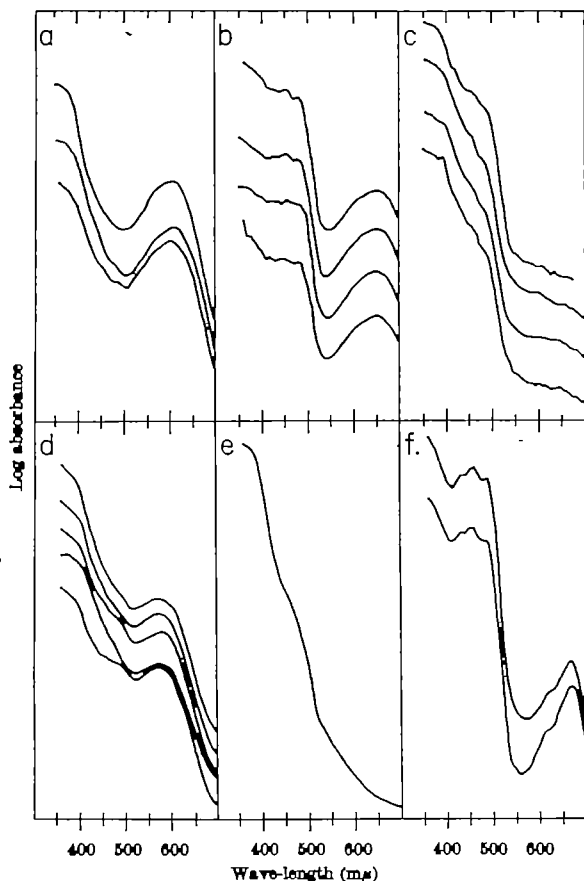


Fig. 2. Egg spectra using the opal glass transmission method. (a) *Diprion simile*; (b) *Gilpinia hercyniae*; (c) *Gilpinia fructorum*; (d) *Neodiprion sertifer*; (e) *Pitonema alaskensis*; (f) *Pitonema dimmocki*.

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## CENTRIFUGAL FIBRES TO THE RETINA IN THE MONKEY AND CAT

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IN normal material stained by the Golgi method, centrifugal fibres have been described in the retinae of birds and mammals<sup>1</sup>. Recently, experimental neurohistological evidence has been provided for the origin, course and termination of such fibres in the pigeon together with the details of the organization of the afferent connexions and projection of their nucleus of origin<sup>2</sup>. There is good evidence from electrophysiological experiments for the presence of such fibres in mammals<sup>3</sup>; but so far it has not been possible to demonstrate them unequivocally with axonal degeneration methods. It is now known that both normal and degenerating nerve endings in certain parts of the central nervous system cannot be stained by silver methods, but that it is possible to recognize them with the electron microscope<sup>4</sup>. For these reasons it was thought that degenerating centrifugal fibres might be seen in ultra-thin sections of the retinae of mammals with the electron microscope. In preliminary experiments the retinae of pigeons, in which the nucleus of origin of the centrifugal fibres had been destroyed, were examined with the electron microscope. After these experiments on the pigeon had shown that electron microscopical techniques can be used to demonstrate degenerating centrifugal fibres in the retina, similar investigations were made in two mammals.

In the pigeons large lesions were placed in the mid-brain, in monkeys the optic tract was divided, and in cats the optic nerve was cut at its junction with the optic chiasma. After survival periods varying from six to fourteen days the animals were anaesthetized, the eyes removed and their anterior halves and vitreous removed before fixation of the retinae with veronal-buffered osmic acid; the retinae of the monkeys and cats were cut vertically close to the optic disk into 'nasal' and 'temporal' halves, and each of these halves was in turn divided vertically into 'inner' and 'outer' halves. All four parts were then cut into small blocks approximately 1 mm<sup>3</sup> in size, and after dehydration and staining with phosphotungstic acid were embedded in 'Araldite'. The brains of all the animals were fixed in formalin, and sections were cut and stained with the method of Nauta<sup>5</sup> in order to determine the site and extent of the lesion.

In the pigeon, evidence of degeneration of fibres and terminals is found only in the retina of the eye on the side opposite to that of the lesion in the brain stem<sup>2</sup>. In the optic nerve fibre layer an occasional fibre, or group of two or three adjoining fibres, are distinctly abnormal in appearance. These altered fibres are larger than the normal optic nerve fibres, contain whorls of electron-dense 'membranes' and have numerous shrunken, opaque mitochondria (Fig. 1). Similar degenerating fibres are seen between the cell bodies of the ganglion cells and at all levels of the inner plexiform layer as far out as the inner aspect of the bipolar cell layer. Degenerating fibres and very dense presynaptic endings are found in relation to the cell bodies of the amacrine cells, a thin process of a Müller cell usually intervening. A degenerating fibre or terminal has been seen directly apposed to the membrane of an amacrine cell; but, because of the absence of localized membrane thickening, it has not been possible to identify with certainty a degenerating presynaptic ending making a direct axo-somatic synapse with an amacrine cell. In some instances, where a degenerating presynaptic ending is opposite the junction between two

very thin Müller cell processes, a thickening of the plasma membrane of the amacrine cell body is evident, suggesting the edge of an axo-somatic synapse. Close to an amacrine cell body degenerating fibres can be seen bifurcating into presynaptic terminals, the fibre and ending together having the shape of a Y. The number of degenerating axons and terminals is very small; sections of many blocks contain none, and not more than three fibres have so far been found on any particular section, even when there are several dozen normal fibres in the layer of optic nerve fibres. These observations on ultra-thin sections are thus in complete agreement with those made with conventional neurohistological methods regarding the number and distribution of the centrifugal fibres.

In the retinae of the eyes of monkeys seven days after section of the optic tract there is also evidence of degeneration of a small proportion of the fibres. In the optic nerve fibre layer the cross-sectional area of these degenerating fibres is several times greater than that of the normal fibres, and they contain numerous mitochondria. In some of these fibres the mitochondria are larger than normal, are clear and have no distinct cristae; in the majority, however, the mitochondria are dense and opaque, and are so numerous that they completely fill the axon (Fig. 2). In a few instances the degenerative process has proceeded further, to the stage seen in the pigeon, so that the normal appearance of the fibre is completely altered owing to the presence of whorls of dense 'membranes' or lamellae. Degenerating fibres, usually at more advanced stages of degeneration, can be seen throughout the inner plexiform layer and on the inner aspect of the bipolar cell layer. In the outer third of the inner plexiform layer very dense remnants of presynaptic endings have been found occasionally (Fig. 4); there is difficulty in identifying the membrane thickenings, however, and it is

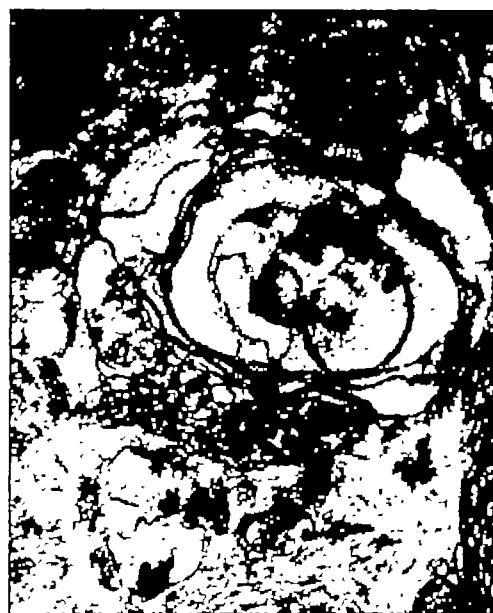


Fig. 1. Degenerating centrifugal fibre in optic nerve layer of retina of pigeon 11 days after destruction of the nucleus of origin of the centrifugal fibres ( $\times 15,000$ )

not possible to be certain of the type of process with which they are related. In the areas of the inner plexiform layer in which degenerating axons and terminals are seen, and most frequently near the outer border of the inner plexiform layer, dense masses are found within the glial cytoplasm. The appearance of these masses is very similar to that described in the cerebral cortex by Colonnier and interpreted by him as phagocytosed presynaptic endings<sup>7</sup>. Because of the sampling difficulties inherent in electron microscopical techniques, any assessment of the number and distribution of the degenerating fibres must be considered to be only tentative, but, with this qualification, it may be stated that they are relatively few, and the observations made so far suggest that their number is of the same order of magnitude as in the pigeon,



Fig. 2. Degenerating centrifugal fibre in optic nerve layer of retina of monkey seven days after section of the optic tract ( $\times 15,000$ )



Fig. 3. Degenerating fibre in inner plexiform layer of retina of cat six days after cutting the optic nerve ( $\times 30,000$ )



Fig. 4. Degenerating endings near outer border of the inner plexiform layer. A and B of monkey ( $\times 40,000$ ), C of cat ( $\times 55,000$ )

where previous work has shown them to form 1 per cent of the total number of fibres in the optic nerve<sup>8</sup>. One to three degenerating fibres have been seen on sections of approximately half the twenty blocks of 'inner' retina which have been examined. They are most numerous on sections which can be identified as being from the perfoveal region, and in sections of one block of this region up to ten such degenerating fibres are seen in the optic nerve fibre layer. In sections from thirty blocks of the 'outer' retina, however, only an occasional degenerating fibre and terminal have been recognized.

Six days after section of the optic nerve in the cat similar, but less marked, changes are seen in a few fibres in the optic nerve fibre layer of the retina. The affected axons are larger than the normal optic nerve fibres, and are filled with numerous mitochondria; the latter are enlarged, pale, and have no distinct internal structure. In the inner plexiform layer occasional degenerating fibres and terminals are seen, and these show a more advanced degree of degeneration than those in the layer of optic nerve fibres. The axons (Fig. 3) contain many small, opaque masses, fused mitochondria, whorls of lamellae, and, in some instances, lamellae arranged in a regular concentric manner around altered mitochondria. The degenerating terminals are especially dense (Fig. 4), and it is not possible to be certain of their precise relationship to other processes. Glial phagocytosis of dense debris is also present.

These electron microscopical observations of the retina following lesions of the central visual pathway may be accepted as valid evidence for the presence of centrifugal fibres to the retina in the mammal for several reasons. The changes which have been observed in the fibres of the optic nerve fibre and inner plexiform layers are similar to those described in other sites in the central and peripheral nervous system<sup>1-3</sup>, and the findings in the monkey and cat are essentially the same as in the pigeon, where the observations made with the electron microscope confirm those obtained with conventional neuro-histological methods. The fact that the degenerating fibres are most numerous in the region of the fovea in the monkey is in agreement with the statement of Ogden and

Brown\* that the P wave, elicited on stimulation of the optic nerve and considered by them to be due to activation of centrifugal fibres, is found consistently only in this part of the retina. It might be argued that the changes which have been described in some of the axons of the optic nerve fibre layer might be indicative of retrograde fibre degeneration, but if this were so it would have been found in the majority of the fibres in this layer, instead of in so few. The degenerative process affects predominantly the mitochondria<sup>8</sup>, and at the survival periods used in this investigation no appreciable increase has been observed in the number of neurofilaments which are normally present in the axons of the optic nerve fibre layer, and there is no evidence of the formation of neurofilaments in the degenerating terminals. In view of the theory that the presence of neurofilaments is necessary for the impregnation of a nerve terminal by silver<sup>10</sup>, this observation might explain the failure to demonstrate such terminals with the paraffin method of Nauta (unpublished observations). The failure to find many degenerating terminals can probably be explained on the basis that the survival periods after operation were too long, as it has been found that, in the lateral geniculate nucleus of the cat, at seven days "fragments that can be clearly identified as former boutons have been difficult to detect by E.M."<sup>11</sup>.

Further work with shorter survival periods should give evidence of the precise synaptic relationship of the terminals of these centrifugal fibres. In addition, it will be of interest to determine, by the method of retrograde cell degeneration, the site of origin of these fibres and of

the relationship of this nucleus to the superior colliculus. In view of the well-organized projection of the optic tectum on the nucleus of origin of the centrifugal fibres in the avian brain it would be reasonable to expect a similar connexion in the mammal. The importance of such a projection from the superior colliculus to the cells of origin of the centrifugal fibres would be two-fold; first, it would serve as a link between the afferent (retina to colliculus) and efferent (centrifugal) limbs of a feedback mechanism acting on the intrinsic neurones of the retina; and secondly, because there is a substantial projection from the visual and other areas of the cerebral cortex<sup>12</sup> to the superior colliculus, there is the possibility of a cortical influence on the activity of the retina.

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## PHOTOSYNTHETIC PHOSPHORYLATION AND ELECTRON TRANSPORT

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THE formation of adenosine triphosphate (ATP), the 'energy currency' of living cells, occurs at the expense of free energy liberated during electron transport. The early type of ATP formation in photosynthesis<sup>1-3</sup>, now known as cyclic photophosphorylation, yields solely ATP and produces no net change in any external electron donor or acceptor. Here the coupling between ATP formation and active electron transport could only be inferred as being of a closed or cyclic type that is hidden in the structure of the chloroplast<sup>4,5</sup>. Direct experimental evidence of a coupling between the light-induced synthesis of ATP and electron transport came from non-cyclic photophosphorylation—a type of photosynthetic phosphorylation in chloroplasts in which ATP formation is stoichiometrically coupled with a light-driven transfer of electrons from water to a terminal electron acceptor and the resultant evolution of oxygen<sup>6</sup>. Both cyclic and non-cyclic photophosphorylation quench chloroplast fluorescence—a recent finding which is consistent with their role as the early energy conversion processes in photosynthesis<sup>7</sup>.

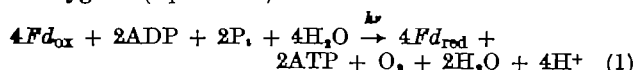
Since cyclic and non-cyclic photophosphorylation generate the assimilatory power in photosynthesis, their mechanisms and interactions are of considerable interest. Previous work led to two hypotheses: (a) cyclic and non-cyclic phosphorylation have a common site for ATP formation<sup>4,5</sup>; (b) non-cyclic photophosphorylation requires the collaboration of two light reactions (connected by a chemical 'bridge' of dark reactions) to bring about the transfer of one electron from water to a terminal electron acceptor<sup>8-10</sup>.

The purpose of this article is to present and discuss new evidence which leads us to abandon both these hypotheses and to re-interpret our earlier findings. We

now conclude, first, that cyclic and non-cyclic photophosphorylation in chloroplasts involve different pathways of electron transfer and do not have a common site for ATP formation. Our second hypothesis is that non-cyclic photophosphorylation involves not two but only one light reaction. According to this hypothesis only one quantum of light is required to transfer one electron from water to the terminal electron acceptor and to produce simultaneously the equivalent amount of ATP and oxygen. The new hypothesis that cyclic and non-cyclic photophosphorylation are bound to distinct electron transport pathways parallels the two modes of ATP formation in non-photosynthetic cells: (a) by substrate level phosphorylation in the course of fermentation; (b) by oxidative phosphorylation during respiration.

**Ferredoxin and photophosphorylation.** Before dealing with the present data, it is necessary to consider briefly recent evidence on the role of ferredoxin in cyclic and non-cyclic photophosphorylation. In non-cyclic photophosphorylation ATP formation was originally thought<sup>4</sup> to be linked with what appeared to be a photoreduction of nicotinamide adenine dinucleotide phosphate<sup>8</sup> (NADP) or an artificial substitute (for example, ferricyanide). But recent evidence has established that illuminated chloroplasts (grana) do not react directly with NADP<sup>11</sup>. The substance which is photoreduced by chloroplasts is not NADP but the water-soluble, iron protein, native to chloroplasts, now called ferredoxin (for the basis of this nomenclature see ref. 11). NADP reduction proper is separated by two dark reactions from the photochemical reduction of ferredoxin: (a) the re-oxidation of reduced ferredoxin by a flavoprotein chloroplast enzyme, ferredoxin-NADP reductase; (b) the re-oxidation of the reduced ferredoxin-NADP reductase by NADP<sup>11</sup>.

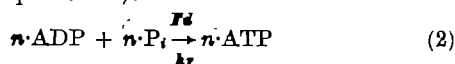
Ferredoxin was identified as the crucial, physiological electron acceptor in non-cyclic photophosphorylation<sup>10</sup>. The oxidation-reduction of ferredoxin was found to involve the transfer of one electron<sup>11,12</sup>. This was confirmed by the stoichiometry of non-cyclic photophosphorylation in which the photo-reduction of 4 moles of ferredoxin was coupled with the formation of 2 moles of ATP and 1 mole of oxygen<sup>10</sup> (equation 1):



The left-hand side of equation (1) shows four moles of water to indicate that we envisage, in the reduction of ferredoxin, each molecule of water (OH<sup>-</sup> at pH 7) yielding only one electron.

In experiments with isolated chloroplasts it is usually more convenient to measure non-cyclic photophosphorylation by using catalytic amounts of ferredoxin and stoichiometric amounts of NADP which, unlike chloroplast ferredoxin, is commercially available and relatively stable to oxygen. However, this operational convenience must not obscure the nature of ferredoxin as the terminal electron acceptor in non-cyclic photophosphorylation and the strongest reductant<sup>14</sup> (about 100 mV more electro-negative than NADP) that has been isolated so far from the photosynthetic apparatus. In experiments with isolated chloroplasts, non-physiological electron acceptors (for example, ferricyanide, benzoquinone) can readily replace ferredoxin to give stoichiometric oxygen evolution (Hill reaction) and, often, a coupled photophosphorylation, but at a great loss in the generated reducing potential.

The role of ferredoxin is not confined to non-cyclic photophosphorylation. Under strictly anaerobic conditions, when the photoproduction of oxygen by chloroplasts is suppressed, ferredoxin also catalyses cyclic photophosphorylation<sup>15</sup> (equation 2):



A characteristic feature of ferredoxin-catalysed cyclic photophosphorylation—not seen when other catalysts are used—is its sensitivity to low concentrations of two inhibitors, antimycin A and 2,4-dinitrophenol<sup>16</sup>. Both these inhibitors are noted for their inhibition of oxidative phosphorylation; hence the likelihood that portions of the enzymatic apparatus of oxidative and photosynthetic phosphorylation are similar. In oxidative phosphorylation inhibition by antimycin is considered to be indicative of the participation of cytochrome *b* in electron transport. It is possible, therefore, that cytochrome *b*, of chloroplasts participates in the ferredoxin-catalysed cyclic photophosphorylation and accounts for its sensitivity to antimycin A. There is no evidence that cytochrome *b*, is involved in the cyclic photophosphorylation catalysed by such exogenous catalysts as phenazine methosulphate.

Another feature of ferredoxin-catalysed cyclic photophosphorylation is consistent with its being the physiological type. Under very low light intensity, ferredoxin is much more effective than other co-factors of cyclic photophosphorylation in catalysing the conversion of radiant energy to the pyrophosphate bond energy of ATP (Fig. 11, ref. 16).

**Desaspidin and ferredoxin-dependent photophosphorylation.** Baltscheffsky and de Kiewiet<sup>17</sup> introduced to the study of photosynthetic phosphorylation a new inhibitor, a phlorobutyrophenone derivative, desaspidin, used in medicine as an anthelmintic agent and found by Runeberg<sup>18</sup> to act as a powerful uncoupler of oxidative phosphorylation. The remarkable property of desaspidin is that at very low concentrations (about 10<sup>-7</sup> M) it inhibited cyclic photophosphorylation catalysed by phenazine methosulphate, menadiene and dichlorophenol indophenol, whereas a similar degree of inhibition of non-cyclic photophosphorylation required desaspidin at about a 100 times

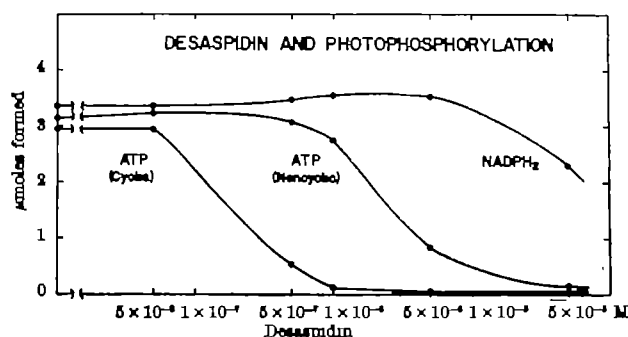


Fig. 1. Effect of desaspidin on ferredoxin-catalysed cyclic and non-cyclic photophosphorylation. In both systems, the reaction mixture (final volume 1.5 ml.) included spinach chloroplasts (equivalent to 250 µg of chlorophyll) and the following in µmoles: Tris buffer, pH 8.0, 50; MgCl<sub>2</sub>, 2.5; ADP, 5; and K<sub>2</sub>HPO<sub>4</sub>, 5. In the non-cyclic system, 0.2 mg ferredoxin and 5 µmoles NADP were also added. In the cyclic system, 8 mg ferredoxin and 2 µg (6.7 × 10<sup>-4</sup> M) of *p*-chlorophenyl-1,1-dimethyl urea (CMU) were added. Desaspidin was added as indicated. Temperature, 20°, illumination, 20,000 lux (yellow light) for 10 min, gas phase, argon. Other methods are given in ref. 10.

greater concentration<sup>17</sup>. In the experiments of Baltscheffsky and de Kiewiet<sup>17</sup> and Gromet-Elhanan and Arnon<sup>19</sup>, sensitivity to inhibition by low concentrations of desaspidin gave a new and unambiguous criterion by which cyclic photophosphorylation was distinguished from the non-cyclic phosphorylation that is associated with the transfer of electrons from OH<sup>-</sup> to NADP (via ferredoxin) or to such Hill reagents as ferricyanide.

In the present investigation, cyclic photophosphorylation catalysed by ferredoxin exhibited the same sensitivity to low concentrations of desaspidin as was observed with the non-physiological catalysts<sup>17,19</sup>. Fig. 1 shows that 5 × 10<sup>-7</sup> M to 10<sup>-6</sup> M desaspidin inhibited almost completely ferredoxin-catalysed cyclic photophosphorylation, but 5 × 10<sup>-6</sup> M to 5 × 10<sup>-5</sup> M desaspidin was required to inhibit non-cyclic photophosphorylation to the same degree.

An interesting effect of desaspidin was observed on ferredoxin-catalysed photophosphorylation in the presence of air. We have previously reported that this type of photophosphorylation is of the pseudocyclic type<sup>10,11</sup>, that is, a special case of non-cyclic photophosphorylation in which electrons are transferred from OH<sup>-</sup> to ferredoxin and thence to molecular oxygen. This non-cyclic electron flow from OH<sup>-</sup> to O<sub>2</sub> via ferredoxin gives the appearance of a cyclic electron flow since manometric measurements give no indication that an electron donor and acceptor are being consumed concomitantly with ATP formation. In pseudocyclic photophosphorylation the consumption of oxygen at the terminal end of the electronic pathway is balanced<sup>10</sup> by the production of oxygen at the site of electron donation by OH<sup>-</sup>.

Table 1 shows that, in the presence of air, ferredoxin-catalysed phosphorylation was 83 per cent inhibited by desaspidin (5 × 10<sup>-7</sup> M) at the lowest concentration of ferredoxin (0.2 mg/1.5 ml.), but ATP formation became progressively more resistant to desaspidin as the concentration of ferredoxin increased (7 per cent inhibition at 3.0 mg ferredoxin/1.5 ml.). Conversely, *p*-chlorophenyl-1,1-dimethyl urea (CMU), the well-known inhibitor of oxygen evolution and hence of pseudocyclic phosphorylation by chloroplasts, gave 11 per cent inhibition at the lowest concentration of ferredoxin and 65 per cent

Table 1. EFFECT OF DESASPIDIN ON FERREDOXIN-CATALYSED PHOSPHORYLATION IN AIR

Ferredoxin added (mg)	ATP formed (µmoles)		
	Control	Desaspidin	CMU
0.2	1.12	0.19	1.00
1.0	2.88	1.06	1.32
2.0	3.18	2.25	1.41
3.0	3.43	3.17	1.20

The experimental conditions were as given for Fig. 1, except that NADP and argon were omitted. The concentration of desaspidin was 5 × 10<sup>-7</sup> M and of CMU, 6.7 × 10<sup>-4</sup> M. Ferredoxin was added as indicated.

inhibition at the highest concentration of ferredoxin (Table 1).

These results indicate that, in the presence of air, ferredoxin catalysed a mixed type of photophosphorylation. At the lowest concentration of ferredoxin, the phosphorylation was predominantly of the cyclic type, whereas at the highest concentration of ferredoxin used, the phosphorylation was predominantly non-cyclic or, more specifically, its pseudocyclic variant.

**Non-cyclic photophosphorylation of the bacterial type.** Deaspidin proved to be especially useful in clarifying the nature of a phosphorylation in chloroplasts that is coupled with an artificial non-cyclic electron flow system, that is, a system in which  $\text{OH}^-$ , the natural electron donor for non-cyclic photophosphorylation in chloroplasts, is replaced by a reduced indophenol dye<sup>11</sup>. The background of this problem is as follows.

Losada *et al.*<sup>8</sup> found that this 'bacterial type' of non-cyclic electron flow in chloroplasts is coupled with a phosphorylation. It was classified as a 'bacterial type' because photosynthetic bacteria cannot use  $\text{OH}^-$  as an electron donor but exhibit a non-cyclic electron flow from other, less-oxidized, electron donors (for example, succinate) to pyridine nucleotide<sup>12,13</sup>. Losada *et al.*<sup>8</sup> also observed that photophosphorylation in chloroplasts occurred when oxygen evolution was suppressed and the dye was maintained in the reduced state by an excess of ascorbate. On the other hand, little phosphorylation occurred when the dye was maintained in the oxidized state by an excess of ferricyanide—an experimental arrangement which did not impair oxygen evolution. The diminished phosphorylation here did not seem to result from an inhibitory or uncoupling effect of the oxidized indophenol dye since the addition of the oxidized dye did not interfere with photophosphorylation when it was coupled to a non-cyclic electron flow from water to NADP (Table 3, ref. 8).

These findings were interpreted<sup>8,14</sup> as a separation of non-cyclic photophosphorylation by chloroplasts into two component light reactions: (a) photo-oxidation of water ( $\text{OH}^-$ ), an auxiliary reaction which did not form ATP but supplied electrons, at an intermediate reducing potential, to be used by (b) a second photochemical reaction, the bacterial type of non-cyclic phosphorylation, in which the reduction of NADP is coupled with photophosphorylation.

This interpretation seemed to receive additional support when Nozaki *et al.*<sup>15</sup> found a similar non-cyclic photophosphorylation in particles of photosynthetic bacteria. Thus, the site of non-cyclic photophosphorylation in plants was considered to be analogous to that in bacterial non-cyclic photophosphorylation and, in accordance with earlier formulations<sup>8,1</sup>, the same site was also shared by cyclic photophosphorylation.

The validity of these conclusions became open to some question after Trebst and Bok<sup>16</sup> found, and other investigators confirmed<sup>17-19</sup>, that dichlorophenol indophenol in a reduced form can catalyse cyclic photophosphorylation. The matter remained complicated because, under the conditions of the experiments of Losada *et al.*<sup>8</sup> with chloroplasts and of Nozaki *et al.*<sup>15,21</sup> with bacterial particles (strict anaerobicity and a great excess of ascorbate), dichlorophenol indophenol failed to catalyse any phosphorylation unless a non-cyclic electron flow from reduced dye to pyridine nucleotide was established and maintained.

The matter was finally resolved by the recent experiments with deaspidin which, at a low concentration, strongly inhibited the bacterial type of non-cyclic photophosphorylation but not the non-cyclic photophosphorylation associated with a flow of electrons from  $\text{OH}^-$  to NADP<sup>17,18</sup>. It became necessary, therefore, to abandon the previous hypothesis<sup>8</sup> and its later elaborations<sup>12,13</sup> in favour of a new hypothesis of cyclic and non-cyclic photophosphorylation which fits the new results and which is not in conflict with the earlier findings on the relation between electron flow and photophosphorylation.

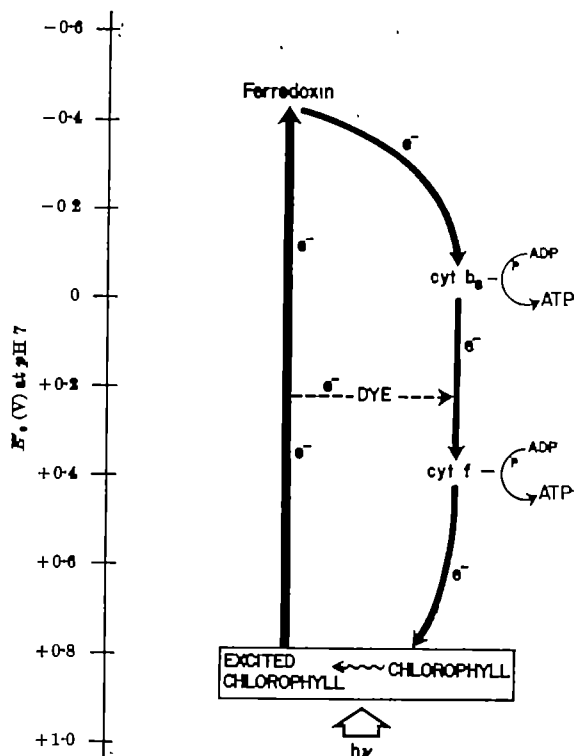


Fig. 2. Scheme for cyclic photophosphorylation

**Cyclic photophosphorylation.** Our new hypothesis envisages cyclic photophosphorylation in chloroplasts as including, under physiological conditions, phosphorylations that are coupled with a flow of electrons from excited chlorophyll to ferredoxin, and then from reduced ferredoxin to cytochromes  $b_6$  and  $f$  and back to chlorophyll (Fig. 2). We specify tentatively two phosphorylation sites in this cyclic electron transport chain but this does not exclude the possibility of additional phosphorylation sites.

We consider the cyclic electron transport chain and its coupled phosphorylation to be distinct from non-cyclic photophosphorylation of the plant type (with  $\text{OH}^-$  as the electron donor) which we will discuss later. This conclusion is based on the fact that ferredoxin-catalysed cyclic photophosphorylation will occur only under conditions when non-cyclic photophosphorylation is excluded: (a) either by the use<sup>18</sup> of such specific poisons of electron flow from  $\text{OH}^-$  as *p*-chlorophenyl dimethylurea<sup>22</sup> (CMU) and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), or (b) by the use<sup>8</sup> of monochromatic light longer than 700 mμ which cannot support the non-cyclic electron flow from  $\text{OH}^-$  and the resultant oxygen evolution.

We include the chloroplast cytochromes<sup>24,25</sup>  $b_6$  and  $f$  as electron carriers in cyclic but not in non-cyclic photophosphorylation although, so far as we are aware, there is no direct evidence for the involvement of chloroplast cytochromes in either type of photophosphorylation. Indirect evidence comes from inhibition of ferredoxin-catalysed cyclic photophosphorylation by antimycin A and 2,4-dinitrophenol, two well-known inhibitors of oxidative phosphorylation where the direct participation of cytochromes is well documented. Antimycin A inhibition of oxidative phosphorylation is considered to be indicative of the participation of cytochrome  $b$  in electron transport<sup>26,27</sup>. It is likely that participation of cytochrome  $b_6$  accounts for the sensitivity of ferredoxin-catalysed cyclic photophosphorylation to antimycin A. As for cytochrome  $f$ , its joint participation in an electron transport chain with cytochrome  $b_6$  of chloroplasts is considered likely by analogy with oxidative phosphorylation in

mitochondria. The span between the redox potentials of cytochromes  $b_6$  and  $f'$  ( $-0.06$  V and  $0.365$  V, respectively) is large enough to accommodate a phosphorylation. Likewise, the span between the redox potentials of ferredoxin ( $-0.43$  V) and cytochrome  $b_6$  is large enough to accommodate at least one phosphorylation in this segment of the cyclic chain.

As discussed elsewhere<sup>12,14</sup>, we consider that in chloroplasts the ferredoxin-catalysed cyclic photophosphorylation is the physiological one. However, experimentally, cyclic photophosphorylation proceeds readily without ferredoxin when catalysed by one of several dyes or other co-factors. Since such artificial cyclic photophosphorylations are resistant to inhibition by antimycin A, it seems reasonable to conclude that they bypass the cytochrome  $b_6$  site (see dotted line in Fig. 2).

**Bacterial type of non-cyclic photophosphorylation.** To account for the results with desaspidin, we now propose a new scheme for the bacterial type of non-cyclic photophosphorylation (Fig. 3). Under strongly reducing conditions (great excess of ascorbate and in the presence of DCMU) no cyclic electron flow is possible since the components of the cyclic chain will be kept in the reduced state (cf. refs. 38, 39, 31). However, under these conditions, electrons will flow unidirectionally via a portion of the cyclic chain to ferredoxin (and NADP). As long as this unidirectional or non-cyclic flow of electrons through a cytochrome is maintained, it would induce a phosphorylation at a 'cyclic' site. This mechanism would explain the two characteristic features of non-cyclic photophosphorylation of the bacterial type: ATP formation at a cyclic site, as indicated by desaspidin inhibition, and the dependence on a non-cyclic electron flow, as indicated by the requirement for the terminal electron acceptor<sup>31,32</sup>.

Fig. 3 (thick arrows) shows how the proposed scheme explains non-cyclic photophosphorylation of the bacterial type induced in chloroplasts when dichlorophenol indophenol is the electron carrier between ascorbate and the cyclic chain. Moreover, the scheme also explains the generation of a non-cyclic photophosphorylation of the bacterial type in chloroplasts with *p*-phenylenediamine which was introduced, with its derivatives, into the study

Table 2. EFFECT OF DESASPIDIN ON THE BACTERIAL TYPE OF NON-CYCLIC PHOTOPHOSPHORYLATION

Treatment	NADP omitted		NADP added	
	ATP formed ( $\mu$ moles)	ATP formed ( $\mu$ moles)	NADP reduced ( $\mu$ moles)	
<i>p</i> -phenylenediamine	0.06	1.23	3.83	
<i>p</i> -phenylenediamine and desaspidin		0.03	3.93	
2,6-dichlorophenol indophenol	0.04	1.69	3.18	
2,6-dichlorophenol indophenol and desaspidin		0.06	3.54	
2,3,5,6-tetramethyl- <i>p</i> -phenylenediamine (DAD)	0.04	2.97	4.06	
DAD and desaspidin		0.05	3.83	
No co-factor added		0.02	1.27	

The experimental conditions were as given for Fig. 1 for the non-cyclic system, except that 100  $\mu$ moles of Na ascorbate and  $2 \times 10^{-4}$  M 2-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) were added to the reaction mixture. 0.1  $\mu$ mole 2,3,5,6-tetramethyl-*p*-phenylenediamine (diaminodurel), 1.0  $\mu$ mole *p*-phenylenediamine, and 0.1  $\mu$ mole 2,6-dichlorophenol indophenol were added as indicated.

Table 3. EFFECT OF DESASPIDIN ON CYCLIC PHOTOPHOSPHORYLATION CATALYSED BY SUBSTITUTED PHENYLENES AND 2,6-DICHLOROPHENOL INDOPHENOL

Treatment	ATP formed ( $\mu$ moles)	
	Control	+ Desaspidin
<i>p</i> -phenylenediamine	1.30	0.03
2,3,5,6-tetramethyl- <i>p</i> -phenylenediamine	4.01	0.07
2,6-dichlorophenol indophenol	1.40	0.04
No co-factor	0.06	

The experimental conditions were as given for Table 2, except that ascorbate and NADP were omitted and the illumination time was 30 min. The reaction mixture was pre-illuminated 1 min before the addition of 2-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU).

of photophosphorylation by Trebst<sup>33</sup>. These compounds are known to interact with the cytochrome chain in oxidative phosphorylation<sup>31</sup>.

Trebst<sup>33</sup> has shown that *p*-phenylenediamine can induce in chloroplasts what, in our terminology, would be a non-cyclic electron flow of the bacterial type to NADP, but he found no accompanying phosphorylation. Table 2 shows that in the presence of a higher concentration of the co-factor and a great excess of ascorbate the non-cyclic electron flow to NADP was accompanied by a significant phosphorylation. Similar results were obtained with 2,3,5,6-tetramethyl-*p*-phenylenediamine (diaminodurel), with which Trebst and Pistorius<sup>34</sup> did obtain a phosphorylation coupled to a bacterial type non-cyclic electron flow to NADP, and with dichlorophenol indophenol. In all three cases phosphorylation was strongly inhibited by  $5 \times 10^{-7}$  M desaspidin and was strictly dependent on the non-cyclic electron flow to ferredoxin and thence to NADP. However, the phosphorylations were poorly coupled to electron flow, resulting in a poor stoichiometry between the NADP reduced and ATP formed.

Table 3 shows that, in the absence of NADP and ascorbate, the phenylenediamine compounds and dichlorophenol indophenol catalysed a cyclic photophosphorylation which was as strongly inhibited by desaspidin as the respective bacterial type non-cyclic photophosphorylations catalysed by the same compounds (Table 2).

We conclude, therefore, that in chloroplasts the non-cyclic photophosphorylation of the bacterial type depends on a unidirectional, non-cyclic electron flow over a portion of the cyclic chain and involves a phosphorylation at a cyclic site. This formulation fits not only the facts in chloroplasts but also those pertaining to the non-cyclic phosphorylation observed with chlorophyll-containing particles from photosynthetic bacteria<sup>31,32</sup>. There again, the phosphorylation occurred in the presence of a great excess of ascorbate which precluded cyclic photophosphorylation and was dependent on a non-cyclic electron flow from ascorbate via dichlorophenol indophenol to NAD.

In the light of present knowledge the observations which led us to the previous hypothesis<sup>9</sup> are also in agreement with the new hypothesis. ATP was not formed in the presence of small amounts of dichlorophenol indophenol and large amounts of ferricyanide because excess ferricyanide kept the dichlorophenol indophenol in an oxidized state known to uncouple phosphorylation. We can also explain now why the addition of oxidized di-

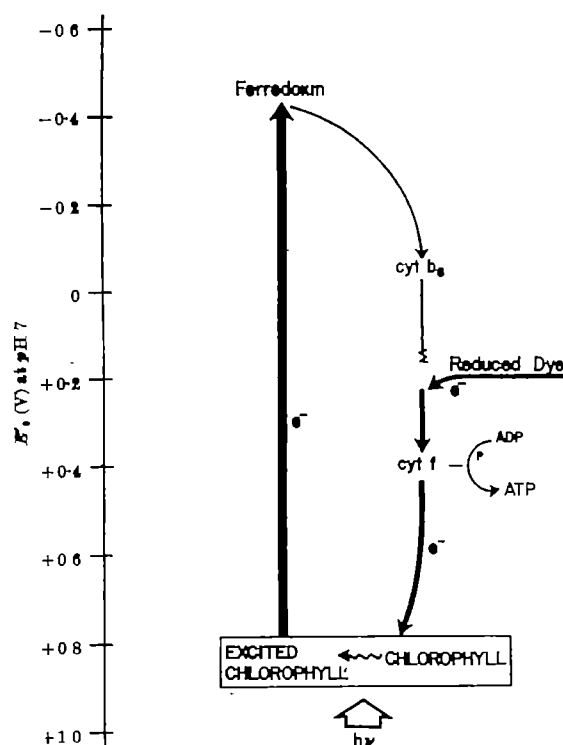


Fig. 3. Scheme for non-cyclic photophosphorylation of the bacterial type



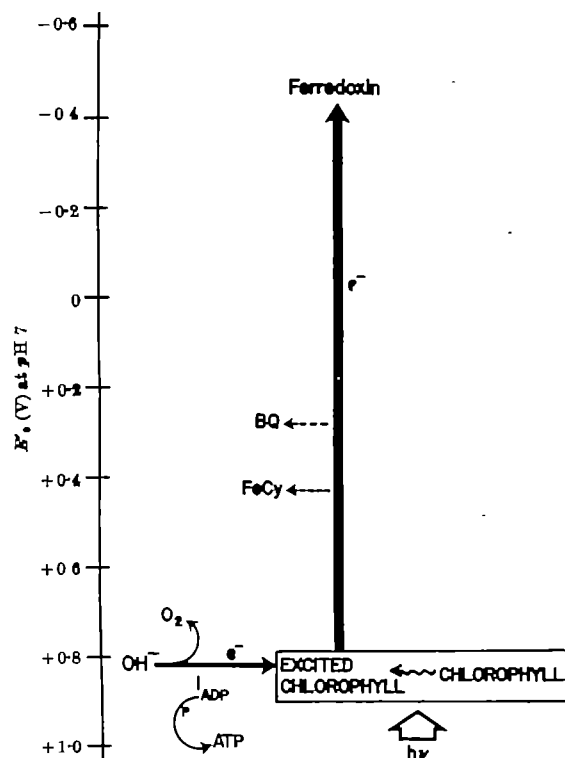


Fig. 4. Scheme for non-cyclic photophosphorylation of the plant type

chlorophenol indophenol to the NADP system did not uncouple phosphorylation: the dye was probably quickly reduced by ferredoxin and, once reduced, it no longer uncoupled phosphorylation<sup>44</sup>.

**Non-cyclic photophosphorylation of the plant type.** Our present concept of non-cyclic photophosphorylation in plants is shown in Fig. 4. The phosphorylation is envisaged as being coupled to the oxidation of  $\text{OH}^-$ , a coupling that would account for the consistent stoichiometry,  $P/2e = 1$ , between oxygen evolution and ATP formation. An electron from  $\text{OH}^-$  is transferred via chlorophyll to ferredoxin in a single light reaction. In isolated chloroplasts, ferredoxin may be replaced by non-physiological electron acceptors (Hill reagents) with an attendant drop in the light-generated reducing potential. Fig. 4 (dotted lines) illustrates this for ferricyanide and benzoquinone (BQ).

The 'chlorophyll' in Fig. 4 represents the complex of chlorophyll *a* and *b* pigments in their various forms and includes those accessory pigments which are involved in light absorption by chloroplasts (see refs. 43, 44). In Nature, photosynthesis occurs usually in white light, hence both cyclic (Fig. 2) and non-cyclic photophosphorylation receive light of the same complete spectral composition. We assume that excitation energy is transferred among the chloroplast pigments with the great efficiency that is well documented in algal cells (see review by Duxens<sup>45</sup>).

In laboratory experiments with monochromatic light, it has been established that cyclic photophosphorylation (Fig. 2) and non-cyclic photophosphorylation of the bacterial type (Fig. 3) can occur at wave-lengths longer than 700 mμ that will not support non-cyclic photophosphorylation of the plant type\* (Fig. 4). Thus, it appears that plant non-cyclic photophosphorylation requires the shorter wave-lengths of light and is perhaps most efficient at wave-lengths not longer than those at which photosynthesis occurs with maximum efficiency. The energy balance given in Table 4 shows that 1 quantum of light at 680 mμ—the longest wave-length that would still support maximum efficiency of plant photosynthesis<sup>46</sup>—contains sufficient energy for the transfer of an electron

from  $\text{OH}^-$  to ferredoxin and the coupled phosphorylation.

The electron pathway from  $\text{OH}^-$  to chlorophyll is the least-understood part in the mechanism of photosynthesis and its elucidation must be left to future research. Only a few of the co-factors and catalysts involved therein are now known: chloride ions<sup>46, 47</sup>, manganese<sup>48, 49</sup> and plastoquinone<sup>50-52</sup> (plastoquinone is also involved in cyclic photophosphorylation in chloroplasts<sup>51, 53</sup>). The nature of the linkage between electron flow from  $\text{OH}^-$  and phosphorylation remains obscure but there is good reason to believe that the two are closely linked in Nature since the rate of electron flow from  $\text{OH}^-$  is greatly increased by the concurrent phosphorylation<sup>5, 44-55</sup>.

The flow of electrons from  $\text{OH}^-$  to ferredoxin, and the resultant oxygen evolution, are easily susceptible to damage when chloroplasts are removed from intact cells (see review<sup>44</sup>). The non-cyclic electron flow of the bacterial type is much more stable but, again, it is also uncertain whether it can give a true measure of the efficiency of electron transport in the intact cell. It is not surprising, therefore, that a requirement of one quantum per electron transferred to ferredoxin (or NADP) has not been obtained in investigations with isolated chloroplasts<sup>57</sup>.

**Cyclic and non-cyclic photophosphorylation as the two light reactions of photosynthesis.** In plant photosynthesis non-cyclic photophosphorylation (equation 1) provides reduced ferredoxin, ATP and  $\text{O}_2$  in a stoichiometry of 4:2:1. Since two moles of reduced ferredoxin are required to give one mole of  $\text{NADPH}_2$ , non-cyclic photophosphorylation gives rise to  $\text{NADPH}_2$  and ATP in a ratio of 1 to 1. Were this ratio adequate for carbon assimilation there would be no need for cyclic photophosphorylation. However, the carbon reduction cycle appears to have a requirement of 2  $\text{NADPH}_2$  and 3 ATP per 1 mole of  $\text{CO}_2$  assimilated to the level of sugar<sup>58</sup>. Additional ATP is required to form starch, the main carbohydrate product of photosynthesis in leaves. (ATP is expended in the formation of adenosine diphosphate glucose from which the glucosyl moiety is transferred to a starch primer<sup>59</sup>.) Moreover, as was pointed out elsewhere<sup>6</sup>, cyclic photophosphorylation may be an important mechanism for providing the large supplies of ATP that are required for protein synthesis and for other endergonic processes in the cell.

If we accept cyclic and non-cyclic photophosphorylation as the two sub-divisions of the energy conversion process of photosynthesis, the relation between them becomes important. We have already cited the evidence<sup>12</sup> that in isolated chloroplasts cyclic photophosphorylation occurs only when non-cyclic photophosphorylation is stopped. As long as electrons move from  $\text{OH}^-$  to ferredoxin, and thence to NADP, cyclic photophosphorylation cannot proceed. We have presented evidence elsewhere<sup>12</sup> that the availability of oxidized NADP may regulate the flow of electrons via the cyclic or non-cyclic pathway.

We shall now examine how our hypothesis can explain some of the observations that are used to support the concept of 'two light quanta per one electron transferred'<sup>43-45</sup>. The 'red drop' (that is, the drop in the quantum efficiency of photosynthesis of intact plant cells in monochromatic light longer than 685 mμ) would mean in our scheme that at the longer wave-lengths of light non-cyclic photophosphorylation became limiting, thereby reducing the availability of  $\text{NADPH}_2$  and decreasing the over-all efficiency of the process. 'Enhancement'<sup>43-45</sup> (that is, the synergistic increase in the quantum efficiency of photosynthesis at long wave-lengths of light that results

Table 4. ENERGY BALANCE OF NON-CYCLIC PHOTOPHOSPHORYLATION OF THE PLANT TYPE

Energy input of 1 einstein, $\lambda = 680 \text{ m}\mu$ , is 43 kcal.
$\text{Ferredoxin}_{ox} = \text{ferredoxin}_{red} + e^-$ ; $E^\circ = -0.43 \text{ V}$ (pH 7).
$1/2 \text{H}_2\text{O} = 1/4 \text{O}_2 + \text{H}^+ + e^-$ ; $E^\circ = +0.82 \text{ V}$ (pH 7).
Potential span between $\text{O}_2$ and $\text{ferredoxin}_{red}$ is $1.25 \text{ eV} = 1.25 \times 23.06 = 28.8 \text{ kcal}$ .
Energy requirement (per 1 electron) to form 1 ATP: $10 \text{ kcal}/2 = 5 \text{ kcal}$ .
Excess of energy input over output. $43 - (28.8 + 5) = 8.2 \text{ kcal}$ .

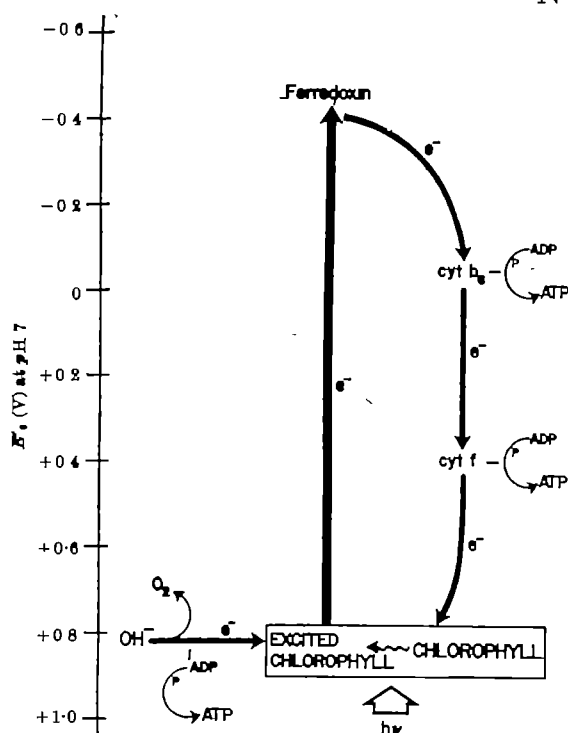


Fig. 5 Joint scheme for cyclic and non-cyclic photophosphorylation in chloroplasts

from the addition of shorter wave-lengths) would mean that the addition of light of a shorter wave-length restored non-cyclic photophosphorylation, removed the shortage of reductant, and thereby enhanced the overall efficiency of photosynthesis.

Among the other observations that would fit the present hypothesis is the oxidation of cytochromes in longer wave-lengths of light and their reduction in shorter wave-lengths of light (see reviews in refs. 43 and 44). The photo-oxidation of cytochromes would be consistent with the onset of cyclic photophosphorylation at the longer wave-lengths and the reduction of cytochromes with the onset of non-cyclic photophosphorylation at the shorter wave-lengths of light. It is possible that electron pressure is so high during non-cyclic photophosphorylation that it tends to keep all the cytochrome components of the cyclic chain in a completely reduced state.

Similarly, some of the observations on the partial loss of photosynthetic activity in algal mutants<sup>45</sup> can be explained as indicating the loss of non-cyclic photophosphorylation of the plant type. The remaining photosynthetic activity would then be cyclic photophosphorylation and the non-cyclic photophosphorylation of the bacterial type.

### Concluding Remarks

The present hypothesis differs from that presented earlier<sup>1-10</sup> in not excluding the possibility that the partial reactions of photosynthesis may proceed with a high degree of efficiency, because of an efficient, resonance type transfer of energy between different photosynthetic pigments<sup>46</sup>, without invoking intervening dark reactions. Since most of our work has been with leaf chloroplasts, it is interesting to note that Warburg's recent experiments<sup>41</sup> show a high efficiency of photosynthesis in leaves: a requirement of 5.5 quanta per CO<sub>2</sub> as contrasted with his best earlier measurements<sup>42</sup> of about 15 quanta per CO<sub>2</sub>.

According to our earlier hypothesis, a transfer of one electron from water (OH<sup>-</sup>) to ferredoxin and NADP required the energy of two photons absorbed by two-pigment systems acting in series. We now consider that the energy of one photon absorbed in a single light reaction may be sufficient.

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## MEASUREMENTS OF PHOTOSYNTHESIS AND RESPIRATION IN A MARINE DIATOM WITH THE MASS SPECTROMETER AND WITH CARBON-14

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FOLLOWING the introduction by Steemann Nielsen<sup>1</sup> of a carbon-14 technique for the measurement of organic production in the sea, widespread discussion has arisen concerning the meaning of data so gathered. Steemann Nielsen (Steemann Nielsen and Hansen<sup>2</sup>) has maintained his attitude that, in short-term experiments, something between net and gross photosynthesis is measured, basing his judgement on observed relative losses of carbon-14 from fully labelled algae in the light and in darkness. Ryther<sup>3</sup>, partly on the basis of similar experiments, concluded that the method measures net photosynthesis. Others (for example, see McAllister, Shah and Strickland<sup>4</sup>) have taken the view that the measurement represents increases in particulate carbon since appreciable amounts of organic substances may be excreted into the extra-cellular environment during the course of experiments. In assessing the carbon-14 technique, it has been usual to make reference to measurements of photosynthesis reflected in changes in dissolved oxygen in the suspending medium and, of necessity, to make the assumption that rates of oxygen consumption in the dark continue unaffected in the light. Evidence derived from mass spectrometer studies using isotopic oxygen by Brown<sup>5</sup> has been relied on for justification of this assumption.

In employing the carbon-14 technique to investigate the influence of temperature and light intensity on the photosynthetic activities of bacteria-free cultures of microalgae from Antarctic sea-ice (Bunt<sup>6</sup>), it was found the data were not fully compatible with any of the described interpretations. Among the evident anomalies, it was apparent that rates of respiration calculated according to the method of Steemann Nielsen and Hansen<sup>2</sup> were far too low when expressed as percentages of saturated rates of photosynthesis. In this respect it seemed that the uncorrected data could not be considered to represent net photosynthesis. Furthermore, the factor used by Steemann Nielsen and Hansen<sup>2</sup> to obtain values for corrected net photosynthesis could not be considered adequate. On the assumption generally applied by Steemann Nielsen, that respiration is 10 per cent of gross photosynthesis, it would have been necessary to assume that not 60 per cent but only around 10 per cent of respiratory CO<sub>2</sub> was in some way identical with that fixed during photosynthesis. Further, acceptance of this assumption indicated compensation intensities well above levels of illumination at which successful growth had been observed to take place. For example, at 7°C and 4°C compensation points of 80 and 35 ft.-candles respectively were indicated for the diatom *Fragilaria sublinearis*. This organism has been grown at light intensities at least down to 40 ft.-candles at 7°C and down to less than 10 ft.-candles at 4°C.

At temperatures of 4°C and down to -2°C, extrapolation of light curves from *F. sublinearis* and physiologically similar species based on intensities between 30 and 500 ft.-candles consistently indicated significant levels of activity in the dark, although corrected for dark activity. To avoid these obviously unacceptable results, it was necessary to measure activities at light intensities down to and below 5-ft. candles. In the ice habitat, light intensities during the summer period when growth takes place are typically around 10-20 ft.-candles and less. Measurements under such conditions and in these terms have limited meaning since the spectral quality of the light penetrating the thick sea-ice layer is quite unlike that of daylight. However, the degree of shade adaptation of these species will be evident.

Data thus obtained, when plotted against light intensity, commonly appeared to display two linear portions very similar in nature to the broken light curves described originally with *Chlorella* in terms of net oxygen exchange by Kok<sup>7</sup> and more recently by Jones and Myers<sup>8</sup>, and by Hoch, Owens and Kok<sup>9</sup>, who offer a rational explanation of the phenomenon from direct experimental evidence.

Finding it necessary to be able to explain the results described, we undertook oxygen exchange measurements with the mass spectrometer system recently devised by Hoch and Kok<sup>10</sup>. *Fragilaria sublinearis* was adopted as the experimental organism. Using the mass spectrometer with the advantages of a modified inlet system, Hoch, Owens and Kok<sup>9</sup> have recently examined the relation between photosynthesis and respiration in the two unrelated algae *Anacystis nidulans* and *Scenedesmus* sp. Their work, in particular with the former organism, has shown that light exerts two effects on the uptake of oxygen: an inhibition of true respiration evident at low intensities and a stimulation of oxygen consumption which increases with the rate of photosynthesis. Results with the diatom from the sea-ice have confirmed these observations (MS. in preparation). Further, it was found that the extent to which inhibition of respiration makes itself evident is dependent on temperature. The conclusion was reached that, at 10°C for this organism, in the absence of stimulated uptake, respiration when fully light-inhibited continues at about 25 per cent of the rate in the dark. Indirect evidence was obtained which indicates that inhibition may occur in light absorbed by accessory photosynthetic pigments, as well as in light absorbed principally by chlorophyll *a*, but that the onset of inhibition requires higher intensity at shorter wave-lengths.

Since the observed ratios of oxygen production to consumption have not been discussed previously, some of this information has been summarized in Table 1. Two separate ratios are given for each wave-length of light and temperature. PT/UT represents the ratio existing between total oxygen evolution (PT) and total consumption in the light (UT). The second ratio is equivalent to that used when changes in dissolved oxygen are measured and when it is assumed that oxygen consumption in the light and in darkness are identical. It will be noted that values of PT/UT are generally lower than P/R and that P/R in particular is influenced quite markedly by temperature and, to a lesser extent, by the wave-length of light used. In view of the differences in spectral quality of submarine light, such an effect could be of some ecological significance, since it will be observed also that at 10°C P/R is higher at 525 mμ than at 678 mμ, whereas the reverse is true at 3°C. The optimum growth temperature for *F. sublinearis* is around 5°C. At 3°C, which approximates the optimum, it should be indicated that dark respiration was approximately 9-14 per cent of gross photosynthesis. It would be expected that the true percentage, with respiration fully inhibited, would be appreciably lower.

Recognition of the fact that true respiration (that associated with the release of CO<sub>2</sub>) may be inhibited in the

Table 1. RATIOS OF OXYGEN EVOLUTION AND UPTAKE IN *Fragilaria sublinearis* AT THREE TEMPERATURES AND TWO WAVE-LENGTHS OF ACTIVATING LIGHT AT SATURATION

Wave-length mμ	3°C		10°C		24°C	
	PT UT	*Net+UT dark UT dark	PT UT	*Net+UT dark UT dark	PT UT	*Net+UT dark UT dark
678	4.7	11.3	3.1	2.8	0.9	0.7
525	2.7	7.3	4.0	5.9	1.0	1.0

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\* Referred to in text as P/R.

light has particular significance in interpreting measurements of photosynthesis based on the uptake of radioactive carbon. It has been mentioned already that both Steemann Nielsen<sup>11</sup> and Ryther<sup>2</sup> have measured losses of carbon-14 from uniformly labelled cell suspensions in order to interpret observed uptake into unlabelled cells. Working with *Chlorella*, the former author found that losses of carbon-14 in the light amounted to 50–70 per cent of losses in the dark, while the latter, using *Dunaliella*, found losses to be insignificant in the light. These, and other considerations, led to a disparity of opinion. With the evidence from the mass spectrometer, it was felt that the results of these two investigators could well be essentially compatible. Accordingly, an attempt was made to confirm the observations with the mass spectrometer using the carbon-14 technique.

For this purpose, *F. sublinearis* was grown in the presence of  $\text{Na}_2^{14}\text{CO}_3$  until labelling was considered to be uniform. The cells were centrifuged and washed several times with fresh medium, allowed to stand in the dark for several hours in accordance with the recommendation of Steemann Nielsen<sup>11</sup>, centrifuged and washed several times further, and finally re-suspended in fresh medium such that the cell concentration, in terms of chlorophyll *a*, was 0.02  $\mu\text{g}/\text{ml}$ . At this concentration, the possibility of mutual shading on exposure to light would be negligible, good uniformity between replicates would be assured, and self-absorption in counting the activity of filtered cells would be unimportant. The cell suspension so prepared was distributed in calibrated 30-ml. Fernbach flasks and then exposed, not to a single light intensity, as had been the practice of Steemann Nielsen and Ryther, but to a range of light intensities as well as in the dark. Cool-white fluorescent lighting was employed. The flasks themselves were housed in a water bath at 7° C with temperature control effective to  $\pm 0.1^\circ\text{C}$ . After 8 h incubation, the cells were recovered on 'Millipore' filters and their activities were determined for comparison with the activities of aliquots filtered at the beginning of the experiment. Since the changes in activity were not expected to be great, accuracy was assured so far as was reasonably possible by recording the numbers of counts after 1 h or 800,000 counts, whichever occurred first.

The results are shown graphically in Fig. 1 with losses of activity in the light referred to losses in the dark. In general character, they were found to parallel the observation of inhibited respiration based on measurement of oxygen consumption in the light with the mass spectrometer. It will be observed that, with this organism and under these conditions, very low light intensities were sufficient to inhibit respiration. At full inhibition, which was probably saturated at 200 ft.-candles, losses of

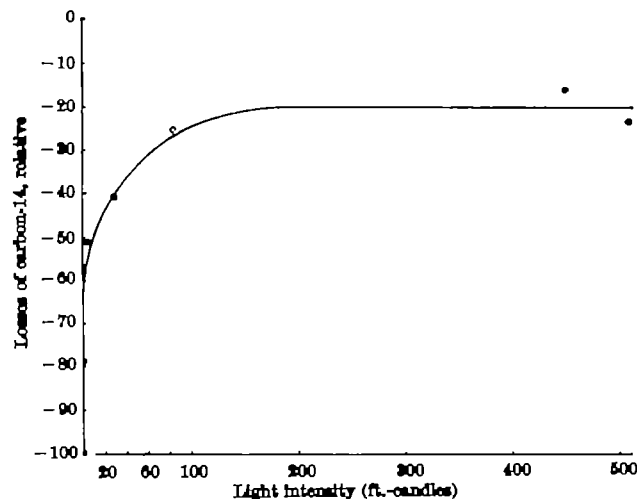


Fig. 1. Inhibition of respiration in the light in terms of losses of carbon-14 from uniformly labelled *F. sublinearis*.

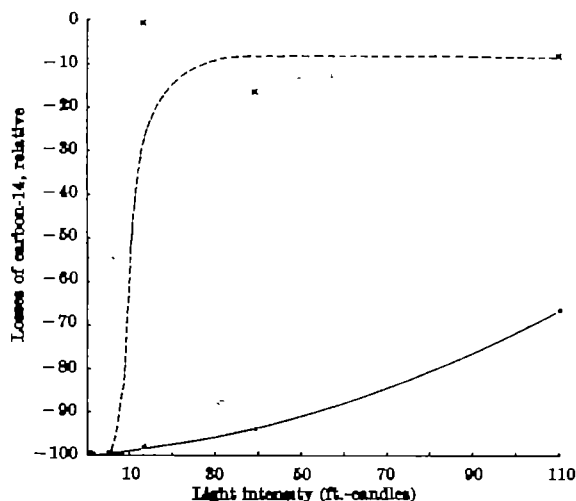


Fig. 2. The effect of adaptation to high and low light intensities on the inhibition of respiration in *F. sublinearis* at  $-2.0^\circ\text{C}$ . —, High light adapted; ---, shade adapted at  $7^\circ\text{C}$ .

carbon-14 were only around 20 per cent of those in the dark. No attempt has been made to apply these findings to values of carbon-14 fixation since to do so reliably would require carefully controlled experiments with labelled and unlabelled cells similarly preconditioned. Nevertheless, the implications of these results are clear and, together with the data from the mass spectrometer, they provide a means of explaining the anomalies described earlier. The fact that Ryther<sup>2</sup> was unable to demonstrate losses of carbon-14 from illuminated cells could have resulted from very marked, perhaps complete, inhibition of respiration under his experimental conditions.

Concerning this possibility, some evidence has been obtained which suggests that, in cells adapted to low light intensities, the onset of inhibition may require less light and may be more fully achieved than in cells accustomed to high light intensities. This effect is demonstrated in Fig. 2, which describes the losses of carbon-14 from uniformly labelled cells at a temperature of  $-2.0^\circ\text{C}$  following adaptation to high (400 ft.-candles) and low (20 ft.-candles) intensities at  $7^\circ\text{C}$ . It should be emphasized, however, that a parallel experiment run at  $7^\circ\text{C}$  throughout yielded less convincing results. While the existence of such an effect would be compatible with the suggestion of Hoch *et al.*<sup>9</sup> that inhibition of respiration may be mediated by the pigment P700 (see Kok<sup>12</sup>), the problem requires more careful study.

In summary, it must be concluded that, where excretion is insignificant, the carbon-14 method gives a measure of net photosynthesis. The precise interpretation of data in terms of gross photosynthesis is not simple, however, and requires a rather full knowledge of the extent to which respiration is inhibited under any given set of conditions. No obvious means of obtaining this information either directly or indirectly from measurements of carbon-14 fixation is evident. The process of inhibition is dependent on a number of factors including the nature of the pigment systems in the material being studied, the spectral quality and intensity of the illumination, and possibly temperature, as well as the general physiological condition of the organisms.

An additional and perhaps more serious complication is presented by algae which excrete significant amounts of organic carbon to the external environment in the course of growth. This process does not appear to be important in *F. sublinearis*, at least in actively growing cultures, and has not been considered in the present discussion.

While this study was based on a single species of organism, the view appears to be justified that inhibition of respiration under appropriate conditions is a general phenomenon among the algae, and presumably in other

groups of plant life also. Consequently, it is expected that the present findings should, in principle, have general relevance to an understanding of processes of primary production in the sea.

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## EVIDENCE FOR NEUROGENIC CONDUCTION IN THE MAMMALIAN HEART

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IT is a widely accepted view that the generation and the conduction of impulses in the mammalian heart are of a myogenic nature. As a result of a comprehensive neuro-histochemical examination, however, we want to express our firm belief that this process is of a neurogenic nature, whereas the conduction of the impulse between the sino-auricular (SA) and the atrio-ventricular (AV) node has a direct nature and takes place through nerve fibres.

We chose the pig as our principal experimental animal, because it has highly differentiated Purkinje fibres. Hearts of dog and cat have also been examined, although in smaller numbers. Since the extent, as well as the structure, of the conductive system in the pig heart was insufficiently known, we examined it with the aid of ordinary nucleus-plasma stains. For the neuro-histochemical examination we used the acetylcholinesterase (AChE) reaction of Koelle and the Champy-Coujard method. Using a modification of the AChE reaction, we succeeded in tracing the course of nervous elements in thicker pieces (up to 5 mm) of tissue. An additional examination of the material has been made by the silver-impregnation method (Bielchowsky-Gros).

From the microscopic and macroscopic examination, it appeared that the conductive system in pig shows the same course and extensions as that in other ungulates<sup>1</sup>. The actual structures of the various parts of the system, however, did not agree with the descriptions to be found in the relevant literature. For example, the dimensions of the cross-sections of the cells of the SA and AV nodes, which are said to be smaller than those of the surrounding myocardial fibres, are, in fact, distinctly larger at many places. Consequently, it appears that, since the retardation of the impulse in the AV node is generally explained by pointing to the smaller dimensions of the cross-section of the nodal fibres, this is no valid argument in itself.

A muscular formation, consisting of thin, interconnected muscle fibres showing a considerable amount of connective tissue in the interfibrillar meshes, occurs in the sulcus terminalis, close to the SA node. In our opinion, this formation is often erroneously referred to as constituting the SA node, and this basic understatement has been a source of controversial statements.

It is impossible to show a direct connexion of specialized muscle fibres between the two nodes, although Purkinje fibres which pass into ordinary cardiac tissue can be sub-endocardially noted in the wall of the right atrium. These observations do not agree with the conclusions formulated by Robb and Petri<sup>2</sup> and by James<sup>3</sup>.

The number of Purkinje fibres in the myocardium is very small as compared with that of the myocardium fibres. This implies that, in the case of a myogenic conduction, the impulse would have to be propagated by ordinary muscle fibres.

The SA node in the adult as well as in the embryonic pig heart was easily marked by the AChE reaction, so that any doubt or inaccuracy concerning the exact location of the SA node has been definitely excluded. In the node, delicate interconnected fibres are marked by the precipitation and, as a whole, the structure gives the impression of a network with swellings at the junctions (Fig. 1). We are of opinion that this nervous network consists of small, interconnected cells. The more delicate structures can be properly studied in Champy-Coujard preparations.

Post-ganglionic nerve fibres in the node have been observed to make contact with the aforementioned network. Moreover, there is a considerable number of pre- and post-ganglionic fibres and ganglion cells in the immediate neighbourhood of the node. The ganglion cells could not be found in the node proper. In Champy-Coujard preparations, the SA node is extremely well marked by the black-stained nervous elements, so that the structure of the network can be seen more readily. It appears to consist of small interconnected nerve fibres, the extensions of which entwine every nodal cell (Fig. 2). In our opinion, this dense network is built up by the autonomic interstitial cells (AIC) of Cajal, which may be looked on as primitive ganglion cells. Moreover, post-ganglionic nerve fibres can be seen in these preparations, which terminate on the network. In microscopic preparations it is very difficult to check the correctness of this interpretation, and electron-microscopic examination is required.

Identical structures may be observed in the Champy-Coujard preparations of the hearts of dog and cat. The



Fig. 1. SA node. Embryo of pig, 70 days. Staining AChE. Positive reaction in and around the SA node. The nervous component of the node is marked by precipitation. This component consists of cells with long extensions (arrow). These cells form a network. Ganglia at the outside of the node (arrow). ( $\times 24$ )

AIC network dominates the picture and there are also post-ganglionic fibres in these animals which probably terminate on the network.

The AV node can also be marked with the AcChE reaction. The microscopic structure of the nerve fibres which have been marked by precipitation differs fundamentally from that of the nervous elements in the SA node. The nerve fibres in the AV node are post-ganglionic and form a very dense plexus (Fig. 3). The use of serial frozen sections of the AV node in the embryonic pig heart showed that the nerve plexus has a different structure in its various layers. The most pronounced plexiform structures have been found in its central layer. In sections cut parallel with the endocardium, nerve fibres could be observed on the basal side of, and entering, the AV node. In sections of the right atrium between the two nodes, nerve fibres could be demonstrated in such numbers that we gained the impression that there is a nervous connexion between the two nodes, but we were unable to verify this impression by frozen sections. We therefore submitted the whole of the right atrium situated between the nodes to the AcChE reaction followed by dehydration and plastic embedding, so that it was made accessible to microscopic examination. These transparent plastic preparations have provided a three-dimensional reconstruction of the course of the nerve fibres. In this way a nervous connexion between the two nodes could be traced with fair certainty (Fig. 4).

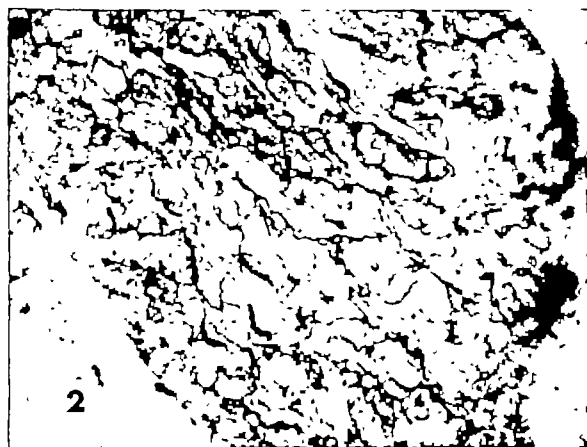


Fig. 2. SA node of adult pig. Staining Champy-Coujard. The brightly stained nodal cells are surrounded by black stained nerve fibres which interconnect and form a network (A.I.C. of Cajal with extensions—arrow). Cut parallel with the epicardium. ( $\times c. 214$ )



Fig. 3. Medial right atrial wall and septum atriorum. Embryo, 80 days. Staining AcChE. Nerve plexus in the AV node. No reaction in the proper nodal cells. Nerve fibres running from SA to AV node, but no uninterrupted connexion. SA node (left upper side) just outside the photograph. Cut parallel with the endocardium. ( $\times 24$ )

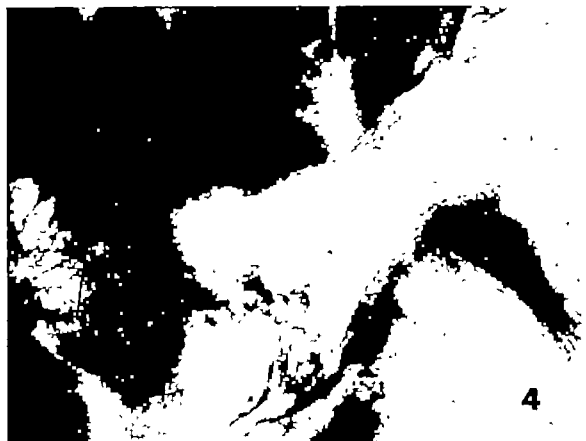


Fig. 4. Stereometric plastic preparation of a part of the right atrium. Embryo, 62 days. Staining AcChE. The nerve fibres between the two nodes may be followed up to the sulcus terminalis (arrow). The AV node and the His bundle are clearly marked by their nervous components.

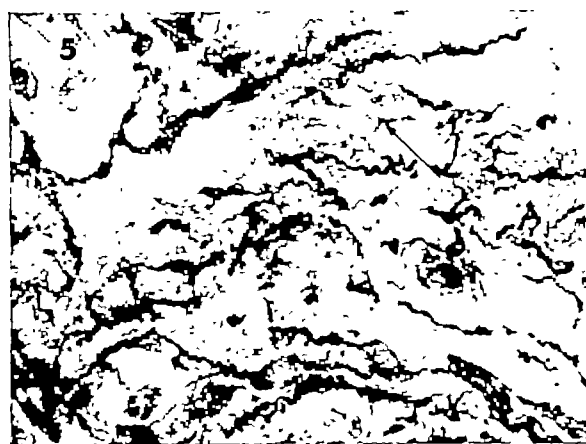


Fig. 5. AV node of adult pig. Staining Champy-Coujard. Post-ganglionic nerve plexus with a number of AIC in its meshes (arrow) ( $\times c. 214$ )

The results obtained by examination of the AV node have been confirmed in Champy-Coujard preparations, which are more suitable for examination at higher magnifications. AIC are also demonstrable in the meshes of the post-ganglionic nerve plexus. Their number is not so great as in the SA node, but there are definitely more AIC here than in the ordinary cardiac musculature (Fig. 5).

With the aid of this method similar nervous elements could be shown to exist in the heart of the dog and cat; in these species, the intensive formation of plexuses is very conspicuous.

Nerve fibres can be marked by the AcChE reaction alongside the His bundle and the peripheral Purkinje fibres. They are predominantly post-ganglionic fibres, which have issued from the nerve plexus in the AV node and run alongside the bundle and the peripheral Purkinje ramifications into the myocardium. Moreover, the bundle is accompanied by some myelinated, probably afferent, nerve fibres.

In the pig heart, ganglion cells in or alongside the bundle could not be found, although a number occur at the basal side of the AV node, alongside the nerve fibres connecting the SA and AV nodes. Such ganglion cells could not be observed at the apical side of the node.

The myocardium is penetrated by nerve fibres accompanying Purkinje fibres as well as by those which accompany the arborizations of the coronary vessels. It appears that a morphological distinction between these two types is possible: the former issue sub-endocardially and reveal a fundamentally aberrant mode of ramification<sup>4</sup>.



Fig. 6. Myocardium of ventricle Embryo, 60 days. Staining Champy-Coujard, A.I.C. with extensions along muscle fibres (arrow) ( $\times$  c. 214)

The result obtained with the aid of the AcChE reaction can be confirmed in Champy-Coujard preparations. Apart from post-ganglionic fibres, AIC can be demonstrated alongside the bundle. Furthermore, some AIC can be shown intramyocardially in Champy-Coujard preparations (Fig. 6). In our opinion these cells form an extensive intramyocardial network, which is interconnected with the muscle fibres. Using the silver impregnation method it has been possible to demonstrate nervous tissue in many parts of the conductive system, but this method does not reveal the exact extent of these elements alongside the conductive system, since its results are too variable. The method is, however, useful in detailed investigation.

It is apparent that many serious objections can be raised against the classic theory of myogenic conduction in the heart. First, the conduction of the impulse in the right atrium has not been satisfactorily explained; the explanation of the wave-like propagation of the impulse through ordinary muscle fibres is illogical and inadequate. Secondly, the histological structure of the two nodes provides no sound basis to explain their function. The peculiar shape of the fibres in the AV node and their smaller cross-section, which have been used as arguments to explain the retardation of the impulse, are not applicable to the situation in the pig heart. Thirdly, the number of penetrating Purkinje fibres in the myocardium, especially in embryos, is very small, so that a single Purkinje fibre would have to activate a considerable number of myocardium fibres. In view of the non-syncretic nature of the cardiac musculature, the occurrence of this type of activation is questionable.

Fourthly, it is highly improbable that the solitary Purkinje fibres in the right atrium have a conductive function. It is logical, therefore, to ask what their function is, as well as that of the Purkinje fibres in other parts of the heart. Our own conclusions may be enumerated as follows:

(1) The generation and conduction of the impulse in the mammalian heart are neurogenic, not myogenic, processes.

(2) The dense network of AIC in the SA node induces the generation of the impulse which leads to contraction. This is probably caused by neuro-secretory products from the AIC. The varicose character of the extensions of these cells points strongly in that direction.

(3) The conduction of the impulse in the right atrium toward the AV node takes place through nerve fibres, connecting the SA and the AV nodes.

(4) The retardation of the impulse in the AV node is caused by the plexiform nervous component in this node; the central layer with its intensive formation of meshes must play an important part in this retardation. The network of AIC between the meshes of this plexus may be responsible for the Tawara-rhythm of this node. Since the network is less dense than that in the SA node, the

Tawara-rhythm will reveal a lower frequency in the AV node.

(5) Beyond the AV node, conduction takes place through post-ganglionic nerve fibres, which run alongside the His bundle and its two branches.

(6) The transmission of the impulse to the muscle fibres takes place through AIC, which are omnipresent between the myocardium fibres.

Apart from the fact that the existence of the required nerve fibres has been demonstrated, the following arguments apply:

(1) Bethe and Boxler<sup>1</sup> pointed to the great physiological resemblance which exists between the rhythmic contractions of the jellyfish and the pulsations of the heart. The marginal nervous organ, constituting the pacemaker in jellyfish, shows a perfect physiological similarity with the SA node. From our examination it appeared that there is a definite morphological resemblance between the network of AIC in the SA node and the network in the marginal nervous organ in the jellyfish. Consequently, it is logical to assume an identical function for both structures.

(2) The place at which we were able to show nerve fibres between the SA and AV nodes coincides with that at which Paes de Carvalho<sup>2</sup> demonstrated electrophysiological pathways of faster conduction.

(3) The shape and the structure of the nervous component in the AV node are compatible with our explanation of its function. A conduction in a plexus will proceed less quickly than that in a longitudinally straightened structure. Moreover, we find the most intensive plexiform structures in the node exactly at the place at which Paes de Carvalho<sup>2</sup> was able to demonstrate the greatest retardation of the impulse. The influence of acetylcholine on the AV node—either a retardation in conduction or a complete block in the case of a lingering or a highly concentrated administration—may be readily explained. It is essential, therefore, to refer to the theory of conduction in axons as has been established by Nachmansohn<sup>3</sup>. According to this author, conduction along axons is not a purely physical but rather a combined physico-chemical process. The liberation of acetylcholine depolarizes the axon-membrane, whereas the enzyme acetylcholinesterase restores the rest-situation by the hydrolysis of the liberated acetylcholine. Administration of additional acetylcholine has the same effect as the use of eserine or DFP, since these compounds prevent the conduction along the axon by blocking the action of AcChE. The action of acetylcholine depends on the permeability of the axon-membrane. In ordinary myelinated nerves, this penetration may be impossible, but, on the other hand, it is quite comprehensible in post-ganglionic unmyelinated fibres in the AV plexus. Any influence of acetylcholine on the nodal fibres must be neglected, since this would involve the occurrence of acetylcholinesterase in these fibres. In the pig, this is not the case, except for the nervous elements around the nodal fibres.

(4) The extension of the nervous elements in the myocardium is such that every muscle fibre contacts nervous elements. According to Meyling<sup>4</sup> and Boeke<sup>5</sup>, AIC may act autonomically at the periphery. This action may give a reasonable explanation for the contraction of small pieces of cardiac tissue which are entirely isolated from the conductive system.

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## STRETCH-INDUCED UTERINE GROWTH, PROTEIN SYNTHESIS AND FUNCTION

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**I**N the work recorded here the question was examined whether chronic stretch alone, without the endocrine support of the ovaries and the placentae, initiates uterine growth, protein synthesis and function.

In the fifteenth century the sawgilders had already noticed that ovariectomy results in castrate atrophy. Later experimental work demonstrated a loss of myometrial function during atrophy, which could be reversed by oestrogen<sup>1</sup>. It was also shown that distension promotes uterine growth and that this growth response is modified by the ovarian hormones. The conclusion was advanced that uterine growth can be generated by tension exerted on the myometrium<sup>2</sup>.

The isolation of the contractile proteins permitted the demonstration that oestrogen regulates the contractile system<sup>3</sup> and working capacity<sup>4</sup> of the myometrium, as well as the protein synthesis in the uterus<sup>5</sup>, while progesterone controls the involution process<sup>6</sup>.

We noted that a physical factor alone, namely, chronic stretch, has a powerful regulatory effect on uterine growth, protein synthesis and function when we ovariectomized parturient rabbits and filled their two uterine horns with balloons of different volumes. The uterine horn in which the parturient volume was restored remained structurally normal and displayed increased function, while the control horn of the same uterus, only carrying an empty balloon, underwent involution, castrate atrophy and a loss of contractile function.

Since in these *post-partum* rabbits both ovarian and placental endocrine functions were suspended, the structural and functional differences in the two horns of the same uterus could only be explained by their different volumes. For even if these uteri were exposed to a hypothetical endocrine effect (of the adrenals or other glands) the two horns must have been equally affected. This argument permitted the tentative conclusion that chronic stretch by itself not only promotes growth but also powerfully regulates uterine biology.

In the experiments reported here we examined non-pregnant as well as parturient rabbits. Rabbits were selected for investigation not only because a suitable method has already been worked out in this animal but also because the two anatomically and functionally independent uterine horns offer a control and an experimental uterus in the same rabbit.

Our operative and recording techniques were described earlier<sup>7</sup>. During surgery we photographed the uterus, introduced condom rubber balloons into both uterine horns and ovariectomized the animal. One balloon in the experimental horn was then filled with 100–120 ml. physiological saline, imitating the parturient volume, if the animal was pregnant, or up to 10 ml. volume if non-pregnant. The other balloon in the control horn was only in-

jected with a few ml. fluid during recording. A polyethylene outlet of the balloon permitted the repeated study of the intra-uterine pressure during 10 days of observation. After 10 days the animals were killed, the uterine horns excised, photographed, weighed and subjected to histological and biochemical analysis (wet and dry weights, protein, DNA and RNA).

After 4 days' pre-treatment of the non-pregnant rabbits with 25 µg/day oestradiol in oil intramuscularly the animals were divided into groups of three, depending on their subsequent treatment. The groups were the following:

(1) *Post-partum*, ovariectomized, untreated; (2) non-pregnant, untreated; (3) non-pregnant, ovariectomized, untreated; (4) non-pregnant, ovariectomized, 25 µg/day oestrogen; (5) non-pregnant, ovariectomized, 5 mg/day progesterone; (6) non-pregnant, ovariectomized, 25 µg/day oestrogen plus 5 mg progesterone.

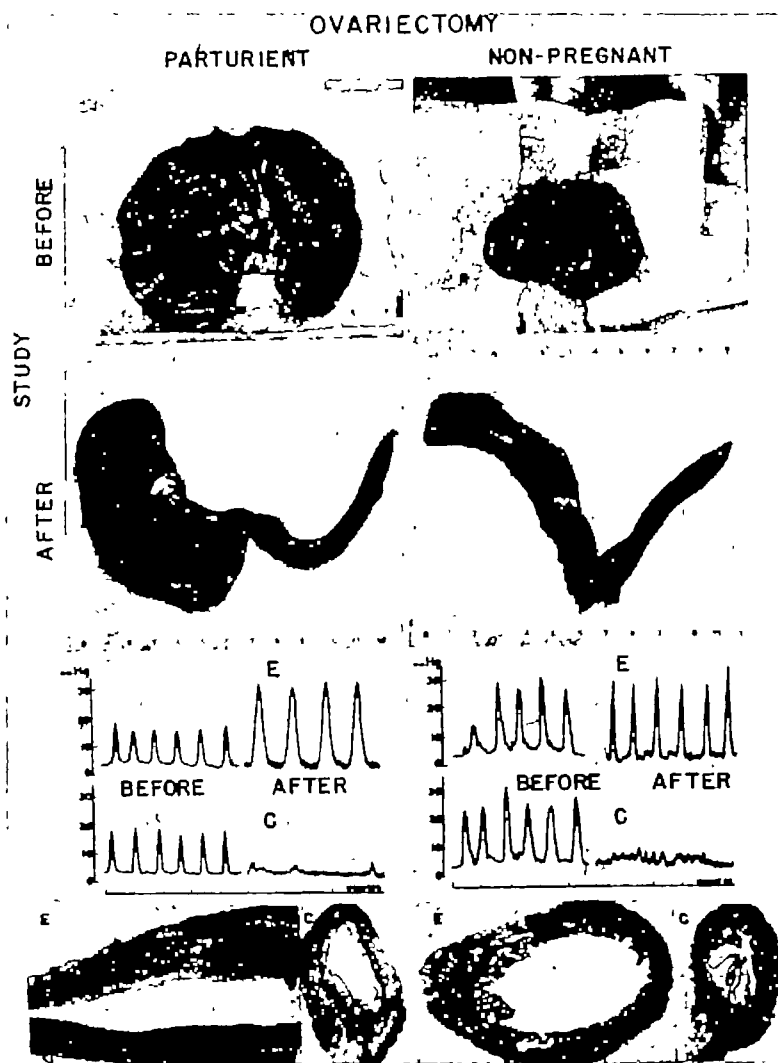


Fig. 1. Anatomical, functional, and structural differences between the two uterine horns of the same uteri induced by chronic stretch.

Fig. 1 illustrates the anatomical, structural and functional differences between the two uterine horns of the same uteri induced by chronic stretch in ovariectomized pregnant and non-pregnant rabbits. All animals, regardless of their treatment, showed this stretch-induced difference between the experimental and control horns.

Table 1 illustrates the differences between experimental and control horns, in wet and dry weights, in the weight ratios: experimental/control horns and in the RNA/DNA ratios. The experimental horn in all non-pregnant rabbits is 83–100 per cent heavier than the control horn. In the pregnant rabbits the difference is 400 per cent. However, while in the pregnant animals the difference is largely due to the involution of the control horn, in the non-pregnant animals it seems to be due to hypertrophy in the experimental horn. It would appear, therefore, that chronic stretch not only prevents involution and castrate atrophy but, like oestrogen, initiates uterine growth.

That this stretch-induced growth is not due to an increased uptake of water is shown by the fact that the wet and dry weight ratios of the experimental and control horns are almost identical. Protein and RNA determinations revealed a 7.8 per cent average protein and 6 per cent RNA/g increase in the experimental horn, as compared to the control horn. The fact (Fig. 1) that the intra-uterine pressure increased with the lapse of time during chronic stretch suggests that this protein increase in the experimental horn is partly due to an increased synthesis of the contractile protein complex, actomyosin.

Significant is the finding that the differences between experimental and control horns developed in spite of a blocking action, imposed on the uterus by progesterone treatment. Thus, stretch-induced myometrial hypertrophy is independent of uterine activity.

The results also suggest that oestrogen and chronic stretch have a synergistic action on uterine growth; that progesterone does not contribute to this process, in fact it may even decelerate it. However, these conclusions are tentative, for final proof rests on further experiments with a highly standardised colony of inbred animals.

Table 1 also presents the ratios RNA/DNA in the experimental and control horns. The fact that the RNA/DNA ratios are higher in the experimental horn is

Table 1. DIFFERENCES BETWEEN EXPERIMENTAL AND CONTROL HORNS IN WET AND DRY WEIGHTS, IN THE WEIGHT RATIOS: EXPERIMENTAL/CONTROL HORNS, AND IN THE RNA/DNA RATIOS

1. Single observation; 2–6, average of 3 animals; E, experimental horn; C, control horn; R, ratio experimental/control horn

	Wet weight (g)	Dry weight (g)	RNA/DNA ratio
(1) E	20.23	3.20	2.53
C	3.06	0.64	0.40
R	5.12	5.2	5.16
(2) E	7.33	1.31	1.68
C	3.72	0.66	0.97
R	1.97	1.98	1.73
(3) E	5.06	0.90	1.24
C	2.06	0.55	0.79
R	2.01	1.60	1.57
(4) E	8.54	1.40	1.71
C	6.85	1.05	1.61
R	1.23	1.33	1.06
(5) E	3.40	0.51	1.47
C	1.85	0.27	0.78
R	1.83	1.88	1.88
(6) E	4.18	0.71	1.86
C	2.25	0.38	0.87
R	1.84	1.86	2.12

considered as evidence that the growth of the experimental horn is due to hypertrophy rather than hyperplasia.

The significance of this study lies in the demonstration that a change in the configuration of the myometrial cell profoundly alters its biology. This relationship between cell structure and function may be operative in a variety of cells, as a common regulatory device. In uterine biology the relationship is interesting in view of the growing uterine contents during pregnancy, uterine tumours, menstrual bleeding, etc. The relationship also suggests a possible mechanism for the action of intra-uterine contraceptive devices. After all, such a device is an imposed intra-uterine resistance which may alter the normal cyclic activity of the uterus during chronic application.

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## GROWTH AND NEUTRALIZATION OF THE TRACHOMA AGENT IN MOUSE LUNGS

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SEVERAL workers have investigated the behaviour of trachoma agents in the mouse lung. Bernkopf<sup>1</sup> found that the susceptibility of mice to the lethal effects of the T'angf strain given intranasally diminished with age until they were 6 weeks old, when they were completely resistant; the Dari strain did not kill mice of any age. By contrast, Watkins and Mackenzie<sup>2</sup> killed mice more than 6 weeks old by inoculating the T'angf strain intranasally. They failed to detect any increase in infective virus during the first week after inoculation and to detect infective virus after the second passage in mouse lungs, although they had some evidence for the presence of virus specific antigens in the lungs up to the ninth passage.

I have found no difference in the susceptibility of mice 3–6 weeks old to the lethal effects of the T'angf strain administered intranasally. Furthermore, I obtained evidence that the agent multiplies in the lungs and that the mice can be protected by previous immunization. The strains used are designated according to Gear *et al.*<sup>3</sup>, and their original names, used in this report, are given in brackets. TRIC/China/Peking-2/OT (ref. 4) (T'ang);

TRIC/SAU/HAR-2/OT (ref. 5) (SA2); TRIC/USA-CAL/CAL-2/OT (ref. 6) (ASGH); TRIC/WAG/MRC-1/OT (ref. 7) (G1); TRIC/WAG/MRC-17/OT (ref. 8) (G17); variants that kill chick embryos comparatively quickly are designated\* by the letter f, as, for example, T'angf.

Virus pools were prepared from heavily infected yolk sacs from live embryos and stored at  $-80^{\circ}\text{C}$ . Mice of the TO strain were used throughout. They were anaesthetized with a freshly prepared mixture of 2 parts ether to 1 part chloroform<sup>18</sup>; when they were breathing deeply and regularly, 0.1 ml. of virus suspension was dropped on to their nostrils. For infectivity titrations, 5–8 mice were inoculated with a number of 3.16-fold dilutions and were observed for 6 days. The titre of each suspension was expressed as the reciprocal of the dilution which killed 50 per cent of the mice within 6 days ( $MLD_{50}$ ) calculated by the method of Reed and Muench<sup>14</sup>. The lungs of all dead mice were completely consolidated, and the mean day of death of the mice was inversely related to dose of virus. The titres obtained from replicate titra-

Table 1. PROTECTION OF MICE AGAINST THE LETHAL EFFECTS OF THE ASGHf AND SA2f STRAINS OF TRACHOMA BY ACTIVE IMMUNIZATION WITH ASGHf

Challenge inoculum		Dose (MLD <sub>50</sub> )*	No. of mice surviving per group		Difference in % survival (b-a)
Strain	Route		Non-immunized (a)	Immunized (b)	
ASGHf	Intranasal	3.3	0/4	3/5	80
		3.3	1/10	10/10	
	Intravenous	1.7	0/4	3/3	67
		1.7	4/8	6/6	
SA2f	Intranasal	Approx. 6.0	0/5	4/5	63
		1.6	6/10	10/10	
	Intravenous	Approx. 1.6	1/4	5/5	63
		1.1	6/10	12/20	
	Totals		0/5	2/3	77
			1/6	4/4	

\*Fifty per cent mouse lethal dose.

tions of the T'angf strain in mice aged 3, 4, 5 and 6 weeks had a standard deviation of  $\pm 8$  per cent and dose response curves did not differ greatly (Fig. 1).

To determine whether the trachoma agent multiplied in the mouse lung, large groups of 3-week-old mice were inoculated intranasally with yolk sac suspensions containing approximately 0.5 MLD<sub>50</sub> of T'angf or of G17. The titre of the inoculum was determined by simultaneous titration in mice and in chick embryos. At intervals, five mice were killed, their lungs removed aseptically, pooled and ground in 5 ml. of phosphate-buffered saline<sup>13</sup> containing 500 µg/ml. of streptomycin sulphate and titrated in the yolk sacs of 7-8-day-old chick embryos. Eggs were incubated at 35° C and candled daily. Titrations were terminated at 13 days, and titres of the lung suspensions were calculated in terms of 50 per cent egg lethal doses (ELD<sub>50</sub>). Fig. 2 shows the percentage of virus recovered from the lung plotted against time. Some mice died in each of the experiments; although the inocula varied from 0.4 to 1.1 MLD<sub>50</sub>, most mice died on the fourth day after inoculation.

Immediately after the anaesthetized mice had revived, all the virus inoculated could be recovered from their lungs. By the second day the titre had increased almost ten-fold, was greatest on the fourth day and slowly decreased thereafter.

By mixing virus suspensions with antiserum before intranasal inoculation, Bernkopf neutralized the T'angf strain with antisera against the T'angf and Dari strains of trachoma<sup>1</sup>. Using this technique, I neutralized the SA2f and G1 strains with both homologous and heterologous antisera.

Mice can be actively immunized against the toxic effects of trachoma strains administered intravenously<sup>12,16</sup>. In order to determine whether mice could be protected

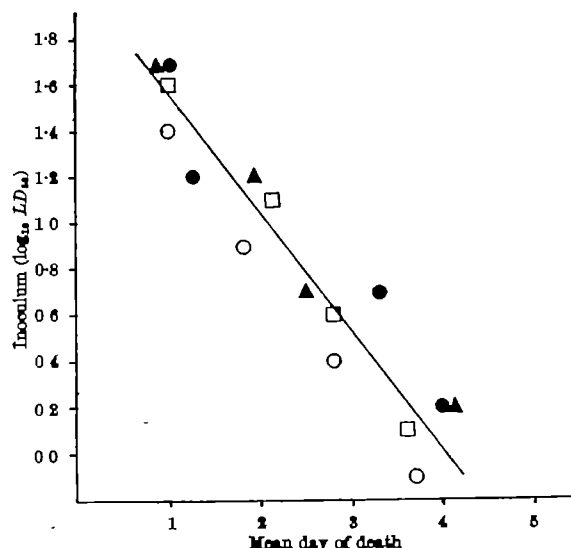


Fig. 1. Relation of time of death of mice of different ages to dose of the T'angf strain inoculated intranasally. Mice aged: 3 weeks, ●; 4 weeks, ○; 5 weeks, ▲.

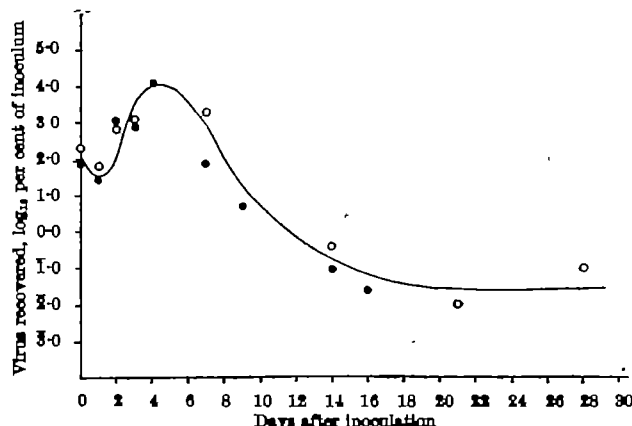


Fig. 2. Growth of two strains of trachoma in the mouse lung. Infecting dose: ●, SA2f 1.1 MLD<sub>50</sub>; ○, G17 0.4 MLD<sub>50</sub>.

from the lethal effects of these agents inoculated intranasally, the technique of intravenous immunization of Chang, Wang and Grayston<sup>15</sup> was used except that about one-quarter of the recommended dose of virus was given. Three-week-old mice received 1/8 of the amount of unpurified virus which killed 50 per cent of the mice when inoculated intravenously (MLD<sub>50</sub>), followed one week later by 1/3 MLD<sub>50</sub>. After a further 7 days, normal and immunized mice were challenged by either the intravenous or intranasal route with an inoculum expected to kill 80-100 per cent of 5-week-old mice. Table 1 shows the results of two such experiments. Mice thus immunized with ASGHf were protected against intravenous and intranasal challenge with the homologous strain, and were cross-protected against challenge with SA2f given by either route.

In contrast with previous reports<sup>1,3</sup>, I find that the trachoma agent multiplies in the adult mouse lung, in which it can be titrated with reproducible results. This positive result, however, may have been due to the different anaesthetic or to the different strain of mice used. All trachoma strains tested, whether of the fast or slow killing type for the chick embryo, multiplied in the mouse lung.

The adult mouse lung thus provides a useful system for investigating immunity to infection—as distinct from immunity to toxic death—induced by these organisms. The cross-protection observed between ASGHf and SA2f appears to differ from the findings of Bell and Theobald<sup>14</sup>. However, both these strains maintained at the Lister Institute grow in HeLa cells, whereas those used by Bell and Theobald do not. This difference in property may have been due to a change in antigenic structure during passage, as suggested by Reeve and Taverne<sup>17</sup>. Preliminary observations of the absence of cross-protection in mice induced by a slow-killing parent strain and a quick-killing variant derived from it support this idea.

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## LETTERS TO THE EDITOR

## ASTRONOMY

## Photographs of Mars taken by Mariner IV

NINETEEN close-up pictures of Mars, showing details down to a few kilometres across, were recorded by *Mariner IV* before the cameras crossed the terminator into the night hemisphere. The first seven pictures are not rich in detail because the Sun was less than 30° from zenith and shadows were absent. Later pictures, taken under a lower Sun, show progressively more relief: what may be afternoon shadows occur even on the ninth frame, composed at a solar altitude of more than 50°.

Contrasts between the shadows and the brightly lit parts of the surface of Mars show that the planet is mountainous and packed with craters and ring-structures strikingly like those of the Moon. Some of the craters have well-developed central eminences: by analogy with the central peaks of lunar craters, they may be volcanoes. Both the central eminences and the walls of the Martian craters appear to slope at steeper angles than those of the Moon. Whereas the mean inner slope of the wall of a well-developed lunar ring structure is 20° or 30°, the corresponding slope on Mars seems to be at least 40°.

Ring structures ranging up to 170 km in diameter are apparent on the eleventh frame recorded by *Mariner IV*. The largest is remarkably similar to the same-sized lunar feature Lacus Mortis, which is probably a lava-flooded, fault-bounded graben structure. The martian ring has a dark floor with crateriform or scalloped edges and may also be a lava-flooded collapse structure.

At present, it is difficult to say whether the majority of the newly discovered craters are the result of impact or volcanism. However, the *Mariner IV* pictures suggest to me that Mars has a volcanic terrain. The pictures are scored by ridge and depression lineaments that are similar to those on the Moon<sup>1</sup>, and there is strong evidence<sup>2</sup> for the growth of the lunar lineaments by volcanic extrusion from fractures and faults. Evidently, Mars has a fracture system of its own, and the indurated ridges may best be explained on the volcanic hypothesis.

The canals drawn on the older maps of Mars are not evident on those *Mariner IV* frames that might have registered them, but I have found a correlation between the trends of the recently photographed martian lineaments and the directions of the mapped canals. A possible explanation is that, as some observers<sup>3</sup> thought previously, the linear canals appear to form under certain conditions of poor seeing by the (subjective) connexion of many discrete patches of dark material arranged linearly on the surface of Mars. The patches might be widely separated ring structures, or groups of craters, arranged in linear chains, with floors of low albedo like many of the large lunar rings. These isolated features might well avoid the particular point in a 'canal' traversed by *Mariner IV*; and, even if they did appear in a picture, one would not necessarily associate them with a canal extending beyond the bounds of the frame.

On the Moon, chains of craters of a given type bear a close relation to the less readily seen Moon-wide lineament system. As, therefore, Mars seems to present volcanic phenomena like the Moon's, it is surely not surprising to find that the lineaments in small patches of Martian surface have trends similar to those of the coarser, larger-scale, canals.

Though full photometric and colorimetric calibration will be necessary before the *Mariner IV* pictures can be interpreted with confidence, it is possible to propose tentatively that the craters are not necessarily impact phenomena, and that the canals are not continuous

markings as drawn on the older maps but may be indicative of fractures or faults that dissect the solid surface of Mars.

This work was supported by a research grant from the Department of Scientific and Industrial Research. I thank the U.S. Information Service for providing the photographs of Mars.

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## Lunar Hot Spots

I suggest that the thermal anomalies found recently in infra-red lunar observations<sup>1,2</sup> could be due to lunar roughness on a centimetre scale rather than due to changes in the thermal composition of lunar rock or localized thermal sources. The idea of roughness of centimetre dimensions<sup>3</sup> has now been directly verified by radar reflexion measurements. In fact, some protagonists of the dust hypothesis now incorporate this type of roughness in their models of the lunar crust.

Infra-red measurements at 11μ of lunar surface temperatures which range from 90° K to 400° K correspond respectively to values of 14.8 and 3.3 for the parameter  $x = hv/kT$  so that throughout the lower part of the temperature range Wein's law:

$$F_1 = 2\pi hc^2 \nu^3 \exp(-hv/kT)$$

is a good approximation to the correct radiation law connecting the radiative energy density  $E_\nu$  from the lunar surface at frequency  $\nu$  with the temperature  $T$ . This result shows that lunar night-time and eclipse observations made through the 8-14μ atmospheric window are extremely sensitive to small temperature changes of the lunar surface. Because of the highly non-linear variation of  $E_\nu$  with  $T$  the brightness temperature, measured by an instrument which sees simultaneously a number of surfaces, will be considerably higher than the mean temperature especially if the surfaces have widely differing temperatures.

Using the model previously proposed<sup>3</sup> and shown in Fig. 1, I have calculated from approximate heat flow considerations the temperatures  $T_1$  and  $T_2$  at the middle of an eclipse in the case for which a smooth surface composed of the same material would give a temperature of 200° K. Values of  $T_1 = 275^\circ$  K and  $T_2 = 195^\circ$  K are obtained from the calculation and these give a brightness temperature of 240° K for telescope observations at zero lunar zenith angle. An increase in the radiative density by a factor of nearly 3 is thus expected when rough and smooth regions are compared during an eclipse. The existence of preferential roughness of centimetre dimensions should thus show up clearly in scans across the lunar disk during an eclipse. It is interesting that the eclipse measurements recently made by Shorthill and Saari<sup>1</sup> show regions of radiation enhancement both greater and less than that calculated from this simple model.

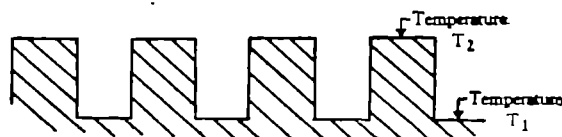


Fig. 1

In the case of hot spots which coincide with craters it is very unlikely that the spots can be attributed to local heat sources since these craters have been shown to be cooler than the surroundings during the lunar day<sup>4</sup>. The case of the crater Tycho is particularly interesting since radar reflexion results<sup>5</sup> give strong back reflexion (indicating a rough or a more dense surface) while the infra-red eclipse measurements show this crater to be a very intense hot-spot. More generally, the spots are found to be associated with maria as well as crater centres<sup>1</sup>. The present hypothesis therefore requires that these regions have preferential roughness. Such an explanation is in good agreement with visual results. It is well known that the albedo for crater centres and maria is in general lower than that for lunar highland while rougher surfaces have in general a lower albedo than smooth ones.

I thank Dr. G. Fielder of the University of London Observatory for his advice.

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## PHYSICS

### Lattice Constants of Gallium at 297° K

THE  $a$  and  $b$  lattice constants of gallium (orthorhombic,  $D_{2h}^{18}$ - $Abma$ ) are almost identical and have often been a source of confusion, as has recently been emphasized by Yaqub and Cochran<sup>1</sup>. Diffractometer data at 4.2° K (ref. 2) (gallium of 99.9999 per cent grade) indicated  $a = 4.5156$  Å,  $b = 4.4904$  Å,  $c = 7.6328$  Å, but at 297° K it appeared that  $a$  is smaller than  $b$  (in disagreement with Bradley<sup>3</sup>), and therefore that at an intermediate temperature (near 255° K)  $a$  should become equal to  $b$ .

An experimental investigation of mechanical twinning at different temperatures, involving compressive and tensile stressing, proved that twins could be produced by compression along  $a$  and tension along  $b$ <sup>4,5</sup>, whereas neither tension along  $a$  nor compression along  $b$  produced twinning, a result interpreted to imply that  $a$  is greater than  $b$  at 297° K, and bringing the 1961 conclusion into question. Furthermore, it was found that tensile stressing along  $a$  at 255° K produced no twins, whereas if  $a = b$  at this temperature, twinning would be expected<sup>4</sup> on the basis that the occurrence of twinning is associated with the elastic anisotropy ( $S_{33} > S_{11}$ ).

The 1961 conclusion for 297° K was independently brought into question by Cochran<sup>6</sup>, who noted that the expansion coefficients for  $a$  and  $b$  were in disagreement with Powell's<sup>7</sup> and could be brought into better agreement if the 4.2° K results of 1961 were accepted but if  $a$  were assumed to be greater than  $b$  at room temperature as well as at 4.2° K.

The 297° K powder diffraction data of 1961 have now been re-computed using new indices for the reflexions, corresponding to  $a > b$ , bringing the calculated value ( $\theta_{204} - \theta_{111}$ ) much closer to the observed value of this significant angle. The new indexing leads to least squares solutions (either with various extrapolation factors or without corrections) that invariably give better overall agreement between calculated and observed  $\theta$  values than the 1961 indexing. The Vogel-Kempter and the Nelson-Riley extrapolation functions both yielded the following results:

	$a$ (Å)	$b$ (Å)	$c$ (Å)
Re-computed values	$4.5258 \pm 0.0007$	$4.5186 \pm 0.0007$	$7.6570 \pm 0.0012$
Bradley (ref. 3)	4.5258	4.5196	7.6602

The agreement with Bradley's values (converted by the factor 1.00202) is much improved. The results now do not conflict with the anisotropic expansion coefficients of Powell<sup>7</sup>, assuming that the  $\theta$ -values of 1961 at 4.2° K are correctly indexed, which we have no reason to question. We therefore conclude that the 1961 values at 297° K should be replaced by the foregoing, that the interpolated values of 1961 for 78° K should be discarded, that  $a > b$  throughout the temperature range, and that the anisotropic thermal expansion is such that  $\Delta b/b > \Delta a/a$ .

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## GEOPHYSICS

### Linear Relationship between Energy and Pressure of Volcanic Explosions

EARLY investigations on the velocity of bombs and the gas pressure of single volcanic explosions by Matuzawa<sup>1-3</sup> led to a means of determining gas pressures which have been generally accepted and used by volcanologists. Matuzawa stated<sup>1</sup> that the initial discharge velocity of volcanic explosions depended primarily on the gas pressure, which could be calculated from the dynamic pressure relationship of Bernoulli's equation:

$$P = \frac{1}{2} \rho v^2$$

where the gas pressure,  $P$ , represents the pressure difference between the interior and exterior of the vent,  $\rho$  is the density of the mass of fragments plugging the vent, and  $v$  is the discharge velocity of volcanic bombs.

Results of investigations by Minakami<sup>4</sup> led to the determination of the amount of kinetic energy necessary to impart the initial velocity to volcanic ejecta (bombs):

$$E = \frac{1}{2} m v^2$$

where  $E$  is the kinetic energy,  $m$  is the mass of ejecta, and  $v$  is the velocity of ejecta at the instant of ejection.

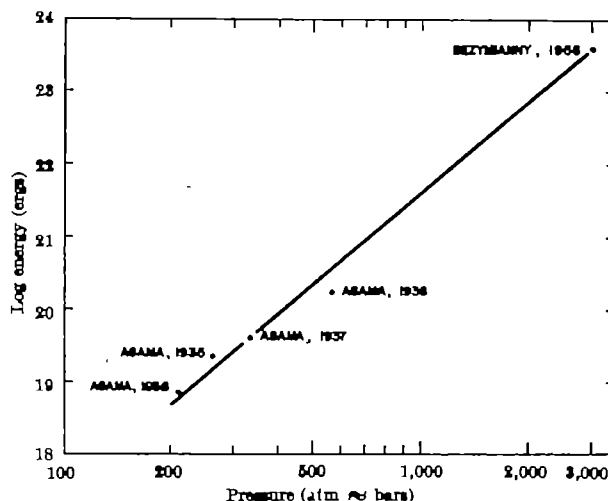


Fig. 1. Relation of the logarithm of kinetic energy of volcanic explosions to the logarithm of the gas pressure at the time of the explosion.

Minakami calculated the energies and pressures for a number of eruptions of Asama Volcano in Japan<sup>4</sup>.

On March 30, 1956, a paroxysmal explosion of Bezymianny Volcano, Kamchatka, occurred. Gorshkov<sup>5</sup> determined the energy of this eruption from the seismic energy connected with the explosion, from the air wave of the blast, and from the equation here, each of which gave approximately the same value. Gas pressure at the time of the explosion was computed from Bernoulli's equation. Values were appreciably higher than for the Asama eruptions and it appeared that a relationship between energy and pressure might be found based on the Japanese and Russian data.

Values published by Minakami and Gorshkov are plotted in Fig. 1, in which is shown an eye-fitted linear relationship between the logarithm of kinetic energy and the logarithm of gas pressure. The equation of the relationship is:

$$E = 10^6 P^{1.2}$$

where energy is in ergs and pressure is in atmospheres or in bars.

Data used in Fig. 1 are few in number, and it would be very desirable for volcanologists to augment them to learn if the given relationship is valid throughout the range of pressures and energies associated with volcanic eruptions. While there are a number of reports on the energies of explosions, for example refs. 6 and 7, and the energy may be determined from barograms<sup>8</sup>, the necessary observations needed to calculate pressures are lacking. It is hoped that this deficiency will soon be rectified.

This work was carried out in 1961 while I held a National Academy of Sciences—National Research Council postdoctoral resident research associateship at the U.S. Navy Electronics Laboratory in San Diego, California.

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## GEOLOGY

### Origin of Australites

THE problem of the origin of tektites, of australites in particular, has been a subject of speculation and controversy for many years. A great body of physical and chemical data on tektites is available and has been used to support various genetic arguments.

According to their present-day terrestrial manner of occurrence, tektites are geological material. Consequently, all their geological properties must be investigated. Physical and chemical data are not sufficient.

During my stay in Australia, I have been fortunate enough to examine the principal public and private australite collections. My investigation was undertaken in order to check the lithological homogeneity of australites, with particular reference to the presence of inclusions of material 'foreign' to them. The number of specimens which I examined totals more than 33,000 (see Table 1). The specimens were initially investigated by means of a 10-power magnifying glass. Any specimens suspected of containing 'foreign' material were put aside and eventually borrowed to be submitted to microscopic investigation. Thin sections were prepared and investigated of several specimens, but 'fingers' were the only internal feature observed.

All the specimens available were checked. Thus, the material investigated consisted of fresh well-preserved

Table 1. SOURCES AND NUMBERS OF LITHOLOGICALLY INVESTIGATED AUSTRALITE SPECIMENS

Source of material	No. of samples investigated
Australian Museum, Sydney	308
South Australian Museum, Adelaide	19,308
National Museum of Victoria, Melbourne	1,621
Western Australian Museum, Perth	1,696
University of Adelaide, Department of Geology and Mineralogy	1,812
University of Melbourne, Department of Geology and Mineralogy	623
University of Queensland, Department of Geology and Mineralogy	248
School of Mines of Western Australia, Kalgoorlie	196
Dr. George Baker, Melbourne, Vic., private collection	2,958
Mr. W. H. Cleverly, Kalgoorlie, W.A., private collection	241
Mr. Dale Tillotson, Kalgoorlie, W.A., private collection	4,227
Mr. C. B. O. Jones and Mr. John Jones, Hampton Hill Station, Kalgoorlie, W.A., private collection	70
Other sources (including Geological Survey, Hobart; Museum, Hobart; University of Western Australia; Queensland Museum)	197
Total	33,696

The australite collection in the South Australian Museum is the largest public collection in existence. Mr. Tillotson's collection, in June, 1965, totalled about 5,300 specimens. The two collections could not be examined in toto, because many samples were being investigated by research workers in the United States. The Jones collection, derived mainly from the Hampton Hill Station area, according to a conservative estimate of the owners, totals about 10,500 specimens but may amount to 11,000 specimens. The 70 specimens investigated were selected at random.

specimens and water- and wind-worn corroded and abraded specimens, whole specimens and fragments, and large and small australites.

During the course of the investigation I was unable to find any material foreign to australites even in a single specimen. Lithologically, the material was exceedingly homogeneous, even to the extent of being monotonous. I was particularly keen on trying to find inclusions of terrestrial sedimentary and igneous rocks, such as shale, sandstone and granite which, because of similarities in chemical composition, are believed by many to be the parent material of tektites; but all my efforts were in vain. Moreover, the museum curators and the owners of private collections assured me that they had never observed any 'foreign' material in australites. Consequently, adding to the 33,700-odd australites which I personally investigated another 1,000 specimens from the Tillotson collection (see Table 1) and the 10,500-odd specimens in the Jones collection (see Table 1), the conclusion follows that no 'foreign' material whatsoever has been observed in a total of about 45,200 australites. This sample certainly represents the majority of australites so far discovered, and the statistical probability of finding inclusions of terrestrial material in any other australite is extremely small. Finally, Chao<sup>9</sup> examined about 2,000 australites under the binocular microscope; his report does not mention the presence of 'foreign' matter in this material.

If the australites originated by an asteroid impact, a giant meteorite impact, or a comet impact on the Earth, as Urey<sup>3</sup>, Barnes<sup>1</sup> and Cohen<sup>4</sup>, among others, have suggested, then they, like genuine impactites<sup>10</sup>, would be heterogeneous. I find it impossible to believe, on the basis of my lithological observations, that australites are terrestrial impactites. Their lithological homogeneity rules out terrestrial origin. The impact producing such a homogeneous material would certainly have been so violent and of such dimensions that several generations of trained geologists could not possibly have failed to observe other effects caused by such a tremendous catastrophe.

If australites formed by an impact on the Moon, their lithological properties suggest that the part of the Moon's surface in which they originated must have been very homogeneous indeed.

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## Upper Cretaceous Fossil Molluscs In South America and West Africa

OVER the past few years there has been a great revival of interest in the theory of continental drift, owing largely to the results yielded by palaeomagnetic investigations. Whereas most geologists a decade ago were inclined to be sceptical about the possibility of the continental land masses having wandered, the evidence now available appears much more convincing.

The close relationships in the floras and in the reptile associations of the Gondwanic continents have long been one of the mainstays of the hypothesis of the existence of Gondwanaland. It seems, however, that little attention has been paid to eventual biological similarities of a later age. The object of this communication is to present observations on some mollusc species that occur in the Upper Cretaceous of the northern half of South America and in Nigeria and Cameroon. Knowledge of (particularly South American) Cretaceous molluscs is still not very advanced and it might reasonably be expected that more common species will show up in the future, for example, when the vasocoeratiid associations of Brazil are investigated.

Table 1 lists the species known to occur in both regions (see also ref. 1).

Table 1. OCCURRENCE OF WEST AFRICAN MOLLUSCS IN SOUTH AMERICA

Species	Age	Occurrence
<i>Pachyaecoceras costatum</i> Reymont (ammonite)	Turonian	Peru, Eastern and Northern Nigeria
<i>Hoplitoides ingens</i> (von Koenen) (ammonite)	Turonian	Colombia, Cameroon, Northern Nigeria, Eastern Nigeria
<i>Benuosites benuosensis</i> Reymont (ammonite)	Turonian	Brazil, Colombia, Northern Nigeria
<i>Benuosites spinosus</i> Reymont (ammonite)	Turonian	Trinidad, Peru, Colombia, Northern Nigeria
<i>Solgerites brancoi</i> (Solger) (ammonite)	Coniacian	Cameroon, Colombia, Peru
<i>Lima pseudohornesi</i> Riedel (pelecypod)	Turonian	Peru, Cameroun, Gabon, Northern Nigeria

The interest in connexion with the geographical distribution of these molluscs lies in the problem of their dispersal which, for the ammonites, has to be viewed in the light of their neoplanktonic buoyancy properties (cf. Reymont<sup>2</sup>), and consequently with respect to oceanic currents.

*Pachyaecoceras costatum* Reymont has a moderately inflated and slightly evolute shell, approximating in form to *Nautilus*. There is little doubt that it, like the living *Nautilus*, could have been posthumously transported by ocean currents over considerable distances.

*Hoplitoides ingens* (von Koenen) has an involute, fairly strongly compressed shell of a shape bordering on that of the type considered by me<sup>3</sup> to have been unable to remain buoyant after the death of the animal, unless the body chamber has been largely broken off. Both the species of *Benuosites* have small involute to slightly evolute and fairly compressed shells with thin walls, and may not have been capable of wide neoplanktonic dispersal.

*Solgerites brancoi* Solger has a round whorl section and is slightly evolute and had certainly good neoplanktonic floating properties. It should finally be mentioned that all these ammonites are "typical West African forms" and, apart from a doubtful report of *H. ingens* from North Africa, seem only to have been found in the northern part of South America, outside West Africa.

To sum up, it seems that only two of the five ammonite species might have survived dispersal across an Atlantic Ocean of the width of the present day, assuming favourable currents existed. The case of *Lima pseudohornesi* Riedel is even more difficult to explain, for if the mechanics of the dispersal of the ammonite shells poses problems, that of a benthonic pelecypod species is very much greater. We have the fact that most of the known occurrences of these species are on 'the wrong side' of South America, but there may have been a shallow sea in existence in northern South America at that time.

Although it is far too early to make anything even approaching a positive statement on the subject, and although this will not be possible until much more published taxonomic information becomes available from South America, it seems that these molluscan distributions might offer some support for the palaeomagnetic results obtained by Creer<sup>4</sup>, Runcorn<sup>5</sup> and others. Their work suggests that Brazil and Nigeria of to-day mark the point of contact of the continents in the Upper Palaeozoic (and thus supports the finding made by fitting coastlines and Pre-Cambrian tectonics<sup>6</sup> and that the rift took place during Upper Permian<sup>7</sup> and widened during the Mesozoic<sup>8</sup>.

Although there is marine Devonian known in Ghana, the first dated marine transgression for the critical central area is the Upper Middle Albian incursion in Nigeria. Older Cretaceous occurs some distance north and south of Nigeria, but there is no marine Jurassic. This might be interpreted as evidence that the two continents were still very close together during early Cretaceous time. The existence of a narrow sea during the Upper Cretaceous, similar to the Red Sea of to-day, would go a long way towards explaining the distribution of the Upper Cretaceous molluscs here discussed.

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## CHEMISTRY

### Octahedral Metal Clusters

THE bonding in the  $\text{Mo}_6\text{Cl}_8^{4+}$  and  $\text{Ta}_6\text{Cl}_8^{3+}$  cations, both of which contain an octahedron of metal atoms, has recently been rationalized in terms of a 40-electron model<sup>1</sup>. The edges of the metal octahedron define the orientation of twelve bonds (type A) while the faces of the octahedron define the position of another eight (type B). In  $\text{Mo}_6\text{Cl}_8^{4+}$  twenty-four metal electrons fill the A orbitals and sixteen chlorine  $\sigma$  electrons fill type B. In  $\text{Ta}_6\text{Cl}_8^{3+}$  the roles of the sixteen metal and twenty-four chlorine  $\sigma$  electrons are interchanged. It is now possible to speculate on the occurrence of other metal octahedra if we require that this 40-electron rule be obeyed.

There are two other possible atomic arrangements with  $O_h$  symmetry. These are: (a) that in which there are ligands above both faces and edges of the metal octahedron; (b) that in which there are no ligands at all. We consider these separately. A possible molecule of type (a) is  $(\text{Al}_6\text{Cl}_{18})^{3-}$  in which both A and B bonding orbitals are filled by chlorine  $\sigma$  electrons. There should be no specific metal-metal bonding and a consideration of ionic radii makes it evident that there would be considerable steric interaction between the chlorine ions. It therefore seems most unlikely that such molecules exist.

If orbitals of types A and B are filled by metal electrons then no ligand need be incorporated (type (b)). There would be no de-stabilizing steric interactions, and if the charge on the octahedron were small it seems highly probable that the species would be stable. The molecule  $\text{Re}_6\text{I}_8$  (actually  $\text{Re}_6^{3+}2\text{I}^-$ ) satisfies these conditions. The iodide anion is suggested since it is large and singly charged; there are many plausible alternatives. Although no such species has been found in the manganese- or rhenium-iodine systems, it is possible that reactions in which the metal appears to have been produced (for example, thermal decomposition of  $\text{Mn}(\text{CO})_5\text{I}$ ) may, in fact, have led to their formation. The 'isoelectronic'



species  $Ru_3I_8$ ,  $Os_3I_8$ ,  $Rh_3I_8$ , and  $Ir_3I_8$  are also potentially examples. The rhodium chloride ' $RhCl_3$ ' reported by Wöhler and Müller<sup>2</sup> might contain a metal octahedron. The chlorine analyses obtained (44.6; 44.0 per cent) agree much more closely with that required for  $Rh_3Cl_{14}$  (44.6 per cent) than with that required for  $RhCl_3$  (40.8 per cent). It should be noted that this formulation gives the cation an improbably large charge, although this could perhaps be offset by a weak co-ordination of the chloride anions.

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## Existence of Half-salt Cations in Aqueous Solutions of Aminophenols

INTEREST in the properties of aminophenols as bifunctional organic substrates in aromatic substitution prompted us to re-investigate the acid-base dissociation constants of these compounds. The method chosen was the measurement of pH in acid-base titrations of sample solutions under a nitrogen atmosphere.

Aminophenols were shown to be ampholytes with no zwitterion structure<sup>1</sup>, and therefore the following two dissociation stages can be distinguished *a priori* in their aqueous solutions:



where  $B$  represents a neutral aminophenol molecule,  $B^+$  the ammonium cation and  $B^-$  the phenolate anion. (It is customary to represent equilibrium (1) inverted; however, all the equilibria are shown and calculated as acid dissociations of the species on the left side, in order to preserve uniformity.) Dissociations (1) and (2) can be measured by titration of neutral aminophenol solutions with hydrochloric acid and sodium hydroxide solutions respectively.

The titrations with sodium hydroxide behaved normally in the sense that the experimental curve of pH versus added volume of reagent solution corresponded to the curves calculated by assuming a certain  $pK_1$  value, as shown in Fig. 1.

Such was not the case when the organic substrate was titrated with acid solution. Fig. 2 shows an experimental titration curve of *p*-aminophenol with 0.1 N hydrochloric acid, which is compared with curves calculated for three different values of  $pK_1$ .

The location of the stoichiometric equivalence point in Fig. 2 is noteworthy, as it lies about one-third in excess of the volume necessary to attain the inflexion point. The

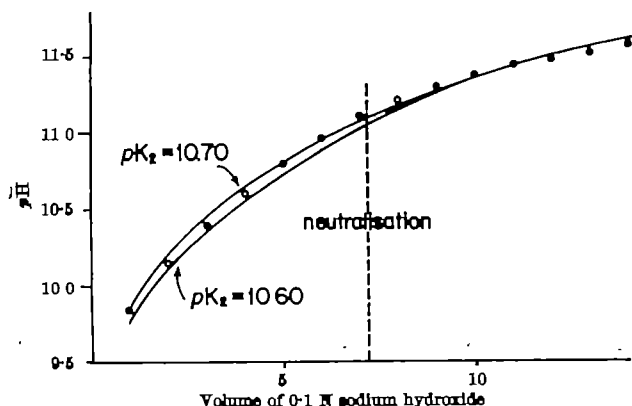


Fig. 1. Titration of 50 ml. of 0.01443 M aqueous *p*-aminophenol with 0.1 N sodium hydroxide, at 40° C (—, calculated; O, experimental)

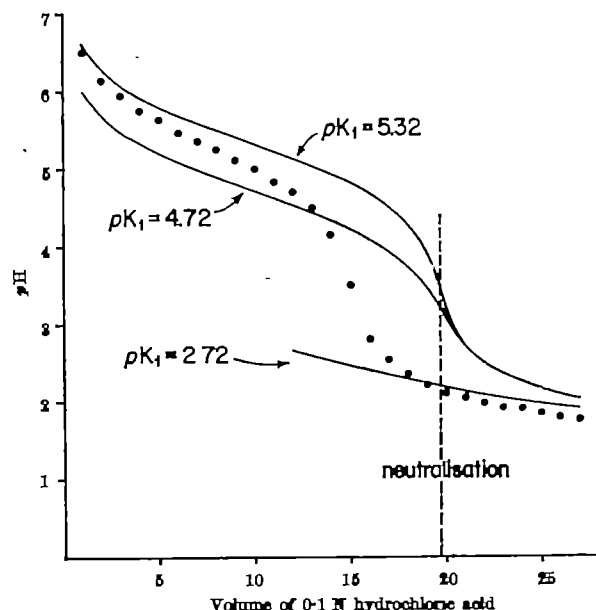


Fig. 2. Titration of 50 ml. of 0.03939 M aqueous *p*-aminophenol with 0.1 N hydrochloric acid, at 40° C (—, calculated; O, experimental)

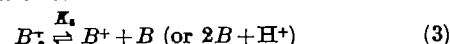
same occurs in the cases of *o*- and *m*-aminophenol, but to a lesser degree. This abnormality is also evident in the very great discrepancy between the values of columns V and VI in Table 1. If the only important equilibrium in acidic solutions is equilibrium (1), columns V and VI should be equal, as they represent  $pK_1$  values calculated at two stages of neutralization during titrations with hydrochloric acid.

Table 1. EQUILIBRIUM CONSTANTS FOR THE DISSOCIATION OF *p*-AMINOPHENOL ( $B$ ) IN AQUEOUS SOLUTION AT 30° (TITRATION WITH HYDROCHLORIC ACID)

$10^4 [B]$	I	II	III	IV	V	VI
0.733	4.875	3.43	4.62	10.77	4.88	3.34
1.072	5.006	3.306	4.71	10.32	5.00	3.55
1.445	4.97	3.23	4.71	10.41	4.97	3.37
2.144	4.97	3.15	4.78	10.32	4.97	3.32
2.961	4.875	2.975	4.46	10.78	4.88	3.03
4.258	5.04	2.94	4.95	10.32	5.04	3.14
5.733	4.97	2.705	5.07	10.68	4.97	2.91
8.577	5.11	2.52	5.05	10.31	5.11	2.73
11.564	5.25	2.38	5.19	11.01	5.25	2.55
17.154	5.006	2.24	4.93	10.42	5.00	2.48
23.123	5.006	2.10	4.82	10.58	5.00	2.32
33.238	5.04	1.96	4.97	11.06	5.04	2.06

Explanation of the columns: (I) pH at half stoichiometric neutralization; (II) pH at stoichiometric neutralization; (III)  $pK_1$ , assuming equilibria (1) and (2); (IV)  $pK_1$ ; (V)  $pK_1$ , calculated for the points in column I assuming equilibrium (1) alone; (VI)  $pK_1$ , calculated for the points in column II assuming equilibrium (1) alone.

In order to account for the observed irregularities an equilibrium (3) is postulated, in which a complex  $B_1^+$  is formed by association of a cation  $B^+$  with one neutral molecule  $B$  as follows:



Qualitatively such type of equilibrium explains the observed "excess of protons" during the titration—in Fig. 2 the excess is shown by the experimental pH being lower than those of the calculated curves—because we have in fact less free base  $B$  than we should have according to equilibrium (1) alone.

Indeed, from the pH values found in experiments in which the initial concentration of *p*-aminophenol was varied over a forty-fold range (columns I and II of Table 1),  $pK_1$  and  $pK_2$  values were calculated. The results are shown in columns III and IV of Table 1, and give an average of  $4.86 \pm 0.20$  for  $pK_1$  and  $10.60 \pm 0.25$  for  $pK_2$ .

These 'manual' calculations could not be successfully applied to the other bases studied, as the equations become unacceptable owing to the lower deviations from an ordinary titration observed in these cases; therefore more

sophisticated numerical methods involving electronic computers would be required.

It is interesting to notice that the  $pK_1$ 's in column III and column V of Table 1 do not differ very much, which suggests that equilibrium (3) becomes extremely important for the stoichiometric balance of the system mainly towards the final stages of the titration, where the largest deviations occur. We believe that other investigators failed to notice these association phenomena for several reasons: determinations of  $pK_1$  were made in mixtures where association passes unnoticed, for example, using equimolecular mixtures of the free base and the hydrochloride<sup>3</sup>, whereas we have already seen (columns III and V of Table 1) that the deviations are not large; or using low pH buffer solutions<sup>4</sup>, where the associated cation  $B^+$  probably undergoes nearly complete dissociation into two ammonium cations  $B^+$ ; another possible reason is the use of ultra-violet spectroscopy<sup>3,4</sup> which might be inadequate for the observation of the associated species. It is possible, therefore, that these types of associated ions in solution were overlooked in many other cases.

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### Identification of Disulphur Monoxide as an Absorbing Species in Carbonyl Sulphide Discharge Products

In a recent article<sup>1</sup> an absorption spectrum in the 2650–3250 Å region, obtained from the products of an electrical discharge through carbonyl sulphide vapour, was described. The carrier of this spectrum was tentatively identified as  $C_2S_2O$ , an analogue of carbon suboxide. This same absorption spectrum was also observed after the flash photolysis of carbonyl sulphide.

It would appear from chemical arguments that  $C_2S_2O$  would not be a likely product of the near-ultra-violet photolysis of COS, since photolysis would yield CO and some CS, both of which are quite resistant to further photolysis. Unless free carbon is formed, it is difficult to find a reasonable mechanism for the formation of 3-carbon chains during flash photolysis. Since free carbon was detected<sup>1</sup> during the electrical discharge through COS, a 3-carbon chain could form under these conditions, although high-energy discharges seem to favour the production of the simpler and more stable molecules<sup>2,3</sup>.

We have now convincingly identified this spectrum as belonging to disulphur monoxide, first prepared by Schenk in 1933 (ref. 4) but identified by him as  $SO$ . Schenk's compound was later ascribed to  $S_2$  by Cordes<sup>5</sup> and to  $S_2O_2$  by Jones<sup>6</sup>. Recently, mass spectrometry<sup>7</sup> and microwave spectroscopy<sup>8</sup> have definitely identified Schenk's compound as the bent molecule,  $SSO$ . The band heads and rough intensities reported by Cordes<sup>5</sup> are in good agreement with the COS discharge product spectrum, as are the long wave-length band heads at 3193 Å and 3234 Å observed by Jones<sup>6</sup>.

Authentic  $S_2O_2$  was prepared by the method of Jones<sup>6</sup>, and a 750-joule discharge through 10-torr COS was used to produce the discharge product. A 180-cm path-length through the gas at about 1 torr was used in obtaining the absorption spectra. High resolution spectra of both  $S_2O_2$  and the COS discharge product were obtained in the

3190–3296 Å region, and several  $S_2O_2$  bands were identified in both samples. Direct comparison of the fine structure in the 3234 Å band shows that the COS discharge product is  $S_2O_2$ . Several shorter wave-length bands, most notably the strong 2907 Å band, were seen in the discharge product, but these were masked by the excess  $SO_2$  absorption when the  $S_2O_2$  sample was used. The products of a Tesla coil discharge in flowing COS also gave the same  $S_2O_2$  absorption spectrum. Since  $S_2O_2$  is known to be a very strong absorber<sup>9</sup>, it may be only a minor product of the discharge.

The mass spectrum of the COS discharge products contains a small mass 80 peak corresponding to  $S_2O_2$ , but no peak at mass 84 which would correspond to  $C_2S_2O$ . The mass 80 peak was mainly  $S_2O_2$ , not  $SO_2$ , since the mass 82 peak due to  $^{34}S$  was about 8.2 per cent of the mass 80 peak, or nearly twice the 4.2 per cent natural abundance of  $^{34}S$ . Mass spectrometry of the  $S_2O_2$  sample gave the same mass 80 and 82 peaks.

No investigation was made of the lifetime of the discharge product, but the mass 80 peak was still present the day after the preparation of the discharge product.

The  $S_2O_2$  absorption spectrum was seen by Wright<sup>10</sup> during the flash photolysis of  $CS_2$  in the presence of  $O_2$  or  $SO_2$ . It is thus not unreasonable to expect some  $S_2O_2$  during the flash photolysis or electrical discharge of COS.

I thank B. J. Killoran for his assistance. Dr. E. D. Loughran did the mass spectrometry. I also thank Drs. N. R. Greiner and P. E. Rouse, jun., for their advice.

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### Oxidase-like Activity of the Copper (II) Poly-L-histidine Complex

SYNTHETIC polyamino-acids have been shown to be instructive protein models<sup>1</sup>. It seems reasonable to assume that their combination with metal ions exhibits some resemblance to metalloproteins. This possibility was investigated by examining the catalytic activity of complexes of copper with polyamino-acids.

Copper-ion-catalysed oxidations of ascorbic acid and quinols by molecular oxygen have been previously suggested as models for copper containing oxidases<sup>2</sup>. This catalytic activity of copper ions is substantially dependent on the nature of the ligands<sup>3,4</sup>. Certain ligands were found to induce an increase in the catalytic activity of copper. On the other hand, certain polydentate ligands like ethylenediamine and ethylenediamine tetraacetic acid were found to act as inhibitors. A similar effect is exhibited by the non-specific binding of  $Cu^{2+}$  by protein molecules such as bovine serum albumin<sup>5</sup>.

The oxidation of ascorbic acid and *p*-hydroquinone by molecular oxygen, catalysed by different complexes of copper (II) with amino- and polyamino-acids, has been investigated. It was shown (Table 1) that histidylhistidine and polyhistidine are the only complexing agents which markedly increase the catalytic efficiency of the copper induced ascorbic acid oxidation. The other amino- and polyamino-acids examined were found to have no positive effect on the activity of copper under the same conditions. Moreover, an inhibitory effect of the complexants was

Table 1. EFFECT OF LIGANDS ON THE CUPRIC-ION-CATALYZED AUTO-OXIDATION OF ASCORBIC ACID

Complexing agent	moles $\text{L}^{-1} \times 10^{-3}$	Reaction rate moles $\text{L}^{-1} \text{min}^{-1} \times 10^4$
None	—	0.24
Imidazole	6–12	0.25
Imidazole	30	0.20
Histidine	6	0.25
Histidine	60	0.20
Glutamine	6	0.24
Glutamine	60	0.17
Methionine	6–60	0.24
Histidylhistidine	2–5	0.40
Polyhistidine*	0.06	1.06
Polylysine†	0.006–0.060	0.34
Polyarginine‡	0.02	0.24
Co-polylysine-tyrosine	110 $\mu\text{g/ml}$	0.25

The reaction mixture contained  $6 \times 10^{-4}$  M  $\text{CuSO}_4$ ,  $1.5 \times 10^{-4}$  M ascorbic acid and the complexing agent at the specified concentration in 0.02 M sodium acetate buffer pH 4.0. The reaction mixture was saturated with pure oxygen before adding the substrate solution. Initial rates of ascorbate oxidation at room temperature were followed spectrophotometrically at 265 m $\mu$  on a Zeiss PMQ-II spectrophotometer. All polyamino-acids were supplied by YEDA Research and Development Co., Rehovoth, Israel.

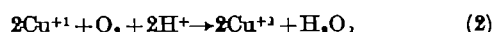
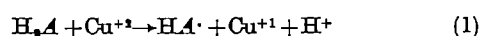
\* molecular weight 10,000  
† " " 75,000  
‡ " " 15,400

encountered in some cases. The kinetic pattern of the copper-polyhistidine catalyzed reaction is substantially different from that of the aquo and acetato complexes. A typical Michaelis-Menten kinetic dependence is demonstrated by the polyhistidine complex with an apparent  $K_m = 1.5 \times 10^{-4}$  M, whereas the kinetics of the aquo-complexes-induced oxidation is first order in ascorbic acid within the range  $10^{-4}$ – $10^{-5}$  M. The value of  $K_m$  is of the same order of magnitude as that obtained for the ceruloplasmin catalyzed reaction<sup>4</sup>. A remarkable difference in the pH dependence between the copper-polyhistidine- and the aquo-complex-catalyzed auto-oxidation was also observed. The ratio of the rates of the two catalytic systems reaches a maximum at pH 4.1, whereas at higher or lower pH values this ratio decreases, reaching a value of unity at pH = 3.3 and 5.8.

At a constant polyhistidine concentration the catalytic efficiency of the copper-polyhistidine complex increases with the amount of copper added to the system, reaching a maximum value corresponding to one copper ion per two histidyl residues.  $\text{Fe}^{3+}$ ,  $\text{Co}^{2+}$  and  $\text{Mn}^{2+}$  ions were also examined in the presence of the aforementioned amino- and polyamino-acids and were found to be without catalytic effect on ascorbic acid oxidation.

The copper-polyhistidine catalyzed oxidation of *p*-hydroquinone exhibits even more conspicuously the unique behaviour of this macromolecular copper complex. This complex induces a rapid oxidation of the hydroquinone within the pH range 3.5–5.5. At pH 4.5,  $[\text{Cu}^{2+}] = 5 \times 10^{-4}$  and  $[\text{polyhistidine}] = 1 \times 10^{-3}$  M the rate of hydroquinone oxidation was  $8 \times 10^{-7}$  moles/l./min. The catalytic activity of the aquo- or acetato-complexes of cupric ions as well as any of the other complexes listed in Table 1 is smaller by more than two orders of magnitude. Under identical experimental conditions catechol was not susceptible to oxidation by either copper (II) or its polyhistidine complex. The kinetics of *p*-hydroquinone oxidation were followed spectrophotometrically at 240 m $\mu$  as well as manometrically by recording the rate of oxygen consumption. At low hydroquinone concentrations, the kinetic behaviour was found to be similar to that of ascorbic acid oxidation. The apparent Michaelis-Menten constant for this reaction was found to be  $3.5 \times 10^{-3}$  M.

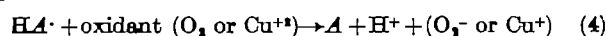
The generally accepted mechanism<sup>5,6</sup> for the cupric-ion-catalyzed auto-oxidation proceeds schematically through the following steps:



followed by



or



Where  $\text{H}_2\text{A}$  stands for the reductant and  $\text{HA}^\cdot$  is the free radical formed by single electron oxidation. In the copper-polyhistidine-catalyzed reaction the only oxidation products of ascorbic acid and *p*-hydroquinone were shown to be dehydroascorbic acid and *p*-benzoquinone respectively. Hydrogen peroxide was the product of the reduction of oxygen and its yield corresponds to the amount of the absorbed oxygen and to that of the oxidized substrate. This suggests that the reaction catalyzed by the copper-polyhistidine complex follows a similar chemical route to that by the cupric ion.

The interpretation of the efficiency of the copper polyhistidine system deserves a thorough investigation. At the present stage we can only make a few comments on the observed phenomena. The binding of copper ions by the imidazole groups of the polyhistidine increases the redox potential of the  $\text{Cu}^{\text{I}}-\text{Cu}^{\text{II}}$  couple by the stabilization of monovalent copper<sup>7</sup>. This alone cannot account for the observed facts since imidazole, histidine and even histidylhistidine do not exhibit catalytic effect to the same degree. The Michaelis-Menten kinetic behaviour observed in the  $\text{Cu}(\text{II})$ -polyhistidine-catalyzed oxidation indicates the formation of a complex between the catalyst and the substrate. The factors favouring the formation of such a complex are likely to be of electrostatic nature, and since the  $\text{Cu}(\text{II})$  polyhistidine complex is positively charged due to the protonated imidazole nitrogens, it should be pH-dependent. Binding of the substrate to the positively charged macromolecule by an electrostatic interaction results in the formation of  $\text{HA}^\cdot$  bound to the  $\text{Cu}^{\text{II}}$ -polyhistidine complex. As  $\text{HA}^\cdot$  is more readily oxidized than  $\text{H}_2\text{A}^\cdot$ , this could possibly account for the enhanced catalytic activity of the polyhistidine complex.

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### Dissolved Silicate and Particulate Iron Content in Different Water Types

CONSIDERABLE information regarding the complex circulation pattern which exists in the waters surrounding the Hawaiian island chain has been obtained through the analysis of surface waters at a station near Koko Head on the Island of Oahu which has been monitored by the U.S. Bureau of Commercial Fisheries since 1956. Temperature and salinity data, together with information gathered in six oceanographic surveys made since 1949 in the vicinity of the high islands of the Hawaiian archipelago, have shown that the islands are surrounded by the cooler and more saline water of the Western North Pacific in winter and by the warmer and intermediate salinity water of the California Current Extension in the summer<sup>1</sup>. The boundary between these two layers of water as shown by a relatively high salinity gradient moves north and south through the island chain and is responsible for the seasonal variations in water type. The Hawaiian waters, being subtropical in nature, are not normally subject to appreciable

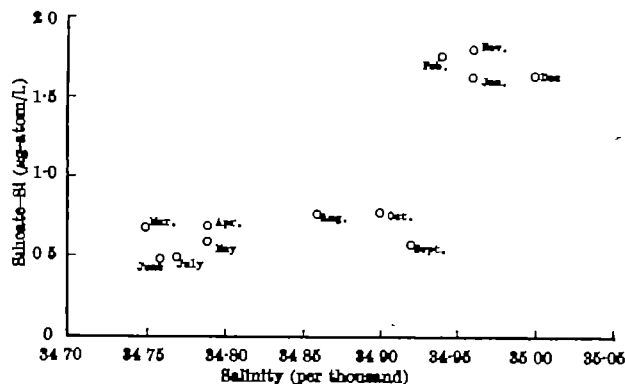


Fig. 1. Silicate-salinity relationship, March 1961-February 1962, at the Koko Head monitoring station, Oahu, Hawaii

changes in standing crops of phytoplankton. Consequently, it seemed of interest to investigate the possibility that variations in trace metals and other chemical species might reflect changes in water type.

Analysis of dissolved silicate and particulate iron was carried out on a weekly basis on surface water samples collected at the Koko Head station from March 1961 through April 1962. The monthly mean concentration of silicate-Si ranged from 0.49 to 1.81  $\mu\text{g-atom/l.}$  and that of particulate iron from 0.50 to 3.44  $\mu\text{g-atom/l.}$

Although seasonal variations of both silicate and particulate iron were discernible, it was not possible to relate these to known local biological activity in the waters. A plot, however, of the monthly mean values of silicate versus the corresponding monthly salinities reveals three distinct distribution groups (Fig. 1). With the studies of Seckel and others<sup>1</sup> on the surface water types of the Hawaiian islands in mind, it can be assumed that the water types moving in and out of the island chain have varying silicon and iron content and that the groups in Fig. 1 indeed represent these different water types. The biological history of each water type is undoubtedly different and hence is responsible for the variation in silicon and particulate iron content. Similar characteristic distribution groups were also observed with particulate iron. Inorganic phosphate and nitrate did not show such distinct groups but rather indicated annual cycles<sup>2</sup>. It is not known whether data on other trace metals will reveal information similar to that obtained from silicate and particulate iron. However, if such proves to be the case, trace element content could be used advantageously under the proper conditions as an additional tracer for distinguishing water types.

We are indebted to the Bureau of Commercial Fisheries (Honolulu) for making available salinity data obtained at the Koko Head monitoring station.

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### Thin-layer Chromatography in S-tanks of Mixtures containing Free Fatty Acids

FREE fatty acids may be readily separated from other lipids by chromatography on thin layers of silica gel. The solvents used generally contain a small amount of acetic acid<sup>1-4</sup> the purpose of which is to suppress dissociation of the fatty acids being chromatographed and thereby prevent serious 'tailing' of the spots.

Stahl<sup>5</sup> has described the separation of phenolic acids with an acid-free developing solvent on silica gel containing a small amount of oxalic acid. Experiments in this laboratory have shown that mixtures containing free fatty

Table 1. *R<sub>F</sub>* VALUES OF VARIOUS FREE FATTY ACIDS AND GLYCERIDES IN AN S-TANK

Oleic acid	0.68
9 and 10-Hydroxy stearic acids	0.13-0.18
9 and 10-Keto-stearic acids	0.40-0.50
1-Monostearin	0.03
1,3-Distearin	0.43
Tristearin	0.93

Adsorbent: Silica gel G (Merck) + 2% w/w oxalic acid. Humidity: circa 55%. 20° C. Solvent: 1:1 cyclohexane: di-iso-propyl ether (purified over alumina, grade 1). 15 cm development. Detection by charring was not affected by the oxalic acid.

acids may also be chromatographed with an acid-free solvent in ordinary rectangular tanks, provided that there is some acetic vapour in the atmosphere of the tank. Presumably this vapour is rapidly absorbed by the dry layer and behaves in much the same way as the oxalic acid in Stahl's experiments.

These observations may usefully be applied to the chromatography of fatty acids in S-tanks. S-tanks<sup>6</sup> have several advantages over normal tanks; but when they are used for chromatographing fatty acids in solvent systems containing a little acetic acid, they have the disadvantage that the fatty acids often move as an irregular spot immediately ahead of a sharp secondary solvent front, some distance below the true solvent front. This secondary front arises from the frontal analysis or demixing of the solvent mixture; the effect is particularly pronounced in S-tanks, in which absorption of solvent vapour by the dry part of the layer is normally at a minimum<sup>7</sup>.

To overcome this problem we have found one of two alternatives necessary. Either the plate may be exposed, just before development, to acetic acid vapour for up to one minute, or about 2 per cent (based on the weight of silica gel) of oxalic acid may be incorporated into the slurry when preparing the chromatoplates. Either course ensures that free acid is dispersed over the whole adsorbent before development begins.

Of the two methods, the addition of oxalic acid is the more reliable and has given satisfactory results with oleic acid (up to at least 50  $\mu\text{g}$  per spot). The oxalic acid has not been observed to lead to isomerization of 1,3 distearin on a chromatoplate or to poor binding of adsorbent.

A suitable system for the separation of free fatty acids and glycerides is given in Table 1.

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### Inhibition of Hyaluronic Acid Degradation by Dimethyl Sulphoxide

THE presence of ascorbic acid in vitreous humour<sup>1</sup> is of great importance in the process of post-mortem degradation, since hyaluronic acid, the major polysaccharide component of vitreous humour<sup>2</sup>, has been shown<sup>3</sup> to be degraded by reducing agents such as ascorbic acid and hydroquinone, in the presence of oxygen (the so-called ORD—oxidative reductive depolymerization—reaction). It is generally held that this depolymerization is brought about by free radical action, since radical scavengers such as sodium diethyldithiocarbamate have been shown<sup>4</sup> to inhibit the reaction, and in an analogous investigation of the degradation of alginate, Smidrod, Haug and Larsen<sup>5</sup> have postulated a free radical reaction with peroxide intermediates. In our examination of the preservation of human vitreous humour a major consideration has been

Table 1

Degrading agent	Volume of DMSO added (ml.)	Reaction time (min)			% Decrease in $\eta_{sp}$
		10	350 $\eta_{sp}$	1,200	
Ascorbic acid	0	0.130	0.036	0.029	79.2
	0.005	0.138	0.123	0.114	17.4
	0.01	0.143	0.137	0.120	15.5
	0.05	0.166	0.158	0.148	10.8
	0.10	0.174	0.175	0.174	0.0
Hydroquinone	0	0.136	0.060	0.040	64.0
	0.005	0.139	0.137	0.120	7.5
	0.01	0.140	0.135	0.133	5.2
	0.05	0.131	0.118	0.115	4.9
	0.10	0.143	0.143	0.143	0.0
Tetracycline	0	0.137	0.123	0.053	61.3
	0.005	0.129	0.120	0.086	33.2
	0.01	0.124	0.123	0.096	21.8
	0.05	0.120	0.121	0.119	0.8
	0.10	0.139	0.140	0.139	0.0

the effects of external agents, such as antibiotic preservatives and their ability to participate in an ORD reaction, together with methods of preventing such degradation.

Solutions (4 ml.) of umbilical potassium hyaluronate (Fluka) (0.045 per cent) in phosphate buffer (pH 7.3, 0.3 M) were mixed in U-tube viscometers at 30° C with aqueous solutions of the following antibiotics: penicillin G (5.4 mM/l.), streptomycin sulphate (5.4 mM/l.), polymyxin B sulphate (5.0 mM/l.), neomycin sulphate (5.4 mM/l.) and tetracycline hydrochloride (5.2 mM/l.). The percentage change in specific viscosity ( $\eta_{sp}$ ) of each digest after 24 h was 2.0, < 1, 4.7, 3.9 and 65.5, respectively. The degradation by tetracycline followed an initial period of about 3 h, during which no degradation was observed. The change in  $\eta_{sp}$  of a control solution of hyaluronate mixed with de-ionized water (2 ml.) was 2.2 per cent, while another mixed with an aqueous solution (2 ml.) of vitamin K (5.4 mM/l.) showed less than 1 per cent decrease.

The considerable decrease in  $\eta_{sp}$  of the hyaluronate solution when mixed with tetracycline suggested that an ORD reaction was taking place. Attempts to show free radical inhibition in this reaction, by addition of sodium diethyldithiocarbamate, failed due to precipitation, caused by interaction between the antibiotic and the inhibitor.

However, we have found that this and other ORD type degradations of hyaluronic acid can be inhibited by dimethyl sulphoxide (DMSO). The latter was added in different proportions (0.005 ml.-0.1 ml.) to standard hyaluronate solutions (4 ml.) as used here. Solutions (2 ml.) of ascorbic acid (0.56 mM/l.), hydroquinone (0.55 mM/l.) and tetracycline (5.21 mM/l.) were then added severally to the separate digests and the changes in  $\eta_{sp}$  determined over 24 h.

In each case the degradative action of the additives was decreased by increasing concentrations of DMSO (Table 1), demonstrating that DMSO is a useful inhibitor of the ORD reaction. The finding\* that DMSO is an anti-inflammatory adjunct and a bacteriostatic agent may depend in part on the ability of DMSO to inhibit free radical biological reactions. It is of interest to note that in recent investigations connected with the storage of cornea<sup>7</sup>, DMSO has been found to be an extremely effective preservative.

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## BIOCHEMISTRY

### Failure of Phenol to remove Residual Protein from Hyaluronic Acid

IN view of the successful removal of protein-containing material from carbohydrates<sup>1</sup> and from nucleic acids<sup>2</sup> by extraction with phenol, we have attempted by this means to remove the protein which remains associated with hyaluronic acid when the latter is prepared from ox synovial fluid by filtration<sup>3,4</sup>. Solutions were extracted at 0°-2° with phenol to give about equal volumes of the phenol and water-rich phases. Phenol was removed from the water-rich phase by extraction with diethyl ether or by dialysis.

Preliminary experiments showed that a single extraction would remove 80 per cent of the protein present in the filtrate obtained from synovial fluid (measured by extinction at 280 m $\mu$  after removing the phenol). Hyaluronic acid (measured as glucuronic acid by the method of Bitter and Muir<sup>5</sup>) was not extracted to a significant extent.

Table 1. ANALYSIS OF HYALURONIC ACID-PROTEIN COMPLEX BEFORE AND AFTER EXTRACTION SIX TIMES WITH PHENOL

Sample	Before extraction	After extraction
Dry wt. (mg/ml.)	0.69	0.42
Total N (mg/ml.)	0.047	0.025
Total N/dry wt. per cent	6.77	5.84
Protein per cent of dry weight	28	21

Accordingly, a sample of hyaluronic acid from which most of the filtrable protein had been removed was subjected to six successive extractions. The extracted solution was freed from phenol and its nitrogen content\* per dry weight was compared with that of the original material (Table 1). The protein contents were calculated on the bases of the analyses of Preston *et al.*<sup>4</sup>. (N content of protein 15.5 per cent; of hyaluronic acid 3.34 per cent.) It is seen that phenol extraction reduced the protein content to, but not below, the value obtained by exhaustive filtration.

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### Dehydrogenation of trans-trans Farnesol by Horse Liver Alcohol Dehydrogenase

Christophe and Popjak<sup>1</sup> have recently reported that a number of prenols<sup>2</sup> could serve as substrates for horse liver alcohol dehydrogenase (LADH) but they could not act as substrates for yeast alcohol dehydrogenase. This high degree of specificity of the prenols for LADH led the same authors to propose that the physiological role of the enzyme is connected primarily with the metabolism of intermediates of sterol biosynthesis and of similar natural products, and only fortuitously with dealing with man's social habit of consuming ethanol.

During the course of a study of the specificity of LADH towards sterols<sup>11</sup> some trans-trans farnesol was prepared and tested as a substrate. This compound was of more interest than the other prenols since it had previously been implicated as being directly involved in cholesterol biosynthesis by rat liver homogenates.

Crystalline LADH was prepared according to Dalziel<sup>1</sup> except that the pure enzyme was washed 6 times with 87 per cent saturation ammonium sulphate and finally recrystallized from 10 per cent methanol. The enzyme preparation was found to be 100 per cent pure on the basis of a specific absorbancy index at 280 m $\mu$  of 0.42 ml./mg.<sup>2</sup> and a molecular weight of 84,000 per 2 coenzyme binding sites<sup>4</sup>. The enzyme concentration was expressed as N, the normality of the co-enzyme-binding capacity per litre, and was determined spectrophotometrically by titrating LADH with NAD<sup>+</sup> in the presence of excess pyrazole<sup>5</sup>. The *trans-trans* farnesol was prepared from a sample of Mann Chemical Co. farnesol by the method of Popjak and Cornforth<sup>6</sup> using a preparative gas-liquid chromatograph. The nuclear magnetic resonance spectrum of the *trans-trans* farnesol agreed with the published spectrum of Bates and Gale<sup>7</sup>.

Fig. 1 shows the Lineweaver-Burke plot for LADH-farnesol. A  $K_m$  value of 0.382 mmoles was obtained which compares quite favourably with the value of 0.600 mmoles for ethanol at pH 9.5. The maximum velocity  $v/s$  is slightly below  $k_2$  obtained with ethanol as substrate, which at pH 9 is 4.9 sec<sup>-1</sup> according to Theorell and McKinley-McKee<sup>8</sup>. The value of  $v/s$  for farnesol is about 0.3 sec<sup>-1</sup> per binding site, which is 16 times lower than the value for ethanol. The  $K_m$  and  $v/s$  values were for the conditions specified. The farnesol was dispersed in glyco-deoxycholic acid, which is a normal liver metabolite, but since a true solution of farnesol was not obtained, its effective concentration is not known and this could cause some doubt about the accuracy of the  $K_m$  value. Until more data are available it is not possible to say if the mechanism of farnesol oxidation is the same as the Theorell-Chance<sup>9</sup> mechanism for ethanol.

This evidence indicates that farnesol might be a normal physiological substrate for LADH. It is known that the human liver can dehydrogenate a maximum of 250 g of ethanol each day<sup>10</sup>; correspondingly it may be calculated that about 15 g farnesol could be oxidized to farnesal per day. Farnesol in the liver arises as a by-product of cholesterol biosynthesis, by hydrolysis of farnesyl pyrophosphate<sup>1</sup>, but it is not known what quantities of this intermediate are diverted to this alternative pathway of metabolism. The daily production of cholesterol in the human liver is about 4 millimoles, requiring the previous conversion of 8 millimoles of farnesyl pyrophosphate into squalene. If it is assumed that one-half of the farnesyl pyrophosphate synthesized acts as sterol precursor, and the other half is metabolized via free farnesol, about 0.9 g of farnesol would be available for dehydrogenation, well within the calculated capacity of the liver. Obviously, these calculations are somewhat speculative since the horse liver enzyme may be different from the human;

nevertheless, the order of magnitude of the calculated values appears to be correct.

It will be of interest to make a detailed study of the kinetics of the LADH-farnesol system and to determine if LADH can play a regulatory part in cholesterol biosynthesis by the liver. For example, one of its normal physiological functions might be to oxidize farnesol to farnesal, but when ethanol is present, it is oxidized instead of the farnesol. Further study will be required to elucidate the significance of the LADH-farnesol system.

This work was done during my tenure of a postdoctoral Fellowship, on leave of absence from Oklahoma State University. I thank Prof. Hugo Theorell for his advice, Dr. Takashi Yonetani for help in preparing the enzyme, Mr. L. E. Goran Eriksson for his help in running the nuclear magnetic resonance spectra, and Dr. George Popjak for stimulating discussions and assistance in preparation of the text.

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### Effects of Pyridoxine Deficiency on the Adaptation of Rat Liver Cystathionase to DL-Ethionine and to Thyroidectomy

The activity of liver cystathionase ('soluble' cysteine desulphydrase) of rats is adaptively increased both after thyroidectomy<sup>1</sup> and after feeding DL-ethionine<sup>2</sup>. Since pyridoxal phosphate (PLP) is the co-enzyme of cystathionase<sup>3</sup>, it was of interest to determine the effect of pyridoxine deficiency on the level of liver cystathionase. Experiments were also conducted to determine the adaptive increase of cystathionase in pyridoxine-deficient rats in response to thyroidectomy and to ethionine feeding.

All experiments were performed using male and female Wistar rats with mean body-weight of 80 g. The rats were fed for 8 days the synthetic diet B (18 per cent casein) already described<sup>4</sup>. Then they were divided among groups of, at least, 6 animals per group. The first group (male rats) and the second one (female rats) were used as controls and were killed immediately. Group 3 was submitted for 8 days to the synthetic diet containing 1.5 per cent of DL-ethionine, while group 4 was thyroidectomized and fed for 10 days the synthetic diet without addition of ethionine. Thyroidectomized animals were given 1 per cent calcium lactate to drink. The other groups were given a synthetic diet similar to diet B but lacking pyridoxine. Some animals (group 5) were maintained for 3 weeks on the deficient diet before being killed, while others were thyroidectomized 10 days after the start of administration of the deficient diet and maintained on the deficient diet for 10 days after surgery. The last group (group 7) was also fed the same diet for 10 days and they received the deficient diet containing 1.5 per cent DL-ethionine for an additional week.

The preparation of the particulate free cell sap of the liver and the determination of enzymatic formation of H<sub>2</sub>S have been described previously<sup>4,5</sup>. The level of enzymatic activity was in each case estimated with and without the addition of PLP. Protein concentrations were measured according to the method of Lowry *et al.*<sup>6</sup>. All activities are expressed as  $\mu$ moles H<sub>2</sub>S produced/g protein/h.

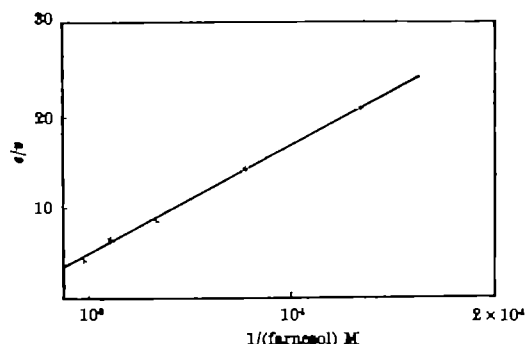


Fig. 1. Lineweaver-Burke plot for LADH-farnesol. The rate determination of the reduction of NAD<sup>+</sup> was carried out in glycine buffer, ionic strength 0.1, pH 9.5 at 25.5°, and was measured fluorimetrically. The LADH concentration was 0.158  $\mu$ M and the NAD<sup>+</sup> concentration was 10  $\mu$ moles in 4.0 ml. total volume. Initial velocities for a period of 90 sec were measured and expressed as moles per litre per second per binding site of LADH. Since farnesol is relatively insoluble in water, it was dispersed in a solution of glyco-deoxycholic acid, pH 9.5. Glyco-deoxycholic acid cannot serve as a substrate for LADH. The dispersion used was 10 mmoles farnesol and 8 mmoles glyco-deoxycholic acid.

Table 1. EFFECTS OF VARIOUS TREATMENTS ON THE LEVEL OF ENDOGENOUS AND PLP-STIMULATED CYSTATHIONASE IN RAT LIVER

Group	Sex	Treatments	Weight of liver (g)	Cystathionase activity		Percentage saturation apo-cystathionase	Stimulation by PLP (%)
				Endogenous	PLP* stimulated		
1	♂	Controls	6.2	163 ± 7.5	310 ± 6.5	50	90
2	♂	Controls	6.0	170 ± 13	380 ± 14.5	47	113
3	♂	Ethionine treated	3.2	856 ± 56	1106 ± 91	78	89
4	♂	Thyroidectomized	5.1	380 ± 13.5	765 ± 84	50	101
5	♂	Pyridoxine deficient	5.2	74 ± 4	230 ± 12.5	32	210
6	♂	Pyridoxine deficient, thyroidectomized	5.5	118 ± 31.5	440 ± 20	28	272
7	♂	Pyridoxine deficient, ethionine treated	3.2	797 ± 63.5	1106 ± 58.5	72	39

Each value given is a mean (± standard error) of results on six rat livers. Activities are expressed as  $\mu$ moles  $H_2S/g$  protein/h.

\* PLP—pyridoxal phosphate.

Percentage of saturation =  $\frac{\text{Endogenous activity}}{\text{PLP-stimulated activity}} \times 100$ .

Stimulation by PLP =  $\frac{\text{PLP-stimulated activity} - \text{Endogenous activity}}{\text{Endogenous activity}} \times 100$ .

The results in Table 1 show that the cystathionase in the liver decreased in pyridoxine deficiency (group 5). This suggests that either the rate of enzyme synthesis is decreased or the degradation of the apo-enzyme is increased in the deficient animals due to decreased availability of the co-enzyme. But although the percentage of saturation of the apo-enzyme in the pyridoxine deficient liver (32 per cent) was low compared to the control liver (group 1, 50 per cent), enzyme activation by PLP was higher in the former (210 per cent) than in the latter group (90 per cent). This suggests that pyridoxine deficiency did not totally suppress the synthesis of apo-cystathionase.

Thyroidectomy resulted in increased level of cystathionase (group 4), but the percentage of saturation of the apo-enzyme and its activation by PLP remained essentially the same as in normal rats. When pyridoxine deficiency was superimposed on the thyroidectomized animals (group 6), the level of the endogenous and the PLP-stimulated cystathionase decreased (380 and 765 respectively in thyroidectomy compared to the corresponding figures of 118 and 440 in thyroidectomy plus pyridoxine deficiency), although these levels were higher than those of the deficient animals without thyroidectomy (group 5). The percentage of saturation of apo-cystathionase with the co-enzyme as well as the degree of activation by PLP were similar in both the deficient and deficient thyroidectomized animals. Thus pyridoxine deficiency did not eliminate but decreased the degree of inducibility of the enzyme in response to thyroidectomy.

Comparison of the data in groups 1 and 3 of Table 1 shows that the higher level of cystathionase in the liver of ethionine-fed animals is also more highly saturated with PLP (78 per cent) and has consequent lower activability by the co-enzyme (29 per cent). Pyridoxine deficiency, superimposed on the ethionine-fed animals (group 7), did not lower the high induced level of cystathionase evoked by ethionine. It is noteworthy that the percentage of saturation of the enzyme and its activation by PLP remained comparable in both the pyridoxine-deficient and normal ethionine-treated rats. These experiments clearly show that pyridoxine deprivation is without effect on the adaptive increase of cystathionase by ethionine.

Lin *et al.*<sup>9</sup> observed that the adaptive increase of the apo-enzymes of tyrosine and tryptophan transaminase in response to hydrocortisone was not impaired in pyridoxine-deficient rats. Similarly, Davis<sup>8</sup> reported that cortisone increased the endogenous level of hepatic decarboxylating activity for tyrosine and 5-hydroxytryptophan in both normal and pyridoxine-deficient rats. The present data demonstrate that while the adaptive increase of liver cystathionase in response to thyroidectomy was somewhat reduced (but by no means eliminated) in pyridoxine deficiency, that due to ethionine administration was not impaired under such conditions. The differential effect may be attributed to separate mechanisms by which the enzyme is induced to increase in the two situations. In the case of tryptophan pyrrolase, it has been suggested<sup>10</sup> that while hormonal action altered the rate of synthesis of the

enzyme, the substrates and co-factors stabilized the existing enzyme. Similarly, it is reasonable to assume that in thyroidectomized animals, the rate of synthesis of cystathionase is increased, but the decrease in the availability of PLP in pyridoxine deficiency might increase the rate of degradation of the enzyme. The sum total is a decrease, but not an abolition, of the response to thyroidectomy—as was in fact observed in the present study. The mechanism of adaptive increase of cystathionase after ethionine feeding is not clear. The high percentage of saturation of the enzyme of the ethionine-treated rats suggests an increase in available PLP in the liver of such animals. We have also recently reported<sup>11</sup> that injections of high doses of pyridoxine increased, after some hours, the percentage of saturation of liver apo-cystathionase with the co-enzyme. But the degree of increase of cystathionase as a result of ethionine administration is not fully explained by the increase of available PLP alone, since ethionine-induced level of cystathionase far surpassed the increase in the level of the enzyme obtained in rats when injected for a week with small doses of pyridoxine<sup>6</sup>.

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### Effect of Ethylenediamine Tetraacetate on the Resistance of *Pseudomonas aeruginosa* to Antibacterial Agents

*Pseudomonas aeruginosa* is a resistant pathogen which is dangerous in ophthalmology<sup>1</sup>, as a hospital cross-contaminant<sup>2</sup>, and as a plant pathogen<sup>3</sup>. We have used the spectrophotometric method of Brown and Richards<sup>4</sup> to investigate the effect of disodium ethylenediamine tetraacetate (EDTA) on the action of several antibacterial agents against cultures of *Ps. aeruginosa*, NCTC 8203, in nutrient broth. It was found that the activity of polymyxin B sulphate, benzalkonium chloride and chlorhexidine diacetate against this organism was substantially increased in the presence of EDTA. Magnesium and calcium ions were found to block this potentiating



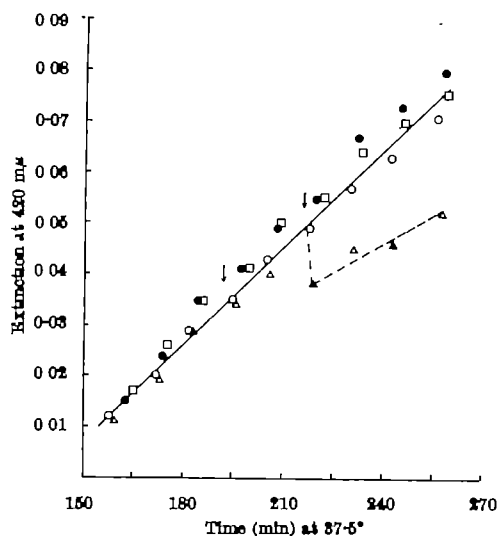


Fig. 1. The effect of EDTA on the action of polymyxin against log phase cultures of *Ps. aeruginosa* grown in nutrient broth.  
○, (A) Control; ●, (B) polymyxin, 1 unit/ml. at 192 min; △, (C) polymyxin, 1 unit/ml. at 192 min; EDTA, 6.5 μg/ml. at 216 min; □, (D) EDTA, 6.5 μg/ml. at 216 min

action. EDTA was also found to potentiate the action of polymyxin against cells grown in undiluted serum.

Replicate cultures A, B, C, and D, in 'Oxoid' nutrient broth at 37.5°, were grown into the log phase. Polymyxin was added to B and C to give 1 unit/ml. Approximately 20 min later EDTA was added to C and D to give 6.5 μg/ml. (Fig. 1). At the time of these additions the colony counts were about  $1.2 \times 10^7$ /ml. and  $2 \times 10^7$ /ml. respectively. Preliminary experiments had shown that these concentrations individually had no measurable effect on growth rate. By this procedure, control culture A was untreated, B received polymyxin 1 unit/ml., C received polymyxin 1 unit/ml. and later also EDTA 6.5 μg/ml., and D received 6.5 μg/ml. EDTA. The use of EDTA alone in higher concentrations caused lysis.

Culture C was the only one in which an effect on the growth rate was observed (Fig. 1). This culture had received doses of both chemicals which individually (B and D) had produced no observable effect. A similar phenomenon was observed when EDTA, 6.5 μg/ml., was added to cultures containing chlorhexidine, 0.7 μg/ml., and also to those with benzalkonium, 35 μg/ml. This potentiating effect of the EDTA on the action of the chemical antibacterial agents was blocked by growing the cultures in nutrient broth with magnesium chloride 50 μg/ml. (Fig. 2). Calcium chloride 18 μg/ml. had a similar blocking effect. The spectrophotometric measurements were correlated with total (chamber) and colony counts which confirmed these phenomena.

A similar experimental procedure was used to test whether the phenomenon of potentiation occurred in serum. Six replicate, log phase cultures, E, F, G, H, I and J, were grown in Burroughs Wellcome recalcified serum No. 2 containing Ca 12.1 mg/100 ml., and Mg 2.7 mg/100 ml. (Fig. 3). Culture E was untreated; EDTA was added to F, H and J at time zero to give 1,000 μg/ml.; EDTA was added to G after 290 min to give 1,000 μg/ml. and also to H and J which then each contained 2,000 μg/ml.; polymyxin was added to I and J after 305 min to give 100 units/ml. By this procedure, the control, E, was untreated, F received 1,000 μg/ml. EDTA at the start, G received EDTA after 290 min (1,000 μg/ml.), H received EDTA at the start (1,000 μg/ml.) and an equal dose after 290 min, I received polymyxin after 305 min (100 units/ml.) and J received 2 doses of EDTA (1,000 μg/ml.) and, later, polymyxin (100 units/ml.).

The addition of 1,000 μg/ml. EDTA at time zero (F) had no appreciable effect on growth rate compared to the control (E) (Fig. 3). A second equal dose of EDTA (H)

merely reduced the growth rate for about 20 min, after which time the rate increased to that of the control (E). The addition of 1,000 μg/ml. EDTA after 290 min (G) resulted in an effect similar to H and the culture recovered its original growth rate. The addition of polymyxin (100 units/ml.) (I) resulted in little appreciable effect for about 45 min, after which time there was significant lysis. J shows that polymyxin (100 units/ml.) added to cells in the presence of EDTA (2,000 μg/ml.) resulted in lysis so rapid that no reading was possible 15 min after the addition of polymyxin. Similar results were obtained using *Ps. aeruginosa*, NCTC 7244, and one strain of *Escherichia coli* with each of the three chemicals tested.

It is interesting to note that MacGregor and Elliker<sup>8</sup> found that the acquired resistance of trained *Ps. aeruginosa* cells to a quaternary ammonium compound could be eliminated, using EDTA. Repeake<sup>9</sup> found that EDTA enhanced the action of lysozyme against *Ps. aeruginosa*. The blocking effect of calcium and magnesium ions may be due to competition by the cations for the active sites; we have found that in the absence of sodium EDTA,

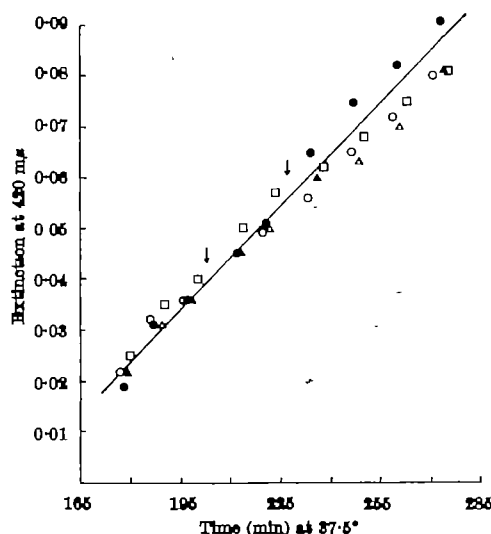


Fig. 2. The effect of EDTA on the action of polymyxin against log phase cultures of *Ps. aeruginosa* grown in nutrient broth, plus magnesium chloride (50 μg/ml.).  
○, (A) Control; ●, (B) polymyxin, 1 unit/ml. at 203 min; △, (C) polymyxin, 1 unit/ml. at 203 min; EDTA, 6.5 μg/ml. at 227 min; □, (D) EDTA, 6.5 μg/ml. at 227 min

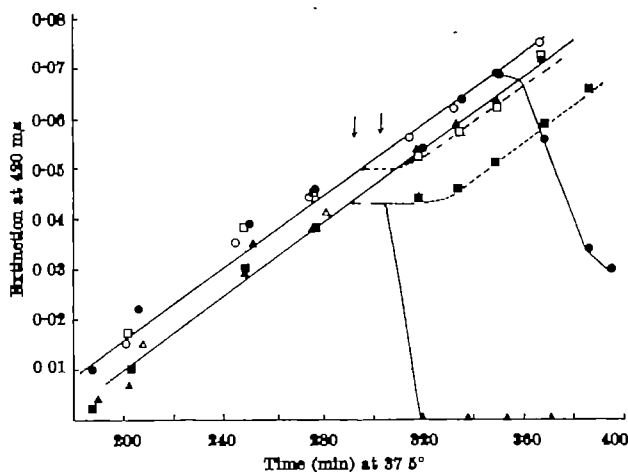


Fig. 3. The effect of EDTA on the action of polymyxin against log phase cultures of *Ps. aeruginosa* grown in undiluted serum.  
○, (E) Control; ●, (F) EDTA, 1,000 μg/ml. at start; □, (G) EDTA, 1,000 μg/ml. at 290 min; ■, (H) EDTA, 1,000 μg/ml. at start, plus further 1,000 μg/ml. at 290 min; △, (I) polymyxin, 100 units/ml. at 305 min; ▲, (J) EDTA, 1,000 μg/ml. at start, a further 1,000 μg/ml. after 290 min; polymyxin, 100 units/ml. at 305 min

calcium and magnesium ions antagonize the action of benzalkonium, chlorhexidine and polymyxin. On the other hand, these ions will chelate with the sodium salt of EDTA and they perhaps prevent chelation with ions in the cell membrane.

Our results suggest that the enhancement of anti-microbial activity by EDTA may be related to its chelating properties. Furthermore, we have found that whereas the sodium salt was effective, the magnesium salt had no apparent effect on the activity of the chemical agents tested nor did it have lytic activity alone. These observations are consistent with the hypothesis that EDTA exerts a lytic action and is synergistic with antibacterial agents by a mechanism involving removal of calcium or magnesium ions or both from the cell membrane. A closely analogous hypothesis has previously been suggested<sup>8</sup> to explain enhanced absorption of heparin from the gastro-intestinal tract of rats when it was given orally with an alkali salt of EDTA. The role of EDTA in reducing permeability barriers in biological systems would appear to have a wide significance.

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### Localization of Creatine Kinase in Microsomes and Mitochondria of Human Heart and Skeletal Muscle and Cerebral Cortex

WITHIN the framework of our investigations of the pathogenesis of progressive muscular dystrophy (Erb)<sup>1</sup>, we assumed as a hypothesis a genetically fixed defect in electrons and energy transport of the cell, to explain the symptoms of the disease, as known so far, such as variation of myoglobin, disorder in the ionic system, enzymes leaving the cytoplasmic and mitochondrial compartment of the muscular cell, etc.

One of the ferments, demonstrable in blood-serum during the illness, is creatine kinase (CK) (E.C.No.2.7.3.2.), the highest specific activity of which appears in diminishing order in human skeletal muscle, heart and cerebral cortex<sup>2</sup>. Since the discovery of CK by Lohmann<sup>3</sup>, Banga<sup>4</sup> and Kuby *et al.*<sup>5</sup>, so far as I know, no details about the localization of this ferment in mitochondria and microsomes of human brain and muscle have been published. This communication describes investigations on CK localization in the mitochondria and microsomes of human heart and skeletal muscle, and cerebral cortex, as well as of rat skeletal muscle.

Microsomes and mitochondria were isolated from heart and skeletal muscle according to the method described by Siekevitz<sup>6</sup>: cell nuclei and bigger tissue parts were centrifuged at 755*g* (2,500 r.p.m.) in the Servall centrifuge RO-2 (rotor SS 34) as well as the mitochondrial fraction at 33,000*g* (16,500 r.p.m.) and 1° C from the 10 per cent suspension (tissue/volume) produced according to Potter and Elvehjem<sup>7</sup> in a 0.88 M saccharose solution. The microsome fraction sedimented out of the supernatant at 105,000*g* (37,500 r.p.m.) in the Spinco L 50 (rotor 50). The last supernatant is called cytoplasmic fraction. Microsome and mitochondrial fractions were

suspended in 0.88 M saccharose solution and preserved at 0° C until further application.

A 10 per cent suspension in 0.88 M saccharose-solution ensued from the nuclear sediment by means of homogenization in the Bühler homogenizer (Bühler, Tübingen, Germany) at 1° C. The supernatant centrifuged at 33,000*g* (16,500 r.p.m.) and 1° C is regarded as the nuclear fraction.

Mitochondria and microsomes were isolated from brain according to the method described by Brody and Bain<sup>8</sup> with the following modifications. A buffer of 0.25 M saccharose, 0.01 M triethanolamine and 0.001 M EDTA, pH 7.4, was used for each operation. From human frontal cerebral cortex a 10 per cent suspension was produced according to Potter and Elvehjem<sup>7</sup>, which was centrifuged at 800*g* (2,800 r.p.m.), as previously reported. The sediment, re-suspended and centrifuged in half the original volume, produced sediment R<sub>1</sub>. Sediment R<sub>1</sub> was centrifuged from the two joined supernatants at 1,500*g* (3,500 r.p.m.) and the mitochondrial fraction from the supernatant at 12,000*g* (10,000 r.p.m.). From this supernatant the heavy microsome fraction was centrifuged at 23,500*g* (14,000 r.p.m.) and the light one in the Spinco L 50 at 105,000*g* (37,500 r.p.m.). The last supernatant is termed the cytoplasmic fraction.

Microsome fractions I and II as well as the mitochondrial fraction were suspended and preserved with 0° C. Sediment R<sub>1</sub> and R<sub>2</sub> were suspended as a 10 per cent tissue suspension in the Bühler homogenizer, as previously described, and from this the supernatant, that is to say the nuclear fraction, was centrifuged at 23,500*g* (14,000 r.p.m.).

Creatine kinase was determined in the optical test by NADH<sub>2</sub> with pyruvate kinase functioning as adjuvant enzyme and lactic dehydrogenase as indicator enzyme according to the modified technique of Tanzer and Gilvarg<sup>9</sup>. The deposit (final volume 3.5 ml.) contained 110 μmoles ATP, 4.2 μmoles ATP, 1.4 μmoles phosphoenolpyruvate, 0.56 μmoles NADH<sub>2</sub>, 1.5 mmoles glycine, 25 μmoles MgCl<sub>2</sub>, 0.1 mg pyruvate kinase and 0.1 mg lactic dehydrogenase, functioning as enzyme protein, as well as 1 ml. test volume, so diluted in glycine buffer pH 9 that it caused an extinction between 0.005 and 0.05 per min. The reaction was started with addition of creatine after a 10-min incubation at 25° C. During this it was measured once per minute with 366 mμ and 25° C in the 'Eppendorf' photometer (*d*=1 cm) against a zero standard (deposit without creatine). In this way inhibitory reactions such as NADH<sub>2</sub> oxidases and various ATPases could largely be excluded. Table 1 shows the averages of double determinations, where the enzyme activities are specified in μmoles creatine min<sup>-1</sup> g<sup>-1</sup> fresh weight, or extracted protein, determined according to the Biuret method<sup>10</sup>.

Relating the enzyme activity to two different quantities we gained two distinct results (Table 1). Values related to 1 g fresh weight are without exception lower and behave in some different way from those related to 1 g extracted protein.

Table 1. SPECIFIC ENZYME ACTIVITIES OF CREATINE KINASE IN FOUR OR FIVE CELL FRACTIONS OF HUMAN SKELETAL MUSCLE, HEART AND CEREBRAL CORTEX OR SKELETAL MUSCLE OF RAT

Organ	Cytoplasmic fraction	Mitochondrial fraction	Microsomal fraction 1	Microsomal fraction 2	Nuclear fraction	Total activity
Rat skeletal muscle	191(97) 1 060(111)	0.8(1) 60(6)		4.7(2) 351(40)		196(100) 959(100)
Human skeletal muscle	125(58) 5 847(153)	0.3(<1) 37(1)		0.3(<1) 107(3)	21(14) 3 359(87)	148(100) 3 829(100)
Human heart muscle	41(98) 2 585(322)	0.01(<1) 0.31(<1)		0.3(1) 439(66)	0.08(<1) 1(1)	43(100) 659(100)
Human cerebral cortex	19(74) 639(353)	1.3(5) 118(52)	0.3(1) 15(7)	0.3(1) 18(8)	5(19) 130(62)	23(100) 223(100)

Enzyme activities in μmoles creatine min<sup>-1</sup> g<sup>-1</sup> fresh weight (first line) and μmoles creatine min<sup>-1</sup> g<sup>-1</sup> extracted protein (second line). In parentheses, percentage of total activity.

The purity of the mitochondrial and microsomal fractions was tested by means of the purely cytoplasmic<sup>11</sup> lactic dehydrogenase, FDP aldolase and glutamate pyruvate transaminase as well as mitochondrial<sup>12</sup> L-glutamate dehydrogenase. Apart from this, the allocation system of the activities of mitochondrial and cytoplasmic<sup>11,12</sup> malate dehydrogenase, glutamate oxaloacetate transaminase and sorbit dehydrogenase was measured in each cell fraction. It was ascertained that an enzymatically pure mitochondrial or microsomal fraction had been produced<sup>13-14</sup>. In this connexion it became obvious that mitochondrial and cytoplasmic components were still to be found in the nucleus fraction of the three organs.

Jacobs, Heldt and Klingenberg<sup>15</sup> demonstrated the relatively high enzymatic activity of CK in the mitochondria of rat and pigeon skeletal muscle, heart and brain. CK activities of the rat quadriceps muscle, shown in Table 1, approximately coincide with those of Jacobs *et al.* In the mitochondria of human skeletal muscle (m. biceps brachii), however, a considerably smaller activity was measured, which might possibly have a connexion with the different mitochondrial contents of the various skeletal muscles or their contents of red and white muscle fibres<sup>16</sup>, as these two kinds of muscles have a clearly distinct metabolism<sup>17</sup>. The negligible CK activity of human heart muscle mitochondria, in contrast to those of rats and pigeons<sup>18</sup>, is surprising, and calls for further investigation.

Whereas greater specific activity (in relation to g fresh weight and g protein) was found in the microsomal fractions of human heart and skeletal muscle than in the respective mitochondrial fractions, the proportions in brain fractions were reversed. The enzyme activity of CK was higher in the mitochondrial fraction than in both microsomal fractions together; the total CK activity, however, quite predominant, was situated in the cytoplasmic fraction of the three organs (Table 1). Thus CK is a mainly cytoplasmic enzyme.

The small mitochondrial fraction of the enzyme seems to have a special correlation with enzymes of the respiratory chain and the citric acid cycle<sup>19</sup>; possibly it mediates in the exchange of phosphates, rich in energy, between intra- and extra-mitochondrial compartments<sup>18</sup>. Further investigations will show whether this constitutes a starting point for the investigation of cationic transport in the cell.

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## Foetal Uptake of Radiocalcium in Tetracycline-treated Rats

In a recent communication we reported that the skeletal uptake of injected calcium-45 in young rats was not affected by dose levels of oxytetracycline appreciably exceeding those generally used in medical practice<sup>1</sup>. We have now extended this line of enquiry to a study of foetal radiocalcium uptake since others have reported that the tetracyclines can markedly interfere with the calcification of embryonic mineralised tissues in a number of animal species<sup>2,3</sup>. The details of this investigation are as follows:

Sprague-Dawley rats weighing 200-250 g were assigned on the first day of pregnancy to four groups (I-IV) and offered a diet previously described<sup>4</sup> containing, per kg, either 0.0 (I), 0.25 (II), 1.0 (III) or (IV) 2.0 g of oxytetracycline hydrochloride (Chas. Pfizer and Co., Inc., New York). All were given intragastrically 1.0 ml. of a solution containing 0.7  $\mu$ Ci of calcium-45 and 20  $\mu$ g of calcium (as the chloride) twice daily from the first through the twentieth day of gestation. On the twenty-first day the foetuses were delivered by Caesarean section and weighed. One femur was removed from each dam and extracted in alcohol (4 h) and in ether (8 h). These femurs and three individual foetuses from each litter were incinerated overnight at 550° C and the ash of each was dissolved in hydrochloric acid and analysed for radiocalcium<sup>5</sup>.

Food consumption and body-weight gain for the 21-day period were, respectively (mean, g,  $\pm$  S.E.): I, 249  $\pm$  16.6 and 56.7  $\pm$  13.8 (3 rats); II, 242.0  $\pm$  6.4 and 62.4  $\pm$  9.7 (5 rats); III, 255.0  $\pm$  10.2 and 73.8  $\pm$  7.3 (4 rats); IV, 247.0  $\pm$  11.5 and 65.4  $\pm$  7.7 (5 rats). None of these values differs significantly ( $P > 0.05$ ) from each other. The number of foetuses in the litters of each group were: I, 9.0  $\pm$  1.4; II, 10.4  $\pm$  0.2; III, 11.0  $\pm$  0.7; and IV, 9.0  $\pm$  0.8. Again there were no significant differences between groups nor was there with regard to the mean weight (g) of these foetuses: I, 5.3  $\pm$  0.2; II, 5.0  $\pm$  0.2; III, 4.9  $\pm$  0.3; and IV, 5.1  $\pm$  0.2. All were viable at delivery and without external developmental anomalies.

Maternal and foetal radiocalcium uptake increased with the tetracycline content of the diet (Table 1). The linear regression coefficient in each case was significantly different ( $P < 0.05$ ) from zero, indicating a positive dose-response relationship.

Table 1. EFFECT OF OXYTETRACYCLINE ON THE MATERNAL AND FOETAL UPTAKE OF RADIOCALCIUM IN RATS\*

Group	Oxytetracycline in diet (g/kg)	No. of litters	Calcium-45 (% dose/mg ash $\times 10^{-5}$ )	<sup>45</sup> Ca foetus/ <sup>45</sup> Ca femur
I	0.0	3	1.197 $\pm$ 0.066	0.593 $\pm$ 0.017
II	0.25	5	1.905 $\pm$ 0.120	0.903 $\pm$ 0.063
III	1.0	4	1.400 $\pm$ 0.066	0.832 $\pm$ 0.019
IV	2.0	5	1.453 $\pm$ 0.048	0.876 $\pm$ 0.038

\* All values expressed as mean  $\pm$  S.E.

† Based on three individual foetuses from each litter.

There is no reason to believe from our previous study<sup>1</sup> that oxytetracycline affects the skeletal fixation of circulating radiocalcium. It follows, then, that the calcium-45 content of the maternal femurs provides a measure of the gastrointestinal absorption of this isotope. On this basis it can be concluded that the antibiotic not only did not interfere with calcium absorption under these experimental conditions, but also slightly enhanced it. Further, the lack of any significant differences in the ratios of foetal-to-maternal radiocalcium (Table 1) permits the conclusion that the placental transfer of calcium was not selectively altered by tetracycline administration.

With regard to the effect of this antibiotic on calcium retention, it is of interest that penicillin has been reported to increase the index of calcium absorption in chicks<sup>6</sup>. It is also noteworthy that the tetracyclines can have a salutary effect on experimentally induced rickets in rats<sup>11,12</sup>. On the other hand, the finding that the incorporation of chlortetracycline into diets adequate in all known dietary

essentials except calcium caused no increase in body calcium retention by growing rats suggests an equivocal role for this group of antibiotics in calcium absorption.

It has been reported that tetracycline can inhibit the developing skeletal anlage of the echinoderm embryo<sup>6</sup> and in the chick embryo can interfere with growth and produce malformed bones deficient in mineral content<sup>7</sup>. Additionally, skeletal deformities have been noted in mice following administration of oxytetracycline from the fifth to the twentieth day of gestation<sup>8</sup>. Marked retardation in foetal weight has also been observed in rats following intramuscular injection of 40 mg tetracycline per kg per day from the tenth to the fifteenth day of gestation<sup>9</sup>.

In the present work up to 100 mg of oxytetracycline per kg per day (Group IV) during essentially the full course of gestation had no effect on foetal weight. This is approximately twice the recommended maximum dose for man. Further, since the percentage of ash based on net weight was the same ( $P > 0.05$ ) for all groups, it follows that tetracycline did not affect the ash content of the foetuses (these ash percentages were: I,  $1.53 \pm 0.024$ ; II,  $1.50 \pm 0.038$ ; III,  $1.55 \pm 0.029$ ; IV,  $1.54 \pm 0.035$ ).

We conclude that the oral administration of oxytetracycline to rats within the therapeutic dose range used in man does not inhibit the maternal absorption of calcium-45 or the uptake of this radioisotope by the foetus.

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### Mitotic Activity in the Skin of Mice Deficient in Essential Fatty Acids

THE most striking difference between the skin of essential fatty acids deficient mice and that of normal mice is the number of layers of cells in the epidermis; the epidermis of essential fatty acids deficient mice is nearly three times thicker than normal<sup>1</sup>. A similar difference has been found in the skin of rats kept on a fat-free diet<sup>2</sup>. The greater thickness of the epidermis under these dietary conditions could conceivably have resulted from either: (a) a decrease in the rate of epidermal keratinization and cell sloughing; (b) an increase in the rate of cellular proliferation; or (c) a combination of these. The accumulation of abnormally large amounts of lipid in the cells of the distal epidermal layers of essential fatty acids deficient mice<sup>3</sup> suggested that these cells may, indeed, keratinize and slough at a slower rate than normal. The mitotic indices of the epidermis of normal and essential fatty acids deficient mice are compared in the present communication in an effort to deal with the second and third of the possible explanations for epidermal thickening.

BUB mice were kept on a fat-free diet for 10–12 weeks. The diet consisted of Fenton's casein No. 4, 5 g; salt mix No. 2, 4 g; vitamin-free test casein, 15 g; dextrose, 72 g; non-nutritive fibre, 4 g. Control mice were kept on

Purina lab. chow. A male and a female essential fatty acids deficient mouse and a control female mouse were killed in the late morning and pieces of skin from different body regions were fixed in Helly's fluid. Serial sections were cut of paraffin embedded material, and stained by the Feulgen reaction.

The results for the ear epidermis are presented in Table 1. The mitotic figures in the first two cell layers of the epidermis and the number of cells in these layers around the entire circumference of the external ear were counted. Five alternating sections per animal were observed. The mitotic indices were transformed to the arcsine for statistical analysis. The mean numbers of mitotic figures per 10,000 cells for the essential fatty acids deficient male and female mice and the control female mouse were 71, 50 and 31 respectively.

Table 1. ANALYSIS OF VARIANCE		
Source of variation	d.f.	Mean square
Total	14	
Animals	2	3025*
Error	12	167

\* Probability of difference arising from purely random variation less than 1 per cent.

Tukey's *D* test<sup>4</sup>, furthermore, showed that the mitotic indices of the female essential fatty acids deficient mouse and the female control differ significantly; the male essential fatty acids deficient mouse, however, did not differ significantly from the female control, suggesting, therefore, a sex difference in the mitotic activity of these mice.

These results thus do not eliminate the second or third possible explanations, mentioned above, for the increased thickness of the epidermis of essential fatty acids deficient mice. It is now clear, however, that the solution to the problem of what cellular activities control the thickness of the epidermis must come from studying the rates of mitosis and of keratinization during the process of thickening.

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### Syntheses of Guanidino-substituted Penicillins and Cephalosporins

THE availability of 6-aminopenicillanic acid<sup>1</sup> and 7-aminoccephalosporanic acid<sup>2</sup> has made possible the syntheses of a number of useful semi-synthetic derivatives<sup>3</sup>. We would like to report the preparation of a new class of penicillins and cephalosporins, which have in common the presence of a guanidino group. These compounds demonstrate quite remarkable *in vivo* potencies which we feel are attributable, at least in part, to low serum binding. The following examples are cited as important analogues of this series.

Phenyldiazomethane<sup>4</sup> reacted with 6-aminopenicillanic acid to yield benzyl 6-aminopenicillanate (I), m.p. 82°–83° (found: C, 59.11; H, 5.95). Prolonged reaction using an excess of phenyldiazomethane gave a dibenzyl derivative which since it could be hydrogenated to I was presumed to be benzyl 6-benzylaminopenicillanate, m.p. 65°–67°, m.p. of hydrochloride 145° (found: C, 60.80; H, 6.48; N, 6.52; S, 7.2; Cl, 8.38). D(–)- $\alpha$ -Aminophenylacetic acid slowly reacted with either *O*-methyl pseudourea hydrochloride or *S*-methyl thiopseudourea sulphate to give

D(-)- $\alpha$ -guanidinophenylacetic acid (II), m.p. 225° (re-solidifies and decomposes at 280°) (found: C, 55.36; H, 6.07). The hydrochloride of II, m.p. 157°–159° (found: Cl, 15.5), reacted with I in the presence of dicyclohexylcarbodiimide, followed by hydrogenolysis of the protective benzyl group gave 6-[D(-)- $\alpha$ -guanidinophenylacetamido]-penicillanic acid (III), m.p. 215° dec. (found: C, 50.71; H, 5.26; N, 17.77). In mice, III is orally as active as oxacillin<sup>8</sup> against several resistant staphylococcal infections, but in addition it is highly effective against penicillin sensitive Gram-positive infections and is also high stable to acid.

*p*-Aminophenylacetic acid was treated with benzoyl cyanamide<sup>6</sup> to give *p*-N<sup>3</sup>-benzoylguanidinophenylacetic acid, m.p. 220° dec. (found: C, 64.45; H, 4.98; N, 14.14), which was hydrolysed with sodium hydroxide to give *p*-guanidinophenylacetic acid, m.p. 320° (found: C, 56.11; H, 5.50; N, 21.65). This acid could also be prepared by reaction with *S*-methyl nitrothiophenol and hydrogenation. Reaction with thionyl chloride afforded the crystalline acid chloride hydrochloride IV which was not further characterized but reacted directly with 6-amino-penicillanic acid to give 6-(*p*-guanidinophenylacetamido)-penicillanic acid (V), m.p. 195°–200° dec. (found: C, 51.96; H, 5.80). The penicillin (V) was more than ten times more effective than benzylpenicillin administered parenterally in mice against a variety of Gram-positive infections, making it the most potent penicillin that we have ever tested. It is nearly as active as ampicillin against an experimental *Salmonella* infection.

Methyl 3-bromo-2,6-dimethoxybenzoate<sup>7</sup> was treated with sodamide in liquid ammonia to give methyl 4-amino-2,6-dimethoxybenzoate, m.p. 143°–144° (found: C, 57.31; H, 6.31; N, 6.56). Reaction with benzoyl cyanamide, followed by hydrolysis, afforded 4-guanidino-2,6-dimethoxybenzoic acid hydrochloride, m.p. 218° dec. (found: C, 43.56; H, 5.04; N, 15.66). The acid chloride of this acid was condensed with I to give, after hydrogenolysis, 6-(4-guanidino-2,6-dimethoxybenzamido)-penicillanic acid (VI) (found: C, 46.96; H, 5.60; N, 14.7). This compound was also as potent as methicillin parenterally in mice against resistant staphylococcal infections.

The acid chloride (IV) also reacted with 7-aminocephalosporanic acid<sup>8</sup> to give 7-(*p*-guanidinophenylacetamido)-cephalosporanic acid (VII), gradually decomposes above 200° (found: C, 50.78; H, 4.6). It is more active than methicillin against a resistant staphylococcal infection in mice and is also more active than benzylpenicillin parenterally against penicillin sensitive infections.

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\* These and subsequent biological data will be the subject of a separate communication by Frost, B. M., Vallant, M. H., Thiele, H. H., and Robinson, H. J.

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## PHYSIOLOGY

### Haemoglobin Type and Reproductive Performance in Australian Merino Sheep

Two haemoglobin types can readily be distinguished in sheep and have been shown to be simply inherited<sup>1</sup>.

The reproductive performance of sheep with these haemoglobin types was investigated in a flock of Scottish Blackface ewes<sup>2</sup>, and although haemoglobin B is not common in this breed<sup>3</sup> there was a suggestion that ewes carrying the B haemoglobin gene produced more lambs at weaning than the other types in the flock. The difference was not, however, significant.

In Merino sheep in Australia, the average gene frequency for Hb.B is approximately 0.6<sup>4</sup>, although values between 0.3 and 0.7 have been noted. The simply inherited red blood cell potassium types<sup>1-3</sup> do not complicate the analysis in this breed, as practically all animals are 'LK' (low potassium)<sup>4</sup>, so the Merino is a particularly suitable breed for a re-examination of the possible association between the haemoglobin type of a ewe and the average number of lambs born and weaned per year. There has been an indication<sup>5</sup> that the actual level of potassium ion concentration in the red blood cells for 'LK' sheep may be associated with lamb production, but any such effect is relatively small and is not considered here.

This relationship has been investigated in four large experimental flocks of Merinos run by C.S.I.R.O., in which pedigrees and performance records are kept. One of these, at Cunnamulla in South-West Queensland, is under selection for various aspects of wool weight and quality<sup>6</sup> but not for any aspect of reproductive performance. Two others, at Deniliquin in the Riverina area (South) of New South Wales, are under selection for high and low twinning rate respectively<sup>6</sup>, while the fourth, also at Deniliquin, was based on a commercial ('Booroola') flock selected for multiple births, in which triplets and quadruplets are common.

Table 1 gives the average number of lambs born and weaned per year during the first 2 lambings by ewes of each Hb type in the four flocks. Ewes in the Cunnamulla flock were born from 1951 to 1957, and in the Deniliquin flocks from 1955 to 1960, so lambings occurred over a range of years in each case.

Ewes of Hb type A had fewer lambs born or weaned than those of the Hb type AB or B in every flock, the difference between AB and B being slight. In the Cunnamulla flock, the difference between Hb.A ewes and the other types was small for lambs born and larger for lambs weaned, whereas in the Deniliquin flocks the difference is approximately the same either for lambs born or lambs weaned. Sheep at both centres are lambed under supervision in yards, but at Cunnamulla the ewes are turned out of the yards into extensive grazing with no supervision, whereas at Deniliquin they are under more intensive husbandry and closer supervision.

The overall average numbers of lambs born or weaned per ewe mated are comparable for the Cunnamulla and the low-twinning flocks, but are considerably higher for the high-twinning flock and higher again for the Booroola flock. The difference in lambs weaned between Hb.A ewes and the remainder increases with the average number of lambs born in the flock. The main source of difference in lambs born in the various flocks lies in the proportion of multiple births, and in Table 2 the number of lambs per birth is given for ewes of each Hb type in the two high-producing flocks. It would seem that the superiority of the Hb.B ewes is associated in part with the production or survival of lambs from multiple births. These results, together with those reported previously from a totally different environment, strongly suggest that Hb.B confers an advantage in lamb production.

It also seems likely, however, that in some environments the advantage conferred by Hb.B with respect to lambing

Table 1. LAMBS BORN AND WEANED PER EWES MATED PER YEAR BY BREDS OF EACH HB TYPE IN FOUR FLOCKS (First two lambings for each ewe)

Hb type	Cunnamulla flock			Deniliquin flocks								
				Low twinning flock			High twinning flock			Booroola flock		
				No. of ewes mated	No. of lambs		No. of ewes mated	No. of lambs		No. of ewes mated	No. of lambs	
	No. of ewes mated	Born	Weaned		Born	Weaned		Born	Weaned		Born	Weaned
A	91	0.88	0.63	9	0.72	0.66	16	0.84	0.72	7	1.14	0.93
AB	374	0.92	0.73	49	0.84	0.74	93	1.10	0.96	18	1.86	1.63
B	512	0.90	0.73	77	0.84	0.76	75	1.15	1.00	1	2.00	2.00
Overall	977	0.91	0.72	135	0.83	0.75	184	1.10	0.96	26	1.67	1.45
$\frac{AB+B}{2} - A$		0.03	0.10		0.12	0.09		0.28	0.27		0.73	0.72

Standard deviations: Lambs born 0.23, lambs weaned 0.35.

Table 2. LAMBS PER BIRTH FOR EWES OF DIFFERENT HB TYPES IN HIGHEST-PRODUCING FLOCKS

Hb type	High twinning flock			Booroola flock		
	per cent of matings giving 0 lamb	1 lamb	2+ lambs	per cent of matings giving 0 lamb	1 lamb	2+ lambs
A	23	63	15	23	50	27
AB+B	16	54	30	15	40	45

is partially outweighed by advantages conferred by Hb.A perhaps at some stage after weaning, and that the gene frequency which is found in any breed in any environment represents a balance between these factors. The gene frequency for Hb.B is known to alter when a breed is taken from its native environment and established in a different environment<sup>7</sup>, suggesting that the relative advantages and disadvantages of Hb.A and Hb.B have also altered.

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## Altitude and Athletic Performance

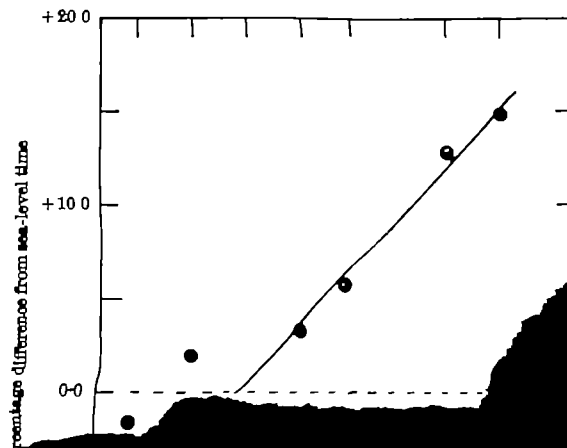
THE decision to hold the 1968 Olympic Games in Mexico City at an altitude of 7,500 ft. above sea-level has aroused interest in the effects of altitude on athletic performance. It has been known for some time that the maximum oxygen intake of acclimatized adults falls off right from sea-level upwards, reaching 50 per cent of the sea-level at 20,000 ft.<sup>1</sup> According to these results the decline in maximum oxygen intake at 7,500 ft. would be 8 per cent and the times for running distances of 1,500 m and more should be longer by approximately this amount. Over shorter distances, an increasingly large fraction of the total energy requirement is covered anaerobically and the decrement should be progressively smaller. In sprint events, factors such as the reduced density of the air at altitude might even lead to improved performance.

In order to obtain further information on the effect of altitude on athletic performance, the performance of the first three competitors in the

each event were averaged, and the differences between the altitude and the sea-level results, expressed as percentages of the sea-level value, were plotted against distance on a one-way logarithmic scale. The results reveal a linear relation between decrement in performance at altitude and log distance (Fig. 1). The increases in the times ranged from 2.62 per cent over 800 m to 14.9 per cent

Table 1. COMPARATIVE TIMES OF THE FIRST THREE COMPETITORS IN THE FINALS OF RUNNING EVENTS AT 7,500 FT. AND AT SEA-LEVEL

Event	Pan American Games Mexico 1965		Olympic Games Melbourne 1966	
	Min	Sec	Min	Sec
100 m	First	10.3	First	10.5
	Second	10.4	Second	10.5
	Third	10.4	Third	10.6
	Mean	10.4	Mean	10.5
200 m	First	20.7	First	20.6
	Second	21.3	Second	20.7
	Third	21.4	Third	20.9
	Mean	21.1	Mean	20.7
400 m	First	45.4	First	45.7
	Second	45.6	Second	46.8
	Third	46.3	Third	47.0
	Mean	45.8	Mean	46.8
800 m	First	1 : 49.7	First	1 : 47.7
	Second	1 : 50.0	Second	1 : 47.8
	Third	1 : 52.4	Third	1 : 48.1
	Mean	1 : 50.7	Mean	1 : 47.9
1,500 m	First	3 : 53.2	First	3 : 41.2
	Second	3 : 53.2	Second	3 : 42.0
	Third	3 : 55.8	Third	3 : 42.0
	Mean	3 : 54.1	Mean	3 : 41.7
5,000 m	First	15 : 30.6	First	13 : 59.6
	Second	15 : 31.4	Second	13 : 50.6
	Third	15 : 59.2	Third	13 : 54.4
	Mean	15 : 33.7	Mean	13 : 48.2
10,000 m	First	32 : 42.6	First	28 : 45.6
	Second	33 : 00.4	Second	28 : 52.4
	Third	33 : 42.6	Third	28 : 53.6
	Mean	33 : 08.5	Mean	28 : 50.5



in the 10,000 m event. Over 100 m and 400 m but not over 200 m the times at altitude were better than at sea-level.

By interpolation on this graph the predicted increase in time taken for a race lasting 6 min would be about 8 per cent, which agrees well with the results predicted from maximum exercise tests referred to here which were of this duration.

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### Response of the Medial Rectus Muscle of the Cat to Succinylcholine

IN contrast to its relaxing action on most skeletal muscles, succinylcholine is well known to contract extra-ocular muscles<sup>1-3</sup>. While examining the effects of drugs on the extra-ocular muscles, it was observed that succinylcholine exerted two basically different effects on the extra-ocular muscles.

Cats were anaesthetized with sodium pentobarbital (36 mg/kg) given by intraperitoneal injection. The trachea, femoral artery and femoral vein were cannulated and artificial respiration started. The medial rectus muscle of the left eye was then separated from the globe and a suture placed through the tendon. Following immobilization of the animal's head in a stereotaxic apparatus, the tendon was attached to a Grass force displacement transducer. Through a parietal craniotomy, the dura mater was opened and the left cerebral hemisphere lifted gently to expose the third nerve. Electrical stimulation of the third nerve was affected by impaling the exposed nerve with a needle electrode delivering supramaximal rectangular pulses, 0.5 msec duration at a frequency of 0.3 c/s.

The intravenous injection of small doses of succinylcholine (less than 10 µg/kg) resulted in an increase in the resting tension of the medial rectus muscle associated with an increase in the twitch height. However, large doses of succinylcholine (30-150 µg/kg) produced a marked increase in resting tension as well as a severe reduction of the twitch response. This reduction in the twitch response was unrelated to the rise in resting tension. A typical example of this response to larger doses of succinylcholine is shown in Fig. 1. It can be seen that inhibition of the twitch persists at a time when the tension of the muscle is returning to normal. In a given animal, the smallest dose of succinylcholine required to depress the twitch height, with respect to the control, was approximately ten times greater than the smallest dose required to increase the resting tension of the muscle. The different pharmacological responses of the extra-ocular muscles to succinylcholine may be explained by anatomical observations of Hees<sup>4</sup> and Hees and others<sup>5</sup>. These authors reported two types of neuro-

muscular systems to be present in the extra-ocular muscles of the cat; a fast or twitch system, and a slow or tonic system. It would, therefore, seem reasonable to attribute the increased muscle tension seen after the intravenous injection of succinylcholine to stimulation of the tonic system, and depression of the twitch response to blockade of the fast neuromuscular system.

By means of the preparation described in this report, it is possible simultaneously to compare and contrast the effect of drugs *in vivo* on the two contractile systems present in the extra-ocular muscles of the cat.

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### Membrane Potentials in Longitudinal Smooth Muscle Isolated from Guinea-pig Ileum

THE activity of the smooth muscle in guinea-pig ileum has been investigated many times. The tissue preparation most often used for such investigations has been the isolated ileum. In a number of cases, however, experiments have been carried out, not with the whole ileum, but with the thin outer layer of longitudinal smooth muscle that can be easily separated from the ileum<sup>1-3</sup>. This communication provides a brief description of membrane potentials which occur in this isolated muscle preparation. The work arose from an interest in correlating electrical activity with the transmembrane movements of potassium ion and the mechanical responses of the muscle that have been examined previously. It was desirable, therefore, to explore the feasibility of estimating changes in electrical activity in this preparation.

Membrane potentials were measured by means of the sucrose gap method<sup>4,5</sup>. An isotonic solution of potassium chloride flowed over an inactive portion of the muscle; and a physiological salt solution, over an active portion of the muscle. The composition of the physiological salt solution was: NaCl, 0.125 M; KCl, 0.0027 M; CaCl<sub>2</sub>, 0.0018 M; NaHCO<sub>3</sub>, 0.024 M; and glucose, 0.011 M. The solution was saturated with a gas mixture consisting of 95 per cent oxygen and 5 per cent carbon dioxide. The temperature was maintained between 35° C and 37° C.

**Potentials of unexcited muscle.** Sections of longitudinal muscle, placed in a sucrose gap apparatus through which the aforementioned solutions flowed, exhibited changes in membrane potentials that are illustrated in Fig. 1. The development of simple, spiked depolarizations which varied in amplitude and frequency were common occurrences. Moreover, each spike was usually preceded by a slower, more prolonged decrease in membrane potential. The slow depolarizations were often followed by a series of pre-potentials that, in turn, were followed by spikes. Slow depolarizations were also followed by complex spikes, which were more



line level. Occasionally, the downward phase of a spike extended below base-line to produce a hyperpolarization of the membrane. The hyperpolarized state was usually dispelled by a gradual return of the membrane potential to base-line level.

No precise statement can be made about the dimensions of the membrane potentials observed. However, a crude estimate of their magnitudes may be obtained by comparing potentials in the longitudinal muscle with the well-characterized potentials in the taenia coli of the guinea-pig<sup>1-7</sup>. In Fig. 2 representative records from a longitudinal muscle and a taenia coli preparation are presented side by side. Both records were obtained under the same experimental conditions. The amplitude of the spikes in the longitudinal muscle were quite small compared with those in the taenia coli.

It is known that records obtained by the sucrose gap method may not reflect the true sizes and configurations of potential changes. Changes would appear to be diminished if current flow from cell to cell encountered resistances that approached in magnitude the resistance of the extracellular space established by the sucrose solution. Asynchronous firing among the fibres of the muscle might also reduce recorded values of potential changes. The extent to which these factors have modified the results of this investigation cannot be assessed at present.

**Potentials in presence of acetylcholine.** The addition of  $5 \times 10^{-4}$  M acetylcholine evoked two groups of changes in membrane potential. One occurred during the increase in tension; the other, during the maintained tension of the muscle. The first series of changes shown in Fig. 3 (upper section) may be characterized as a gradual depolarization of the membrane on which were superimposed spiked discharges of variable amplitudes, fired at a high frequency. As peak-level of depolarization was approached the spiked discharges became less frequent or disappeared entirely. For a brief period the membrane potential remained relatively stable at the reduced level of polarization and then began to exhibit a second series of fluctuations which are illustrated in Fig. 3 (lower section). The outstanding characteristic of the latter series was the development of oscillations in potential which occurred concomitantly with a slow partial repolarization of the membrane. Occasionally, spiked dis-

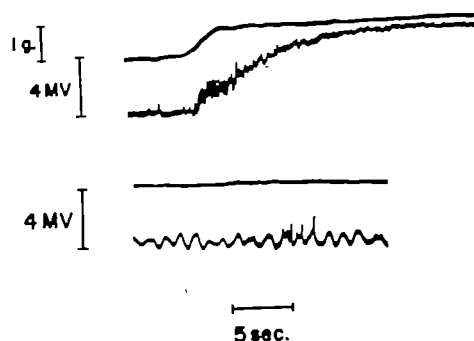


Fig. 3. Changes in membrane potential and tension of longitudinal smooth muscle in the presence of acetylcholine. The record in the upper section was taken shortly after  $5 \times 10^{-4}$  M acetylcholine was added to the bathing medium. A short lag-period occurred before the drug came into contact with the muscle fibres. The record in the lower section, taken 5 min later, was obtained from the same muscle preparation. The tension recording in the lower section is at the same level as the highest level of tension shown in the upper section.

any point in the oscillatory cycle. When the drug was removed, muscle tension declined, the oscillations gradually disappeared, and the membrane potential returned to its original level or to a somewhat lower base-line.

Although the sucrose gap technique does not allow quantitative measurements of potential changes to be made, this work has shown: (1) that the isolated longitudinal muscle is capable of generating prepotentials and spiked depolarizations; (2) that acetylcholine can elicit two different series of changes in membrane potential, each associated with a different phase in the mechanical response of the muscle.

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The presence of similar resorption cavities in bones of patients suffering from von Recklinghausen's disease of bone, caused by overactivity of the parathyroid glands<sup>6</sup>, suggested the hypothesis that these glands may be overactive in severe skeletal fluorosis.

To test this hypothesis, four pairs of twin lambs have so far been used. One lamb received distilled water containing sodium fluoride 200 p.p.m., *ad lib.*, while its twin received distilled water alone. One pair of lambs was killed after a week on fluoride, the remaining three pairs after a month. The parathyroid glands were removed under 'Pentothal' anaesthesia, fixed in glutaraldehyde, post-fixed in osmium tetroxide, and then dehydrated and embedded in 'Araldite'. Sections were cut with glass knives on a Huxley microtome and examined by a Siemens Elmiskop 1 electron microscope. Samples of bone and kidney were also examined.

The ultrastructure of the normal sheep parathyroid gland is essentially similar to that of the three species, man<sup>7</sup>, macaque<sup>8</sup> and rat<sup>9</sup>, so far described in the literature. Unlike the primate parathyroid glands, however, there is only one cell type. The sheep parathyroid cells, furthermore, frequently contain large lipid bodies, only an infrequent finding in the other three species. These bodies, which are preserved by osmic fixation, are membrane-bound, measure up to  $5\mu$  in diameter and have a random distribution within the cell.

The ultrastructural criteria for assessing the activity of the parathyroids have been described in the glands of rats following nephrectomy<sup>10</sup> and in the rat gland grown in organ culture in a low calcium medium<sup>11</sup>.

The parathyroid glands of the lamb killed after a week's exposure to fluoride showed slight changes only. In the parathyroid glands from the lambs killed after a month's exposure, however, these changes had become quite marked: these glands were larger than the controls and the hypertrophy resulted from an increase in the size and number of the parenchymal cells and caused a reduction in the width of the intercellular space. The ergastoplasm was more extensive; the rough-surfaced endoplasmic reticulum was dilated and the normally flattened sacs were more rounded. The perinuclear space, which was normally narrow, was much wider in those



Fig. 2. Parathyroid gland from a sheep that received fluoride for one month. Two parenchymal cells are separated by a narrow intercellular space (is). The endoplasmic reticulum (er) is dilated, the perinuclear space is wider and the Golgi apparatus (G) more extensive. (Lead citrate  $\times 15,000$ )

more extensive, and the plasma membranes of the parenchymal cells had become extremely tortuous, increasing their interdigitations with those of neighbouring parenchymal cells, but not with those of adjacent endothelial cells. There was no significant alteration in the size and number of the lipid bodies.

All these changes have been observed in the parathyroid glands from nephrectomized rats<sup>10</sup>, and some of them in parathyroid glands grown in organ culture<sup>11</sup>. An increase in the size of the cells, ergastoplasmic profiles and Golgi complex are changes that were first demonstrated in the gonadotrophic and thyrotrophic cells of the pituitary gland after castration<sup>12</sup> and thyroidectomy<sup>13</sup> respectively, and are now well recognized as being indicative of increased activity in most cells.

The immunoassay of the amount of parathyroid

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## PHARMACOLOGY

### Inhibition of Gastric Motility by a Commercial Duodenal Mucosal Extract containing Cholecystokinin and Pancreozymin

IN the course of the search for the hormone which results in inhibition of gastric secretion and motility after fat has entered the duodenum, the ability of duodenal extracts to inhibit the motility of denervated gastric pouches has become crucial. The presence of fat in the duodenum inhibits the spontaneous motility of Heidenhain pouches<sup>1</sup>, but many duodenal extracts which inhibit acid secretion will not do this. Extracts of duodenal mucosa containing secretin<sup>2</sup> and even more conclusively extracts containing cholecystokinin/pancreozymin<sup>3</sup> inhibit gastric acid secretion.

We have studied, in the conscious animal, the effect of these two extracts on the motility of Bickel (vagally and sympathetically denervated) pouches of the stomach in six dogs and on one transplanted pouch. Each pouch was filled with saline and connected to a pressure transducer by a water-tight indwelling stainless steel cannula. Resting motility was regular; on average contractions occurred 3-4 times per min and developed a pressure of 70 cm water. Infusion of secretin (cholecystokinin/pancreozymin, 'Vitrum'—Stockholm, Sweden) into a vein in doses of 1 unit/kg altered neither the frequency nor the force of contraction of the gastric pouch. Cholecystokinin/pancreozymin ('Vitrum'), in doses above 0.3 units/kg, on the other hand, invariably depressed the Bickel pouch. Higher doses (1.0 unit/kg) produced complete cessation of contraction for the duration of the infusion and for 10-15 min afterwards (Fig. 1).

Motility was recorded from the intact stomach by means of an air-filled balloon and again cholecystokinin/pancreozymin inhibited and secretin did not in the doses used in the Bickel pouch animals. Bile or pancreatic juice was introduced into the duodenum in a number of animals. Neither bile nor pancreatic juice depressed pouch motility, but olive oil always did. It is not possible yet to say whether the inhibition of motility observed by us or the inhibition of gastric secretion is a property of either cholecystokinin or pancreozymin or of some other substance present in the extract, but certainly this extract reproduces

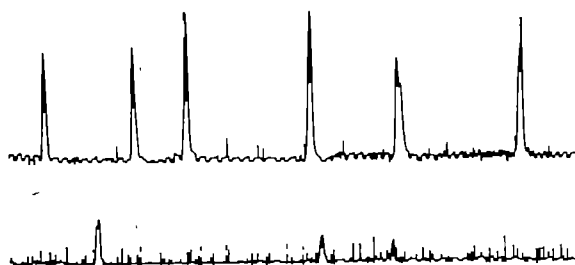


Fig. 1. Upper tracing: spontaneous contractions in saline-filled denervated fundic pouch of stomach. At the end of the upper tracing the cholecystokinin/pancreozymin infusion was started and continued for the duration of the lower tracing. The contractions can be seen to get progressively smaller and eventually to disappear in the lower tracing. The duration of each tracing is 10 min.

on the stomach the physiological effect of fat in the duodenum.

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### An Inhibitor of Oxytocin from the Urinary Bladder of the Toad, *Bufo marinus*

A PREVIOUS investigation has shown that the isolated bladder of the toad, *B. marinus*, releases an inhibitor of oxytocin into the bathing Ringer's solution<sup>1</sup>. More recently a similar inhibitor has been invoked to explain the inhibition of the effects of vasopressin and of adenosine-3', 5'-monophosphate on the toad bladder<sup>2</sup>. In the former investigation it was shown that inhibitor could be obtained by gently stirring isolated toad bladders in a modified Ringer's solution, and that the inhibitor so obtained was not readily dialysable. In the investigation reported here the previous results were confirmed; however, it was found that, following heat-treatment of the inhibitor solution, the inhibitor became dialysable. We report a modified preparation of the inhibitor based on this finding.

The assays for oxytocin and for inhibitor were as previously reported<sup>1</sup>, and were a modification of Bentley's method<sup>3</sup>. Synthetic oxytocin ('Syntocinon', Sandoz) was used in these experiments, usually at a concentration of 2 milliunits per ml. This concentration produced about 75 per cent of the maximal response.

The previous method of preparation of inhibitor was repeated. Two whole bladders were excised, briefly washed, and stirred in 100 ml. Ringer's solution for 2 h at 4° C. The resulting solution was dialysed for 24 h at 4° C against three changes of 20 vol. distilled water. The contents of the dialysis bag were assayed for inhibitor. At a final dilution corresponding to the inhibitor from 20 bladders/l., the response to oxytocin at a concentration of 2 milliunits/ml. was inhibited by approximately 50 per cent.

It was found that if the bladders were homogenized instead of being merely stirred, about twice the amount of inhibitor was obtained per bladder. In this case the bladders were washed in distilled water, minced with a pair of scissors, and homogenized for 1 min at medium speed in a 'Vir Tis' homogenizer. The homogenate was centrifuged at 30,000g for 15 min, and the supernatant was dialysed as before.

Heating the dialysed inhibitor solution obtained by either of the foregoing methods to 100° C did not cause an appreciable loss in inhibition. Moreover, it was found that following the heating of the solution at 100° C for 10 min the inhibitor would readily pass through a 'Viking' dialysis membrane. These observations led to the following

method of preparation: 12 bladders were briefly washed and then homogenized in 100 ml. distilled water. The homogenate was centrifuged as above, and the supernatant was dialysed against three changes of 20 vol. water. The contents of the dialysis bag were made up to 0.01 M acetic acid and kept at 100° C for 10 min. The solution was cooled and then centrifuged at 30,000*g* for 15 min to remove precipitated material. The supernatant was then dialysed against 5 vol. of water for 18 h at 4° C. The solution outside the dialysis bag contained roughly 80 per cent of the total inhibitor. This solution, about 500 ml., was concentrated in a flash evaporator to 25 ml. The concentrated solution was either frozen and stored, or taken to a powder by lyophilization. The yield of powder was quite small, and it was usually more convenient to keep the inhibitor in the concentrated solution. At a final dilution corresponding to the inhibitor from 5 to 10 bladders/l. the inhibition of 2 milliunits/ml. oxytocin was at least 50 per cent.

We conclude that the inhibitor is a molecule small enough to pass a 'Viaking' dialysis membrane and hence has a molecular weight of the order of 10,000 or less<sup>4</sup>. Its inability to dialyse before heating is possibly due to its binding to a larger molecule, this binding being sensitive to temperature. The two dialyses undoubtedly effected a partial purification of the inhibitor since, in the first, small molecules were removed and, in the second, large molecules were removed, while the overall recovery of inhibitor was apparently quite high. In some further experiments, the details of which will be reported later, it was found that this preparation also inhibited the response of the toad bladder to arginine vasotocin and of the rat uterus to oxytocin.

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## HAEMATOLOGY

### Effect of Phytohaemagglutinin on Synthesis of 'Rapidly-labelled' Ribonucleic Acid in Human Lymphocytes

THE treatment of human peripheral blood leucocyte cultures with phytohaemagglutinin (PHA) causes a transformation of small lymphocytes to primitive blast cells after morphological and cytochemical changes<sup>1-4</sup>. The cells undergoing the transformation acquire the ability to synthesize DNA<sup>5,6</sup> and the morphological changes observed after 24-72 h of incubation are accompanied by an increase of RNA synthesis<sup>7,8</sup>.

In the present work the mechanism of transformation was examined by observing cell metabolism during the early stages of transformation, before any morphological change has occurred.

Thirty-minute, or one-, two- or four-hour cultures of normal human peripheral blood leucocytes ( $10^6 \pm 10$  per cent cells/ml. in TC 199 medium with 20 per cent of the subject's own plasma, at 37° C), control or PHA-treated

('Bacto'-phytohaemagglutinin M, Difco, batch No. 469791, 0.05 ml. standard sol./ml.), were given <sup>3</sup>H uridine (Amarsham, specific activity 2.7 c./mmole) at a final concentration of 2  $\mu$ c./ml. The incubation with <sup>3</sup>H uridine was interrupted after 20 min by label-diluting with non-radioactive uridine ('Sigma') to a concentration of 1 mmole and collecting by centrifugation (1,000 r.p.m. for 5 min). The cells were washed twice with TC 199 containing 20 per cent human AB+ serum, and after the last centrifugation were suspended in a drop of medium. The viability of lymphocytes was controlled by means of trypan blue staining.

The smears were made on gelatinized slides, air dried, fixed in ethanol:acetic acid mixture (3:1) and then treated for 5 min with 5 per cent trichloroacetic acid at 0° C. After 20 min washing in running water, the smears were coated with 'AR 10' Kodak emulsion and exposed at 4° C for 10 days. After the photographic procedure, autoradiographs were counterstained with Giemsa<sup>9</sup> and subjected to a quantitative analysis. Two thousand lymphocytes from each sample were analysed. The experiment was repeated twice. Results are presented in Table 1 and Fig. 1. During the first 4 h incubation no DNA synthesis in small lymphocytes has been demonstrated<sup>1-7</sup>, so methylated <sup>3</sup>H uridine incorporation into DNA can be excluded. The increase of labelled cells in PHA-treated cultures shows that more cells synthesize 'rapidly labelled' RNA. The rate of RNA synthesis, estimated from intensity of labelling, is the same in the control as in the PHA-treated cultures.

Under present experimental conditions, when pulse labelling with <sup>3</sup>H uridine was performed, one may assume that the label is incorporated in the prevalent amount into messenger RNA<sup>10</sup>. It should be emphasized that the observed phenomenon, that is, a start of RNA synthesis,

Table 1

	Number of silver grains above the cell	Time of incubation			
		30 min	60 min	120 min	240 min
Control	0-2	1,961	1,907	1,825	1,754
	3-5	32	80	120	170
	6-8	6	8	25	44
	9-11	1	4	12	14
	12-15		1	6	10
	16-20			7	2
	20-∞			5	6
Mean value of silver grains above labelled cells		4.82	4.62	6.26	5.84
PHA treated	0-2	1,962	1,904	1,512	1,189
	3-5	36	130	200	440
	6-8	8	30	102	200
	9-11	2	14	40	71
	12-15	2	16	23	68
	16-20		2	13	21
	20-∞		4	10	11
Mean value of silver grains above labelled cells		5.17	6.21	6.33	6.71

The table presents results of quantitative analysis of autoradiographs. 2,000 small lymphocytes of each group were analysed. 0-2 silver grains in nuclear emulsion above the cell were taken as a background, and these cells were not taken into account in calculation of the mean value of grains above labelled cells.

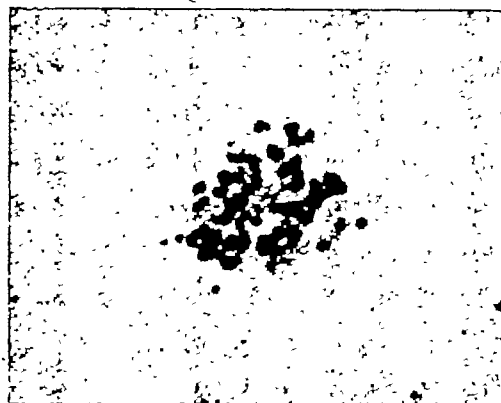


Fig. 1. Intensely labelled lymphocyte after pulse incubation with <sup>3</sup>H uridine and 240-min pre-incubation with phytohaemagglutinin.

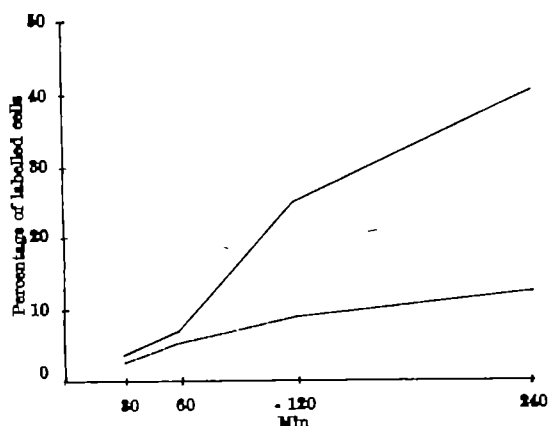


Fig. 2. Increase of percentage of labeled lymphocytes in controls and under phytohemagglutinin treatment. Dashed line, control; solid line, PHA-treated.

may be related to the beginning of lymphocyte transformation. The transformatory mechanism of the PHA action on lymphocytes is obscure. From the present experiment one may consider the possibility that PHA initiates gene transcription in lymphocytes. If this agent affects cell membrane, its action may be connected with a change in membrane permeability to contain substances<sup>11</sup> and a subsequent imbalance of gene-histone complexes<sup>12</sup>. On the other hand, if PHA molecules with polyanionic groups in the mucoprotein fraction<sup>13</sup> are allowed to penetrate into the cell and reach the nuclear region<sup>14</sup>, they may directly affect the cationic groups of histones, changing the cistron activity. The increase in lymphocytes synthesizing RNA, in cultures without PHA, may express their environmental adaptation and may be preparatory to the blastogenic auto-transformation which is observed after longer periods of cultivation.

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## Appearance of Rat Slow Alpha-two Globulin after Irradiation

Slow alpha-two globulin<sup>1</sup> (SA<sub>2</sub>G), although not demonstrable by the usual method in the sera of healthy, non-pregnant, adult rats, appears in rat sera under a large number of conditions involving growth, regeneration, necrosis, inflammation, infection or the administration of bacterial lipopolysaccharide<sup>2,3</sup>. Consequently, it was considered of interest to determine whether or not SA<sub>2</sub>G could be demonstrated in the sera of rats subjected to irradiation alone or to irradiation followed by the administration of bacterial lipopolysaccharides.

Sera were obtained from Sprague-Dawley rats of either sex as previously described<sup>4</sup>, and the presence or absence of SA<sub>2</sub>G was demonstrated by vertical starch-gel electrophoresis using the method of Smithies<sup>5</sup> with minor modifications<sup>6</sup>. The rats were exposed to approximately 600 r. of  $\gamma$ -radiation from a caesium-137 source (0.662 MeV) at a dose rate of approximately 10,000 r./h. Lipopolysaccharides from either *Salmonella abortus equi* or *S. typhosa* (Westphal type, Difco Laboratories, Detroit, Michigan) were administered intraperitoneally at the rate of 2.5–3.5 mg per rat in 0.25 ml. of 0.9 per cent sterile sodium chloride solution. Control animals were given 0.25 ml. of 0.9 per cent sodium chloride or left uninjected. The injections were made 3 or 6 days after irradiation.

The effectiveness of the radiation dosage may be judged by the observation that, while 47 unirradiated rats showed an average total white blood cell count of 16,250 per mm<sup>3</sup> (standard deviation 3,610), 40 animals examined 3 days after irradiation had an average count of 710 per mm<sup>3</sup> (standard deviation 314) and 38 rats examined 6 days after irradiation showed an average count of 451 white blood cells per mm<sup>3</sup> (standard deviation 402). The large standard deviations are due, at least in part, to the difficulties of securing accurate counts in these extreme cases of leucopenia.

The results obtained are shown in Table 1. One hundred and sixty-four examinations were made, within the first 15 days after irradiation, of sera from irradiated but uninjected rats. In 7 of these, or 4.3 per cent of the cases examined, SA<sub>2</sub>G was detected. This may be compared with 2 cases, or 1.4 per cent, of demonstrable SA<sub>2</sub>G in the sera of a consecutive series of 141 normal, untreated animals from our colony. The difference between these two rates of incidence is not statistically significant, and it may be concluded that irradiation at the dose-level here administered does not evoke the production of SA<sub>2</sub>G to the degree needed to permit its demonstration by vertical starch-gel electrophoresis.

Table 1. APPEARANCE OF SLOW ALPHA-TWO GLOBULIN AFTER IRRADIATION AND INJECTION OF LIPOPOLYSACCHARIDES

Days after irradiation	Irradiated only	No. of rats showing SA <sub>2</sub> G over No. of rats tested	
		Injected 3 days after irradiation	Injected 6 days after irradiation
0	0/23*	—	0/24*
1	0/15	0/25†	0/8
2	2/11	20/20	—
3	0/12	—	0/24†
4	2/19	15/15	12/13
5	1/18‡	4/9	11/11
6	0/8	8/8	—
7	0/12	0/1	4/5
8	0/2	0/1	—
9	0/1	0/2	—
10	2/12	0/1	2/3

\* Blood sample taken immediately before irradiation.

† Blood sample taken immediately before injection of lipopolysaccharide.

‡ Very weak SA<sub>2</sub>G band.

Of further interest is the question as to whether or not irradiated rats, despite their extreme leucopenia, are still capable of responding normally to the administration of lipopolysaccharide by the production of sufficient SA<sub>2</sub>G to permit its demonstration by starch-gel electrophoresis. The results are again shown in Table 1. None of the 49 irradiated rats showed SA<sub>2</sub>G after irradiation but before injection of the lipopolysaccharide. All such rats tested within 4 days after the administration of the lipopolysaccharide showed high levels of SA<sub>2</sub>G in their sera. The percentage of sera with demonstrable SA<sub>2</sub>G decreased thereafter approximately at the rate we have learned to expect from previous work<sup>3</sup>.

It may be concluded that, first, exposure of rats to 600 r. of  $\gamma$ -radiation does not lead to the production of SA<sub>2</sub>G demonstrable by vertical starch-gel electrophoresis and, secondly, that rats injected with bacterial lipopolysaccharide 3 or 6 days after such irradiation still respond with the production of demonstrable quantities of SA<sub>2</sub>G in a manner closely similar to that of unirradiated rats. Therefore, at least the major part of the white blood cell

system does not appear to be involved in the SA<sub>2</sub>G response to bacterial lipopolysaccharide.

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### Plasminoplastin Generation Test of Normal, HF<sup>-</sup>, PTA<sup>-</sup>, X<sup>-</sup>, PTC<sup>-</sup> and AHF<sup>-</sup> Platelet-poor Plasmas: Evidence that only HF<sup>-</sup> Plasma has an Abnormal Fibrinolytic Activity

SINCE Hageman factor (HF) was found to participate in fibrinolytic activity<sup>1,2</sup> several investigations have been carried out to explore its role in fibrinolysis. Iatridis and Ferguson<sup>3</sup>, in a detailed analysis of this phenomenon, suggested that Hageman factor has a lysokinase activity which converts plasminogen-proplasminoplastin (pro-activator) into plasminoplastin (activator). Aznar *et al.*<sup>4</sup> investigated the fibrinolytic activity of Hageman factor by means of thrombelastography and concluded that the active form itself has a similar character to that of the plasminogen proactivator. Haanen *et al.*<sup>5</sup> showed that the fibrinolytic activity of a purified Hageman factor is only measurable in the presence of plasminogen, and therefore Hageman factor is an activator of plasminogen. But Iatridis and Ferguson<sup>3</sup> had shown that chicken plasma, which is deficient in proplasminoplastin (proactivator) and in Hageman factor, did not develop any plasminoplastin (activator) activity, despite the addition of streptokinase or kaolin-SF (surface factor or activation product). Thus, from this experiment and from others using normal or Hageman deficient plasmas, they concluded that activated Hageman factor is only efficient for the generation of endogenous plasminoplastin when its precursor (the proplasminoplastin) is available.

Recently Holemans and Roberts<sup>6</sup> showed that in normal persons, venous occlusion of the forearm results in an increase in fibrinolytic activity of the blood within the occluded segment, while three subjects with Hageman trait had less fibrinolytic activity. In addition they found normal fibrinolytic activity in a patient with antihaemophilic factor (AHF) deficiency before and after venous occlusion. Kamel *et al.*<sup>7</sup> showed that patients with haemophilia A, Christmas disease and von Willebrand's disease had normal fibrinolytic activity. Moreover, Phillips *et al.*<sup>8</sup> presented two cases of familial hypofibrinogenaemia with normal fibrinolytic activity.

In the work recorded here we present further evidence that plasma from Hageman trait cases is deficient in fibrinolysis too, whereas plasmas from XI<sup>-</sup>, X<sup>-</sup>, IX<sup>-</sup> and VIII<sup>-</sup> have normal fibrinolytic activity.

The method used during this investigation was the plasminoplastin generation test (PGT) of Iatridis and Ferguson<sup>3</sup> which may be briefly reviewed as follows. Two ml. of intact platelet-poor plasma diluted with 38 ml. of distilled water was adjusted to pH 5.3 with 1 per cent acetic acid, and incubated at 37° C. At successive time-intervals, 4-6 ml. samples were removed, centrifuged at 3,000 r.p.m. for 15 min at 4° C, the precipitate (euglobulin) dissolved in 0.23 ml. of borate buffer (pH 7.8) and tested for fibrinolytic activity by the euglobulin lysis time method<sup>9</sup>. The surface factor (SF) was prepared according to the previously described technique<sup>2</sup>. Well-studied cases of (XII<sup>-</sup>) Hageman trait (L.C.), (O.E.) and (A.M.), (VIII<sup>-</sup>) haemophilia A, (IX<sup>-</sup>) haemophilia B, and

(X<sup>-</sup>) Mr. R. Stuart respectively, provided bloods with these specific clotting factor deficiencies. (XI<sup>-</sup>) PTA deficiency was an artificial preparation according to Nossel's<sup>10</sup> method.

Fig. 1 shows that only plasmas from the three subjects with Hageman trait were deficient in the generation of plasminoplastin, while XI<sup>-</sup>, X<sup>-</sup>, IX<sup>-</sup> and VIII<sup>-</sup> deficient plasmas had normal plasminoplastin generation. These findings (VIII<sup>-</sup>, IX<sup>-</sup>) confirm the results of others<sup>3,7</sup> that haemophilia A and B had normal fibrinolytic activity. Also we found that X<sup>-</sup> and XI<sup>-</sup> deficient plasmas had normal fibrinolytic activity.

Fig. 2 shows the PGT on normal and Hageman deficient plasmas. The controls were run in silicone and in glass, while all other tests were in silicone. Active surface factor was added to both plasmas, and the results show a striking normalization of Hageman deficient plasma. When inactive SF (which was prepared from an exhausted plasma<sup>11</sup>) was added, no increase in the plasminoplastin generation was detected. (Compare the results with their

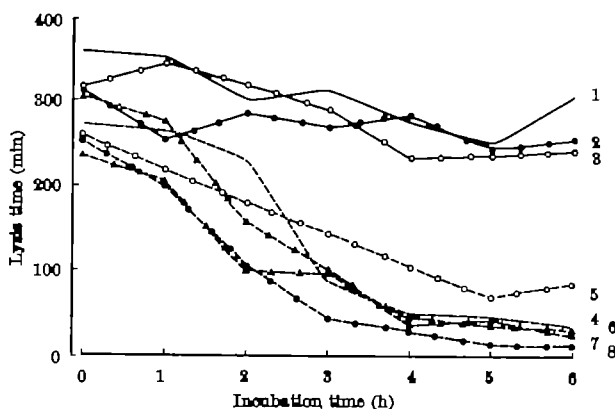


Fig. 1. PGT in glass on three Hageman deficient (curves 1, 2 and 3), normal (curve 4), PTA<sup>-</sup> (curve 5), PTC<sup>-</sup> (curve 6), AHF<sup>-</sup> (curve 7) and Stuart<sup>-</sup> (curve 8) plasmas

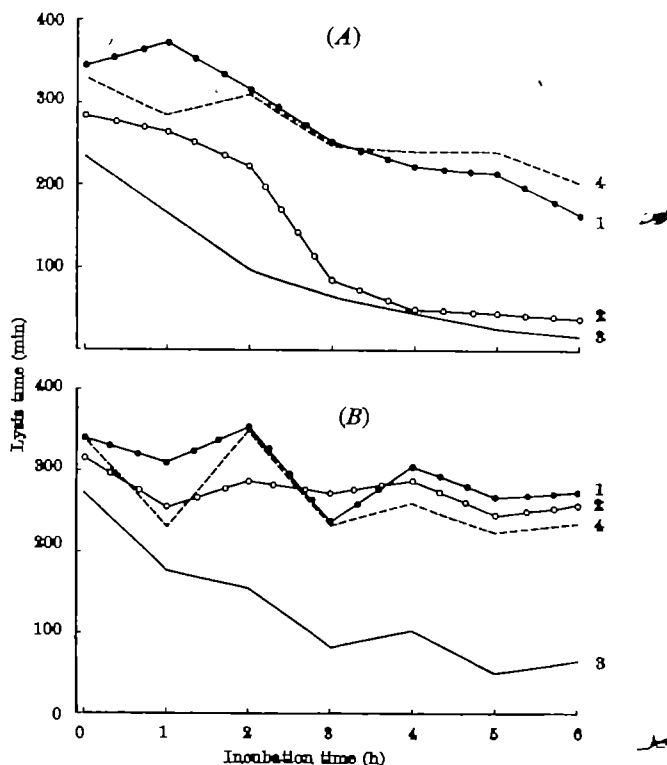


Fig. 2. PGT on (A) normal and (B) Hageman deficient plasmas. Curve 1 is a silicone control without any additive and curve 2 is a similar control in glass. The plasmas are pre-incubated, in silicone, with an active SF (curve 3) or with an inactive SF (curve 4)

respective silicone controls.) On the other hand, the control PGT performed in glass showed an increase of the plasminoplastin generation only in normal plasma, not in the Hageman deficient.

It seems, therefore, that when Hageman factor is activated, this triggers both systems: (a) the clotting mechanism; (b) the fibrinolysis. What can be demonstrated in the test-tube could very well occur *in vivo*<sup>11</sup>. Since XI, X, IX and VIII deficient plasmas, in which factor XII is normal, behave in the PGT like normal plasmas, we may conclude that factors XI, X, IX and VIII have no fibrinolytic activity and only factor XII is the link between coagulation and fibrinolysis.

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## IMMUNOLOGY

### Immunological Significance of Lysosomes within Lymphocytes *In vivo*

Recent reports<sup>1,2</sup> have demonstrated the presence of lysosome-like particles in human peripheral blood lymphocytes and have shown that these increase in size and number in tissue culture following stimulation with phytohaemagglutinin. These changes appear to precede the transformation of the small lymphocytes into cells which synthesize RNA and eventually undergo mitosis. In both these reports<sup>1,2</sup> it is suggested that these lysosomes are in some way involved in the initiation of cell division.

In this work we have investigated the relative number of small lymphocytes *in vivo* which contain lysosomes, and the average number of lysosomes within lymphocytes in lymph nodes draining the site of application of a chemical sensitizing agent, at various stages during the initiation of delayed-type hypersensitivity. The development of delayed-type hypersensitivity has been shown to be associated with massive proliferation of small lymphocytes in the cortex of the draining lymph node<sup>3,4</sup>. This has been shown to occur through an intermediate large pyroninophilic cell which has many features in common with the transformed lymphocyte found following stimulation with phytohaemagglutinin *in vitro*. It was therefore essential to find out whether lymphocyte lysosomes responded *in vivo* to an immunological stimulus which would induce lymphocyte proliferation in the lymph node. An investigation was also undertaken to see whether any changes could be detected in the lysosomes within small lymphocytes in the circulation and in an inflammatory exudate in an established hyperimmune state. The system chosen was one where the animals were in an exquisitely sensitive delayed hypersensitive state to tuberculin, following the injection of dead tubercle bacilli in water-in-oil emulsion.

Guinea-pigs of the Hartley strain bred in the Institute of Dermatology were used throughout. They were exam-

ined either fresh from stock at a weight of between 400 and 500 g, or following sensitization either by one application of the chemical sensitizing agent 2-phenyl-4-ethoxymethylene-5-oxazolone (oxazolone) or by the injection of heat-killed *Mycobacterium tuberculosis* (tubercle bacilli) in water-in-oil emulsion.

Sensitization to oxazolone was with 0.2 ml. of 10 per cent oxazolone dissolved in ethanol and painted on the left ear<sup>5</sup>. Sensitization with tubercle bacilli was by injection of 1 mg of heat-killed dried bacilli (obtained from the Ministry of Agriculture Central Veterinary Laboratory, Weybridge) suspended in water-in-oil emulsion using Freund's incomplete adjuvant (Difco). Guinea-pigs were injected intramuscularly with a total of 0.8 ml. divided equally between four sites in the thighs and the nape of the neck between three and four months before the experiment. They were selected because they gave a necrotic reaction 24 h after the intradermal injection of 1.3 µg tuberculin (human PPD, obtained from the Ministry of Agriculture Central Veterinary Laboratory, Weybridge). Imprints of the left auricular lymph nodes were made from normal guinea-pigs and 2, 4 and 6 days after the application of oxazolone to the left ear.

Peritoneal exudates containing large numbers of mononuclear cells were obtained by washing out the peritoneal cavity with Hanks's balanced salt solution four days after the intraperitoneal injection of 30 ml. of sterile light liquid paraffin B.P. The cells were washed twice, diluted to a concentration of  $3 \times 10^6$  cells/ml. in 5 per cent bovine albumin (Armour, fraction V) and centrifuged lightly on to glass slides<sup>6</sup>.

Peripheral blood lymphocytes were prepared from the blood of guinea-pigs injected intravenously with 10 mg of heparin. The erythrocytes were allowed to settle following the addition of an equal volume of 5 per cent polyvinylpyrrolidone ('Polyvidone', May and Baker) in 0.15 M sodium chloride. The supernatant was first centrifuged at 150g to remove polymorphonuclear leucocytes and the residual cells in the supernatant (~90 per cent mononuclear cells) were centrifuged on to glass slides in 5 per cent bovine albumin as outlined here.

Acid phosphatase was demonstrated by the Gomori method as modified by Holt<sup>7,8</sup> using sodium-β-glycerophosphate as the substrate. The incubation medium was prepared as follows: 0.1 M sodium-β-glycerophosphate (B.D.H.), 0.003 M lead nitrate and 0.05 M acetate buffer (pH 5). The mixture was kept in the incubator at 37° C for 18 h and the precipitate was filtered. Fresh unfixed cell preparations were incubated in the substrate solution at 37° C for varying periods up to 2 h. An incubation time of 1-2 h was found to be necessary to obtain definite reactions in small lymphocytes. The slides were then immersed in a saturated solution of hydrogen sulphide prepared by bubbling hydrogen sulphide gas from a Kipp's apparatus into distilled water. They were then mounted in 8 per cent gelatine in an equal volume of glycerol and water. Control slides were incubated in the same substrate medium but containing 0.01 M sodium fluoride to inhibit the enzyme.

Lysosomes within cells of the lymphoid series were identified as granules which stained for acid phosphatase. These could be activated by pre-incubation in an acid buffer at pH 5.0 and the enzyme activity was stripped off by treating the preparation with 'Triton X 100'. Pre-treatment with distilled water increased the enzyme activity of the granules, which was also inhibited by 0.01 M sodium fluoride. The granules were therefore found to possess the property of latency of enzyme activity. By these criteria it was considered that these granules could definitely be identified with lysosomes which have been defined previously<sup>9</sup> by biochemical criteria.

An investigation was undertaken of cells in imprints of the auricular lymph nodes of normal guinea-pigs and those which had been painted with oxazolone on the left ear. Three types of cells were seen containing acid



phosphatase staining granules. One group consisted of large cells containing granules which stained after only 5-min incubation with the substrate mixture. These would appear to be cells of the macrophage series and were similar to cells in the peritoneal exudate which had ingested oil; the cytoplasm of these cells was not basophilic. A second group of large cells which had an intensely basophilic staining cytoplasm and corresponded to the large pyroninophilic cells in previous studies<sup>3,4</sup> had a much smaller number of granules which only began to stain for acid phosphatase after 20 min incubation with the substrate mixture. Finally, there were small lymphocytes which contained granules which stained optimally for acid phosphatase following 2-h incubation. As can be seen in Table 1, there was a marked increase in the proportion of small lymphocytes containing lysosomes in the lymph nodes draining an area of application of a chemical sensitizing agent. The average number of lysosomes per cell also increased, reaching a peak 4 days after sensitization, falling off on the sixth day after sensitization.

Table 1. EFFECT OF SENSITIZATION TO OXALOPHORE ON THE ACTIVITY OF LYSSOMES IN LYMPHOCTES WITHIN THE DRAINING LYMPH NODE

Auricular lymph node	Small lymphocytes Maximum per- centage of cells containing lysosomes	Mean number of lysosomes per cell	Large lymphoid cells Per cent cells containing lysosomes
Normal	10	3	19.5
2 days after sensitization	78	6.3	77
4 days after sensitization	90	9.1	93.5
6 days after sensitization	85	5.1	77.5

All figures are the mean of those found on examination of the material from six different animals. The 'large lymphoid cells' were found to have a strongly basophilic cytoplasm. Highly active macrophages which were not basophilic were not included in this series.

About 35 per cent of small lymphocytes in the peripheral blood or an oil-induced peritoneal exudate from normal guinea-pigs were found to contain lysosomes (Table 2). In guinea-pigs which had been sensitized with dead tubercle bacilli in water-in-oil emulsion 3-4 months previously, the number of small lymphocytes containing lysosomes had increased to between 80 and 90 per cent. Cells from sensitized animals contained about 10-11 lysosomes per cell as compared with 3.5-5 lysosomes per cell in normal animals. Lysosomes in small lymphocytes from sensitized animals were seen to be markedly swollen when compared with those from normal animals. Incubation of cells from peritoneal exudates with the substrate mixture for only 5 min showed that macrophages from tuberculin-sensitive animals had more active lysosomes than macrophages from normal animals.

Table 2. EFFECT OF SENSITIZATION WITH TUBERCLE BACILLI ON THE ACTIVITY OF LYSSOMES WITHIN SMALL LYMPHOCTES IN THE PERIPHERAL BLOOD AND IN A PERITONEAL EXUDATE

Source	Normal guinea-pigs		Tuberculin sensitized guinea-pigs	
	Per cent active cells	Mean No. of lysosomes per cell	Per cent active cells	Mean No. of lysosomes per cell
Peripheral blood	35 (3)	5.1	80.6 (3)	11.5
Peritoneal exudate	35.2 (5)	3.5	92.2 (5)	9.8

Figures in parentheses indicate the number of animals examined.

In an investigation of this type it is important to differentiate cells of the lymphocyte series from macrophages. Macrophages can be distinguished easily because their lysosomes appeared to have a more fragile lysosomal membrane and consequently the lysosomes stain for acid phosphatase after a shorter incubation time than cells of the lymphocyte series. Lymphocyte lysosomes would appear to be more stable than those of macrophages. Large cells of the lymphocytes series with a basophilic cytoplasm (immunoblasts) were seen in lymph nodes draining the area of application of a chemical-sensitizing agent, reaching a peak in concentration on the fourth day after sensitization. A high proportion of these cells contained lysosomes. However, these lysosomes were present in smaller numbers and were far less fragile than

those seen in macrophages. These cells would appear to be similar to those described by previous authors<sup>1,2</sup> in studies on the lysosome content of lymphoid cells in tissue cultures containing phytohaemagglutinin.

On the days immediately following the application of a chemical sensitizing agent to the skin, there was a marked increase in the number of small lymphocytes containing lysosomes in the draining lymph node. The number of active lysosomes in an active cell also increased, reaching a peak on the fourth day after sensitization. It is not known whether these cells were precursors of the immunoblasts, or were derived from them by cell division. However, they were still present in large numbers on the sixth day after sensitization, at a time when it was known that the number of immunoblasts had fallen considerably<sup>3,4</sup>. It would therefore be unlikely that these cells were about to differentiate into immunoblasts.

It may also be that small lymphocytes containing lysosomes are immunologically competent cells. It is therefore interesting that there is an increased number of small lymphocytes with lysosomes in animals injected with tubercle bacilli in water-in-oil emulsion and that these are also much larger in size and increased in number. This may also have some bearing on the increased immunological responsiveness of animals injected with Freund's adjuvant. One might also speculate on whether the small lymphocytes which contain lysosomes are the effector cells in delayed-type hypersensitivity. However, in preliminary experiments in which tuberculin has been injected intraperitoneally in a dose sufficient to cause aggregation of macrophages (10 µg PPD)\* no significant change was found in the lysosomes of either small lymphocytes or macrophages.

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### Precocious Anti-diphtheria Response Induced by RNA Immuno-carrier

In previous papers an increase of the RNA content in the serum of rabbits immunized with horse serum albumin and rat or guinea-pig red blood cells was observed; the greatest increase was in the gamma-globulin fraction. The RNA extracted from immune rabbit sera (RNA Immuno-Carrier or RNA-I-C) was able to induce in normal rabbits a precocious antibody production against the same antigens used for immunizing the animals' source of the RNA<sup>1,2</sup>.

It was then found that RNA-I-C from the serum of animals immunized with heterologous RBC was able to induce rapid antibody production in normal animals of different species<sup>3</sup>. The possibility was also demonstrated of obtaining a precocious auto-haemantibody response in normal rats treated with a suitable amount of RNA-I-C from serum of rabbits immunized with rat RBC<sup>4</sup>.

The following investigations were undertaken to ascertain whether it is possible to induce a precocious antibody response in normal animals with RNA from serum of animals immunized against diphtheria toxin. Further-

more, because in preliminary experiments an attempt to obtain an effective RNA-I-C from 'stored' antitoxic horse serum was unsuccessful, we tested whether the RNA content of 'stored' immune sera was changed, and whether the capacity of inducing immunity in normal animals was modified in RNA extracted from 'stored' immune sera.

Female rabbits weighing 1.8-2.0 kg, fed on a standard diet, were immunized with 200 Lf of diphtheria toxoid ('Anadifterall' Solavo) given by four intravenous injections at four-day intervals; seven days after, a fifth injection of 50 Lf was made and, nine days afterwards, the blood of the animals was collected. One part of the serum was 'stored' at +2° C for 40 days, the remainder was used at once.

The RNA was estimated and extracted from anti-diphtheria rabbit sera 'fresh' or 'stored', according to the technique described in a previous paper<sup>3</sup>; as a control, anti-diphtheria horse serum (from Solavo, Siena, Italy) 'stored' for 40 days was used as a source of RNA.

The RNA so obtained was administered by a single intravenous injection to normal rabbits, 1.5 mg/kg body-weight; the animals were bled 48 h after the RNA injection and the sera were used in the diphtheria toxin neutralizing tests.

Four groups of male guinea-pigs weighing about 250 g (with the abdominal skin previously shaved) were used: to the first group was given subcutaneously, in a final volume of 4 ml, a mixture of various amounts of diphtheria toxin (1 ml. = 1,000 MLD; from Solavo, Siena, Italy) corresponding to 1; 2.5; 5; 7.5; 10; 15; 20 MLD, pre-incubated for 1 h at 37° C with 0.05 ml. of serum from rabbits treated with RNA-I-C from 'fresh' antitoxic serum. The second group was treated in the same way but using serum of rabbits treated with RNA-I-C from 'stored' antitoxic serum; the third and fourth groups were used as controls, using normal and hyperimmune rabbit's serum respectively.

The MRD was determined in a group of rabbits by several dilutions of toxin according to Jensen<sup>4</sup>. The MRD so determined was injected in a final volume of 0.1 ml. into the previously shaved abdominal skin of the rabbits treated 24 h before with RNA-I-C and, as a control, of hyperimmune rabbits.

Table 1. EFFECT OF 'STORAGE' ON THE RNA CONTENT OF IMMUNE SERUM

RNA mg/100 ml.	Fresh serum 9.64 ± 0.57	Stored for 40 days 5.50 ± 0.75
Per cent	100.00	58.00

Table 2. EFFECT ON GUINEA-PIGS OF VARIOUS AMOUNTS OF DIPHTHERIA TOXIN NEUTRALIZED BY VARIOUS ANTITOXIC SERA *in vitro*

Guinea-pigs treated with toxin neutralized by:	MLD of diphtheria toxin						
	1	2.5	5	7.5	10	15	20
Serum from rabbits treated with RNA-I-C from 'fresh' serum	—	—	—	—	—	—	local hyper- aemia
Serum from rabbits treated with RNA-I-C from 'stored' serum	—	—	local oedema	death	death	death	death
Normal rabbit serum	death	death	death	death	death	death	death
Hyperimmune rabbit serum	—	—	—	—	—	—	—

Table 3. *In vitro* NEUTRALIZATION OF THE DIPHTHERIA TOXIN MRD IN RABBITS BY VARIOUS ANTITOXIC SERA

	Dilutions								
	1:500	1:1,000	1:2,000	1:4,000	1:8,000	1:16,000	1:32,000	1:64,000	1:128,000
Serum of rabbits treated with RNA-I-C from 'fresh' serum	—	—	—	—	—	—	—	—	—
Serum of rabbits treated with RNA-I-C from 'stored' serum	—	—	—	—	—	—	—	+	+
Hyperimmune rabbit serum	—	—	—	—	—	—	—	+	+
Normal rabbit serum	—	—	+	+	+	+	+	+	+

Key: —, no skin reaction within 48 h; +, 8 mm erythematous halo within 48 h.

In addition, MRD neutralizing tests were made: dilutions of various antitoxic sera from 1:500 to 1:128,000 were incubated for 4 h at 37° C with the MRD of toxin and then injected, in a final volume of 0.1 ml., into the shaved abdominal skin of normal rabbits. Four groups of animals were used: to the first group was administered MRD pre-incubated with serum of rabbits treated with RNA-I-C from 'fresh' antitoxic serum, to the second the MRD pre-incubated with serum of rabbits treated with RNA-I-C from 'stored' antitoxic serum; in the third group and in the fourth the MRD pre-incubated with hyperimmune and normal rabbit serum was used.

The immune serum 'stored' for several days at +2° C undergoes a perceptible decrease of its RNA content so that, after 40 days, it is about half that of 'fresh' serum (Table 1).

RNA from 'fresh' anti-diphtheria toxin rabbit's serum, when injected into normal rabbits, elicits an antibody response which can protect guinea-pigs from the effects of the diphtheria toxin (neutralization test *in vitro*): in all the animals injected with toxin pre-incubated with serum of rabbits treated with RNA-I-C from 'fresh' antitoxic serum no paresis was found, and a hyperaemic and oedematous halo at the point of injection was observed only at the highest dose of toxin; the animals of this group were still living sixty days after treatment. Similar results were obtained in the fourth group in which antitoxic hyperimmune serum was used. All the animals of the third group, in which toxin neutralized with normal serum was used, were dead within 96 h, surviving according to the dose injected. Finally, in the guinea-pigs of the second group, in which toxin neutralized with serum of rabbits treated with RNA-I-C from 'stored' antitoxic serum was used, only those animals treated with 1, 2.5 and 5 MLD survived sixty days after treatment; the others were dead within 96 h (Table 2). Similar results were obtained using RNA-I-C from 'stored' antitoxic horse serum.

The MRD of diphtheria toxin did not induce the formation of a hyperaemic halo within 48 h in the rabbits which had received antitoxic RNA-I-C 24 h previously, or in the rabbits immunized with toxoid, used as a control. The MRD pre-incubated with normal rabbit's serum did not give a typical reaction within 48 h until serum dilutions reached 1:1,000; in the rabbits which had received the MRD neutralized with serum from animals treated with 'fresh' RNA-I-C, it was neutralized by serum diluted 1:128,000; in the rabbits treated with the MRD neutralized by serum from rabbits injected with 'stored' RNA-I-C the highest dilution able to prevent the typical hyperaemic reaction was 1:32,000 (Table 3).

As observed in our earlier work, it appears that it is possible to obtain from hyperimmune anti-diphtheria rabbit serum an RNA (RNA-I-C) that can induce a precocious anti-diphtheria response when administered by a single intravenous injection to normal rabbits. The serum of animals treated with this RNA could neutralize the diphtheria toxin *in vitro*. Storage of immune sera at +2° C affects their RNA-I-C content so that the 'stored' immune sera have a greatly decreased capacity to induce a rapid antibody response by their RNA-I-C; it is very probable that the responsibility for that is the high ribonuclease content of the serum<sup>5</sup>. Further investigations are in progress.

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## PATHOLOGY

### Localization of Tumours and Drugs on the Chorio-allantoic Membrane of the Chick Embryo

BECAUSE the chick embryo is immunologically immature during the first two weeks of its development, heterologous tissues such as human and rat tumours can be grown on the highly vascular chorio-allantoic membrane (CAM) of this organism without the necessity of pre-treating it in order to suppress the immune response. Tumours grown in this way have been used to assess the effects of potential anti-tumour drugs<sup>1-4</sup>.

The technique, although seemingly very simple, is not without difficulties, and some of these are sufficiently severe to make interpretation of the results, if not impossible, certainly problematical. Two difficulties in particular have prevented the wider use of this method in testing for anti-tumour drugs: the large variation in growth rate of implanted tumour fragments, which results in uncertain control values in relatively small groups of eggs, and the sharp increase in operative mortality when successive doses of the drug under test are administered into the yolk sac or intravenously, which are the usual routes of drug administration in this system.

We sought to overcome these disadvantages by applying disks of the kind shown in Fig. 1 to the CAM and filling the six peripheral compartments with tumour cells, and the larger central well with the drug to be examined.

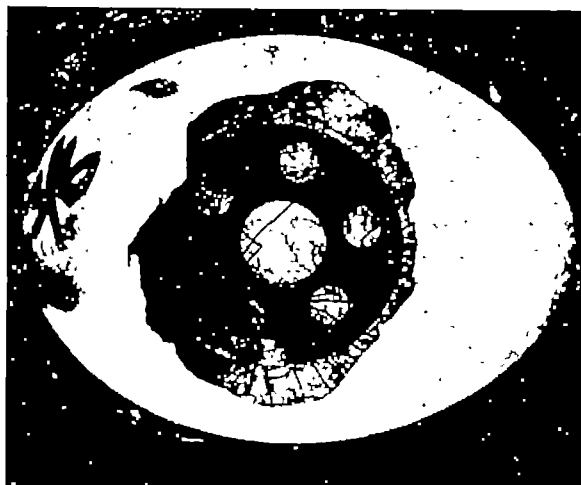


Fig. 1. Disk in place on the vascular CAM. The window in the shell is much enlarged in order to show the whole of the disk in position.

Our aim was twofold; first, if an approximately equal number of tumour cells were to fill the compartments of the disks, then it seemed possible that tumour nodules of approximately equal size might develop. Depression of size or weight of the nodules by a drug would then be amenable to relatively simple assessment. If, on the other hand, the tumours were found to be of different sizes, then because six tumours instead of the usual one could be grown on one CAM, statistical evaluation again of size, weight, or possibly number of 'takes' might be on somewhat firmer ground.

A second aim was to be able to give drugs in a technically simple and consistent way without at the same time applying them directly to the tumour cells, as might be done in tissue culture. It will be seen that the method we describe goes some way towards achieving these aims. (An outline of this work was given to the British Pharmacological Society, Bristol, 1964, ref. 7.)

Fertilized White Leghorn eggs were incubated at 103° F and implantation was carried out on the 8th day of incubation. The method of approaching and dropping the CAM was essentially that of Ballantyne<sup>5</sup>.

Disks were placed on to the 'dropped' CAM through the window made by removing a portion of the shell and the underlying shell membrane. They adhered closely to the CAM, the holes in the disks forming six tight outer compartments and a central well, all having the vascular CAM as their base. The disks were cut from black polythene sheet 0.0125 mm thick and each weighed 40 mg. Before use they were heat sterilized between sheets of filter paper to prevent them from curling. The disks were used once only.

In order to see how watertight the compartments were when the disks were in position on the CAM, methylene blue was dropped into them in one experiment and eosin in another. Any tendency for liquids to run together under the disks could then be followed with the naked eye when methylene blue was used, or under ultra-violet light in the case of eosin.

In the present experiments, only the Walker 256 rat carcinosarcoma in the form of a suspension of cells was used, though in another series of experiments to be reported later, other tumours were also examined.

The inoculated cells were derived from either the solid or ascitic forms of the tumour. Cells from the solid Walker tumour were packed by centrifugation and administered either in this highly concentrated form (100 per cent packed cells) or as a 10 per cent cell suspension in sterile saline. Cells from the ascitic tumour were concentrated in a similar manner; the dense upper layer of the cell pellet being withdrawn into a 0.25-ml. syringe and used directly for inoculation (100 per cent ascites cells). All suspensions were applied dropwise through a 26-gauge needle to each of the six compartments in rotation, until the total volume had been given. This varied from 0.003 to 0.03 ml. according to the cell concentration. After inoculation of the cells the eggs were sealed with 'Sellotape' and returned to the incubator.

Solutions of drugs used, in the concentration indicated in Table 1, were applied to the central well at intervals between 0 and 96 h after tumour inoculation through a small temporary slit in the 'Sellotape' seal. Drugs used were bis(2-chloroethyl) methylamine hydrochloride (mustine hydrochloride; HN2) and cyclophosphamide. They were given in a total volume of 0.05 ml. per egg. All drugs were given as a single dose except in the last experiment (see Table 1).

Eggs were inspected daily and embryos that had died before the 6th day of incubation were discarded. At that time all the embryos were killed. A segment of the CAM sufficiently large to include the disk and about 5 mm of the surrounding membrane was cut out and placed in neutral buffered formalin. The fixed membranes were then examined for the presence of tumour in the six compartments. Tumour growth in any one compartment

Table 1. EFFECT OF DRUGS ON NUMBER OF TUMOUR 'TAKES'

Tumour inoculum	Tumour (ml.)	Total dose/egg Drug	Total 'takes'	Total 'non-takes'	Variance
10% cell suspension	0.03	none (control)	10	20	0.0105
	0.03	25 $\gamma$ HN2 (72 h)	7	35	
	0.03	none (control)	21	21	
	0.06	20 $\gamma$ HN2 (0 h)	2	22	0.0151
	0.03	none (control)	21	21	
100% packed cells	0.03	500 $\gamma$ OPA (0 h)	4	14	0.0196
	0.03	none (control)	14	23	
	0.03	25 $\gamma$ HN2 (72 h)	10	20	
	0.003	none (control)	9	39	0.0038
	0.003	25 $\gamma$ HN2 (24 h)	2	52	
100% ascites cells	0.01	none (control)	16	2	0.0319
	0.01	50 $\gamma$ HN2 (48 h)	4	8	
	0.01	none (control)	15	9	0.0114
	0.01	2 $\times$ 25 $\gamma$ HN2 (48 and 96 h)	2	46	

Total 'takes' = The sum of the number of 'takes' on each disk in the group.  
Total 'non-takes' = The sum of the number of 'non-takes' on each disk in the group.

OPA = Oxyphosphamide.

All disks had 6 inoculation sites. Evaluation was made at 8 days. Cell suspensions were made from solid Walker tumour and ascites cells were derived from the ascitic form of the tumour which included, beside tumour cells, red, white and peritoneal cells.

was rated as 1 'take' irrespective of whether one or more than one tumour nodule had developed in that compartment.

Representative samples of control and treated membranes were subjected to histological examination.

Twenty-four hours after applying the disks to the CAMs the mortality rate of the chick embryos was approximately 25 per cent of the total number of eggs operated. This rather high rate was similar to that of sham-operated eggs. Initial survival was thus not impaired by the presence of the disks on the CAMs and neither was their survival after a further 5 days incubation. Reaction of the membrane to the disks was minimal and confined to a slight thickening around the cut edges of the disks.

The number and size of blood vessels that ran across the bed of each compartment varied considerably (Fig. 1). Equally variable, taking all experiments together, appeared to be the number of tumour 'takes', but in those experiments where 100 per cent cell suspensions were used, greater uniformity of tumour development seemed to be achieved. The tumours, although derived from cell suspensions, showed a high degree of localization (Fig. 2), and while the cells might have been expected to be carried to distant sites, they grew only at or near the site of implantation. Macroscopically they did not metastasize either to other areas of the CAM or to the embryo and rarely spread beyond the outer perimeter of the disks. The experiments with methylene blue and eosin showed that a considerable degree of localization could also be obtained with solutions. When disks were properly applied, solutions were largely retained within the compartments in which they had been placed.

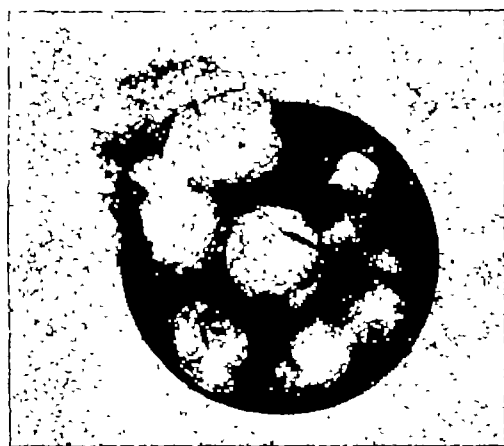


Fig. 2. Disk still attached to the membrane in order to show the localization of tumour nodules. Tumour derived from ascites cells

Histological examination of membranes on which disks only had been placed for six days revealed a little stromal thickening as the sole abnormality. In contrast the membranes on which nodules had developed as a result of tumour cell inoculation had numerous large nests of rapidly dividing cells of rat origin in the mesenchymal layer.

Because of the variation in numbers and size of the tumours which developed in different compartments, an analysis was made of 'takes' and 'non-takes' in the control and drug-treated eggs. The variance calculated for each of the experiments is given in Table 1. The method of comparison for all seven experiments taken together was that used by Lea<sup>8</sup> and gives a total variance of 0.1052.

The standard error is therefore  $\sqrt{0.1052} = 0.3243$ . Differences in proportion =  $2.1505 \pm 0.3243$ , that is 6.6 times the standard error, and  $P$  is less than 0.001.

Several facts emerge from these preliminary studies. First, that the CAM of the chick embryo is able to tolerate a disk of the dimensions and weight that has been applied without deleterious effects. Secondly, multiple localized foci of tumour growth can be produced by implantation of living tumour cells in the compartments of the disks, thus increasing the statistical value of each egg. Since application of drugs in the central well is also largely localized, little reaches the tumours by direct diffusion; and most of it is probably absorbed into the blood vessels or lymphatics which traverse the base of the well. Since applications are quick and technically easy, drugs can be given in equally or unequally divided doses, thereby reducing toxic effects of large single doses. This is all the more desirable since we have observed that the maximum dose tolerated by the embryo increases rapidly with increasing age, and while the maximum tolerated dose on the 1st day of the experiment may be ineffectual against the tumour, that on the 2nd day may have reached effective levels.

Thirdly, a comparison of 'takes' and 'non-takes' shows that known inhibitory substances produce a significant reduction in the number of 'takes' in the disks. This would appear to eliminate the necessity for weighing tumours from individual compartments or even the total tumour mass on each disk. It would also appear justifiable therefore to assess the potential value of a substance as an anti-tumour agent by its ability to depress the number of 'takes' in this system.

It is of course possible to vary the scheme and to test six different tumours in the peripheral compartments against one drug in the central well or indeed six different drugs against one tumour in the central well. Or one could of course use small fragments of solid tumour instead of the suspension that we have used.

Finally, six or seven different drugs may be placed into the compartments without tumour in order to assess their teratogenic effects alone or in combination.

We thank Dr. A. J. Lea for the statistical analysis of our results, E. V. Willmott and G. D. Leach for the photographs, and D. I. Duke and Ian Binks for their assistance.

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## ANATOMY

## Interrelationships of the Visual Cortex and Superior Colliculus in the Cat

It is well known that in the mammal the retina is related in a well-organized manner with the lateral geniculate nucleus of the thalamus and with the visual cortex<sup>1</sup>, on one hand, and with the superior colliculus<sup>2</sup> of the midbrain, on the other. In addition, a projection from the visual and other cortical areas to the tectum has been demonstrated<sup>3</sup>. Apart from a recent investigation in the rat<sup>4</sup> there is no evidence, however, to indicate whether the projection of the cortex on the tectum is topically organized and in particular whether the parts of the cortex and tectum related to a specific part of the retina are interconnected. This problem of the interrelationships of the visual cortex and the superior colliculus has been investigated in the cat in three stages by determining, first, the detailed projection of the retina on the lateral geniculate nucleus and the superior colliculus; secondly, the organization of the projection of the lateral geniculate nucleus on the visual cortex (and thereby the representation of the retina in the cortex); and finally, the manner of termination of the cortical efferent fibres to the superior colliculus.

Following the enucleation of an eye, fragmented fibres can be traced by the method of Nauta and Gygyax<sup>5</sup> to several sites<sup>6</sup>. On the ipsilateral side, these are laminae A1, the dorsal and ventral components of the central interlaminar nucleus and the central part of the medial interlaminar nucleus of the dorsal nucleus of the lateral geniculate body, the ventral nucleus of the lateral geniculate body, the whole extent of the pretectal area (particularly the nucleus of the optic tract) and the strata opticum, griseum superficiale and griseum intermediale of the superior colliculus. On the contralateral side, terminal degeneration is found in laminae A and B, the dorsal and ventral components of the central interlaminar nucleus and the medial and lateral parts of the medial interlaminar nucleus of the dorsal lateral geniculate body, the ventral lateral geniculate nucleus, the pretectal area and nucleus of the optic tract, the same layers of the superior colliculus, and in the lateral and medial terminal nuclei of the accessory optic tract. The density of the terminal degeneration in the superior colliculus is considerably greater on the contralateral side.

The details of the representation of the retinal quadrants on the dorsal lateral geniculate nucleus<sup>7</sup> and the superior colliculus<sup>8</sup> were examined by placing small electrolytic lesions in different parts of the retina. That part of the retina lateral to the area centralis, the temporal retina, projects to the above sites on the ipsilateral side, while fibres from the retina on the nasal side of the area centralis are distributed only to the contralateral nuclei. The superior quadrants of the retina project to the anterior part of the lateral geniculate nucleus and to the posterolateral part of the superior colliculus, while the inferior quadrants project posteriorly and antero-medially respectively. In the lateral geniculate nucleus the horizontal retinal meridian is represented obliquely from posterolateral to antero-medial just behind the middle of its antero-posterior extent as viewed from the dorsal aspect, while on the colliculus this meridian is disposed obliquely from postero-medial to antero-lateral; the representations of the vertical meridian are co-extensive with the medial and anterior margins of the lateral geniculate body and superior colliculus respectively. Very small lesions in the immediate vicinity of the area centralis have shown that it projects to a little behind the middle of the medial margin of the lateral geniculate body and to the antero-lateral edge of the superior colliculus, and also that the degeneration from such lesions occupies a disproportionately large area compared with similar lesions at the periphery of the retina. There is also a well-defined

organization in the retinal projection to the interlaminar nuclei of the lateral geniculate body. In the central interlaminar nucleus the terminal degeneration found in both its dorsal and ventral components on each side always adjoins that present in the main laminae. In the medial interlaminar nucleus the upper and lower parts of the retina project respectively anteriorly and posteriorly; the central area is represented in the more dorsal parts of the nucleus, while peripheral retinal lesions cause degeneration in the ventral half. As the temporal hemiretina projects to these nuclei on the ipsilateral side, and the nasal on the contralateral, it is clear that there is an additional representation of the entire retina in each of the nuclei<sup>9</sup>.

The organization of the projection of the lateral geniculate nucleus on the cortex has been defined by correlating the site and extent of retrograde cellular changes in the thalamus following lesions of various sizes in the visual cortex as recently defined<sup>10</sup>. The nucleus projects ipsilaterally to areas 17, 18 and 19 in such a way that its anterior and posterior parts project to the corresponding portion of these areas<sup>11</sup>; in the medio-lateral dimension, however, there is a reversal in the thalamo-cortical relationship, in that medial and lateral parts of the nucleus project to the lateral and medial portions respectively of the cortex. The cellular degeneration in the central interlaminar nucleus is found to be co-extensive with that in the main laminae. Cellular degeneration is found in the medial interlaminar nucleus only when the lesion extends beyond area 17, but whether this nucleus is connected with area 18 or with area 19 or both has not been determined. The retina is therefore represented in the visual cortex with the superior quadrants anteriorly and the inferior quadrants posteriorly; the area centralis is represented near the junction of the lateral and posterolateral gyri, and the periphery of the retina along the anterior, medial and posterior borders of the visual cortex. The representation of the area centralis is relatively greater than that of the rest of the retina. The finding of a separate retinal representation in the medial interlaminar nucleus together with the differential projection of this nucleus to area 18 or area 19 (or both) may provide the anatomical basis for the additional retinal representation recently found in these areas<sup>11</sup>.

Following lesions of the visual cortex, terminal fibre degeneration is found in several subcortical nuclei<sup>12</sup>; of particular relevance are the dorsal<sup>13</sup> and ventral nuclei of the lateral geniculate body, the posterior thalamic and pulvinar complex, the pretectum and nucleus of the optic tract, and the superior colliculus. The nucleus of the optic tract receives fibres from all parts of the visual cortex, but the rest of the pretectum is found to be related only to a small inferior part of the posterolateral gyrus<sup>14</sup>. The visual cortex projects on those layers of the superior colliculus which receive retinal fibres and to the stratum zonale<sup>5</sup> in a well-organized manner, the anterior part of the cortex being related to the posterolateral half of the colliculus, and the posterior cortex to the antero-medial half. Furthermore, any part of the cortex related to a specific part of the retina sends fibres to that part of the tectum which is directly related to the same part of the retina. The degeneration in the lateral geniculate nucleus after lesions in the visual cortex is localized to those areas which project to the same parts of the cortex. Fibres are found to project to the deeper layers of the superior colliculus from other neocortical areas<sup>8</sup>.

The observations recorded here, using experimental neuroanatomical techniques, have confirmed that there is a precise representation of the retina on both the visual cortex<sup>15</sup> and the superior colliculus<sup>8</sup> in the cat and, in addition, have shown that the visual cortex projects in an equally well-organized fashion on the same layers of the tectum. Thus, every part of the retina is related to a particular part of the tectum directly, and also indirectly via the thalamus and cortex. It may be noted that,

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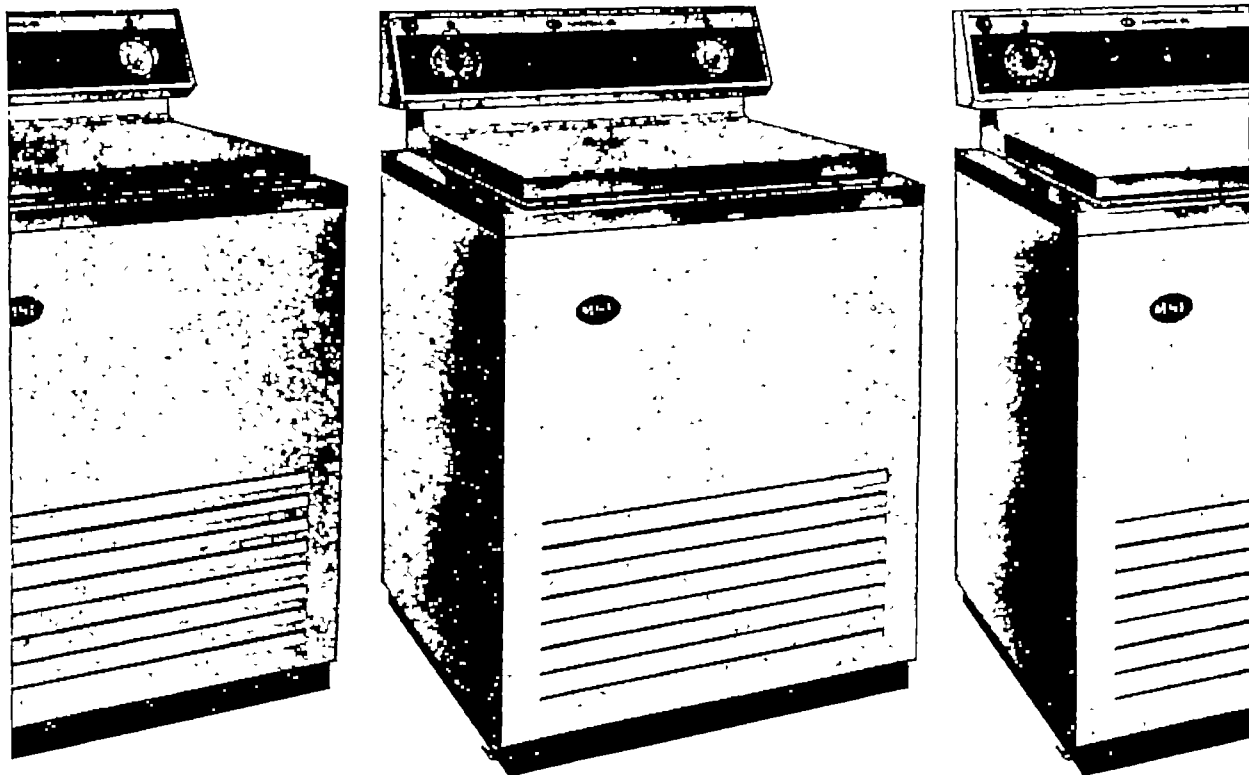
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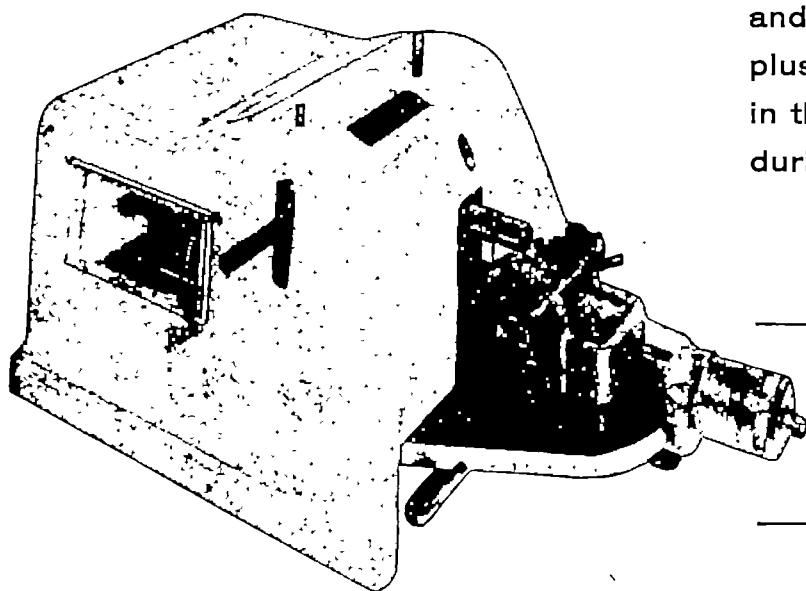
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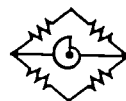
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RADIOBIOLOGY

Relation of Cell Size to the Effect of X-rays on Poliovirus Production by Monkey-kidney Cells

In several systems of animal cells in tissue culture, irradiation with either X- or gamma-rays progressively reduces the ability to produce virus (that is, the 'capacity') until it is between 1 and 10 per cent of the capacity of non-irradiated control cultures. The remaining capacity is less radiosensitive, so that a graph depicting residual capacity of the test population as a function of radiation dose tends to level off<sup>1-3</sup>.

Such a dose-response curve could indicate that the cells in the test population were of two types, with differing radiosensitivity. It was suggested that those cells the capacity of which was less radiosensitive might be larger than the other cells. Since virus production per cell increases with the size of the cell, the larger cells would synthesize more virus and might therefore require more irradiation to stop their virus synthesis<sup>4</sup>.

The evidence presented here supports that suggestion. X-irradiation reduced poliovirus yields from monkey-kidney cells of regular size more than it reduced the yields from giant monkey-kidney cells.

For these experiments, all test monolayers were prepared by plating suspensions of cells obtained from monolayers of primary monkey kidney, and those intended for giant-cell monolayers were then irradiated, as previously described<sup>5</sup>. Both the irradiated and the non-irradiated plates were incubated at 37° C in an atmosphere of 4 per cent CO<sub>2</sub> in air for 6 or 7 days, because previous experiments had demonstrated that by that time the average volume of the irradiated cells could be expected to be 4-6 times the average volume of the non-irradiated cells<sup>6</sup>. During the incubation period the medium was renewed every second day.

After incubation, the 2 groups of monolayers were separated into sets which were subjected to different doses of X-ray, ranging from 10,000 r. to 193,000 r. (see Table 1). The X-ray machine used for this irradiation was that used to prepare the giant cells and was operated at the same kV and amp (140 kV, 9 m.amp), but this time the beam was unfiltered. As controls, each experiment included one non-irradiated set of plates from each group of monolayers. In these experiments the X-ray dose corresponding to a 37 per cent survival point (the D<sub>0</sub>) was approximately 160 r. for cell multiplication and 120,000 r. for virus infectivity (Levine, unpublished data).

Table 1. EFFECT OF X-RAY DOSES ON POLIOVIRUS YIELDS FROM MONOLAYERS OF REGULAR AND OF GIANT MONKEY-KIDNEY CELLS

Experiment No.	Radiation dose (r.)	P.F.U. produced per cell		Percentage of yield from control monolayers	
		Regular	Giant	Regular	Giant
1	0	366	990	100	100
	10,000	187	1,000	51	113
	50,000	106	1,060	29	110
	100,000	106	1,060	29	110
2	0	200	425	100	100
	50,000	180	425	66	100
3	0	380	800	100	100
	100,000	72	480	20	60
4	0	37	160	100	100
	143,000	5	37	14	55
5	0	230	750	100	100
	193,000	50	465	19	62

After irradiation of the monolayers, the growth medium was removed and they were washed once with phosphate buffered saline<sup>7</sup>. Then test and control monolayers were infected with type 3 poliovirus (Leon strain) having a titre of 1.2 × 10<sup>6</sup> plaque forming units (P.F.U.) per ml. Each inoculum consisted of 0.5 ml. virus in 0.5 ml. of phosphate buffered saline. The infected monolayers were incubated at 37° C for 1 h to allow the virus to attach to the cells, and were then washed 3 times with phosphate-buffered saline, overlaid with Eagle's medium<sup>8</sup> enriched with 2 per cent calf-serum, and returned to the carbon-dioxide incubator until cells were destroyed, which was

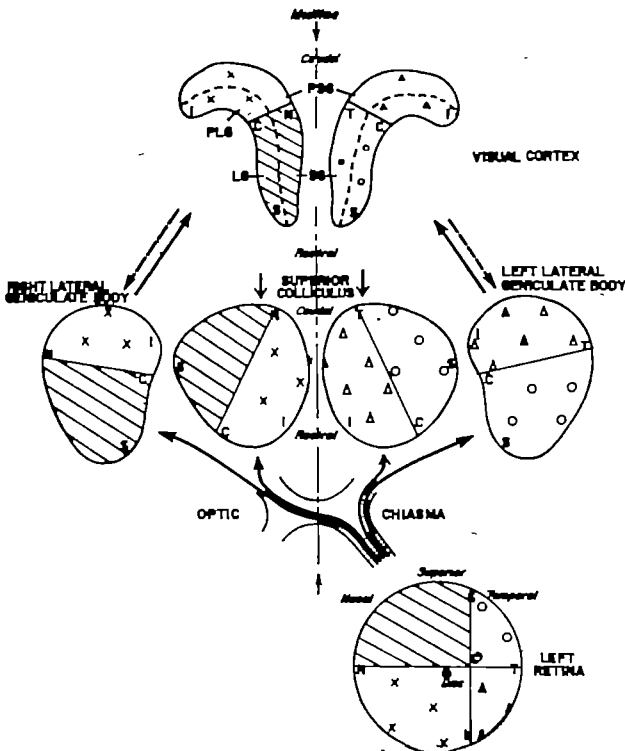


Fig. 1. Schematic diagrams of the organization of the retinal projection on the lateral geniculate nucleus (and visual cortex) and the superior colliculus, and of the visual cortical projection on the superior colliculus. Anterior view of left retina; dorsal orthographic reconstruction of lateral geniculate body and superior colliculus; schematic view of dorsal and medial surfaces of visual cortex (PLG, postlateral gyrus; LG, lateral gyrus; PSG, postsupralateral gyrus; SG, supralateral gyrus).

although the fibres from the retina and the visual cortex end in essentially the same layers, fibres from other cortical areas terminate predominantly in the deeper 'efferent' layers<sup>9</sup>. The functional significance of the high degree of organization of these connexions is not clear; it seems probable that it is of importance in the known role of the superior colliculus in the control of eye movements and also in the centrifugal system of fibres to the retina<sup>10</sup>. Considering the well-defined organization of the projection of the optic tectum on the nucleus of origin of the centrifugal fibres to the retina of the pigeon<sup>11</sup>, the retino-tectal fibres in the cat may serve as the afferent limb of a reflex loop. Through the cortico-tectal fibres, there is also the possibility of a cortical influence on the retina.

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usually 36–48 h after infection, when the virus was collected.

Virus yields were determined by plaque assay on monolayers of monkey-kidney cells. The number of cells per monolayer was determined for representative monolayers from each set before infection and was used to determine the average yield of virus per cell.

The effects of various doses of X-ray on poliovirus yields from monolayers of regular and of giant cells were compared (Table 1). Virus yields are expressed as the number of P.F.U. produced per cell and as the percentage of the yield from control monolayers. Although both the yield and the effect of the same radiation dose on the yield varied among the experiments, virus production by the giant-cell monolayers was always more radio-resistant than virus production by the regular-cell monolayers.

Although these results do not prove that the residual capacity of a cell population as a function of its radiation dose is related to the proportion of large cells in that population, they are compatible with such a relationship.

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### Electron Spin Resonances in Formicidae

THE investigation of radiation phenomena in living systems, where the diffusion processes can occur in a 'normal manner', is, as recently pointed out by Zimmer *et al.*<sup>1</sup>, a most important task in present electron spin resonance research. With this in mind, investigations on the effects of ionizing radiation on survival and behaviour of ants were extended into the field of microwave spectroscopy.

So far the following species of ants have been examined in their electron spin resonance responses: *Lasius umbratus* (Nyl.), *Tetramorium caespitum* (L.), *Formica exsectoides* Forel, *Formica fusca* L., *Camponatus castaneus* (Latr.), and *Pogonomyrmex occidentalis* (Cress). The electron spin resonance spectra were recorded for each species with a Varian 4,500 series, 100 kc/s field modulation spectrometer, using a single cavity resonator and spin resonance glass tubings of 5 mm diameter as container for the living ants. DPPH and pitch were used in dual sample cavity recordings as reference standards for evaluation and comparison of the spectra. In one case, *Tetramorium*, a quantitative determination of the *g*-value could be made by frequency and magnetic field measurements with calibrated equipment at the National Bureau of Standards.

Each ant species was checked repeatedly for its electron spin resonance spectrum at different settings of the spectrometer, at different time periods and for different batches of ants. *Lasius umbratus* (Nyl.) was also examined for changes in the signal after exposure to cobalt-60  $\gamma$ -radiation (dose rate 1,550 r./min), and *Formica exsectoides* was analysed for effects of annealing on the spectrum. As expected for ants as living systems and as found in seeds and other biological objects<sup>2,3</sup>, ants exhibited in the normal state already typical electron spin resonance signals. On the basis of these signals the investigated ants could be divided into two groups: Group 1—species which showed a strong, relatively narrow signal in the free radical region (the quantitative determination of the *g*-value of the *Tetramorium* signal gave a value of 2.012) and a weaker signal in the *g*-value range of 3.5–4 (Fig. 1); Group 2—species which gave a strong 6-peak signal

covering a range of about 800 gauss with peak-to-peak distances of about 100 gauss, modulated by a more or less pronounced signal in the free radical region (Fig. 2).

Irradiation—studied in Group 1 ants—caused an increase in height of the signal, followed by a slow decay with time after irradiation (Fig. 3), and annealing, explored in ants of Group 2, changed the 6-peak signal dramatically. Annealing for 2 h at 104° C completely depressed the 6-peak signal and enhanced the free radical region signal (Fig. 4), while samples treated more gently in the biological range showed smaller changes in the shape of the signal, which returned to its original form after standing in air for several hours at room temperature.

Interpretation and identification of the observed electron spin resonance spectra are possible on the basis of existing data on some other biological objects, on

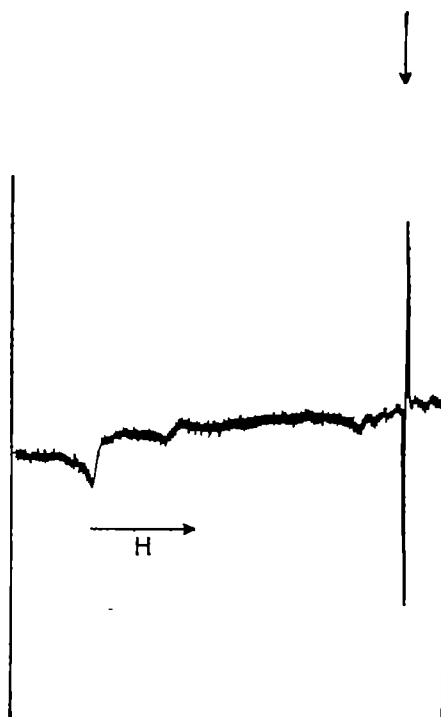


Fig. 1. Electron spin resonance pattern of normal ants. Group 1. Genera *Lasius*, *Tetramorium*, *Pogonomyrmex*

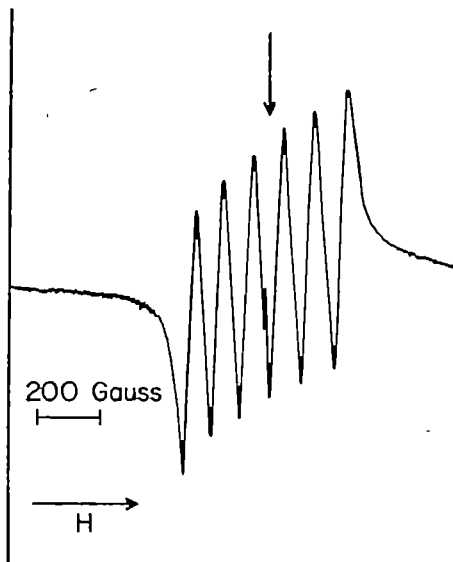


Fig. 2. Electron spin resonance pattern of normal ants. Group 2: Genera *Formica*, *Camponatus*

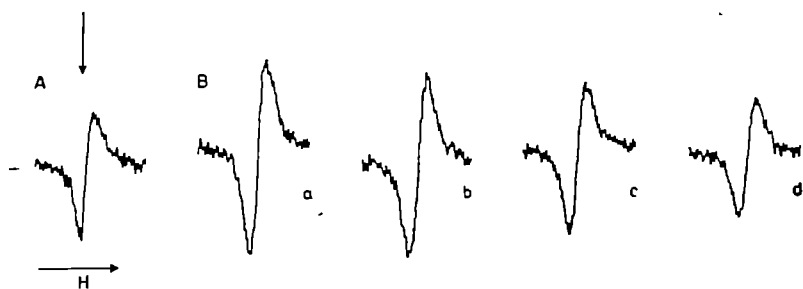


Fig. 3. Electron spin resonance pattern of  $\gamma$ -irradiated ants. Genera *Lasius*, *Tetramorium*, *Pogonomyrmex*. A, Control; B,  $\gamma$ -irradiated; a, 30 min after exposure; b, 60 min after exposure; c, 90 min after exposure; d, 120 min after exposure.

electron spin resonance studies in chemical and related systems as well as on survival studies with irradiated ants. The relatively narrow signal in the  $g = 2$  area without pronounced fine structure observed in ants of Group 1 compares favourably with the signals recorded for different kinds of seeds. In the seed experiments this signal has been interpreted as the result of the interaction of several processes occurring in dormant seeds, and a similar interpretation may be possible in the case of ants the chemical system of which is quite complex. Randolph and Haber<sup>4</sup> found in lettuce seeds a resonance with a  $g$ -value of perhaps 3.5, but emphasized its qualitative nature and did not give an explanation for it. Newer studies by Maling *et al.*<sup>5</sup> showed in nucleic acid complexes a signal with a  $g$ -value between 3.5 and 4, which could be related to iron. Cotzias<sup>6</sup> directed attention to the possible role of pigments in the genesis of the electron spin resonance spectrum.

The response of the ant signal to ionizing radiation has its counterpart in electron spin resonance examination with seeds. Here also the signal changed after irradiation. Increasing doses caused increase in height of the signal (number of magnetic centres), followed by a slow decay of the signal after end of the exposure. It would be of interest to investigate the relationship between the slow decay of the signal and the high radioresistance of ants, which in the case of *Tetramorium* was found to be in the order of 124,000 r. for  $LD(95)_{13}$ .

The 6-peak signal found in ants of Group 2 identifies itself by its length (600 gauss) and the peak-to-peak distance (100 gauss) as the manganese signal attributed to the  $Mn^{++}$ -ion<sup>7</sup>. This identification is supported by electron spin resonance investigations of living, manganese-containing biological objects and biochemical-chemical systems. Manganese-containing *Chlorella pyrenoidosa* exhibits a typical manganese signal which is dependent

on iron content and which changes reversibly under illumination<sup>8</sup>. Reversible annealing effects were observed in paramagnetic resonance spectra of heat-treated manganous preparations where the  $Mn^{++}$ -signal, weakened by the treatment, restored to its original form after standing for several hours in air at room temperature<sup>9</sup>, similar to the responses observed in the spectra of heat-treated *Formica*.

The work by Cohn and Leigh<sup>10</sup>, and by Mildran and Cohn<sup>11</sup>, using the paramagnetic properties of manganous ion for the examination of interaction mechanisms in enzyme-metal-substrate complexes, also invites speculation on the relationship

between these processes and similar responses in ants, which as living systems offer some intriguing challenges to electron spin resonance research.

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## BIOLOGY

### A Direct Response of the Crab *Carcinus* to the Movement of the Sun

ALTHOUGH it is at first sight unlikely that a crab should take note of the direction of the Sun's movement directly, two lines of evidence already suggest this conclusion. Crabs follow with their eyes the movements of a striped drum which is revolved around them at speeds even lower than one revolution per day<sup>1</sup>, and several species of lower Crustacea<sup>2</sup> as well as the Pacific shore crab *Hemigrapsus*<sup>3</sup> utilize some feature of the path of the Sun as a basis for directional escape movements in relation to the local direction of the sea.

An advantage in working with the crab *Carcinus* is that it responds by eye movements to the movement of a single small light in a dark room. When the crab is firmly held by the carapace the crab's eyestalks follow both horizontal and vertical movements of the light. For movements which subtend angles of  $0.02^\circ$  to  $1.0^\circ$  at the crab's eye at a lamp velocity of  $0.01^\circ/\text{sec}$  the horizontal movement is 5–45 per cent of the light's movement in the horizontal plane. The light used (1.5 V, 45 mamp, 'Pinlight', Kay Electric Co., Fairfield, New Jersey) gives an illumination of approximately 0.0005 lux on a surface in the position of the crab's eye. Further work is being carried out to analyse the main attributes of the response to small lights, but the figures given here make it evident that the crab's visual system is quite able to respond directly to the movements of the Sun, full Moon ( $0.24$  lux) and perhaps some of the brighter stars.

Since the movement of the Sun has continuously changing horizontal and vertical components, the straightforward demonstration of the crab's response in the

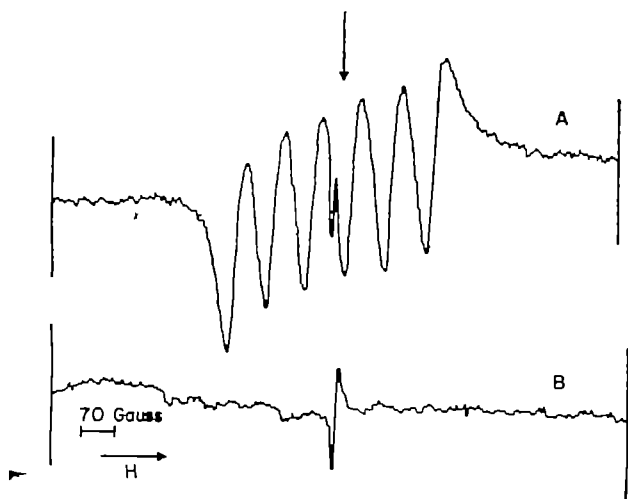


Fig. 4. Electron spin resonance pattern of heat-treated ants. Genera *Formica*, *Osaponatus*. A, Normal; B, heat-treated.

horizontal plane has been restricted to the position at noon on midsummer day when the rate of change of altitude of the Sun passes through zero and the rate of change of azimuth (which is the projection to the horizontal plane) was  $25.3^\circ/\text{h}$  at St. Andrews (lat.  $56^\circ 20'$ ). Movement of the crab's eye was recorded with a light backwardly directed flag (weight 3 mg) mounted on the eye, and the flag moved over a horizontally mounted photocell (Texas, type LS.221). The crab's carapace was clamped facing due east at the inclination typical of normal posture, so that the Sun's azimuth moved from front to back across the right eye. The left eye faced a large sheet of black paper which continued right around the crab so that, apart from the support for the recording photocell at the rear end of the crab, no contrasting objects were present in the visual field except the horizontal artificial horizon so formed. This is essential for the experimental demonstration, because any contrasting object in the visual field cuts down the movement of both eyes, though the apparent velocities are greater for eyes which do not follow the moving object.

The horizontal movements of the right eye in the situation already described are shown in Fig. 1 with the movement of the Sun's azimuth drawn as a continuous line at half the vertical scale. As in the response to a striped drum, the eye movement is slower than the Sun's movement, being 10–40 per cent of it when few stationary contrasting objects are in the visual field. That the movements truly arise from the Sun was shown by reversing the apparent movement with a mirror. To do this, the Sun was shaded from the right eye by a large piece of black paper and a mirror to the north of the crab was used to deflect the Sun's rays on to the left eye. For the crab as a whole the motion is then reversed and the right eye is driven by the movement of the left eye. The response fails when the Sun is altogether shaded from the eyes for a short time, as in Fig. 1.

The latency of the response to the movement of the Sun, and therefore the minimum time taken by the animal to realize its escape direction, is near 10 sec of time, as shown by direct measurement from the records of the eye movements. This means that a movement of the Sun of 2 min of arc is necessary before a response is apparent above the noise-level, which is mainly caused by tremor of the eyestalk muscles.

The function of this response is not known. The reaction may be no more than a useful indicator that the animal can perceive the celestial movement directly. A possible relation to the ability of the crab to make towards the sea<sup>4</sup> when stranded on land is suspected but not yet proved. The horizontal movement of the eye cuts down the horizontal movement of the Sun across the eye, so that the rate of change of altitude of the Sun becomes a relatively more pronounced variable for the crab. This is for two reasons: first, because vertical eye movements take place over a limited range of about  $5^\circ$  in the crab;

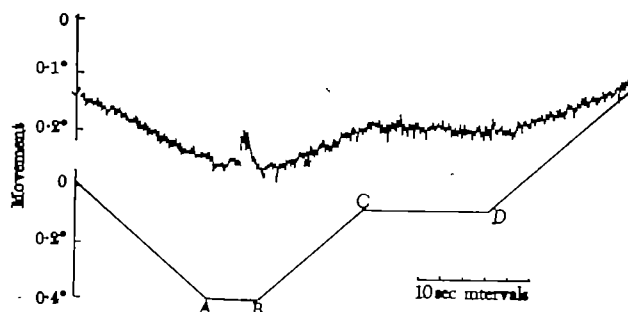


Fig. 1. Horizontal movements of the right eye of a crab *Carcinus* facing east in response to the Sun's movement at noon. At A the Sun was obscured with black paper and at B was reflected from a mirror on to the left eye. Over the period C D the mirror was turned so that the crab had no direct or indirect view of the Sun. Note that the scale of the eye movements is twice that of the horizontal projection of the Sun's movement. Downward movement on the record is towards the west.

the eye then fails to make any more vertical response so that the vertical component of the Sun's speed becomes the vertical slip speed; and secondly, because the vertical component of the Sun's movement is too slow during most of the day to elicit a following movement by the eye. If efficient horizontal optomotor responses reduce or eliminate the horizontal component of the celestial movement, the change of Sun's altitude remains as the feature which stimulates the receptors in succession across the retina. Measurement of rate of change of altitude requires a reference to the vertical and for *Carcinus* regulation by the statocyst reflex in the pitching plane results in an eye movement of only about  $1^\circ$  for a  $10^\circ$  displacement of the crab's body on its transverse axis. For an animal without sense of time of day an error of  $\pm 1^\circ/\text{h}$  in estimation of the vertical velocity of the Sun in summer would result in an error of approximately  $\pm 9^\circ$  in the estimation of direction of escape relative to the Sun's azimuth at that moment. By using such a method the animal could substitute the Moon for the Sun with a further error which is at most equal to the inclination of the two orbits,  $5.18^\circ$ . Such a mechanism would also explain the orientation of the shore amphipod *Talitrus saltator* by moonlight<sup>5</sup> without reference to an intrinsic lunar rhythm. More sophisticated navigational abilities are not known in Crustacea; but it is possible that other animals which move towards home or in definite compass directions have the crab's ability to follow rather accurately the celestial movements.

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### *Cercospora herpotrichoides* Fron on Gramineous Hosts in Ireland

CLIMATIC factors are known to affect greatly the prevalence of cereal eyespot caused by *Cercospora herpotrichoides*, leading to great variation in disease intensity in different seasons. The work of Sprague<sup>1</sup> shows that susceptibility of various gramineous species is a function of the environment; many grasses became infected in near optimum greenhouse conditions, whereas only a few species were found to be naturally infected during an intensive 7-year search in the north-western United States. These included *Bromus* and *Agropyron* species. *Agropyron repens*, however, was uninfected in the field though susceptible in the greenhouse. The very pronounced climatic effect on disease incidence and on the host range of the causal agent is shown by the prevalence of eyespot on spring-sown cereals in Scotland compared with the drier parts of southern England<sup>2</sup>, and on oat crops in the humid conditions prevalent in Ireland<sup>3</sup>. There are few records of the natural occurrence of *Cercospora herpotrichoides* on grasses in Europe, but in Holland the organism was isolated from *Alopecurus myosuroides*, *Poa pratensis* and *Apera spica-venti*<sup>4</sup>. Hosts found in Finland included *Festuca* and *Poa* species as well as *Alopecurus pratensis* and *Agropyron repens*<sup>5</sup>, while an isolate was obtained from *Agrostis stolonifera* at Rothamsted<sup>6</sup>.

In the Columbia Basin in the United States some of the prevailing grasses are claimed to be native hosts of this fungus<sup>1</sup>. The role of possible susceptible pasture and weed grasses in the more disease-prone areas of Europe cannot be overlooked. Such grasses could increase the inoculum level in the intensive cereal-producing districts while being the main if not the only factor responsible for survival of the fungus where cereals are grown infrequently.

During the summer and autumn of 1961, 1962 and 1964, studies were made of the incidence of eyespot infections on grasses in Ireland. Various grasses were collected in and adjacent to eyespot-infected wheat crops. Stems showing lesions were surface-sterilized and plated on potato dextrose agar. When a fungus resembling *Cercospora herpotrichoides* grew from the tissue, disks were obtained from pure culture and set to sporulate for identification.

Other than the cultivated cereals the only species found infected in 1961 and 1962 was *Agropyron repens* (couch grass). The only occurrence of the fungus on couch in 1961 was from an intensively cropped Co. Kilkenny wheat field, where one infected plant was found among 340 examined. This isolate, grey-black in colour, was darker than 12 isolates obtained from wheat, these varying from medium to very light grey. This colour difference persisted when the isolates were grown on different media and re-isolated after cross-infection on to wheat and couch in the greenhouse. In comparative studies on the isolate from *Agropyron repens* and a typical isolate from *Triticum vulgare* it was found that the latter had a somewhat greater growth rate (though not significant at the 5 per cent level), that it sporulated more rapidly on colonized oat grains, and caused earlier lesion development on wheat seedlings. There was no significant difference between spore length measurements of the 2 isolates. The isolates were compared for pathogenicity on *Agropyron repens* and *Triticum vulgare* in a replicated experiment (Table 1).

The couch isolate was significantly more pathogenic on couch at the 0.1 per cent level than was the wheat isolate, while the greater resistance of couch than wheat even to the isolate from couch was significant at the 1 per cent level.

In 1962, *Cercospora herpotrichoides* was isolated from *Agropyron repens* from wheat fields in counties Wexford, Waterford and Carlow. In all cases fewer than 5 per cent of shoots were infected, but the isolates in culture were variable in colour and indistinguishable from isolates obtained from wheat. It was not possible to undertake pathogenicity studies.

In 1964, a severe eyespot year, a high incidence of infected *Agropyron repens* was found in wheat crops examined in counties Meath, Waterford, Wexford and Carlow. The pathogen was also isolated from *Avena fatua*, *Lolium perenne*, *Phleum pratense* and *Cynosurus cristatus*.

A detailed study was made of an intensely couch-infected plot in Co. Waterford on which the fifth successive wheat crop was grown. On August 15, 6 random samples were taken using a circular hoop 18 in. in diameter. From each sample all wheat and couch stems bearing lesions were plated for subsequent identification of causal agents (Table 2).

Lesioned straws of wheat and couch not containing *Cercospora* most usually had *Rhizoctonia solani*, *Fusarium culmorum* or an unidentified Basidiomycete. The fact that the wheat straws were senescent by the time of isolation and may have been dominated by secondary organisms, whereas the couch shoots were still green, may be partly responsible for the practically equal recovery rate of *C. herpotrichoides* from couch and wheat. The result, nevertheless, illustrates the hitherto unrealized potential of a common species other than the cultivated cereals to act as host in a season favourable for infection in Ireland.

Table 1. MEAN PERCENTAGE INFECTION OF WHEAT AND COUCH STEMS BY ISOLATES OF *Cercospora herpotrichoides* FROM WHEAT AND COUCH

Source of isolate	<i>Triticum vulgare</i>	<i>Agropyron repens</i>
<i>T. vulgare</i>	96.1	20.8
<i>A. repens</i>	99.4	84.3
Control	0	0

Standard error of means = 2.1

Table 2. MEAN PERCENTAGE EYESPOT INFECTION OF 6 SAMPLES OF WHEAT AND COUCH FROM A COUCH-INFESTED WHEAT PLOT

	<i>Triticum vulgare</i>	<i>Agropyron repens</i>
Percentage infection	71.4	62.3

Standard error of means = 4.0

While strain examination of isolates collected in 1964 has not yet been made, the existence of different pathogenic types of the fungus shown in the 1961 studies cannot be ignored, more especially in view of the susceptibility of wheat. Different pathogenic forms of this organism relative to host specificity have been reported to date elsewhere<sup>4,7,8</sup>. Furthermore, the existence of a typical wheat isolate at least somewhat pathogenic on couch, depending on climatic conditions, illustrates the danger of higher levels of inoculum, resulting from narrow as well as broad host range types on grasses, within areas and during seasons conducive to disease development.

In tillage rotations and short-term leys the inoculum may be mainly in the form of colonized debris of the earlier-infected stubble crops. This is scarcely the case after the ploughing-up of a twenty-year-old pasture where the second spring-sown cereal on such ground often suffers severely and the first may have moderate infection. The possible role of the parasitic phase on Graminae in contributing to the inoculum-level requires thorough examination of the numerous unsown and wild species often found in such old pastures. The findings in the present studies are particularly relevant since eyespot lodging in cereal fields is often more pronounced within about 20 yards of the fence where there is greatest abundance of weed grasses such as couch.

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## Petrichor and Plant Growth

It is commonly believed that the advent of rain in regions long subject to drought or desert conditions is followed by an abnormally rapid response in natural seed germination in the areas concerned. As such climatic conditions are universally favourable to the accumulation and liberation of petrichor<sup>1</sup> from clays and other silicate minerals, as components of soils, it seemed possible that some of the substances which accompany the argillaceous odour of petrichor might favourably influence the germination of seeds. Our experiments have shown that there is a marked effect but that it is unexpectedly characterized by a retardation rather than an acceleration of germination and early plant growth. It was observed that minute amounts of the oily extracts prepared from various mineral sources significantly delayed the germination and growth of cereals, mustard and mixed grass seeds. Further, it was found that seeds, shown on moistened kaolinized granite or other source material which had been previously exposed to the atmosphere under the warm, dry conditions necessary for the development of petrichor, took longer to germinate and had a slower growth rate than seeds sown on source material that had not been exposed or that had been steam-distilled and oven-dried before use. An exposure period of 2-3 weeks at 60°-75° F with a relative humidity of less than 70 per cent was sufficient to produce a small difference in growth rate. This inhibitory effect increased with prolonged exposure of the source material.

Teo<sup>2</sup> has shown that fatty acids will inhibit the auxiliary bud development of *Nicotiana glauca* plants and that the methyl esters of acids C<sub>8</sub>-C<sub>14</sub> were particularly effective in this capacity.

The acid fraction of petrichor-containing extracts, which has been analysed in some detail, consists of a series of normal fatty acids  $C_8$ - $C_{18}$  (ref. 3) and a number of nitrophenolic compounds. Identification of the latter has not yet been completed, but as relatively substantial amounts of the acids  $C_8$ ,  $C_9$  and  $C_{10}$  are present, the extracts were fractionated and the basic neutral and acid portions tested separately for growth-regulating properties.

The tests were made by treating a layer of cotton wool in a Petri dish 3.75 in. in diameter with a light petroleum (b.p. <40° C) solution of the fraction in question. The petrol was allowed to evaporate at room temperature, after which the cotton wool was moistened with 30 ml. water and sprinkled with seeds. The germination and growth rates of seeds on test trays were compared with those of seeds on control trays where light petroleum only had been added before watering and seeding.

When 1-2 mg of test samples were used (that is, 30-70 p.p.m. of water added) all three fractions decreased the growth rate of cress, mustard and grass seeds, but the effect was much stronger with the basic fraction and with some acid fractions.

Fig. 1 compares the effect of 2.5 mg of a crude basic fraction on cress seeds (No. 11) with an untreated tray (No. 12). In this case the cress withered and died before reaching maturity. 0.6 mg of basic fraction (20 p.p.m. of water) was sufficient to retard growth by 24 h or more, but did not otherwise alter the habit of growth. When a pasture mixture of perennial rye grass, wild white clover, ladens clover, cocksfoot, cowgrass and *Phalaris* was treated with the basic material, the foliated species—which germinate several days earlier than the grasses—were slow growing and remained stunted, but the grass after germination grew strongly.

The nitrophenolic compounds were separated from the fatty acids by esterifying the acids with a 12 per cent solution of  $BF_3$  in methanol and extracting the acidic nitrophenolic compounds from a light petroleum solution of the esters with sodium hydroxide. The nitrophenols were recovered from the acidified aqueous alkali by extraction with petrol. The shoot growth of all seeds tested was retarded strongly by 0.5 mg or more of the mixed nitrophenols, while root growth, particularly in the case of grasses, was very vigorous.

Reference samples of stearic and palmitic acid when applied in amounts of 1-2 mg did not produce any noticeable retardation, but the same quantity of octanoic, nonanoic and decanoic acid did so. The methyl esters of the combined acids and a reference sample of methyl nonanoate tended to increase growth rates. This suggests that in this instance the inhibitory effect of acids may be a function of pH.

Petrol solutions of the total petrichor extracts and neutral fractions aged on standing a few weeks and lost their potency. This could result from either oxidation or interaction of the compounds in solution, but as the isolated basic and nitrophenolic fractions did not age readily, interaction appears to be the major factor.

It can be seen that the growth-regulating properties of these extracts are not due to a single inhibitory principle or to the acids alone, but to the combined action of a

number of components in the mixture. As the composition of extracts from source materials exposed in widely separated areas has been found to be similar, it seems likely that compounds associated with the formation of petrichor will have a significant effect on seed germination and early plant growth in areas intermittently subjected to prolonged dry periods.

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## MICROBIOLOGY

### Use of Streptomycin in the Separation of Nucleic Acids from Protein in a Bacterial Extract

It is well known that in bacterial extracts, in contrast to extracts from animal tissues, nucleic acids interfere markedly with the isolation and separation of proteins, for example in chromatographic separation on substituted cellulose columns. Protamine sulphate and manganese chloride<sup>1</sup> have been used to precipitate selectively nucleic acids from the protein; these methods have often proved difficult and have resulted in much loss of protein. More recently, streptomycin or dihydrostreptomycin<sup>2</sup> have been used. In order satisfactorily to separate the protein components of an extract of *Lactobacillus plantarum* (N.C.I.B. 6376) it was necessary to reduce the nucleic acid content of these extracts. The protamine sulphate method was found to be unreliable due to lack of reproducibility, even under rigidly controlled conditions. Accordingly, the streptomycin method was investigated.

The ratio of the 280 to 260 m $\mu$  absorbance gives an indication of the protein:nucleic acid ratio. In the untreated extracts from *L. plantarum* this ratio is usually 0.55 to 0.57. Substantial removal of the nucleic acids is indicated when the ratio is above 1.0. For purposes of comparison, a 280/260 m $\mu$  ratio of 0.7 is equivalent to about 0.12 mg nucleic acid/mg protein, and a ratio of 1.0 to about 0.033 mg nucleic acid/mg protein<sup>3</sup>. However, as these figures are only approximate, it is considered better to use the 280/260 m $\mu$  ratio.

The bacterial extracts were prepared in a Linnane blender<sup>4</sup>. The protein obtained by the addition of ammonium sulphate to 80 per cent saturation was dissolved in 0.1 M phosphate buffer, pH 7.0, and dialysed in a rotary dialyser against about 1,200 times the volume of 0.0016 M phosphate buffer, pH 7.0, at 5° C. This dialysis step was found to be of crucial importance and accordingly a highly efficient dialyser was constructed. With this apparatus and using 'Visking' dialysis tubing size 8/32 in., equilibrium was achieved for small ions (for example,  $PO_4^{3-}$ ,  $SO_4^{2-}$ ,  $Na^+$  etc.) in about 3 h. Thus, the buffer was changed after the first 3 h of dialysis and dialysis continued for a further 28 h.

The precipitation of nucleic acids with streptomycin depends on the salt concentration of the solution<sup>5,6</sup> and, to a first approximation, the salt concentration is inversely proportional to the specific resistance of the solution. At intervals the extract was removed from the dialyser and its specific resistance measured with a Philips conductivity meter (type GM 4249/01) and cell (PR 9510/00) at 0° C. Fractions of the extract were then diluted to 10 mg protein/ml. with dialysing buffer and streptomycin sulphate (10 per cent w/v solution, pH 7.0,

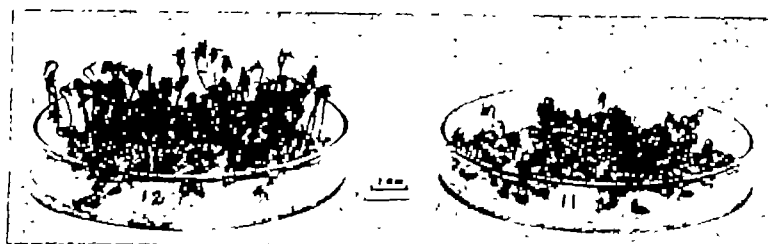


Fig. 1

Evans Medical Australia (Pty.) Ltd.) was added in the amounts indicated in Fig. 1. The extract was stirred continuously with a magnetic stirrer and kept at 0°C during the slow addition of the streptomycin. The solutions were then centrifuged and the absorbances of the supernatants at 280 and 260 mμ were measured in a Unicam 'S.P. 500' spectrophotometer.

Fig. 1 shows that the degree of selective precipitation of nucleic acids depends on the salt content of the solution. As the salt concentration is lowered, and the specific resistance rises, so the degree of selective precipitation improves. The optimal salt concentration depends on the salts present<sup>1,2</sup> and probably on the source of the extract as well.

Two additional features were noted in these experiments: (1) The precipitation is not affected over the pH range from 6.0 to 8.0. It should be noted that this is most probably due to the very low concentration of phosphate buffer used. Donovan *et al.*<sup>3</sup> found that the precipitation of DNA by streptomycin could be prevented by phosphate buffer and the concentration needed was dependent on pH. However, their concentrations of phosphate were about 10 times higher than that used here. (2) The higher the amount of streptomycin added, compared with the protein content of the extract, the smaller the loss of protein. With the specific resistance of the extract =  $26.3 \times 10^3 \Omega$ , and the ratio of mg streptomycin sulphate/mg protein = 0.5, the loss of protein was 35 per cent using the biuret method, and when this ratio was 1.0 the loss was 24 per cent. This latter figure compares favourably with the best results obtained by the older protamine sulphate precipitation method<sup>4</sup> and is more easily reproduced.

At concentrations of streptomycin sulphate greater than 3 mg/mg protein, the 280/260 mμ ratio of all extracts approaches a value of 0.9 (see Fig. 1). The reason for this may be that at these concentrations of streptomycin, relatively more protein than nucleic acids may be precipitated than at lower concentrations. This point was not checked, as the biuret estimation is unreliable in the presence of high amounts of streptomycin and the use of the absorbance at 280 and 260 mμ to estimate protein<sup>5,6</sup> is too inaccurate while this ratio is changing.

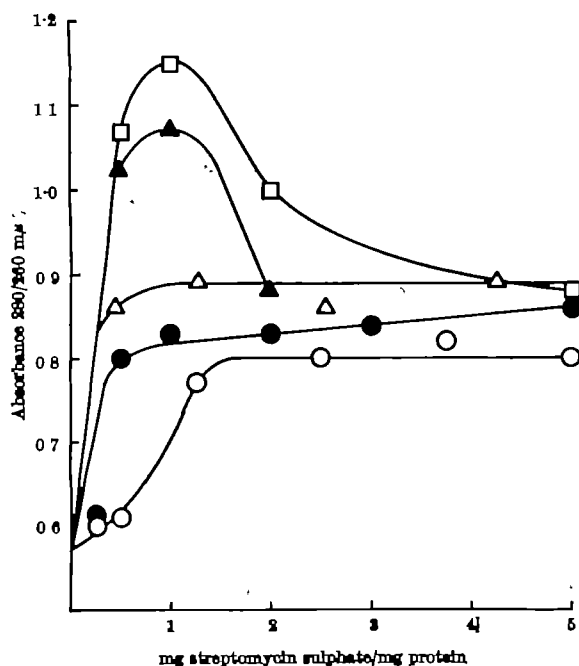


Fig. 1. Addition of 10 per cent (w/v) streptomycin sulphate, pH 7.0, to a dialysed extract from *Lactobacillus plantarum*. ○, Specific resistance (SR),  $1.12 \times 10^3 \Omega$ ; ●, SR,  $5.98 \times 10^3 \Omega$ ; △, SR,  $16.5 \times 10^3 \Omega$ ; □, SR,  $41.9 \times 10^3 \Omega$ .

Thus the use of streptomycin sulphate in the selective precipitation of nucleic acids from bacterial extracts, as outlined here, appears to have many advantages over the older methods. These include reproducibility, moderately low loss of protein, pH independence, combined with a convenient method of monitoring the crucial dialysis step.

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### Culture *in vitro* of *Giardia* Trophozoites from the Rabbit and Chinchilla

*Giardia* may well rank among the earliest described intestinal protozoa. Dobell<sup>1</sup> contends that these were the organisms that Leeuwenhoek in 1681 described from his own faeces. *Giardia* may cause intestinal disease in man. The presence of large numbers of these organisms in the small intestine is often associated with a syndrome that includes diarrhoea, epigastric pain and loss of appetite. Oral administration of atabrine usually results in the prompt disappearance of the symptoms and of the organisms. *Giardia* are also thought to be a cause of enteritis in chinchillas<sup>2</sup>.

The genus *Giardia* includes a variety of binucleate flagellate protozoan parasites which inhabit the intestinal tract of mammals (including man), birds, reptiles, amphibians and fish. *Giardia* occur in trophozoite and cyst form. Each trophozoite has eight flagella and a sucking disk, with which it attaches to the epithelium of the small intestine of its host. Metamorphosis to the cyst form occurs as trophozoites are swept down the intestine. It is as cysts that these organisms are transferred from host to host. Excystation takes place after swallowed cysts reach the small intestine. In a suitable environment, trophozoites multiply repeatedly by binary fission.

Despite their ubiquity, antiquity and possible pathogenicity, no member of the genus *Giardia* had been cultured *in vitro* until quite recently. In 1960, Karapetyan<sup>3</sup> described a method with which he had cultured *Giardia* trophozoites from man for seven months. Karapetyan established his culture in a complex medium in the presence of chicken fibroblasts and *Candida guilliermondii*. Once the *Giardia* were established, they continued to multiply even though the fibroblasts died out and were not replaced; in the absence of *Candida*, however, the *Giardia* failed to survive. Later, Karapetyan<sup>4</sup> reported culturing *Giardia* trophozoites from the rabbit for five months in a complex medium to which *Saccharomyces cerevisiae* was added at the time of inoculation; fibroblasts were not used. This report concerns our experience with Karapetyan's method of culturing *Giardia* trophozoites; to our knowledge no other workers have reported any attempts to use it.

We have not been able to culture *Giardia* from the rabbit using Karapetyan's method. Repeatedly, many trophozoites persist in the culture tube for 4 to 7 days, after which time their numbers decrease; no trophozoites survive more than 11 days.



Karapetyan's method involves an initial addition of yeast to the culture, then the daily replacement of two-thirds of the culture fluid with fresh medium. Reasoning that a certain number of metabolizing yeast may be necessary for *Giardia* survival, and that Karapetyan's strain of *S. cerevisiae* may have differed from ours in multiplying more rapidly in this medium, we modified the method by daily adding viable yeast as well as culture medium. This modification proved successful; using it, we have cultured *Giardia* trophozoites from the rabbit for 18 months, and *Giardia* trophozoites from the chinchilla for 14 months. Our success suggests the possibility that these two organisms can be cultured indefinitely with this method.

*Giardia* trophozoites for inocula were obtained from animal intestine with both the methods fully described by Karapetyan<sup>4</sup>. Stock cultures were kept and examined in 16 × 125 mm Leighton type tissue culture tubes (Bellco Glass, Inc., Vineland, New Jersey) plugged with silicone rubber stoppers. Organisms to be examined with the oil immersion objective or the phase microscope were cultured in tissue culture chambers of the type developed by Rose<sup>5</sup>. The procedure for the establishment of cultures is shown in Table 1.

Day	Removed from tube	Table 1 Added to tube
0	—	2.0 ml. Karapetyan's medium No. 1 (K) 0.2 ml. yeast suspension (Y) 0.1 ml. inoculum 1.0 ml. K
2	2.0 ml. culture fluid	0.1 M NaHCO <sub>3</sub> to bring pH to 7.2-7.4 1.0 ml. K 0.2 ml. Y 0.1 M NaHCO <sub>3</sub> as above, if necessary
3	1.7 ml. culture fluid	1.5 ml. K 0.2 ml. Y 0.1 M NaHCO <sub>3</sub> as above, if necessary

The procedure used on day 3 is used on all subsequent days.

As described by Karapetyan<sup>4</sup>, medium No. 1 contains chick embryo extract, human serum, a tryptic meat digest and Hanks's solution. The yeast suspension is made by dispersing one loopful of a 48-h culture of *S. cerevisiae* from a Sabouraud dextrose agar slant in 1.0 ml. of medium No. 1. Incubation temperature is 37° C.

The trophozoites attach to, and eventually completely populate, the flat inner surface of the Leighton tube. This surface is scanned microscopically each day, and the approximate number of organisms per low-power field is noted. Tubes which contain about ten trophozoites per low-power field on the day after inoculation often have about 1,000 trophozoites per low-power field 5 to 8 days later. Culture fluid from such tubes contains many trophozoites and can be used as inoculum to make subcultures by the method described above. The formation of *Giardia* cysts has never been observed in 18 months of daily examination.

We have tried repeatedly to maintain established cultures of *Giardia* trophozoites in Karapetyan's medium without added viable yeast. Consistently, the *Giardia* in such cultures have died in 1-2 weeks. Resumption of daily additions of yeast to tubes almost devoid of trophozoites results in vigorous growth and division of the protozoa until many thousands of the organisms are present per low-power field. We have tested three strains of *S. cerevisiae* in the above system. When added daily, all proved capable of supporting the growth of *Giardia*. No yeast derivative that we have tested will substitute for the living yeast in this system. Morphologically, *Giardia* trophozoites from the rabbit (Fig. 1) and chinchilla are indistinguishable. Organisms from both animals average about 8 mc in width and 13 mc in length. Considerable size variation is observed, since many of these organisms are in the process of division.

The culture of *Giardia* trophozoites *in vitro*, described here, is one step in an effort to culture members of this genus axenically. Though there are obvious limitations to the amount of physiological information to be obtained



Fig. 1. Photomicrograph of living *Giardia* trophozoites from the rabbit (× c. 960)

from *Giardia* growing in mixed cultures, it is, nevertheless, possible to investigate a number of characteristics of these organisms, such as morphology, response to chemotherapeutic agents and preservation at low temperatures.

We thank the Medical Research Foundation of Oregon, Inc., for grants in aid of this work, and Judith Chadd and Loraine Schwartz for their help.

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## GENETICS

### Atypical Duffy Blood Group Inheritance and Chromosome Abnormalities

Baikie, Garson and Birrell report in the following communication their discovery of a child who was a mosaic of cells trisomic for a chromosome of the group 17-18 and cells trisomic for a chromosome of the group 13-15. Three cells of a total of 60 were trisomic for both chromosomes. The child had many congenital abnormalities most of which are associated with trisomy 17-18, but microphthalmia and posterior prominence of the heels which occur in trisomy 13-15 were also present.

Table 1 shows the blood group results obtained by one of us (R. T. S.) on blood samples from the father, mother and child.

	ABO	MNSs	Rh	P <sub>1</sub>	Le <sup>a</sup>	Fy <sup>a</sup>	Fy <sup>b</sup>	K	Jk <sup>a</sup>	Lu <sup>a</sup>	Lu <sup>b</sup>
Father	A <sub>1</sub>	MNSs	rh	—	—	+	—	—	—	—	+
Mother	A <sub>2</sub> B	MNSs	Rh <sub>1</sub> Rh <sub>2</sub>	—	—	—	+	—	+	—	+
Child	A <sub>1</sub>	MNSs	Rh <sub>1</sub> rh	+	—	—	+	—	—	—	+

An abnormality of inheritance of the Duffy group was found, in that the father was apparently Fy(a+b-), the mother Fy(a-b+) and the child Fy(a-b+). Thus, while the father appears to carry two Fy<sup>a</sup> genes, the child is Fy(a-).

The present finding is the sixth reported observation of an anomaly of the Duffy blood group inheritance in a

family in which chromosomal abnormality also occurred. The first report was that of Chown, Lewis and Kaita<sup>1</sup>, who found, among 22 families with a mongol child, three with atypical inheritance of the Duffy blood group system. One of the families also showed a possible abnormal inheritance of the ABO system. In all three families a parent of the phenotype Fy(a+b-), whose genotype would ordinarily be assumed to be Fy<sup>a</sup>Fy<sup>b</sup>, had at least one Fy(a-b+) child, while agglutination times indicated that some of the Fy(a+b-) individuals, both parents and children, had only a single dose of Fy<sup>a</sup>. Only one mongol had been recognized in each of the three families studied by Chown *et al.*<sup>1</sup> and the anomalies of blood group inheritance were not found in mongol offspring only. It was thought possible that there may be a genetic factor affecting the expression of a Duffy gene, and perhaps an ABO gene too, which may predispose to non-disjunction of chromosome 21. The same authors<sup>1</sup> have since reported results in 53 more families with a mongol propositus, among whom they found two more families with the same Duffy anomaly. The parents whose red cells had reacted as Fy(a+b-) were later re-tested with a very high-titre incomplete anti-Fy<sup>b</sup> serum and a very weakly positive indirect Coombs reaction resulted. It was then shown that after sensitizing the same red cells with the potent anti-Fy<sup>b</sup> serum an eluate could be obtained which reacted specifically as anti-Fy<sup>b</sup>. It thus appeared that the occurrence of non-disjunction affecting chromosome 21 may be related in some families to either suppression of Fy<sup>b</sup> or the presence of another allele in the Duffy blood group system.

In the present family the father's apparently Fy(a+b-) red cells were carefully re-tested with both complete and incomplete potent anti-Fy<sup>b</sup> sera using three techniques, namely indirect Coombs, agglutination enhancement, and elution. The presence of a small amount of Fy<sup>b</sup> antigen was indicated by each of the three methods used. Our findings are thus analogous to those of Chown *et al.* The important difference is that in the present family non-disjunction involved chromosomes other than chromosome 21 and occurred several times, if not a great many times. The possible association between errors of cell division giving rise to chromosomal abnormalities, and atypical inheritance of the Duffy blood group system, is thus strengthened.

Race and Sanger<sup>2</sup> presented details of six Caucasian families whose Duffy phenotypes could not be explained by the genes Fy<sup>a</sup> and Fy<sup>b</sup> alone. Two of the anomalies relate to one parent who was Fy(a+b-) and did not transmit Fy<sup>a</sup> to some children, while in three others, one parent who was Fy(a-b+) did not transmit Fy<sup>b</sup> as expected. In the sixth Caucasian family living in Texas, a son was found to be Fy(a-b-) and there was no known Negro ancestry. Race and Sanger consider that a Fy(a-b-) condition must be present in Caucasians and that the genetical background is the same as that responsible for the Fy(a-b-) type commonly found in Negroes. The Fy(a-b-) type has also been found in Yemenite and Iraqi families by the same authors.

We have ourselves, like Chown *et al.*, been able to prove that a parent apparently Fy(a+b-) who did not transmit Fy<sup>a</sup> did, in fact, possess some Fy<sup>b</sup> antigen demonstrable only with potent anti-Fy<sup>b</sup> antisera, and using special techniques for its detection. It would appear that the male parent of the present doubly trisomic baby possesses a third Duffy allele or, alternatively, the expression of Fy<sup>a</sup> in the child has been suppressed either as a consequence of the chromosomal abnormalities, or possibly as a concomitant of a special liability to errors of cell division. This last possibility is in accord with the finding<sup>1</sup> of anomalous Duffy inheritance in individuals who were themselves of normal chromosome constitution. The present observations do not allow us to choose between these possibilities, and the study is proceeding

to determine whether the male parent possesses a Duffy antigen which can be shown to be identical with any of the low-frequency blood antigens not at present known to be part of any particular blood group system.

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<sup>1</sup> Chown, B., Lewis, M., and Kaita, H., *Amer. J. Hum. Genet.*, **14**, 301 (1962).

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### Mosaicism for Trisomy 17-18 and Trisomy 13-15 in Man

TRISOMY for two chromosomes of the normal chromosome complement has been found in several human subjects. The chromosomes most often involved in these double trisomic states have been the X-chromosome<sup>1,2</sup> and chromosomes of the pairs 13-15<sup>3</sup>, 17-18<sup>1,4</sup> and 21<sup>2,4</sup>. In addition, male subjects with 48 chromosomes have been described in whom one extra chromosome was an X-chromosome, giving the sex chromosome constitution XXY, together with true trisomy for an autosome<sup>5,6</sup>. Hitherto, trisomies 13-15 and 17-18 have not been found in one individual and all the reported cases of double trisomy have apparently had only one cell line.

We recently examined a new-born female child with multiple congenital abnormalities. Chromosome investigations on cultures of peripheral blood leucocytes showed that of 60 dividing cells 39 were trisomic for a chromosome of the pairs 17-18 and 18 were trisomic for a chromosome of the group 13-15. Three cells had 48 chromosomes and were apparently trisomic for a chromosome of both these groups. Buccal mucosal cells were normally chromatin positive. Ten drumsticks were found in 4,000 polymorphonuclear leucocytes in the peripheral blood. It thus seems possible that the child developed from a doubly trisomic zygote of 48 chromosomes and that loss of one or other additional chromosome occurred very early in development. Since no normally constituted cells were found it seems less likely that loss of the additional chromosomes occurred selectively and progressively throughout intra-uterine development.

The child was born at term after a normal pregnancy to a mother aged twenty-six who has had no other pregnancies. The father was aged thirty-one and has one normal child of a previous marriage. Both parents are of normal chromosome constitution. The obvious congenital abnormalities were micrognathia, left microphthalmia, malformed ears, folds of redundant skin and subcutaneous tissue around the neck, dislocation of the left hip, prominent heels, short and dorsiflexed great toes, and partial syndactyly of other toes. In addition, a loud systolic heart murmur was heard and the arms were held in the 'surrender position' with fingers flexed and the index fingers overlapping the others. Most of these abnormalities are associated with trisomy 17-18, the exceptions being microphthalmia and undue prominence of the heels which are commonly found in trisomy 13-15. The child died at the age of six weeks. At autopsy the heart had a high interventricular septal defect and the ductus arteriosus was incompletely closed. The brain was not grossly abnormal apart from an unusually small cerebellum.

The results of blood group examination of the parents and child indicate a possible anomaly of inheritance of

the Duffy group system. These are reported in the previous communication.

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## PSYCHOLOGY

### Learning in the Polychaete *Nereis*

THE rapid withdrawal reflex of the polychaetes *Nereis diversicolor*, *N. pelagica* and *Platynereis dumerilii* habituates rapidly to sudden stimuli, such as changes in illumination or mechanical stimuli, but if a foreign stimulus is interpolated in a series of stimulus presentations it is usually followed by greater reactivity to the original stimulus. Responses can sometimes be induced to sudden decreases in light intensity in completely habituated worms by the interpolation of a single tactile stimulus. This process of reflex sensitization has already been described in the polychaete *Meroierella enigmatica*<sup>1</sup>.

Most species of *Nereis* are tubicolous or burrowing animals which come to the ends of their tubes or burrows in order to feed. When feeding they are highly susceptible to attack from a wide variety of predators including fishes, seabirds and crustaceans. Habituation of the rapid withdrawal reflex is usually interpreted as allowing for a compromise between the conflicting needs of the animal: to respond to stimuli caused by predators but not to respond to repeated innocuous stimulation such as shadows caused by passing clouds and seaweed floating in the water<sup>2</sup>. Sensitization may be of biological importance because it enables habituated *Nereis* to react to a previously innocuous stimulus if there is a sudden change in the background stimulation.

Recently it has been shown that either sensitization or similar processes due to repeated stimulation by an unconditioned stimulus (US) can be important in other learning situations. In procedures designed to demonstrate classical conditioning and avoidance learning in *Nereis* the effects of the US have been responsible for modifications in behaviour which might otherwise have been interpreted as associative learning.

Attempts to condition the rapid withdrawal reflex in *N. diversicolor* have been made by pairing a sudden change in illumination (CS), to which *Nereis* habituates, with an electric shock (US), which invariably elicits withdrawal. Controls subjected to the CS alone habituate to it whereas worms subjected to a series of US-CS pairings become more reactive to the CS. However a similar effect can be produced by presenting the US between trials so that it is not paired with the CS (Fig. 1). Furthermore, controls habituating to CS presentations become more reactive if one or more groups of ten consecutive electric shocks are given between trials. Sensitization of the withdrawal reflex by repeated effects of the electric shocks is clearly a more tenable interpretation of these data than one of classical conditioning.

The repeated effects of US stimulation have also been shown to have an important effect in a learning situation

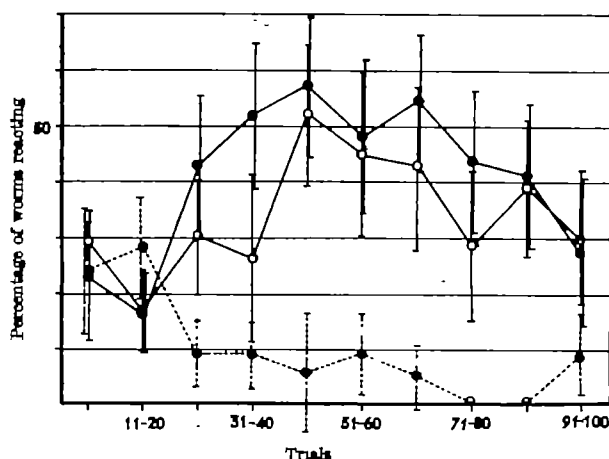


Fig. 1. Percentage of worms *N. diversicolor* reacting to sudden decreases in light intensity (CS) during the following training procedures in which an electric shock has been used as a US: CS-US pairing (continuous line, open circles); US between CS presentations (continuous line, filled circles); CS presented alone (broken line). Standard errors are indicated by the vertical lines.

previously described by the author<sup>3</sup> in which individual *N. diversicolor*, *N. virens* and *P. cultrifera* could learn to avoid an electric shock at the end of a 'Perspex' channel. Worms introduced into the channel crawl rapidly to the end and continue to do so indefinitely as long as they are not punished in any way. However, the behaviour of worms given slight electric shocks, whenever they reach the end of the channel, becomes modified with training so that they either reverse in the channel before reaching the electrodes or refuse to crawl along it. It has now been shown that electric shocks given outside the apparatus are as effective in inducing this modified behaviour as shocks received at the exit of the channel, and that shocks given to worms with no experience in the channel have the same effect on subsequent performance as shocks given in the apparatus. Strong tactile stimulation as a punishment has been shown to have a similar effect. Although an explanation of fatigue can be excluded because trained worms crawl actively outside the apparatus and refusal itself often involves an active reversal by the worm, there is no doubt that the acquired behaviour cannot satisfactorily be regarded as associative learning. It is more likely to be the result of a process similar to sensitization of the reversal response, which is part of the worm's normal tubicolous behaviour<sup>4</sup>, and a mechanism which inhibits forward movement in a confined space, such as a channel or burrow, when the worm is harmfully stimulated.

The importance of sensitization and processes producing similar effects has yet to be evaluated. The effects of these processes can be regarded as learning in the sense that they are modifications of the animal's behaviour as a result of experience but clearly cannot be regarded as manifestations of associative learning. Although similar processes have not been demonstrated in *Nature*, the possibility of widespread sensitization and non-associative learning mechanisms in the lower invertebrates cannot be excluded. Indeed, it has already been suggested<sup>5</sup> that the conditioning claimed in flatworms<sup>6</sup> may be due to the sensitization effects of electric shocks. A study of these processes may be valuable because they may have been important in the evolution of the more refined learning mechanisms of vertebrates and higher invertebrates.

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## FORTHCOMING EVENTS

## Tuesday, September 28

INSTITUTE OF MECHANICAL ENGINEERS, GRADUATES' AND STUDENTS' SECTION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6.30 p.m.—Prof. R. R. Lathwaite: "Propulsion Without Wheels".

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP AND APPEAL AND TEXTILE CHEMICALS GROUP (at 14 Belgrave Square, London, W.1), at 6.30 p.m.—Mr. D. M. Woodford: "Some Aspects of the Emulsion Polymerization of Vinylidene Chloride and the Properties of its Copolymer".

## Wednesday, September 29

INSTITUTE OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.B.E.-I.M.E. COMPUTER GROUPS (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.O.1), at 10.30 a.m.—conference on "Airborne Computers".

AFRICAN STUDIES ASSOCIATION OF THE UNITED KINGDOM (at University College, London, W.O.1), from 10.30 a.m. to 5 p.m.—Symposium on "The Oil Resources of Tropical Africa".

INSTITUTE OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.B.E.-I.M.E. MEDICAL ELECTRONICS GROUP (at 9 Bedford Square, London, W.O.1), at 6 p.m.—Dr. P. M. McGuff: "Treatment of Experimental Animal and Human Malignant Tumours by Laser Radiation".

## Thursday, September 30

ROYAL MICROSCOPICAL SOCIETY, ELECTRON MICROSCOPY SECTION (in the lecture Theatre, Baden-Powell House, Queen's Gate, London, S.W.1), at 1.30 a.m.—Symposium on "The Correct Use of the Electron Microscope in the Study of Biological Specimens".

INSTITUTE OF PETROLEUM, EXPLORATION AND PRODUCTION GROUP (at 1 New Cavendish Street, London, W.1), at 5.30 p.m.—"Underwater research" (A Shell film).

TELEVISION SOCIETY (in the I.T.A. Conference Suite, 70 Brompton Road, London, S.W.3), at 7 p.m.—Mr. I. F. MacLennan: "An Introduction to Television-Testing Methods in Television".

## Thursday, September 30—Friday, October 1

INSTITUTE OF BIOLOGY (in the Lecture Hall, Royal Geographical Society, Kensington Gore, London, S.W.7), at 10 a.m. daily—Symposium on "Man-made Lakes".

HUGHES SOCIETY (in the Botany Lecture Theatre, University College, Lower Street, London, W.C.1), at 10.30 a.m. on Thursday and 10 a.m. on Friday—Symposium on "Genetic and Environmental Factors in Human Ability".

## Friday, October 1

ROYAL MICROSCOPICAL SOCIETY, ELECTRON MICROSCOPY SECTION (in the lecture Theatre, Baden-Powell House, Queen's Gate, London, S.W.1), at 0.30 a.m.—Symposium on "Recent Advances in the Technique for Preparing Biological Specimens for Electron Microscopy".

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Prof. Frans Sondheimer: "The Influence".

## Monday, October 4

BRITISH BIOPHYSICAL SOCIETY, PHYSICAL BIOCHEMISTRY GROUP (at the school of Pharmacy, Brunswick Square, London, W.O.1), from 11 a.m. to 3.30 p.m.—Meeting on "Light Scattering".

INSTITUTE OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—discussion Meeting on "The Teaching of Mechanics of Machines".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (in the Shell Centre theatre, Shell Centre, London, S.E.1), at 6.30 p.m.—Scientific Film Evening.

## Monday, October 4—Wednesday, October 6

IRON AND STEEL INSTITUTE AND THE INSTITUTE OF METALS (at the Royal Institution, Northumberland Avenue, London, W.O.2)—Joint conference on "Machinability".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER IN PSYCHIATRY IN THE DEPARTMENT OF PSYCHOLOGICAL MEDICINE—The Secretary, The University, Edinburgh (September 30).

SENIOR LECTURER and a LECTURER (preferably with research experience) IN THE DEPARTMENT OF MATHEMATICS—The Acting Registrar and Clerk to be Governors, Portsmouth College of Technology, Hampshire Terrace, Portsmouth (September 30).

SENIOR LECTURER (with an honours degree) IN MATHEMATICS—The Director of Education, The Polytechnic, Regent Street, London, W.1 (October 1).

ASSISTANT (honours graduate) IN THE DEPARTMENT OF GEOGRAPHY—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (October 4).

CHAIR OF MATHEMATICS—The Secretary, Royal College of Advanced Technology, Salford 5 (October 4).

RESEARCH ASSISTANT IN THE DEPARTMENT OF PSYCHOLOGY to work with Dr. R. Lynn on a developmental investigation of autonomic reactivity and adaptation—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (October 8).

DEMONSTRATOR (registered member of the veterinary profession) IN VETERINARY MEDICINE in the Royal (Dick) School of Veterinary Studies—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh, 8 (October 9).

LECTURER or JUNIOR RESEARCH FELLOW IN PHYSIOLOGY IN THE DEPARTMENT OF PHYSIOLOGICAL SCIENCES, University of Lagos Medical School—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.O.1 (October 10).

RESEARCH ASSISTANT (graduate in engineering or applied mathematics with a higher degree or equivalent research experience) IN THE DEPARTMENT OF CIVIL ENGINEERING, to investigate the possibilities of solving field problems in various branches of applied mechanics, by utilizing finite-element solutions developed for analogous problems in stress analysis—The Registrar, The University, Leeds, 2 (October 11).

LECTURER IN CLINICAL VIROLOGY AT THE UNIVERSITY DEPARTMENT OF INFECTIOUS DISEASES, Rushlin Hospital—The Secretary of University Court, The University, Glasgow (October 15).

LECTURER (with a distinguished academic record) IN THE DEPARTMENT OF PHYSICS, Wollongong University College, University of New South Wales—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 15).

SENIOR LECTURER and a LECTURER (with a Ph.D. degree and postdoctoral research experience) IN ORGANIC CHEMISTRY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, October 15).

LECTURER IN THE PHYSIOLOGY DEPARTMENT—The Secretary, Royal Free Hospital School of Medicine (University of London), Hunter Street, London, W.O.1 (October 18).

LECTURER/ASSISTANT LECTURER (2) IN COMPUTING (for one post a knowledge of numerical analysis would be an advantage)—The Assistant Registrar (Science), The University of Birmingham, Birmingham, 15 (October 18).

S.R.C. RESEARCH ASSISTANT (with a good honours degree and research experience in experimental physics, and preferably a Ph.D.) IN GEOPHYSICS IN THE DEPARTMENT OF GEOLOGY, for research concerned with properties of rocks and mineral crystals at high temperatures and pressures relevant to deep-seated processes which occur in the earth—The Secretary, University College, Gower Street, London, W.O.1 (October 18).

SENIOR RESEARCH ASSOCIATE and a RESEARCH ASSOCIATE (with research experience in genetics, preferably in the fields of biometrical genetics, population genetics, genealogy or fungal genetics) in the Department of Genetics—The Assistant Registrar (Science), The University of Birmingham, Birmingham, 15 (October 18).

ASSISTANT LECTURER IN CHEMISTRY IN THE DEPARTMENTS OF BOTANY AND ZOOLOGY—The Registrar, The University, Manchester, 13, quoting Ref. 174/65 (October 20).

SENIOR LECTURER or LECTURER IN GEOLOGY WITHIN THE FACULTY OF SCIENCES, Ahmadu Bello University, Northern Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.O.1 (October 20).

SENIOR LECTURER (ELECTRONICS) (graduate with considerable industrial or other postgraduate experience in semiconductor applications) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING; and a LECTURER (graduate with industrial or other postgraduate experience, preferably with a special interest in hydrology) IN THE DEPARTMENT OF CIVIL ENGINEERING, University College, Nairobi (University of East Africa)—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.O.1 (October 25).

SENIOR LECTURER or LECTURER IN MATHEMATICS at the University of Basutoland, Bechuanaland Protectorate and Swaziland—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.O.1 (October 25).

LABORATORY TECHNICIAN or SENIOR LABORATORY TECHNICIAN IN THE DEPARTMENT OF BIOCHEMISTRY AND NUTRITION—The Registrar, University of New England, Armidale, New South Wales, Australia (October 25).

READER IN INFORMATION PROCESSING; and a READER IN COMPUTER SCIENCE in the Institute of Computer Science—The Academic Registrar, University of London, Senate House, London, W.O.1 (October 29).

SENIOR LECTURER and a LECTURER IN PHYSICS at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 29).

CHAIR OF ORGANIC CHEMISTRY—The Registrar, University of Kent at Canterbury, Canterbury (October 30).

SENIOR DEMONSTRATOR (with an honours degree in science with biochemistry as a major subject) IN BIOCHEMISTRY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, October 30).

LECTURER IN PLANT PHYSIOLOGY—Prof. O. P. Whittingham, Botany Department, Imperial College, Prince Consort Road, London, S.W.7 (October 31).

LECTURER or SENIOR LECTURER (specialized in mineralogy and geochemistry); an ASSISTANT LECTURER (with basic training in geology and a special knowledge of geomorphology); and a DEMONSTRATOR (with a special knowledge of mineralogy and petrology) IN THE DEPARTMENT OF GEOLOGY AND MINERALOGY, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 31).

LECTURER (with an active interest in educational sociology or educational philosophy) IN EDUCATION at Massey University of Manawatu, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 31).

READER and a SENIOR LECTURER IN MICROBIOLOGY; and a READER and a LECTURER IN ZOOLOGY at the University of Adelaide, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, October 31).

HEAD (specialist in plant breeding) OF THE DEPARTMENT OF PLANT SCIENCE, Institute of Animal Science, Havana, Cuba—The Chargé d'Affaires, The Cuban Embassy, 22 Mount Street, London, W.1 (November 1).

POST-DOCTORAL RESEARCH ASSISTANT (BIOCHEMIST) to take over and develop a programme of research on the effects of gene suppressor mutations on protein synthesis in yeast—The Administrator, Botany School, The University, Oxford (November 1).

LECTURER (with a degree in engineering or in science or an equivalent qualification, and preferably practical experience in the mineral industry) IN MINERAL TECHNOLOGY, University of Otago, Dunedin, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or the Registrar of the University (New Zealand and London, November 30).

SENIOR LECTURER (with a good honours degree in engineering or science with several years of research and/or industrial and/or teaching experience) IN THE DEPARTMENT OF CHEMICAL ENGINEERING, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 30).

**ASSISTANT EXPERIMENTAL OFFICER** (with a pass degree, H.N.C. or equivalent, or if under 22, 2 G.O.R. "A" levels or maximum) in the Department of Radiobiology, for work involving the assay, synthesis and study of the metabolism of isotopically labelled compounds—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Berkshire, quoting Ref. 64/15.

**ASSISTANT EXPERIMENTAL OFFICER** (with a suitable degree, Dip. Tech., H.N.C. (or equivalent) or, if under age 22, two G.O.R. "A" levels in scientific subjects, a sound knowledge of physics and a keen interest in astronomy) for research into the physics of stars and galaxies—The Secretary, Royal Observatory, Edinburgh, 9.

**ASSISTANT LECTURER IN THE DEPARTMENT OF MOLECULAR BIOLOGY** to participate in the work of an M.R.C. Research Group on bacterial enzyme variation, involving the application of biochemical, biophysical and chemical techniques to the study of the specific control of enzyme biosynthesis in bacteria—Prof. M. R. Pollock, Department of Molecular Biology, University of Edinburgh, The King's Buildings, Edinburgh, 9.

**ASSISTANT TRIALS OFFICER** (with training in botany or agriculture and a recognised degree or national diploma) at the NATIONAL INSTITUTE OF AGRICULTURAL BOTANY TRIALS OFFICER, Seale-Hayne, Newton Abbot, Devon, for work on small-scale field trials on new varieties of farm crops—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

**CHEMISTS** (with a good honours B.Sc. degree and preferably of Ph.D. standing) to work in fields such as relation between structure and properties in polymeric materials, modification of polymer properties, high temperature resistant surface coating agents, and the application of reverse osmosis to the desalination of water and to non-aqueous systems—The Director, Arthur D. Little Research Institute, Inverkeithing, Midlothian.

**ENTOMOLOGIST (THESE)** (national of the United Kingdom or the Republic of Ireland, with an honours degree in natural science, entomology or zoology) in Zambia (Northern Rhodesia), to plan and assess control measures involving surveys of fly incidence and its relation to local ecological factors, to determine the most suitable methods of control, and to supervise Tsetse Control Staff—The Appointment Officer, Ministry of Overseas Development, Room 301, Mand House, Stag Place, London, S.W.1, quoting Ref. BO 517/132/01.

**GRADUATE BIOCHEMIST or CHEMIST** to assist in the study of enzymes occurring in cerebral tumours—The Secretary, Institute of Psychiatry, The Maudsley Hospital, Denmark Hill, London, S.E.5.

**HOSPITAL BIOCHEMIST** in the Area Laboratory—The Secretary, Whipps Cross Hospital, London, N.11.

**JUNIOR RESEARCH OFFICER or RESEARCH OFFICER** (preferably post-doctoral) in the DEPARTMENT OF PHYSICS to assist in the analysis of rocket and satellite data—The Registrar, University College of Wales, Aberystwyth.

**LECTURER IN INORGANIC CHEMISTRY**—The Academic Registrar, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

**LECTURER** (preferably with a special interest in thermodynamics and applied mechanics or strength of materials) in MECHANICAL ENGINEERING at the University of the West Indies (Trinidad)—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1.

**POST-DOCTORAL FELLOW IN ORGANIC CHEMISTRY** to work in collaboration with Dr. N. A. J. Rogers, on a study of the protonation of conjugated enolic derivatives—The Secretary, University of Lancaster, Bailrigg House, Lancaster.

**RESEARCH ASSISTANT** (with a qualification, not necessarily a degree, in physics and/or chemistry) in the DEPARTMENT OF INORGANIC AND STRUCTURAL CHEMISTRY, for duties which will include the operation of electronic equipment for X-ray crystallography—Dr. M. R. Truter, School of Chemistry, The University, Leeds, 2.

**SENIOR LECTURER** (preferably with interests in mathematical logic or numerical analysis or control theory) in the DEPARTMENT OF MATHEMATICS—The Registrar, Hatfield College of Technology, Hatfield, Hertfordshire.

**SOIL SURVEYOR** (honours graduate in natural science, agricultural science, or agriculture, with experience in soil surveying, preferably in the tropics) in the REGIONAL RESEARCH OFFICER, Trinidad (University of the West Indies)—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

Ministry of Overseas Development. Overseas Development: The Work of the New Ministry. (Cmd. 2786.) Pp. 74. (London: H.M. Stationery Office, 1965.) 5s. 6d. net.

Bulletin of the British Museum (Natural History). Zoology. Vol. 12, No. 8: Acanthodrilidae and Budrilidae (Oligochaeta) from Ghana. By B. W. Sims. Pp. 233-311. 10s. Vol. 12, No. 9: The Ophid Fishes of Lake Nabugabo, Uganda. By P. H. Greenwood. Pp. 313-357. 15s. Vol. 12, No. 1: Primitive Cryptostigmatid Mites from Rhododendron Forests in the Nepal Himalayas. By J. G. Shearer. Pp. 1-35. 18s. The Ophiote Mosquitoes of the Indomalayan Area. Part 6: Genus *Aedes* Meigen. Subgenus *Stegomyia* Theobald (Groups A, B and D). By P. F. Mattingly. Pp. 67. 24s. (London: British Museum (Natural History), 1965.)

Proceedings of the British Ceramic Society, No. 4 (July 1965): Load-bearing Brickwork. Pp. 121. (Stoke-on-Trent: British Ceramic Society, 1965.) 25s.

Royal Observatory Bulletin. No. 95: Photometry of the Cluster NGC 6522. By S. V. M. Clube. Pp. B383-B405+2 plates. 4s. 6d. net. No. 96: Time and Latitude Service 1964, July-September. Pp. 1+B371-B385. 2s. net. (London: H.M. Stationery Office, 1965.)

Camlab (Glaxo). Ltd. Camlab Guide to Thin-layer Chromatography. Second edition, including Column Chromatography and Electrophoresis. Pp. 80. (Cambridge: Camlab (Glaxo), Ltd., 1965.)

The Wildfowl Trust. Sixteenth Annual Report 1963-64. Illustrated by Peter Scott. Pp. 136+22 photographs. (Brimbridge, Gloucestershire: The Wildfowl Trust, 1965.)

National Society for Clean Air. Clean Air Year Book, 1965-66. Pp. 120. (London: National Society for Clean Air, 1965.) 5s.

Commonwealth Agricultural Bureaux. Technical Communication No. 16: The Meat Production Potential of Wild Animals in Africa—a Review of Biological Knowledge. By Leo M. Talbot, W. J. A. Payne, H. P. Ledger, Lorna D. Verdcourt and Martha H. Talbot. Pp. vi+42. (Farnham Royal, Bucks: Commonwealth Agricultural Bureaux, 1965.) 12s. 6d.

European Ceramic Association. Report of a Meeting to consider Particle Size Measurement arranged by the Basic Science Committee, held at the British Ceramic Research Association, Stoke-on-Trent, Friday and Saturday,

1st and 2nd November, 1963. Pp. 20. (Stoke-on-Trent: The British Ceramic Society, 1965.) 5s.

Oiba (A.R.L.), Ltd. Technical Notes (July, 1965): Analite in 1 Restoration of Roman Mosaics. Pp. 12. (Dunford: Oiba (A.R.L.), Ltd., 1965.)

Forestry Commission. Forty-fifth Annual Report of the Forestry Commission for the year ended 30th September, 1964. Pp. 67+8 plates. 2s. 6d. net. Bulletin No. 57: Experiments on Nutrition Problems in Forest Nurseries. Vol. 1: Pp. xi+251+25 plates. 50s. net. Vol. 2: Pp. v+204 20s. net. Forest Record No. 53: Studies on the Mineral Nutrient Status of Heather, *Calluna vulgaris*. By J. R. Aaron. Pp. 23. 2s. net. Forest Record No. 55: Death of Pedunculate Oak and Variations in Annual Radial Increments Related to Climate. By O. W. T. Young. Pp. 16. 2s. net. Library Review, No. 1, March 1965. Pp. 27. (London: H.M. Stationery Office, 1965.)

### Other Countries

Bulletin of the American Museum of Natural History. Vol. 129, Article Hybridization in the Flickers (Colaptes) of North America. By Lester Short, Jr. Pp. 307-428. (New York: American Museum of Natural History, 1965.) 2 dollars.

Republic of South Africa: Department of Commerce and Industries Division of Sea Fisheries Investigation Report No. 51: South Africa Contribution to the International Indian Ocean Expedition. (3): Cru. 251 of R/S *Afronius II* during June/July 1961. By R. W. Rand. A Preliminary Report on the Planktonic Crustaceans. By A. de Deel and F. J. Mombbeck. (Reprinted from *Oceanus and Industry*.) Pp. (Pretoria: Government Printer, 1965.)

Food and Agriculture Organization of the United Nations. World Food Program Studies, No. 5: The Role of Multilateral Food Aid Programs. J. Jan Demau. Pp. v+28. (Rome: Food and Agriculture Organization of the United Nations; London: H.M. Stationery Office, 1965.) 5s.; 1 dollar.

Organization for Economic Co-operation and Development: European Nuclear Energy Agency. O.N.E.A. High Temperature Reactor Project "Dragon"—Sixth Annual Report, 1964-1965. Pp. 120. (Paris: Organization for Economic Co-operation and Development, European Nuclear Energy Agency; London: H.M. Stationery Office, 1965.)

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Bulletin of the Florida State Museum—Biological Sciences. Vol. 9, No. 6: The Pleistocene Follies of Florida. By Bjorn Kurten. Pp. 215-277. Vol. 9, No. 7: The Cranial Anatomy of the Hog-nosed Snake (*Heterodon*). By W. G. Weaver, Jr. Pp. 275-304. (Gainesville: Florida State Museum, 1965.)

Southern Rhodesia. Report of the Trustees of the Museums of the Nations. Southern Rhodesia for the year ending December, 1964. Pp. 25. (Salisbury: National Museums of Southern Rhodesia, 1965.)

Australia: Commonwealth Scientific and Industrial Research Organization. Land Research Series, No. 14: Land Use in the Murrumbidgee-Kalbarri Area, Territory and Papua and New Guinea. Papers by J. A. Mabbitt, P. O. Heyliger, B. M. Scott, J. G. B. Smith, A. Fitzpatrick, J. R. MacAlpine and B. Pullen. Pp. 123+12 plates. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1965.)

World Health Organization. Public Health Papers, No. 26: Domestic Accidents. By Prof. B. Maurice Backlund. Pp. 137. (Geneva: World Health Organization; London: H.M. Stationery Office, 1965.) 6 Sw. franc. 10s.; 2 dollars.

Metropolitan Life Insurance Company. Statistical Bulletin, Vol. (May 1965): Marriages Continue to Increase. Births in Plural Confinement Perinatal Mortality Decreasing. More Than 5,000 Drowning a Year in the United States. Pp. 12. (New York: Metropolitan Life Insurance Company, 1965.)

Proceedings of the California Academy of Sciences—Fourth Series Vol. 22, No. 12 (July 23, 1965): Studies on the Atlantic American Pipefishes with Descriptions of New Species. By Earl S. Herald. Pp. 363-376. Vol. 22, No. 1 (July 23, 1965): The Brachyuran Decapod Crustaceans of Offington Island. By John B. Garth. Pp. 1-46. Vol. 22, No. 2 (July 23, 1965): On the Identification of *Schizoglyphus californiensis* Conrad, a Californian Pliocene Gastropod. By W. O. Addicott. Pp. 47-68. (San Francisco: California Academy of Sciences, 1965.)

Forest Research in India, 1961-62. Part 1: The Forest Research Institute Dehra Dun. Pp. ii+181. Rs. 2.90; 6s. 10d. Forest Research in India, 1963-64. Part 1: The Forest Research Institute, Dehra Dun. Pp. ii+16. Rs. 3.75; 8s. 9d. (Delhi: Manager of Publications, 1965.)

Records of the Australian Museum, Vol. 26, No. 12 (4th December, 1964): Metrical Features of Aboriginal Crania from Coastal New South Wales Australia. By L. Freedman. Pp. 309-323. (Sydney: The Australian Museum, 1964.) 4s.

Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem. Heft 115 (Juni 1965): Deutsche Pflanzen schutz-Tagung der Biologischen Bundesanstalt für Land- und Forstwirtschaft in Wiesbaden, 12-18 Oktober 1964. Pp. 236. (Berlin-Dahlem: Biologische Bundesanstalt für Land- und Forstwirtschaft, 1965.) 44 D.M.

National Academy of Sciences—National Research Council. Publication 1276: Geochronology of North America. (Nuclear Science Series—Report No. 41.) Pp. v+816. (Washington, D.C.: National Academy of Sciences—National Research Council, 1965.) 6 dollars.

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